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# STUDIES ON THE EVOLUTION OF THE WINE LABEL, FROM ART TO BRAND

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***Keywords***: *label, wine, grape crop, quality, classification*

## ABSTRACT

*Wine label is the first item with which we come into contact when looking at a bottle of wine. It has a long history and it has gradually started to have a major importance in the process of knowing the wine we drink.We propose to approach this interesting subject here by presenting a history of wine label and reaching the current legal standards dealing with all specific situations in this field. The emphasis is laid on the rightful standards of intellectual property and the information included in the wine label, more and more complete and interesting. We shall ponder on a more special label pattern, i.e. the German wine label pattern, because it is one of the most complete patterns from the point of view of the information contained.*

## INTRODUCTION

Label is for wine a true autobiography, an open book intended to convey everything there is to know about the living being behind it.

As it is an instrumental item to the win bottle, label has a history almost as old as wine. The oldest wine label dates from the late 1600's- the beginning of the 1700 years and it was created by a French monk, named Pierre Perignon.

Beside the importance and the winemaker's mastery for the consumer is also very important the wine presentation, what it is inside that bottle, and the information given by the producer being essential for the consumers choice.

## MATERIAL AND METHODS

This approach's goal is to study wine label via the information it contains on the one side, and from the legislative standpoint on the other side. Also, specific legislation is also an objective of our research.

The methods used are logical – legal methods as means to analyze the rightful standards regulating the wine label field and also means to interpret by comparison. The material submitted for analysis is constituted by the legal standards regulating the wine label, as a brand, submitted to the intellectual property laws. Also, we have taken into account the artistic side of wine labels, and to that effect we have analyzed a few specific samples of wine labels as material.

## RESULTS AND DISCUSSIONS

The information contained by wine label was for the most part the same even since ancient times. What is important is that one of the oldest versions of label was found even since antiquity, and not anywhere, but at the grave of Tutankhamun who died in 1325 B.C., containing enough information about the product it features. Of course, we are talking about ancient relics which were very hard to recompose, the information reaching us only with the help of remarkable historians, archeologists and restorers.The way in which it was designed on a piece of parchment where the crop year and wine region were entered and related to the bottle's neck actually conveys what is essential, i.e. the fact that ever since the oldest times, wine had to have its birth document with all the elements containing enough information about it.

Later on, moulds made of stone were created on which data was engraved, and after that an ink roll was passed over them, after which the label was printed. After 1798, along with lithography being discovered by Alois Senefelder, wine labels enter modern age, and they are printed on large scale in a greater and greater number. The major change was, of course, that as of this period, label begins comprising more and more characteristic elements.

After the 1900's, wine label may easily have the valence of works of art. An eloquent example from our country is represented by Stirbey (Figure 1) wine labels in Dragasani, some of them executed in an art deco style.


Figure 1. Wine label in Art Deco style.


Figure 2. German Wine Label.

Beginning with the 1960s, a true progress is registered in the wine world. The consumer gets more and more educated and wine begins to be more and more associated to gastronomy, cultural environment, Bohemian encounters.

Presentations of wines start to appear in magazines, or humor or satirical publications, sketches that show representative social aspects of those times, connected with alcohol beverages and wine especially, some of them being able to be considered the first commercials for wine in our country. As it was only natural, the first legal standards began arising to regulate wine brands, wine labels content and, generally, all aspects with regard to vineyard and wine. Laws on vineyard and wine began being passed in all producing countries. Currently, label is a key element with regard to wine presentation. The features it has may be general, going to more and more specific information. No matter how we look at things, they represent an important source of information for consumers, providing graphic elements and on paper about wine type, origin and quality. Label may have a strong visual impact on the consumer and it may influence his choice.

Lately, wine producers have laid accent more and more on labels' design, investing large sums of money to change them from one crop to the other. Some wine producers, especially those from the old Wine World (Europe, especially France, Italy, Spain, Portugal) are more conservative and preserve the same graphical shape of the label for decades. However, when carefully looking at the international market, the conclusion is that the success wine from the New World is registering may also be due to audacious, unconventional labels. This is why wine producers in Europe have begun to consider a change in attitude with regard to labels' design.

The famous French producer, Mouton Cadet, had a label for every crop separately since 1945. Wines in Romania, Spain and Portugal are classified in relation to their quality level and they have a hologram on the label or on the cap certifying to this. The hierarchisation system is, however, different from one country to the other, and in France, wines can also be classified depending on the wine regions they come from. Labeling for wines produced in Romania is regulated by the methodological standards to enforce the law on wine and vineyard 244/2002, subsequently amended by law 164/2015, regulations completed by the legal standards in the intellectual property field nationally and on European level.

The standard label of a wine is to contain the producer name, bottler and import merchant, trade mark, crop year, quality category, geographical indication, wine preparation manner, as well as its alcoholic degree. As a general rule, producer's name and brand are the most criteria in selecting a wine.

An important element is the wine's crop year, because crops change from year to year. The crop year is the year the grapes were harvested, not the one the wine was bottled. For wines made by blending several wine varieties, crop year may be absent from the label. Also, wine labels include the quality category and geographical indication (wine region). Another element which may also be included in the label which provides information is the one concerning the type of wine depending on the sugars content (dry, medium dry, medium and sweet).

We believe it is necessary to introduce to the legislation the compulsoriness to state on the label the exact quantity of sugars, not just the features "dry", "medium dry", "medium" and "sweet". The fact that sugars level determining the wine's classification into one these categories is different from one country to the other is well known. For instance, in Italy, sugars level set out by legislation is a bit higher as compared to the rest of Europe. This mention would have a significant impact, having regard to the incidence of diabetes, as well as the struggle against obesity worldwide. Hence, there are various systems for mentions and labels, usually differing from one country to the other. Generally, the French system is the most widespread and many producers have inspired from this pattern.

The German pattern has special particularities and this is why we have chosen to present them next. The label of a German wine generally has the following distinctive elements: the name written in bigger letters is always the producer name. Next, there is a combination of two names, the first out of which ends in "-er" corresponding to the village, and the following is the vineyard name (for example: Kanzemer Altenberg), then the grape variety, the mention "pradikat" and crop year. Sometimes, there is also the indication "Trocken" (dry), "Halbtrocken" or "Feinherb" (medium dry). The basic particularity of German labels is the introduction of a system of mentions based upon quality which is very

thorough, so that they leave no doubt on what is about to be tasted. This rigorous system has been instituted by wine law in the year 1971 and it classifies German wines as follows: tafelwein (table wine), landwein (region wine) corresponding to the French ”vin de pays”, qualitatswein bestimmter anbaugebiete- qba (quality wine from a well delineated production region and from a single variety of grapes approved for that region, the wine may contain added sugar) (Figure 2).

The highest quality grade – qualitatswein mit pradikat – (very good quality wine, no added sugar) is accompanied by mentions detailing grape state and ripe degree on harvesting time: kabinett (grapes harvested at full ripeness grade), spatlese (grapes harvested late and generally healthy), auslese (grape selection potentially partly fortified), beerenauslese (selection of fortified grapes), trockenbeerenauslese (selection of raisined grapes), eiswein (grapes harvested after they freeze on the spindle). In 2001, the Association of German Quality Wine entered mentions for dry wine emphasizing the vine estate value too, similar to the French classification system. The appellation ”erstelage” regards class wines made from the best vineyards, called Grosse gevachse (GG), following the wines made from classified vineyards and basic wines of upper quality - gutswein. It should be noted that given the communist period, we may say we are currently benefiting from a new beginning in the field and we have the possibility to customize too, using the more advanced and well-established countries’ patterns in terms of wine producing.

## CONCLUSIONS

The wine label is nowadays the most important element for a consumer’s choice when they buy a bottle of wine, because of all the information provided. Although the label usually provides very precious information and its impact in choosing a wine is quite important, just some of those information are mandatory, being regulated by laws. We recommend that the label must also contain information regarding the wine qualities and its composition, like for example the exact sugars level of the wine.

The wine label has also become an important marketing factor, therefore the legal provisions in the field of intellectual property law are becoming increasingly important and widely applicable.

## REFERENCES

Legea nr. 244 din 29 aprilie 2002 -Legea viei şi vinului în sistemul organizării comune a pieţei vitivinicole - publicat în M.Of. nr. 333/20 mai. 2002

Legea viei şi vinului în sistemul organizării comune a pieţei vitivinicole nr. 164/2015 publicată în M.Of. nr. 472/30 iun. 2015

Legea nr. 84/1998 privind mărcile şi indicaţiile geografice, republicată în M.Of. nr. 337 din 8 mai 2014

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# BIOMETRIC CHARACTERISTICS OF VINE PROPAGATION MATERIAL AFTER TREATMENT OF VINE NURSERY WITH GARDOPRIM PLUS GOLD

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***Keywords***: *herbicide, grafted vine cuttings, vines, mature growth, roots*

## ABSTRACT

*With the aim to identify opportunities for application of Gardoprim plus Gold (312,5 g/l s-metolachlor + 187,5 g/l terbuthylazine) against annual weeds in the production of vine propagation material, reaction of six varieties grafted on rootstock Berlandieri X Riparia SO4 was studied at the Institute of Viticulture and Enology (IVE), Pleven, Bulgaria. Immediately after planting of cuttings the vine nursery was treated with testing herbicide at dose of 4 l/ha. At the end of the vegetation was made assessment of the Gardoprim plus Gold impact on some biometric indicators (length and weight of mature growth, number of roots). The results were grouped by hierarchical cluster analysis.*

*The vines of the treated variants form mature growth with greater length and weight than those of the controls. Gardoprim plus Gold does not inhibit the formation of roots and does not lead to reduction of the number of roots of a vine.*

## INTRODUCTION

Weed control in vine nurseries is performed mainly by manual weeding. The application of chemical preparations however is widely used as a preventive measure - the previous year the areas intended for the production of vine propagation material are treated with glyphosate. The number of sprays and the doses are determined depending on the species diversity and the level of weed infestation (Tonev et al. 2007). During the second half of the 20$^{th}$ century a series of studies have been carried out for defining other active substances (simazine, atrazine, trifluralin, oryzalin, oxyfluorfen, fluorhloridon, etc.) that could be applied directly to the soil surface and suppress the growth of weeds propagating by seeds for a long enough period (Bravenec and Miŝa 1978; Calastru 1982; Chelebiev and Katerova 1988; Lange et al. 1969; Litvinov et al. 1987; Prabha Challa 1987; Todorov 2005).

On the basis of data for the applicability of Gardoprim Plus Gold in fruit-bearing plantations (Sarpe et al., 2007), since 2011 at the Institute of Viticulture and Enology (IVE) - Pleven experiments have been carried out for determining the action of Gardoprim Plus Gold both on the harmful vegetation in the nursery and on the grafted cuttings during the period of their rooting. The results have shown efficient control of the annual weeds (except *Xsantium strumarium* L.) and lack of

adverse effects after treatment of Muscat Kaylashki cuttings (Prodanova - Marinova et al. 2014).

Cluster analysis as an opportunity for comprehensive assessment based on all measured indicators has not been applied in the assessment of the herbicidal effect in the production of vine propagation material. This method has been successfully used to group objects by similarities and differences based on biometric indicators in a number of crops - soybeans, green beans, etc. Cluster analysis allows better objectivity in assessing them, but alone is not sufficient to explain the reasons for the formation of different groups (Kalaydzhieva et al. 2015; Kuneva et al. 2015).

The objective of this study was by following the impact on biometric indicators of the grafted vines of different varieties the possibility of applying Gardoprim Plus Gold to be determined in the production of vine propagation material. The similarity and distance of the impact of the tested herbicide to be assessed, and grouping of the varieties based on this evaluation to be established.

## MATERIAL AND METHODS

The experiment has been carried out at the Institute of Viticulture and Enology-Pleven, Bulgaria during the production of vine propagation material in nurseries without mulching the soil surface. The herbicide Gardoprim Plus Gold *(312.5 g/l s-metolachlor + 187.5 g/l terbuthylaine)* was applied once at a dose of 4 l/ha, after planting the grafted cuttings, immediately before spray irrigation. It was tested its effect on the varieties: Storgozia (red wine, with increased resistance to low temperatures and some diseases); Naslada (white wine, with increased resistance to low temperatures and some diseases); Merlot (red wine), Cabernet Sauvignon (red wine), Muscat Plevenski (red, table grapes) and Mechta (white, table grapes).

The treatment variants of the study were six - V1 (Storgozia), V2 (Naslada), V3 (Merlot), V4 (Cabernet Sauvignon), V5 (Muscat Plevenski) and V6 (Mechta). The assessment was made by comparison with untreated controls of the same varieties - K1 (Storgozia), K2 (Naslada), K3 (Merlot), K4 (Cabernet Sauvignon), K5 (Muscat Plevenski), K6 (Mechta). The cuttings of the tested varieties were grafted to Berlandieri X Riparia (SO4) rootstock.

The experiment was set by the block method in four repetitions. The nurseries were located on leached chernozem soil type.

After the end of vegetation and taking out the propagation material from the nursery the length of the mature part of the shoots on the average per vine (cm) and the weight of the mature part of the shoots on the average per vine (g) were measured for determining the size of the mature growth. The total number of roots was calculated as the sum of the roots with a diameter below and over 2 mm.

The grouping of the studied variants was performed by hierarchical cluster analysis. The method of intergroup binding was used (Duran and Odelle 1977; Ward 1963), while the data were processed with software package SPSS-19.

## RESULTS AND DISCUSSION

The results from the biometric measurements for 2014 and 2015 were unidirectional and their average values were indicative for the studied period. The shoot length and the rate of ripening were determined by the specifics of the variety. The greatest length of mature growth had vines from Storgozia variety (V1

and K1), followed by the other variety with increased resistance - Naslada (V2 and K2) - Table 1. Merlot (V3 and K3) and Cabernet Sauvignon (V4 and K4) occupied an intermediate position and both table grape varieties - Muscat Plevenski (V5 and K5) and Mechta (V6 and K6) had the smallest length. The shoot mass was to a great extent in correlation with their length however some differences were observed depending on the thickness. The differences between the first four varieties were insignificant and Muscat Plevenski and Mechta had again the lowest values for that indicator.

Eliminating the competition with weeds Gardoprim Plus Gold provided favorable conditions for the growth and development of the grafted cuttings in the nursery (Prodanova-Marinova 2015). Thus it ensured the opportunity for obtaining significant rate of mature annual growth. The vines from all treated variants exceeded those of the controls in length and weight of mature growth. The differences in the length ranged from 24.4 cm for Cabernet Sauvignon to 56.1 cm for Storgozia, as they were the most significant for the varieties with increased resistance to low winter temperatures and mildew (Storgozia and Naslada). The data for the growth weight correlated similarly - the major differences were accounted for in favor of the treated variants of both resistant varieties.

Gardoprim Plus Gold in the applied dose did not affect the rooting of the grafted cuttings from the studied varieties and the vines obtained from them had relatively equal number of roots (Table 1). Significant differences between the treated variants and the controls were not observed referring the roots with a diameter above 2 mm, and those having a diameter below that limit. The smaller number of roots in Mechta variety corresponded to the smaller length and weight of mature growth.

Table 1

Biometric characteristics of vines on the average for the period 2014 – 2015

| Variant | Variety | Mature growth length (cm) | Mature growth weight (g) | Roots number | | |
|---|---|---|---|---|---|---|
| | | | | d>2mm | d<2mm | Total number |
| V1 | Storgozia | 171.1 | 26.92 | 6.1 | 9.8 | 15.9 |
| K1 | Storgozia | 115.0 | 19.22 | 4.7 | 8.2 | 12.8 |
| V2 | Naslada | 147.9 | 29.52 | 6.3 | 8.4 | 14.6 |
| K2 | Naslada | 98.4 | 18.00 | 5.9 | 8.2 | 14.1 |
| V3 | Merlot | 123.6 | 26.34 | 6.1 | 9.2 | 15.3 |
| K3 | Merlot | 97.5 | 20.76 | 5.2 | 7.3 | 12.5 |
| V4 | Cabernet Sauvignon | 129.3 | 30.63 | 5.1 | 8.0 | 13.1 |
| K4 | Cabernet Sauvignon | 104.9 | 24.93 | 6.1 | 7.4 | 13.5 |
| V5 | Muscat Plevenski | 101.6 | 18.64 | 5.9 | 9.0 | 14.9 |
| K5 | Muscat Plevenski | 74.4 | 13.90 | 5.7 | 7.8 | 13.3 |
| V6 | Mechta | 101.8 | 16.31 | 5.1 | 6.4 | 11.5 |
| K6 | Mechta | 71.6 | 8.94 | 5.5 | 6.7 | 11.2 |

The cluster analysis to evaluate the similarity and distance of the herbicide effect on the different varieties on the basis of their biometric characteristics found that they were grouped in one basic cluster with relatively equal Euclidean distances between the variants (Figure 1). The biggest similarity for the three indicators had V2 (Naslada) and V4 (Cabernet Sauvignon). V5 (Muscat Plevenski) could be attached to them, as it approached them for the indicator – number of roots. The variants V1 (Storgozia) and V3 (Merlot) were similar for weight of mature growth and number of roots. The most distant from the rest of the variants for all considered indicators was V6 (Mechta) with the lowest rates for all biometric characteristics.

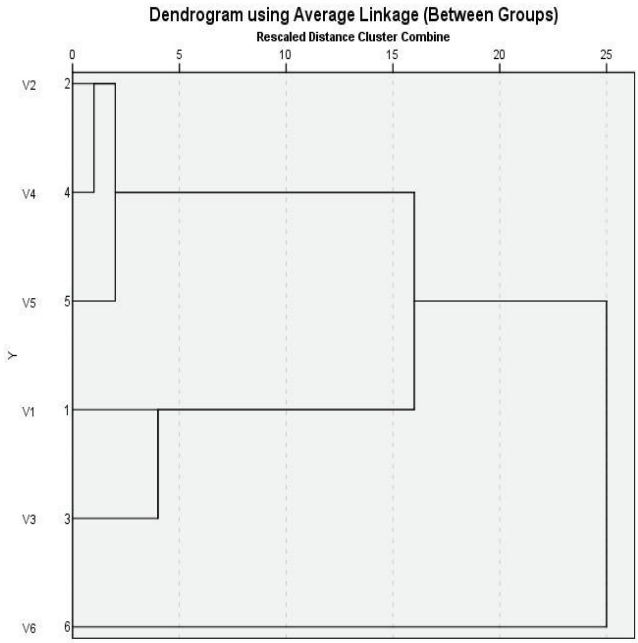


Figure 1. Hierarchical cluster analysis depending on the effect of Gardoprim Plus Gold on the average for the period 2014 – 2015. Dendrogram based on the average intergroup distances.

The results, presented in the second dendrogram (Figure 2) showed that this grouping was mainly due to the varietal characteristics. The distribution of the untreated controls was again in one main cluster, but with well outlined two sub-clusters. The first one included K2, K6 and K4 and confirmed the similarity between the varieties Naslada and Cabernet Sauvignon. The second sub-cluster was formed by K1, K3 and K5, showing the similarity of varieties Storgozia and Merlot as in the dendrogram for the treated variants (Figure 1).

Grouping of Muscat Plevenski (K5) and Mechta (K6) in two different sub-clusters probably expressed the interaction of the specific for the variety biometric characteristics of the propagation material and the impact of the tested herbicide on them.

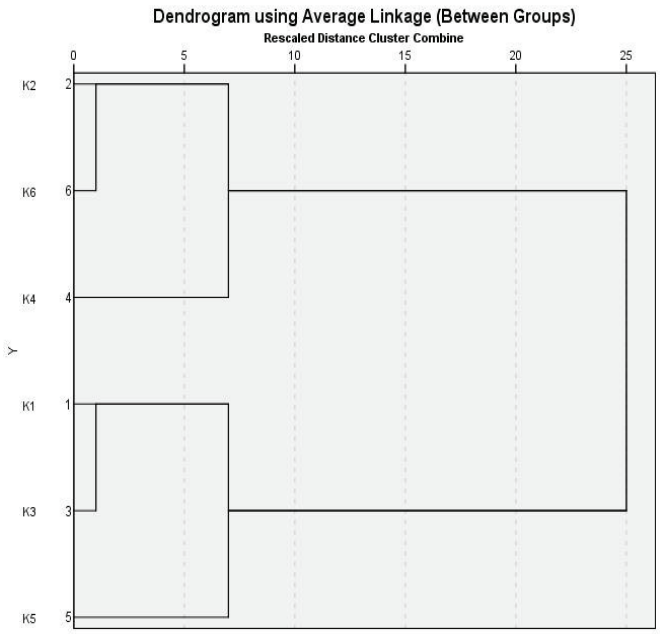


Figure 2. Hierarchical cluster analysis of the control variants on the average for the period 2014 – 2015. Dendrogram based on the average intergroup distances.

## CONCLUSIONS

Vines from Storgozia, Naslada, Merlot, Cabernet Sauvignon, Muscat Plevenski and Mechta varieties obtained after treatment of the nursery with Gardoprim Plus Gold had mature growth with greater length and weight. The tested herbicide did not inhibit rooting and did not reduce the number and diameter of the roots.

The cluster analysis objectively displayed the lack of negative impact of Gardoprim Plus Gold at a dose of 4 l/ha on the quality of the propagation material and confirmed the opportunity for the application of the herbicide in vine nurseries.

## REFERENCES

Bravenec P., Miŝa D., 1978, Herbicidy proti plevelum v vévovich ŝkolkach. Vinohrad, 16, 12, 274-275.

Calastru Gh., 1982, Erbicide preemergente recomendata in cultura vitelor in solaxii. Productia vegetate - Horticultura, 31, 3, 32-37.

Chelebiev M., Katerova L., 1988, The effect of some herbicides on weeds and vines in the nursery. Lozarstvo I Vinarstvo, 3, 43-46.

Duran B., Odelle P., 1977, Cluster analysis. Moscow.

Kalaydzhieva R., Kuneva V., Matev A., 2015, Using cluster analysis to evaluate the impact of irrigation regime on yield and its structural elements in the garden beans. Scientific Papers of University of Ruse, 54, series 1,1, 89-92.

Kuneva, V., Kalaydzhieva R., Nikolova M., Matev A., 2015, Evaluation of irrigation regime impact based on main biometric indicators in soybeans using cluster analysis. Scientific Papers of University of Ruse, 54, series 1,1, 83-88.

Lange A., Lider L., Fischer B., Agamalian H., 1970, Herbicide - Variety studies of Ioung grapevines. American Journal of Enologie and Viticulture, 21, 2, 85-93.

Litvinov P. I., Grushkova S.A., Syrel'shchikova D.P., Chebanovskaya A.F., 1987, Chemical control in grapevine rootstock nurseries. Zashchita Rasteni., 3, 32.

Prabha Ch., 1987, Chemical weed control in grape (*Vitis vinifera* L.) nurseries. Pesticides, 21, 11, 27-29.

Prodanova-Marinova N., 2015, Biological efficiency of some herbicides in vine nursery. Journal of Mountain Agriculture on the Balkans, 18, 4, 702-713.

Prodanova-Marinova N., Dimitrova V., Peykov V., Tsvetanov E., 2014, Study on the technological improvement of vine growing in nursery. Annals of the University of Crajova, XIX (LV), 287-292.

Sarpe N., Radulescu I., Poienaru S., 2007, Research with classical herbicides and new herbicides (Merlin duo and Gardoprim Plus Gold) applied on the chernozem from Fudules Fetesti and the bown soil - Luvic from Stefanesti Arges. Lucrări Ştiinţifice, Anul XXXXX VOL. 9 (50), 307-312.

Todorov I., 2005, Production of Vine Propagation Material. Dionis, Sofia, 303.

Tonev T., Dimitrova M., Kalinova Z., Zhalnov I., Spasov V., 2007, Herbology. AU - Plovdiv, 227.

Ward J.H., 1963, Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association, 58, 236-244.

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# COMPARATIVE EVALUATION OF LUMAX 538 SC AND GARDOPRIM PLUS GOLD IN VINE NURSERY

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**Keywords:** *herbicides, grafted vine cuttings, vines, germinate, phytotoxicity*

## ABSTRACT

*The influence of Lumax 538 SC (375 g/l s-metolachlor + 125 g/l terbuthylazine + 337,5 g/l mesotrione) and Gardoprim plus Gold (312,5 g/l s-metolachlor + 187,5 g/l terbuthylazine) on cuttings of the Bolgar variety grafted on the rootstock Berlandieri X Riparia SO4 was studied at the Institute of Viticulture and Enology (IVE), Pleven (Bulgaria). The herbicides were applied at doses of 4 and 6 l/ha. Their impact on the grafted vine cuttings was assessed by the percent of germinated shoots after treatment, yield of the standard rooted vines and some biometric indicators. Cuttings showed sensitivity to both herbicides, most pronounced at the beginning of vegetation. There were no significant differences in the yield of the standard rooted vines, as well as between the different variants and between the variants and technological control. The values of the biometric indicators (length and weight of the mature growth, number of roots) are aligned with those of the vines obtained from untreated cuttings. Unlike Lumax 538 SK, Gardoprim plus Gold does not cause phytotoxic reaction on the vine leaves.*

## INTRODUCTION

The cultivation of vine propagation material without mulch system requires more attention to maintain the soil surface free of weeds. Over the years experiments have been made for using different herbicides as many of them have shown good efficacy and sufficient selectivity for vines (Calastru 1982; Chelebiev and Katerova 1988; Lange et al. 1969; Litvinov et al. 1987; Prabha Challa 1987). Simazine, oryzalin, trifluralin, etc. have been recommended in different periods. (Chelebiev 1981, Tonev et al. 2000; Encheva and Chelebiev 2002; Todorov 2005). The development of technology requires constant updating of the list of active substances suitable for use in vine nurseries. Lumax 538 SC, as herbicide with a broad spectrum of action has been studied at IVE - Pleven since 2007. It has been found that after its single application after planting the cuttings in the nursery it ensures efficient control over the annual weeds throughout the whole period of rooting. Characteristic for that herbicide was that it caused pronounced phytotoxic effect in Muscat Plevenski and Bolgar varieties (Prodanova-Marinova 2012; Prodanova-Marinova 2016).

There has been evidence that Gardoprim Plus Gold could be used successfully in vineyards (Sarpe et al. 2007). Studies of our have shown that it has

manifested great persistence and effectiveness against weeds in vine nurseries (Prodanova -Marinova et al. 2014; Prodanova-Marinova 2015).

The objective of this paper is to make comparative assessment of the action of both herbicides, to establish the one more suitable for use in the production of vine planting material from the more sensitive to pesticides Bolgar variety, as well as to be determined safe doses for application on the basis of the obtained results.

## MATERIAL AND METHODS

The study was carried out in the period 2011 – 2013 at the Institute of Viticulture and Enology (IVE), Pleven, Bulgaria. The trial was set in four repetitions on leached chernozem soil without application of mulching with polyethylene wrap or plant materials. It was tested the impact of herbicides Lumax 538 SC (*375 g/l s-metolachlor +125 g/l terbuthylazine + 337.5 g/l mesotrione*) and Gardoprim Plus Gold (*312.5 g/l s-metolachlor + 187.5 g/l terbuthylazine*) on Bolgar variety cuttings grafted to Berlandieri X Riparia SO4 rootstock. The variety was selected because of the available data for its high sensitivity to some pesticides and a strong response to adverse soil and weather conditions (Bulgarian Ampelography, 2010). The variants were as follows: V1 - Lumax 538 SC (dose of 4 l/ha); V2 - Lumax 538 SC (dose of 6 l/ha); V3 - Gardoprim Plus Gold (dose of 4 l/ha); - V4 - Gardoprim Plus Gold (dose of 6 l/ha). The treated variants were compared between each other and also with manually weeded out, untreated with herbicides control - K.

The spraying was performed immediately after planting the cuttings in the nursery just before the first sprinkling. Impact assessment of both herbicides was made for the indicators: ratio of germinated grafted cuttings (%), yield of standard rooted vines (% compared to he planted cuttings), average length of mature growth per vine (cm), average weight of mature growth per vine (g), average length of internodes (mm) and number of roots (including with the diameter above and below 2 mm). Visual examinations were carried out during the vegetation period for signs of phytotoxicity.

The data were processed by analysis of variance (Dimova and Marinkov, 1999).

## RESULTS AND DISCUSSION

The most indicative for the buds development of the grafted cuttings after their planting in the vine nursery were the results for their germination during the first ten-day and the fifth ten-day periods. The ratio of germinated cuttings reported at the end of the first ten-day period after treatment with the herbicides ranged from 47.8% (V2) to 57.25% (K) - Figure 1. The most significant germination delay was reported for V2 - Lumax 538 SC at a dose of 6 l/ha, and for V3 - Gardoprim Plus Gold at a dose of 4 l/ha. For these variants the differences compared to the control were proven at $GD_{5\ \%}$ = 7.991; $GD_{1\ \%}$ = 11.626; $GD_{0.1\ \%}$ = 17.469. Over the next ten-day periods the cuttings were germinating intensely and somewhat compensated for the delay in the first days. During the last reporting (at the end of the fifth ten-day period), the highest ratio was found for V4 - Gardoprim Plus Gold at a dose of 6 l/ha (75.68%). The differences compared to the control (74.75%) and between the variants were insignificant and unproven (at $GD_{5\%}$ = 10.024; $GD_{1\%}$ = 14.583; $GD_{0.1\%}$ = 21.912).

The application of Lumax 538 SC and Gardoprim Plus Gold resulted in minimal delay of Bolgar variety cuttings ratio of germination that was not related to the tested doses – the correlation between the variants of the different doses for the same herbicide changed during the period of accounting.

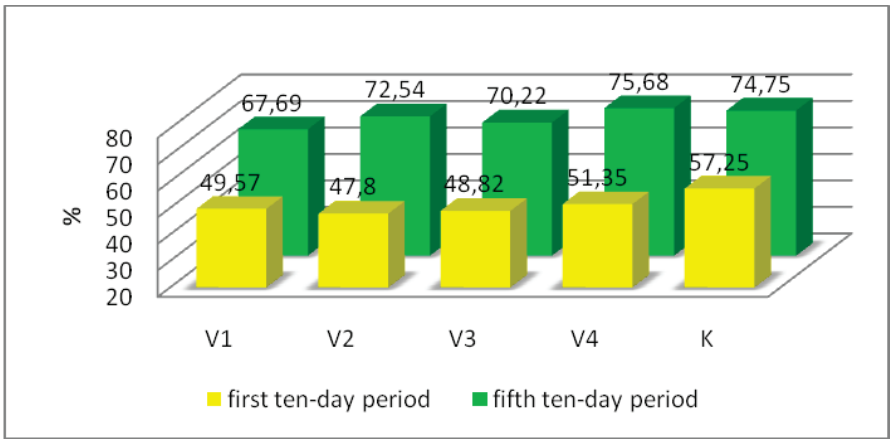


Figure 1. Germination ratio of planted Bolgar variety cuttings after treatment of the nursery with LUMAX SC 538 and Gardoprim Plus Gold, on the average for period 2011 - 2013.

The present study has confirmed the results of our previous work on the signs of phytotoxic response to Lumax 538 SC (Prodanova-Marinova, 2014). In this case it has also been observed the typical whitening of the leaves induced by mesotrione action (Jdziak and Woznica, 2008). Often chlorosis (particularly at the leaf blade periphery) developed into necrosis (Figure 2). Between the thirtieth and the sixtieth day after treatment the plants overcame the herbicidal stress and with their active growth compensated the initial delay.

Gardoprim Plus Gold includes s-metolachlor and terbuthylazine. As mesotrione is absent from the combination of the active substances, in any of the three years of the trial, there were no indications of phytotoxicity after treatment of the vine nursery.

The indicator determining the overall effect of the treatment is the yield of standard rooted vines. The highest yield of quality vine propagation material (46.18%) was obtained from the grafted cuttings treated with Lumax 538 SC at a dose of 6 l/ha (V2) - Figure 3. For variants V1 and V4 the yield was lower compared to the control (43.01%), however the differences were insignificant. The results showed a slight variation for this indicator both between the different treated variants as well as between them and the control. The unproved differences (at $GD_{5\%}$ = 9.113; $GD_{1\%}$ = 13.259; $GD_{0.1\ \%}$ = 19.921) indicated that there was no inhibitory effect on the yield of standard rooted vines. There was no reason to believe that any of the two herbicides exhibited more negative action compared to the other one, regardless of the dose.

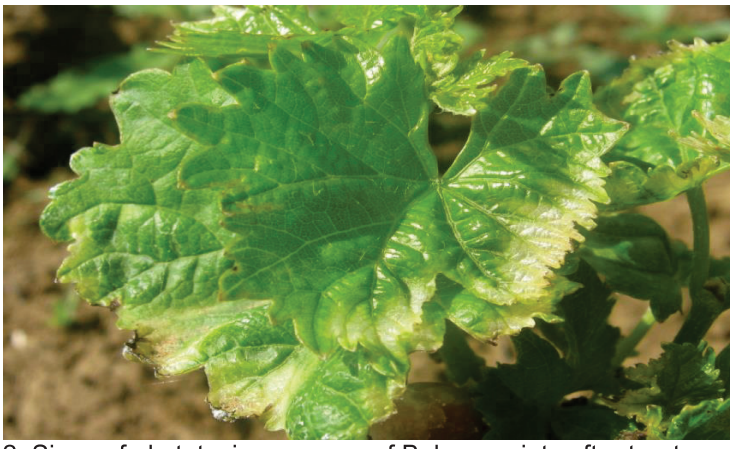

Figure 2. Signs of phytotoxic response of Bolgar variety after treatment of the nursery with Lumax 538 SC.

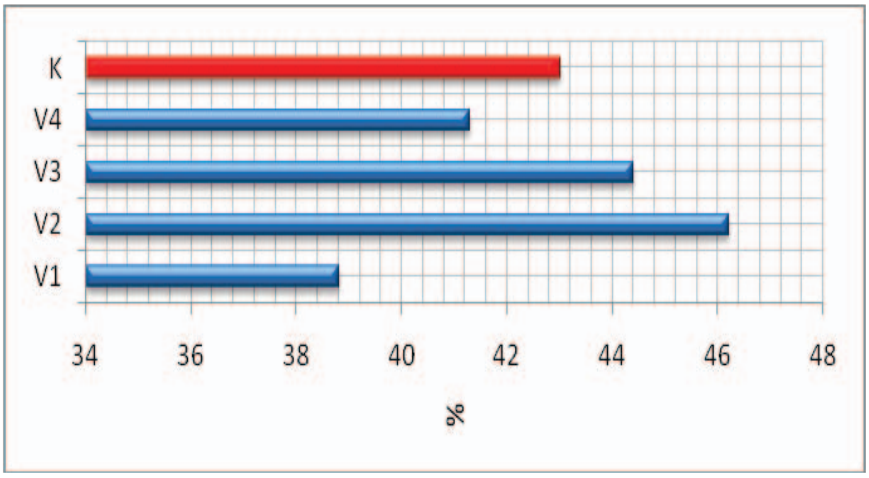


Figure 3. Yield of standard rooted vines of Bolgar variety after treatment of the nursery with Lumax 538 SC and Gardoprim Plus Gold, on the average for the period 2011 - 2013.

The biometric characteristics of the rooted vines were indicative of the vegetation processes activity in the nursery as well as of the vine propagation material quality. The mature growth length, on the average per vine did not exceed 111.0 cm (V1) and the differences between the variants and between them and the control were insignificant and did not show a negative effect of the herbicides on the shoot growth and maturation (Table 1). In confirmation of that, even greater uniformity was observed in the weight of the mature growth – ranging from 19.34 g (V4) to 22.90 g (K). The length of internodes as genetically determined trait has been relatively constant and a typical varietal sign that could serve for determining the impact of different factors (Lilov 1979). The data in Table 1 showed that the application of Lumax 538 SC and Gardoprim Plus Gold at the tested doses did not result in changing the values of this indicator. Similarly, both herbicides did not

cause variations in the number of roots - with a diameter above and below 2 mm. It has not been found any inhibitory effect of both products on the root formation of the grafted cuttings.

Table 1

Biometric characteristics of the grafted rooted vines obtained after treatment of the nursery with Lumax 538 SC and Gardoprim Plus Gold, on the average for the period 2011 - 2013

| V | Length (cm) | Weight (g) | Average length of the internodes (mm) | Number of roots | | |
|---|---|---|---|---|---|---|
| | | | | d>2mm | d<2mm | Total number |
| V1 | 111.0 | 21.97 | 3.2 | 5.2 | 7.1 | 12.3 |
| V2 | 103.5 | 21.26 | 3.2 | 5.4 | 6.0 | 11.4 |
| V3 | 100.0 | 20.96 | 3.1 | 5.3 | 5.8 | 11.1 |
| V4 | 97.1 | 19.34 | 3.2 | 5.0 | 6.6 | 11.6 |
| K | 109.7 | 22.90 | 3.3 | 5.5 | 7.2 | 12.7 |

## CONCLUSIONS

The herbicides Lumax 538 SC and Gardoprim Plus Gold caused minimum delay of germination in the first ten-day period after treatment of Bolgar variety grafted cuttings, however it did not affect negatively the next vegetation processes in the nursery.

The yield of quality propagation material was not affected significantly either by the tested products or the doses of application. Regardless the manifestations of phytotoxic effect, the highest ratio of standard rooted vines (46.18%) was reported for the variant of Lumax 538 SC at a dose of 6 l/ha.

Introduced in the soil, before sprinkling Gardoprim Plus Gold did not cause signs of phytotoxicity during the vegetation.

The tested herbicides in the applied doses did not affect adversely the quality of the propagating material – the vines from the treated variants had mature growth of approximately the same length and weight as well as internodes with characteristic length for the variety. Lumax 538 SC and Gardoprim Plus Gold did not inhibit root-formation.

With a view of the similar course of the biological processes during the vegetation, the small differences in yield and almost identical biometric characteristics of the vines, it could be concluded that both herbicides (at doses of 4 and 6 l/ha) were equally applicable in the production of vine propagation material from Bolgar variety.

## REFERENCES

Calastru Gh., 1982, Erbicide preemergente recomendata in cultura vitelor in solaxii. Productia vegetate – Horticultura, 31, 3, 32-37.

Chelebiev M., 1981, The application of herbicides in vine nursery. Viticulture and Enology, 5, 15-18.

Chelebiev M., Encheva H., 2002, Chemical weed control in vineyards. Coll. by Jubilee session with international participation "100 Years Institute of Vine and Wine" - Pleven - 2002. SPS Print, Sofia, 220-227.

Chelebiev M., Katerova L., 1988, The Effect of some Herbicides on Weeds and Vines in the Nursery. Lozarstvo I Vinarstvo, 3, 43-46.

Dimova D., Marinkov E., 1999, Experimental work and biometrics. Academic Publishing House of HIA, Plovdiv, 263.

Jdziak R., Woznica Z. Wplyw adinvantow na skutecznosc chwastobojoza oraz selektywnosc herbicydu mezotrion stosowanego w upravvie kukurudzy. Progress in Plant Protection rostepy w Ochronie roslin, 48 (1), 2008, 268-271.

Lange A., Fischer B., Agamalian H., Kempen H., 1969, Weed control in young grapes. Californian Agriculture, 23, 11-12.

Lilov D., 1979, Biological basis of affinity at the vine. Sofia, Bulgarian Academy of Sciences, 156.

Litvinov P.I., Grushkova S.A., Syrel'shchikova D.P., Chebanovskaya A.F., 1987, Chemical control in grapevine rootstock nurseries. Zashchita Rasteni., 3, 32.

Prabha Ch., 1987, Chemical weed control in grape (*Vitis vinifera* L.) nurseries. Pesticides, 21, 11, 27-29.

Prodanova-Marinova N., 2012, Study on the efficiency and selectivity of soil herbicides in vine nursery. Dissertation thesis for award the degree of PhD, 161.

Prodanova-Marinova N., 2015, Biological efficiency of some herbicides in vine nursery. Journal of Mountain Agriculture on the Balkans, 18, 4, 702-713.

Prodanova-Marinova N., 2016, Impact on BURALL manifestations grafted cuttings after treatment of vine nursery with Stomp new and Lyumaks 538 SC. Russian grapes, 3, 88-93.

Prodanova-Marinova N., Dimitrova V., Peykov V., Tsvetanov E., 2014, Study on the technological improvement of vine growing in nursery. Annals of the University of Crajova, XIX (LV), 287-292.

Sarpe N., Radulescu I., Poienaru S., 2007, Research with classical herbicides and new herbicides (Merlin duo and Gardoprim Plus Gold) applied on the chernozem from Fudules Fetesti and the bown soil – Luvic from Stefanesti Arges. Lucrări Ştiinţifice, Anul XXXXX VOL. 9 (50), 307-312.

Todorov I., 2005, Production of Vine Propagation Material. Dionis, Sofia, 303.

Tonev T. 2000, Manual for integrated weed control and crop farming AU-Plovdiv, vol. 2, 270.

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# COMPARATIVE STUDIES ON THE ACCUMULATION OF SOME METABOLITES AT *ORIGANUM MAJORANA* PLANTS MULTIPLIED BY *IN VITRO* CULTURE AND BY CONVENTIONAL METHODS

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**Keywords:** *conventional methods, herba, "in vitro" culture, metabolites, Origanum majorana*

## ABSTRACT

*The aim of this study was to carry out comparative studies concerning accumulation of primary and secondary metabolites at Origanum majorana plants obtained by "in vitro" culture and by conventional methods. Biochemicals investigations revealed no major differences between the plants obtained by the two culture technologies. The difference between the two multiplication techniques consist in superior cost price in the case of „in vitro" culture, price which is compensated by obtaining a higher number of plants than in the case of conventional multiplication, in a very short time, plants from which can be obtain a higher quantity of primary and secondary metabolites.*

## INTRODUCTION

The *Origanum* genus belonging to *Lamiaceae* family and comprises approximately 38 species, most of which are originate in Mediterranean region. Many of them are commercially exploited, their demand is increasing (Schiuma, 1993). One of these species is also the marjoram (*Origanum majorana*), a valuable medicinal and aromatic perennial plant. The presence of the flavones, the polyphenolcarboxylic acids and the volatile oil confers of marjoram antiseptic, stomachic and spasmolytic properties (Hinore et al., 1989; Konakchiev et al., 2004; Vokou et al., 1993; Paster et al., 1993).

Considering the economic importance of this species, the researches conducted in this work were oriented in the direction of quantitative evaluation of some primary and secondary metabolites at *Origanum majorana* plants obtained by *in vitro* culture and by conventional methods.

## MATERIALS AND METHODS

The biological material derived from the *Origanum majorana* species has been multiplied *in vitro* and by conventional methods.

The plants obtained formed two comparative lots:

1. the lot with plants obtained *in vitro* - micropropagation technology (V1);
2. the lot with plants obtained by conventional techniques (V2).

For biochemical determinations was sampled the plant material (*herba*) after blooming, at maturity. Option for analysis of plant material from *herba* left from

the presence of phytocomplex that characterize it for a series of active principles conferring the possibility of a comparative study of material obtained (conventional cultures and plants regenerated *in vitro*).

Biochemical investigations on plant material were designed to measure:

- *the dry matter content* (%) by dehydration of the plant material at 105 °C, up to a constant mass;
- *the content of chlorophyll and carotenoid pigments*; their extraction was performed with 80% acetone and was followed by reading the optical density at three wavelengths: 440.5 nm, 644 nm and 662 nm. In the measurement of the pigment content in mg/g of green substance, the Tvet calculation formulas were used;
- *the soluble carbohydrate content* (spectrophotometric dosage determination after the colour reaction with anthrone reactive);
- *the phenolic compounds* (colorimetric determination based on the reaction with Folin-Ciocalteu reactive);
- *the content of total flavonoids* (colorimetric dosage with aluminum chloride);
- *the phosphorus content* (spectrophotometric method with ammonium molybdate);
- *the potassium content* (flam photometric method).

Each biochemical analysis was performed in three repetitions, the results represent the average/variant.

## RESULTS AND DISCUSSIONS

Among the most important vegetal compounds from biological point of view are vegetal pigments. The content in vegetal pigments depend on species, environmental conditions and culture technology.

The most spreading from the vegetable kingdom are the chlorophyllian and carotenoidic pigments.

In the experiments carried out by us, in the first stage was determined the content of the plants obtained by the two methods in chlorophyllian pigments. The graphic representation of that (figure 1) shows that, at biological material obtained *in vitro* (V1), both chlorophyll "a" and chlorophyll "b" has higher average values (0.779 mg chlorophyll „a”/g green substance, respectively 0.433 mg chlorophyll “b”/g green substance) compared to the values registered at plants obtained by the conventional method (0.598 mg chlorophyll „a”/g green substance, respectively 0.312 mg chlorophyll „b”/g green substance).

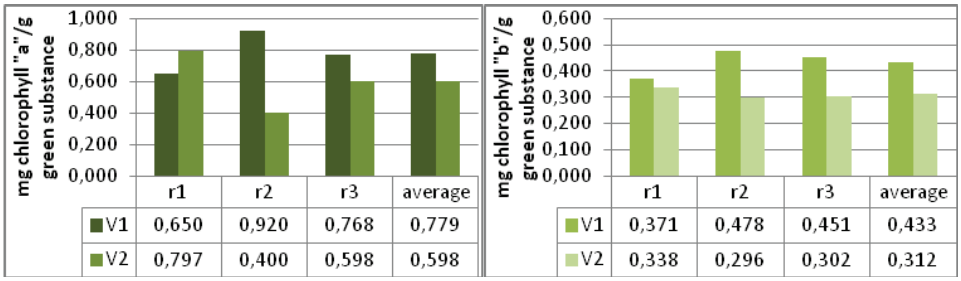



Figure 1. The content of the chlorophyllian pigments from the plant depending on the method of obtaining the biological material.

The quantitative evaluation of the carotenoids pigments at *Origanum majorana* species depending on the method of obtaining the biological material is shown in figure 2. It is noted that, in average, the values obtained in the case of *in vitro* regenerated plants are higher (0.474 mg/g green substance) than those obtained by conventional methods (0.321 mg/g green substance).

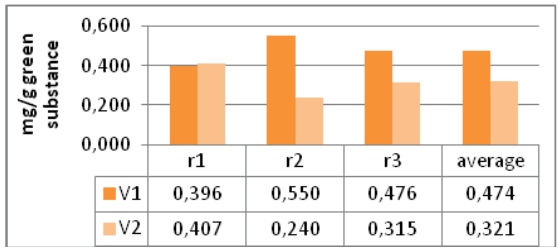



Figure 2. The content of the carotenoids pigments from the plant depending on the method of obtaining the biological material.

It calculated the main indices of the chlorophyllian assimilation in plants, namely the report of chlorophyll "a" and chlorophyll "b", as well as the report chlorophyll/carotene. The report chlorophyll "a"/chlorophyll "b" has the maximum value at the beginning of the vegetation period (reaching value 2.5) and decreases towards the end of September around 1.7, this modification being due to the decrease the content in chlorophyll "a " during the vegetation period.

In the case of the experiences made by us, both indicators show the preponderance of chlorophyll "a", respectively of the pigments chlorophyllian in the plants analyzed (figure 3).

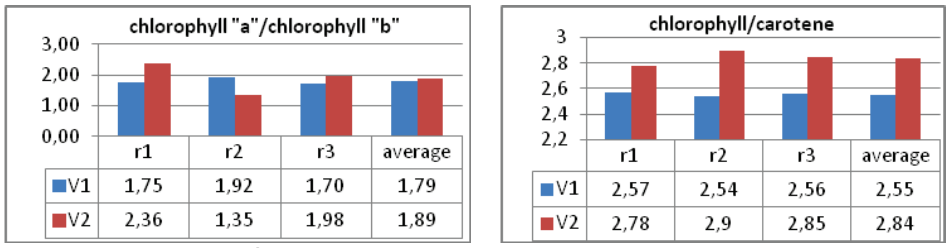
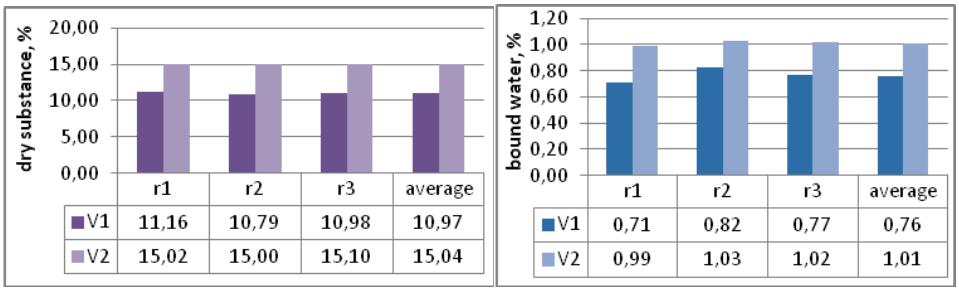



Figure 3. Indicators of the chlorophyllian assimilation within the two experimental variants.

Evaluation of dry matter and bound water content from the plant within the two experimental variants highlights superior values of the vegetative mass and of the bound water quantity in the case of the plants obtained by conventional methods compared with those obtained *in vitro* (fig. 4). On average, the dry matter content is 10.97% at the plants obtained *in vitro* and 15.04% at the plants obtained by conventional methods. The average value of the bound water quantity is 0.76% in the case of variant V1, respectively 1.01 in the case of variant V2. Within each variant (V1/*herba* of plants regenerated *in vitro* - V2/*herba* of plants from conventional cultures), we find that there is a direct correlation between the dry matter and bound water content from the plant.



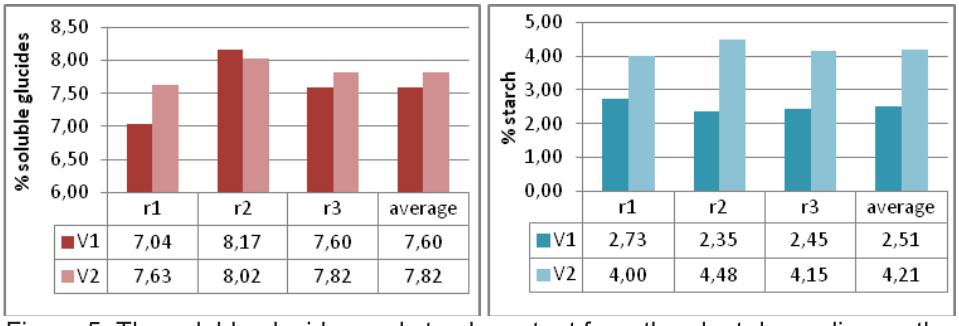

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 11,16 | 10,79 | 10,98 | 10,97 |
| V2 | 15,02 | 15,00 | 15,10 | 15,04 |

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 0,71 | 0,82 | 0,77 | 0,76 |
| V2 | 0,99 | 1,03 | 1,02 | 1,01 |

Figure 4. The dry matter and bound water content from the plant within the two experimental variants

The glucides, primary products of photosynthesis, have been determined within of plants results from the two culture techniques. The content in soluble glucides is almost identical at the samples from the plants in conventional cultures (V2) and regenerated *in vitro* (V1), with few exceptions. On average, the soluble glucides content registered values by 7.60% at the plants obtained *in vitro* and 7.82% at the plants obtained by conventional crultures.

The polymerization of monoglucides was more intense at the plants obtained by conventional culture, as evidenced by higher content of the starch thereof. On average, the content of starch in the case of variant V1 is by 2.51% and in the case of variant V2 by 4.21% (figure 5).



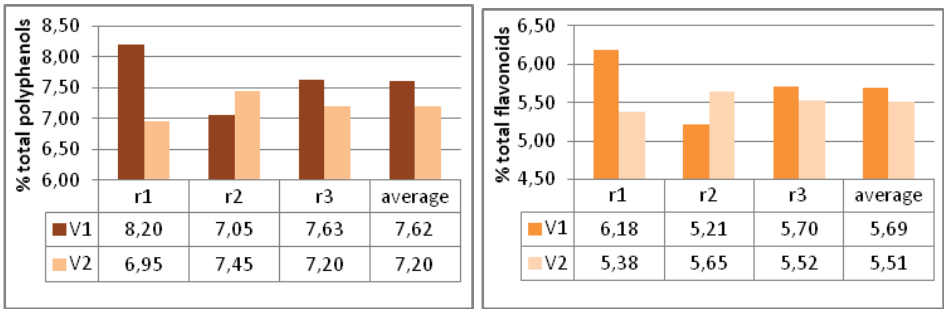

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 7,04 | 8,17 | 7,60 | 7,60 |
| V2 | 7,63 | 8,02 | 7,82 | 7,82 |

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 2,73 | 2,35 | 2,45 | 2,51 |
| V2 | 4,00 | 4,48 | 4,15 | 4,21 |

Figure 5. The soluble glucides and starch content from the plant depending on the method of obtaining the biological material.

Because the characteristics of bioproduction that interested to be kept especially at the *Origanum* plants refers to the content in active principles, the ethanolic extracts derived from the plants investigated biochemically were analyzed quantitatively for evaluating the content in polyphenols and flavonoids.

In the case of the experience made by us, *herba* of *Origanum* was characterized by a balance between the polyphenolic and flavonic compounds, aspect reflected also in quantitative analysis. The highest mean quantity was highlighted in the case of variant V1 (7.62% polyphenols, respectively 5.69% flavonoids) (fig. 6).



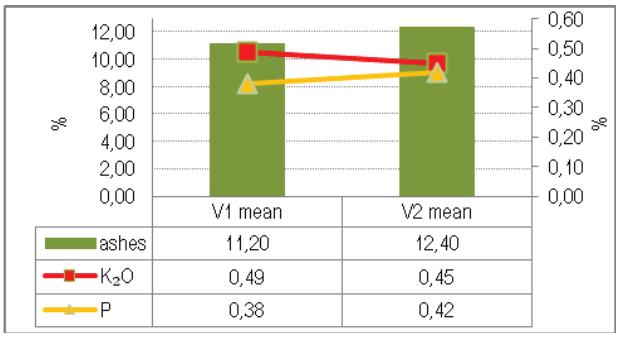

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 8,20 | 7,05 | 7,63 | 7,62 |
| V2 | 6,95 | 7,45 | 7,20 | 7,20 |

| | r1 | r2 | r3 | average |
|---|---|---|---|---|
| V1 | 6,18 | 5,21 | 5,70 | 5,69 |
| V2 | 5,38 | 5,65 | 5,52 | 5,51 |

Figure 6. The total polyphenols and total flavonoids content from the plant depending on the method of obtaining the biological material.

By mineral point of view, the content in potassium from plant tissues varies within very wide limits (0.1 - 11%) depending on the species, the development stage of the plant, the organ analyzed. The values corresponding to a normal state of supply with potassium are 3-5% at the young plants and 1.5-2.5% in the phases of forming generative organs.

The results relating to the phosphorus and potassium content of the plants obtained by the two experimental methods are presented in figure 7. While the ash content ranged between 11.2% (V1) and 12.4% (V2), the content in potassium has recorded similar values to the two experimental variants (0.49% - V1 and 0.45% - V2). The samples of vegetal material analyzed revealed a slightly higher content of phosphorus within the conventional variant (0.42%) compared with *in vitro* variant (0.38%).




| | V1 mean | V2 mean |
|---|---|---|
| ashes | 11,20 | 12,40 |
| $K_2O$ | 0,49 | 0,45 |
| P | 0,38 | 0,42 |

Figure 7. The content in P and K of the plant obtained by the two experimental methods.

The comparative study of the samples (*herba* of plants from the conventional culture and *in vitro* regenerated) confirms the bioproductive qualities of both the origin material and the *in vitro* regenerants.

## CONCLUSIONS

- The quantitative evaluation of chlorophyllian and carotenoids pigments to *Origanum majorana* species depending on the method of obtaining biological material showed that, on average, the values obtained in the case of *in vitro* regenerated plants were superior to those registered at the plants obtained by conventional method.
- Regarding the content in active principles (polyphenols and flavonoids) from plant, the differences were insignificant at the two experimental variants (*herba* from plants obtained from conventional culture and regenerated *in vitro*).
- Biochemicals investigations revealed no major differences between the plants obtained by the two culture technologies (*in vitro* multiplication and conventional propagation). The difference between the two multiplication techniques consist in the cost price superior in the case of *in vitro* culture, price which is compensated by obtaining a much higher number of plants than in the case of conventional multiplication, in a very short time, plants from which can be obtain a higher quantity of primary and secondary metabolites.

## REFERENCES


Hinore J.B., Harvala C.E., Hinon E.B., 1989, Antimicrobial activity of 32 common constituents of essential oils. Pharmazie 4:302-303.

Konakchiev A., Genova E., Couladis M., 2004, Chemical composition of the essential oil of *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart in Bulgaria. Comptes redus de l'Academie bulgare des Sciences 57(11):49-51.

Lichtenthaler H.K., Wellburn A.R., 1983, Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions 11:591-592.

Pánczél M., Eifert J., 1960, Die Bestimung des Zuckerund Stärkegehaltes der Weinrebe mittels Anthronreagens. Mitteilungen Klosterneuburg 10:102–110.

Paster N., Menasherov M., Shaaya E., Juven B., Ravid U., 1993, The use of essential oils applied as fumigants to control mycotoxigenic fungi attacking stored grain. Hassadeh 74:25-27.

Schiuma G., 1999, Propuesta de reglamentacion para la proteccion de la flora silvestre de aromaticas y medicinales. In: du Tucuman SM (ed) Congreso Nacional de Recursos Naturales, Aromaticas y Medicinales, SAIPA, Argentina pp 20-23.

Singleton V.L., Rossi J.A., 1965, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 16:144-158.

Vokou D., Kokkini S., Bessiere J.M., 1993, Geographic variation of Greek oregano (*Origanum vulgare* ssp. hirtum) essential oils. Biochemical Systematics and Ecology 21:287-295.

Zhishen J., Mengcheng T., Jianming W., 1999, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64:555-559.

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# A HARMFUL INFLUENCE OF LOW TEMPERATURES ON GROWING GRAPEVINE IN SOUTH-WESTERN OLTENIA

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***Keywords:*** *sandy soils, negative minimum temperature*

## ABSTRACT

*The organs of vines grapes resist, during the winter, until temperature* -18$^0$C… -21$^0$C*. The risk that the organs of vine grapes for wine to be affected by negative minimum temperatures during active rest, increased in 2007-2016 period compared with the 1956-2016 period, from 34.4 to 60 per cent in the case of winter bud, from 22.9 to 50 per cent in the case of annual wood, and from 8.2 to 30 per cent in the case of multiannual wood.Table grape varieties are more sensitive to negative minimum temperatures, and as a result, in the 61 years analyzed, vine organs were not affected in 16 years (26.2 per cent). Winter buds were at risk of extinction in 45 years (73.8 per cent), annual wood was affected in 21 years (34.4 per cent) and multiannual wood was affected in 19 years (31.1 per cent).Therefore, adaptation measures must be taken. Thus, the use of varieties tolerant to harmful minimum temperatures, but this is only possible at temperatures down to -18…-21$^0$C, or the protection of elements of fruit, in the form of leading classical (low) hubs by placing, in autumn, of 2-4 young wooden elements on the ground and covering with soil.*

## INTRODUCTION

Climate change is not merely increase or decrease annual temperature and temperature shocks, both during the growing season and during rest period.

The rise of temperature shock, especially during active rest, it might be a consequence of the increase of average temperature.

Also, in the category of climate change full and lack of rainfall, but this can be decided throught irrigation, but also very abundant precipitation during the growing season, in important phases for the vines as well as blooming period, or the period of ripening of grapes, ehen the danger of occurence of main diseases is large and catastrophic, leading to compromise the production of grapes from that year.

Analysis of climatic conditions in Romania has led to the employment of sandy soils in the area very favourable for vines (Martin et al. 1974; Bishtawi A. & Popa 2005). However, the period of the life of the tree stumps is shorter due to the mainly negative minimum temperatures in winter that enhance plant and increase the risk of bacterial cancer (Agrobacterium tumefaciens), (Baniță, 1983).

Vines, is a perennial plant, and this is in to weather conditions of external environment in all seasons. Thus, it is subject to risks to diminish or to endanger growing season or production in respectively year or over a longer period. Although

it features survival strategies (deep rooting, effective control of stomate, or can be aided with shading nets, anti-hail nets or tilt against rainfall abundant, during the growing season, vines is heavily dependent on climate and during active rest when temperatures coming down in the sandy soils of South-Western Oltenia, up to values of -30$^0$C.

Minimum temperatures below -18$^0$C, has a negative influence on the viability of the buds and the annual and multiannual wood (Martin 1968; Oprean 1975; Oşlobeanu 1980; Ţârdea & Dejeu, 1995; Stroe et al., 2012).

## MATERIAL AND METHODS

Were taken into account minimum temperatures recorded negative during the period 1956-2016. For the classification of the degree of resistance of the various organs from vines, the minimum temperatures, have been taken into account resistance thresholds established by various authors, existing in the literature.

## RESULTS AND DISCUSSIONS

Along time, the level of minimum temperatures, recorded at the mteorogical station of Research – Development Center for Plant on Sands Dăbuleni, standing oscillated from one year to another. Meteorological data records began in Dăbuleni in 1956 year.

Vine resists different from minimum temperatures, depending on the variety (table 1). The table grape varieties the diaphragm resists up to - 12$^0$C, winter buds resists up to -15.1$^0$C…-18$^0$C, annual wood resists up to -18.1$^0$C…-21$^0$C, and multiannual wood can withstand up to -21,1$^0$C. In the case of wine grape varieties with resistance threshold is -15$^0$C for diaphragm, -18.1$^0$C…-21$^0$C for winter buds, -21$^0$C…-24$^0$C for the annual wood and -24$^0$C for a multiannual wood.

Table 1

Level of the temperatures affecting the organs of the vines to the species Vitis vinifera (Martin 1968 and 1972)

| Vine organ | Temperature level ($^o$C) | |
|---|---|---|
| | Table grapes | Wine varieties |
| Diaphragm | Under -12$^0$C | Under -15$^0$C |
| Winter buds | -15.1$^0$C…-18$^0$C | -18.1$^0$C…-21$^0$C |
| Annual buds | -18.1$^0$C…-21$^0$C | -21.1$^0$C… -24$^0$C |
| Multiannual buds | Under -21$^0$C | Under -24$^0$C |

To varieties of wine grapes, the vines organs, during the period 1956-2016, have escaped the harmful effect of minimum negative temperatures in 40 years (65.6 per cent), (table 2). The negative effect of these minimum temperature was manifested in 21 years (34.4 per cent), for the winter buds, in 14 years (22.9 per cent), for annual wood and in 5 years (8.2 per cent), for multiannual wood. The absolute minimum temperature in this period was – 30.5$^0$C, recorded in 1963.

During the period 1956-1975, the vines organs have not suffered losses in 13 years (65 per cent), the winter buds were destroyed in 7 years (35 per cent), annually wood suffered losses in four years (40 per cent), and multiannual wood was destroyed in one year (5 per cent).

In the period 1997-2016, the vines organs were not affected in 12 years (60 per cent). During the same period the winter buds perished in 8 years (40 per cent), wood annually has been affected in 7 years (35 per cent), and multiannual wood was destroyed in 3 years (15 per cent).

Table 2

Minimum harmful effect of negative temperatures for wine grape varieties in the South -Western Oltenia

| Temperature level | 1956-2016 period | | 1956-1975 period | | 1997-2016 period | | 2007-2016 period | |
|---|---|---|---|---|---|---|---|---|
| | Number years | % | Number years | % | Number years | % | Number years | % |
| Until -18$^0$ C | 40 | 65.6 | 13 | 65 | 12 | 60 | 4 | 40 |
| -18.1$^0$C…-30.5$^0$C | 21 | 34.4 | 7 | 35 | 8 | 40 | 6 | 60 |
| -21.1$^0$C…-30.5$^0$C | 14 | 22.9 | 4 | 20 | 7 | 35 | 5 | 50 |
| -24$^0$C…-30.5$^0$C | 5 | 8.2 | 1 | 5 | 3 | 15 | 3 | 30 |
| Total years analysed | 61 | | 20 | | 20 | | 10 | |

The situation of the last 10 years (2007-2016) indicates that the organs of fructification of the vine have escaped the danger minimal negative temperatures in 4 years, only and 40 per cent, respectively. During the same period the winter buds perished in 6 years (60 per cent), the annual wood was destroyed in 5 years (50 per cent), and multiannual wood was affected in 3 years (30 per cent).

The risk that the organs of the vines with wine grapes being affected by negative lows during the active rest, increased in the period 2007-2016, compared with the period 1956-2016: in case of winter buds from 34.4 to 60 per cent, in the case of annual wood from 22.9 to 50 per cent and in the case of multiannual wood from 8.2 to 30 per cent.

To varieties of table grapes in those 61 years analysed, the vines were not affected in 16 years, 26.2 per cent, respectively (table 3). Winter buds were subjected to the risk of perishing in 45 years (73.8 per cent), annual wood has been affected in 21 years (34.4 per cent), and multiannual wood was affected in 19 years (31.1 per cent).

In the first 20 years of this period (1956-1975), to varieties of table grapes, vine organs were not in danger only 4 years (20 per cent). In the same period, the winter buds were at risk of extinction in 16 years (80 per cent), the annual wood has been affected in 7 years (35 per cent), and multiannual wood was affected in 4 years (20 per cent).

In the last 20 years (1997-2016), the situation is relatively similar to the period 1956-1975 (Table 3). Vine organs were not affected in 4 years (20 per cent), winter buds were at risk in 16 (80 per cent), annual wood was affected in 8 years (40 per cent), and the multiannual wood was affected in 7 years (35 per cent).

The situation is totally different past 10 years. Of the 10 years, in two years, only (20 per cent) were not losses organs vine. Winter buds were affected in 8 years (80 per cent), wood annually suffered losses in 6 years (60 per cent) and the multiannual wood was affected in 5 years (50 per cent).

Table 3

The adverse effect of negative minimum temperatures for varieties of table grapes in South-Western Oltenia

| Temperature level | 1956-2016 period | | 1956-1975 period | | 1997-2016 period | | 2007-2016 period | |
|---|---|---|---|---|---|---|---|---|
| | Number years | % | Number years | % | Number years | % | Number years | % |
| Until -15$^0$ C | 16 | 26.2 | 4 | 20 | 4 | 20 | 2 | 20 |
| -15.1$^0$C…-30.5$^0$C | 45 | 73.8 | 16 | 80 | 16 | 80 | 8 | 80 |
| -18.1$^0$C…-30.5$^0$C | 21 | 34.4 | 7 | 35 | 8 | 40 | 6 | 60 |
| -21$^0$C…-30.$^0$C | 19 | 31.1 | 4 | 20 | 7 | 35 | 5 | 50 |
| Total years analysed | 61 | | 20 | | 20 | | 10 | |

## CONCLUSIONS

The risk that the organs of vine grapes for wine to be affected by negative minimum temperatures during active rest, increased in 2007-2016 compared with the period 1956-2016, from 34.4 to 60 per cent in the case of winter bud, from 22.9 to 50 per cent in the case of annual wood, and from 8.2 to 30 per cent in the case of multiannual wood.

Table grape varieties are more sensitive to negative minimum temperatures, and as a result, in the 61 years analyzed, vine organs were not affected in 16 years, 26.2 per cent, respectively. Winter buds were at risk of extinction in 45 years (73.8 per cent), annual wood was affected in 21 (34.4 per cent) and multiannual wood was affected in 19 years (31.1 per cent ).

Therefore, adaptation measures must be taken. Thus, the use of varieties tolerant to harmful minimum temperatures, but this is only possible at temperatures down to -18…-21$^0$C, or the protection of elements of fruit, in the form of leading classical (low) hubs by placing, in autumn, of 2-4 young wooden elements on the ground and covering with soil.

## REFERENCES


Baniţă P., 1983, Viticultura pe nisipuri. Editura CERES București.

Bishtawi M.A.A.M., Popa A., 2005, Comportarea unor soiuri de masă în condițiile ecopedoclimatice din centrele viticole Dăbuleni, Banu Maracine, Drăgășani. Analele Universității din Craiova, vol X (XLVI), 39-44.

Martin T., 1968, Viticultură. Editura didactică și pedagogică București.

Martin T., 1974, Strugurii de masă**.** Editura CERES, Bucureşti.

Oprean M.,1975, Viticultură generală. Editura didactică și pedagogică București.

Oşlobeanu M., 1980, Viticultură generală și specială. Editura didactică și pedagogică București.

Țârdea C., Dejeu L., 1995, Viticultură. Editura didactică și pedagogică București.

Stroe M.V., Bucur G.M., 2012, Study regarding the influence of low winter temperatures between 2011-2012 on the viability of winter buds of some table grape varieties in the conditions of the didactic experimental field in Bucharest. Scientific papers of University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Series B. Horticulture, Volume LVI, 181-184. Publishing UASVM Bucharest.

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# PLEVENSKI FAVORIT – NEW WHITE INTERPSECIES TABLE GRAPES VARIETY

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***Keywords***: *vine, table grapes variety, interspecies hybridization*

## ABSTRACT

*Plevenski Favorit is an early, white table grapes variety obtained by the method of interspecies hybridization at the Institute of Viticulture and Enology – Pleven. The leaf blade is large, medium thick, light green, medium to slightly notched, indented, five-lobed. Cluster is medium to large, conical, winged, semi-compact. Berry is medium to large, elongated, egg-shaped, yellow-green to amber in colour with a reddish hue. Skin is medium thick, quite tender with abundant wax coating. Berry texture is succulent-fleshy; the taste is neutral, harmonious, slightly fresh. Grapes have good transportability. Vines grow vigorously, having high fertility and yield. The variety has increased resistance to low winter temperatures, downy mildew and powdery mildew. It is suitable for organic table grapes production.*

## INTRODUCTION

Regardless the large number of new varieties of cultural vine (Vitis vinifera L.), obtained by man for thousands of years, they could not fully meet the current requirements due to their sensitivity to low winter temperatures, phylloxera, downy mildew, powdery mildew and other stressors. The method of interspecies hybridization in vine selection allows both from scientific and applied aspect, the obtaining of new immune and resistant varieties and clones. Crossing of European varieties producing high quality grapes with American species and hybrids characterized by resistance to mildew, phylloxera and low winter temperatures was the basis of vine interspecies hybridization (Ivanov & Valchev 1971; Ivanov 2011).

In Bulgaria the method of interspecies sexual hybridization had also practical application for obtaining sustainable wine and table grapes varieties. By the end of the last century at IVE – Pleven, 13 new original interspecies vine varieties with increased resistance to low winter temperatures and fungal diseases were approved by the State Variety Commission, among which five white wine – Pomoriyski Biser (Ivanov et al., 1984), Srebrostrui (Ivanov et al., 1984a), Muskat Kaylashki (Valchev et al., 1984b), Dunavski Lazur (Valchev et al., 1984a) and Slava (Roychev, 2012); five red wine - Mizia (Valchev et al., 1984d); Plevenski Kolorit (Valchev et al., 1984e); Nikopolski Mavrud (Valchev et al., 1984e), Storgozia (Ivanov et al., 1984e) and Dunavska Gamza (Ivanov et al., 1984c); two varieties for double use, for fresh consumption and for white wines and juices - Naslada (Valchev et al., 1984g) and Druzhba (Ivanov et al., 1984b) and one black

table grapes variety Lyubimets (Valchev & Ivanov, 1996). Most of these varieties have been efficiently planted on thousands of decars of vineyards.

In 2009 the Executive Agency of Plant Variety Testing, Approbation and Seed Control approved five interspecies varieties, new generation. Three of them were wine: Plevenska Rosa (Ivanov et al., 2011), Kaylashki Rubin (Ivanov et al., 2011) and Trapezitsa (Ivanov et al., 2012) and two table grapes varieties - Garant and Plevenski Favorit.

*The objective of this study* is to present detailed ampelographic characteristics of the newly-selected white table grapes interspecies variety Plevenski Favorit.

## MATERIAL AND METHODS

The ampelographic study of the variety was carried out during the period 1990-2009, at the vine plantation (hybrid area) in the Experimental Base of ILV-Pleven. Vines were planted at a distance of 3.00 m and 1.30 m in the row. They were grown on modified Moser training system. The climate in this region is pronouncedly continental. The soils are slightly leached black earth, formed on loam. The analysis of soil and weather conditions of the region had shown that they were favorable to vine growing and researches for obtaining new varieties. The methodology described in Bulgarian Ampelography vol.1 (Katerov et al., 1990) was used in the ampelographic study of the variety.

## RESULTS AND DISCUSSIONS

**ORIGIN**. Plevenski Favorit variety was obtained from crossing of SV 12375 x Muskat Plevenski in1983 in the Experimental field of IVE Pleven by M. Ivanov et al. It was recognized by Order No. RD 12-22/16.04.2009 of MAF and patented by the Patent Office as a new plant variety with certificate No. 10874/30.07.2010.

### BOTANIC DESCRIPTION

**Young shoot:** Crown is bare, light green. Young leaves are bare with copper red hue. The young shoot is fully open. The pubescence on the apex is very rare. The apex pubescence lacks anthocyanin coloring. The internode dorsal and ventral side is green with reddish stripes. There are no bristles and pappus along the internodes. The number of tendrils on the shoot is less than three. The available tendrils are long. The shoot position before tying is semi-upright.

**Mature shoot:** The internodes are medium long (8.6 cm). Mature shoot is medium thick, almost cylindrical; its bark is dark brown in colour. The surface relief is petty indented. The tendrils are long, medium branched and often fall.

**Leaf:** Leaf blade is large (19.4/18.8 cm). It is medium thick; light green, slightly indented, five-lobed. The cross section profile is V-shaped. The top side of the leaf blade is slightly vesiculated. The bottom surface is slightly bristle and bare. The top notches are deep, open in the form of a fissure. The bottom notches are hardly noticeable, open in an acute angle or hardly noticeable. The tail notch is medium to wide open. It is not bordered by the nervation. The top teeth are big and long while the end ones are medium big. Both are straight, triangular with wide base. The anthocyanin coloring of the main veins on the top side of the leaf blade is average. The bristles density on the main veins on the bottom side of the leaf blade is average. The leaf petiole is long. Compared to the median vein length, it is slightly longer.

**Inflorescence:** The inflorescence is androgynous with six, very rarely with five or seven stamens, with an average long, thin, upright stems. The pistil is medium large, bottle-shaped. The stalk is medium long while the stigma is big, enlarged and oval.

**Cluster:** The cluster is medium to large (19.50/14.20 cm), conical, winged, semi-compact. The average mass of the cluster is 505.4 g. The rachis is green. The cluster stem is long, wooden. The berry stem is medium long, thick, with a large cone-shaped bed. The brush is medium long (Fig. 1).

Fig. 1. Variety Plevenski Favorit.

**Berry:** The berry is medium large to large (19.50/13.20 mm), elongated, ovoid, yellow greenish to amber colored, with reddish hue. The berry is easily detached from the stalk. The skin is medium thick, rather fragile with abundant wax coating. The texture is fleshy, juicy with neutral, harmonious and fresh taste. The average berry mass is 6.56 g.

**Seeds:** The seeds are medium large to large (6.2/4.4 mm), elongated, olive green to brownish. The beak is medium long, conical in shape. The chalaza is well outlined, oval. The raphe is slightly convex.

## AGROBIOLOGICAL CHARACTERISTIC

**Vegetation period.** Plevenski Favorit variety belongs to the group of early ripening varieties. Its grapes ripen during the second half of August. The duration of the period from budding to the grapes maturation is about 130 days. The first leaf appears around 20 April while the first inflorescence around 26 April (Table 1).

Table 1

Stages of vegetation of variety Plevenski favorit average from 1990-2009 periode

| Beginning of sap movement | Beginning of budding | Flowering | | Softening of berries | Technological ripeness | Fall of the leaf |
|---|---|---|---|---|---|---|
| | | beginning | final | | | |
| 08.04. | 18.04. | 26.05. | 08.06. | 20.07. | 31.07. | 25.10. |

**The maturation degree of the annual growth.** The annual shoots ripen very well until the end of the growing season. The shoot maturation starts around 20 August and ends by 5 October.

**Vine growth strength.** Vines have an intense growth. They form a small number of side shoots and are slightly leafy.

**Fertility and yield.** Under ground and stem training systems the variety has high fertility and high yield. The ratio of developed eyes is high. The fruit shoots ratio is 70.00% while the fertility rate – 0.96. Fruit shoots with two and more clusters are prevailing. The yield per vine is 5.500 kg, and per decar - 2200 kg.

**Putting forth catkins and milerandage.** The variety is not liable to putting forth catkins.

**Disease resistance.** Plevenski Favorit variety has increased resistance to downy mildew and powdery mildew under field conditions (score 1-2).

**Agrotechnical characteristics.** With ground training systems, depending on the vine growth, it can be used single or double Guillot pruning. Mixed fruit units are left on stem training.

**Suitable rootstocks.** Vines grow and have good fruit-bearing capacity grafted to rootstocks Shasla x Berlandieri 41 B, Rupestris du Lot and CO4.

**Response to environmental conditions.** Plevenski Favorit variety has increased resistance to low temperatures. In the studies on the resistance degree to low temperatures in refrigerating chambers at t= -24$^0$C in some of the years over 20 % of the main and 30 % of the substituting buds had remained not damaged.

**TECHNOLOGICAL CHARACTERISTICS**

**Grapes mechanical composition.** According to the mechanical composition of the cluster and berry, Plevenski Favorit variety is typically table grapes. The berry ratio in a cluster is 97.26%, while skins are 4.28%, seeds 1.53%, and the mesocarp–94.19% (Table 2).

Table 2

Mechanical analysis of the grapes of variety Plevenski Favorit average from 1990-2009 periode

| Mechanical analysis of cluster | | |
|---|---|---|
| 1. | Weight, g | 505.40 |
| 2. | Length, cm | 19.50 |
| 3. | Width, cm | 14.20 |
| 4. | Rachis, % | 2.74 |
| 5. | Berries, % | 97.26 |
| Mechanical analysis of berry | | |
| 1. | Weight, g | 6.56 |
| 2. | Length, mm | 19.50 |
| 3. | Width, mm | 13.20 |
| 4. | Skins, % | 4.28 |
| 5. | Seeds, % | 1.53 |
| 6. | Flesh, % | 94.19 |

**Must chemical composition.** When grape is mature for consumption, it contains 17.9% sugars and 6.65 g/dm$^3$ titratable acids. It is characterized by

harmonious, neutral taste. Grapes tasting score over 7.60 (on a 10-score scale) (Table 3).

**Use of the grapes and the produce assessment.** Grapes have good transpor-tability, as the detachment resistance of the berries is 593.0 g, and to pressure 1901.0 g. It is suitable for satisfying the market demands with high-quality white table grapes at the end of August.

Table 3

Physicochemical analysis of grapes from variety Plevenski Favorit average from 1990-2009 periode

| Sugars, % | Titratable acids, g/dm$^3$ | Endurance of berries: | |
|---|---|---|---|
| | | of pressure, g | of picking up, g |
| 17.90 | 6.65 | 1901.00 | 593.00 |

## GENERAL ASSESSMENT OF THE VARIETY

The newly-selected Plevenski Favorit is an early, white table grapes variety. It has bare light green crown and large leaf blade, medium thick, light green, medium to slightly notched, indented. Vines grow vigorously, having average to high fertility and yield. Cluster is medium to large, conical, winged, semi-compact. Berry is medium to large, elongated, egg-shaped, yellow-green to amber in colour with a reddish hue and abundant wax coating. Berry texture is succulent-fleshy; the taste is neutral, harmonious, slightly fresh. Grapes have very good transportability. The variety has increased resistance to low winter temperatures, downy mildew and powdery mildew. It is suitable for organic table grapes production. It can be grown in all vitucultural regions in Republic of Bulgaria.

## REFERENCES


Ivanov M., Nakov Z., Simeonov I. 2011, Plevenska rosa – a new white wine grapevine variety, Agricultural Science, Vol. 44, №3, 80-85 pp.

Ivanov M., Nakov Z., Simeonov I., Iliev A. 2011, Kaylashki Rubin – a new red wine grapevine variety, Agricultural Science, Vol. 46, №4, 60-65 pp.

Ivanov M., Simeonov I., Nakov Z.. 2012, Trapezitsa – a new red wine grapevine variety, Agricultural Science, Vol. 45, №1, 57-62 pp.

Ivanov Y., Valchev V.. 1971, Cold tolerance and resistance to mildew of hybrids between varieties of vine. Genetics and Selection, 3, 139-146 pp.

Ivanov Y., Valchev V., Petkov G., 1984a, New white wine grapevine variety - Srebrostrui. Horticulture and wine science, 5, 65-69 pp.

Ivanov Y., Valchev V., Petkov G., 1984b, New vine variety - Drujba. Horticulture and wine science, 2б, 78-83 pp.

Ivanov Y., Valchev V., Petkov G., 1984c, Dunavska Gamza - a new vine variety. Horticulture and wine science, 3б, 70-74 pp.

Ivanov Y., Valchev V., Petkov G., 1984d, New white wine grapevine variety – Pomoriiski Biser. Horticulture and wine science, 46, 60-64 pp.

Ivanov Y., Valchev V., Petkov G., 1984e, Storgozia - a new vine variety. Horticulture and wine science, 2, 84-88 pp.

Ivanov, M. 2011, Results from interspecific hybridization with table varieties. Dissertation of PhD, AU-Plovdiv, 181 p.

Katerov K., Donchev A., Kondarev M., Getov G., Nachev T., Hershhovich E., Valchev V., Markova M., Braykov D., Todorov H., Мамarov P., Ivanov Y., Zankov Z., Tsankov B., Radulov L., Ivanov M., Jekova M., 1990, Bulgarian Ampelographia, General ampelographia, Publisher of BAS, V. I, 296 p.

Roychev, V., 2012, Ampelographia. Academic publisher of the Agricultural University of Plovdiv, 574 c.

Valchev V., Ivanov Y., Petkov G., 1984a, New white wine grapevine variety - Dunavski Lazur. Horticulture and wine science, 8, 105-110 pp.

Valchev V., Ivanov Y., Petkov G., 1984b, New white wine grapevine variety – Muskat Kaylashki, Horticulture and wine science, 7, 76-80 pp.

Valchev V., Ivanov Y., Petkov G., 1984c, Nikopolski Mavrud – a new wine grapevine variety. Horticulture and wine science, 6, 84-88 pp.

Valchev V., Ivanov Y., Petkov G., 1984d, Mizia –a new wine grapevine variety, Horticulture and wine science, 4, 65-69 pp.

Valchev V., Ivanov Y., Petkov G., 1984e, Plevenski Kolorit – a new wine grapevine variety, Horticulture and wine science, 5, 70-74 pp.

Valchev V., Ivanov Y., Petkov G., 1984g, Naslada – a new vine variety, Horticulture and wine science, 3б, 75-79 pp.

Valchev V., Ivanov M., 1996, Lubimets – a new table grapevine variety. Viticulture and Enology, 3, 3-4 pp.

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# PLANTATION PRODUCTIVITY OF QUINCE ZAREA AND CODREANCA VARIETY DEPENDING ON THE PLANTING DISTANCE

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**Keywords:** *Quince, planting distances, number of fruits, average weight*

## ABSTRACT

*The research was conducted in plantation quince enterprise „Schit Agromex " Ltd. founded in spring 2009 year with fruit shaped rod. Trees are led by improved system of natural crown with low volume. The research was carried out during 2012-2014. As an object of study were varieties Zarea and Codreanca grafted on rootstock BA29 and planting distance between rows 3.5; 4.0 and 4.5 m, and between trees in row 1.5; 2.0 and 2.5m. As witness was taken the planting distance of 4.0 x 2.0 m. It was established that the number of fruits and average weight depend of biological peculiarities of the varieties and planting distance. Planting distance influences on productivity on a tree and unit area. Versions with a greater planting distance register higher productivity values to a tree, but lower to a unit area than traditional setup with higher density planting.*

## INTRODUCTION

Quince tree is a species whose fruits are highly valued as a raw material in the food industry. Quinces can be processed in a wide assortment: honey, jam, jelly, marmalade, compote etc. (Cimpoieş 2002, Масюкова 1963).

The surface of nutrition is the unit area of land of a certain shape and size and adequate living space underground air and they offered a herbal plantation (Синягин 1970).

The size and shape of the nutrition surface determines the density and uniformity of plants in the plantation, factors of which largely depends the system of crop, harvest and production competitiveness (Babuc 2000, Balan 2003).

In the modern horticulture, there are different suggestions about the planting distance and culture system of quince. From the studies made in Romania by Popescu et al. (1992), it is recommended the distance between the quince trees with medium and low growth vigor to be 3.0-3.5x2.0-2.5 m.

Ghena and Branişte, (2003) recommends the distance of 4.0x4.0 m and 3.5x2.0 m, Cepoiu et al. (2005) recommend 3.5-4.0x1.5-2.0 m, and Drăgănescu and Mihuţ (2005) suggest a planting distance of 4.0 m between rows and 2.5-3.0 m on the row.

Quince fruit yield and quality are closely connected with the agricultural technique used in the orchard. The most important processes that enable stable

and high harvests are associated with the right choice of variety/rootstock, distance of planting, location of trees, forming and cutting of crowns according to the requirements in force (Babuc 2012; Balan et al. 2001; Cimpoieş 2002; Grădinăriu 2002; Клименко 1993).

The results of studying these indicators form the basis of planting distances argument on the quince trees in a plantation industry.

## MATERIALS AND METHODS

The research was conducted in the quince plantation at the „Schit Agromex" Ltd, founded near the village Hartopul Mic, district Criuleni. Planting was carried out in spring of 2009 with Zarea and Codreanca variety trees shaped as a stick grafted on rootstock BA29.

The distance between rows is 3.5; 4.0 and 4.5 m, and between the trees on the row is 1.5; 2.0; 2.5m. As control was taken the planting distance of 4.0 x 2.0 m. The trees are led by natural improved crown system of low volume.

Each variant include four rehearsals located on the ground after latin square system and the number of trees in a rehearsal is eight.

The research was conducted by approved methods of experiences in the field and laboratory conditions to study growing plants (Мойсейченко и др. 1994).

The number of fruits, the average weight of a fruit, the production per tree and per unit area was established during harvest.

## RESULTS AND DISCUSSIONS

In the early years, the greatest amount of fruits is done on annual branches and on the second and third degree of branching. We cannot count on the crop from the snail horns, because the potential fruitfulness of knobs on them is reduced and the fruits fall early (Cimpoieş, 2002; Горин, 1961; Масюкова, 1963).

Quince plantation productivity depends on the number of fruits in the trees crown, the average weight of a fruit and the planting density of plants per unit of area.

Investigations conducted indicate that the highest amount of fruits is obtained from Zarea variety, and less from Codreanca. If in the control variant where the planting distance was 4.0 x 2.0 m, in the crown of Zara variety were 31-129 fruits, then on Codreanca variety only 22-84 fruits. These index values are in direct correlation with the biological particularities of the studied variety (tab. 1).

During the research years, the highest number of fruits was obtained in 2013 being 120-134 pcs/tree on the Zarea variety, and 74-93 pcs/tree of Codreanca.

The lower number of fruits (21-34 pcs/tree) obtained in 2012 is explained by the fact that the trees were in their fourth year of vegetation and this is optimal for growth and fruiting period of quince.

In 2014, the number of fruits decreased being 28-32 pcs/tree on Zarea variety, and 23-27 pcs/tree on Codreanca. The abundant rainfall caused this during flowering and the flowers placed on the formations in the various multiannual branches were affected by bacteriosis.

The number of fruits depends and on the density of trees. The higher the density of trees is, the lower the amount of fruits obtained from the trees crown is. The lowest amount of fruits was obtained when the distance of planting was 3.5x1.5 m where the density of tree was 1904 pcs/ha. At Zarea variety, in 2012, the

mentioned index was 120 pcs/tree, and on Codreanca variety 74 pcs/tree. In the years 2012 and 2014, large deviations between the numbers of fruit was not recorded.

Increasing the distance of planting, heels increase the number of fruits in the trees crown. When the distance of planting was 3.5x2.0 m, the number of fruits increased by 5% on Zarea variety compared with the planting distance 3.5x1.5 m, but in the variant where the planting distance was 3.5x2.5 m with 8.3%. On Codreanca variety, the mentioned index increased with 6.7 and 12.2%.

Once the planting distance between rows changed from 3.5 to 4.0 m and the distance between trees in the row changed from 2.0 to 2.5 m, the number of fruits increased being on Zarea variety 8.4 to 12.6% and on Codreanca variety 9.1 to 16.9%.

Table 1

The number of fruits and their average weight based on the distance of planting trees quince

| Planting distance, m | Number of trees, pcs/ha | Quantity of fruits, pcs/tree | | | Average weight, g | | |
|---|---|---|---|---|---|---|---|
| | | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 |
| Zarea variety | | | | | | | |
| 3.5 x 1.5 | 1904 | 28 | 120 | 28 | 190 | 185 | 237 |
| 3.5 x 2.0 | 1428 | 28 | 126 | 28 | 190 | 188 | 240 |
| 3.5 x 2.5 | 1142 | 31 | 130 | 30 | 194 | 190 | 242 |
| 4.0 x 1.5 | 1666 | 30 | 119 | 28 | 192 | 188 | 241 |
| 4.0 x 2.0 | 1250 | 33 | 129 | 31 | 194 | 193 | 246 |
| 4.0 x 2.5 | 1000 | 34 | 134 | 32 | 194 | 202 | 244 |
| 4.5 x 1.5 | 1481 | 31 | 124 | 29 | 192 | 189 | 243 |
| 4.5 x 2.0 | 1111 | 33 | 127 | 32 | 195 | 201 | 245 |
| 4.5 x 2.5 | 888 | 34 | 131 | 32 | 195 | 202 | 246 |
| Average | - | 31,3 | 126,6 | 30 | 192,9 | 193 | 242,6 |
| Codreanca variety | | | | | | | |
| 3.5 x 1.5 | 1904 | 21 | 74 | 23 | 270 | 255 | 275 |
| 3.5 x 2.0 | 1428 | 21 | 79 | 24 | 270 | 257 | 274 |
| 3.5 x 2.5 | 1142 | 22 | 83 | 24 | 276 | 261 | 278 |
| 4.0 x 1.5 | 1666 | 22 | 77 | 25 | 267 | 255 | 274 |
| 4.0 x 2.0 | 1250 | 22 | 84 | 27 | 271 | 257 | 277 |
| 4.0 x 2.5 | 1000 | 22 | 90 | 27 | 274 | 263 | 275 |
| 4.5 x 1.5 | 1481 | 21 | 79 | 25 | 271 | 257 | 275 |
| 4.5 x 2.0 | 1111 | 23 | 86 | 24 | 275 | 260 | 277 |
| 4.5 x 2.5 | 888 | 23 | 93 | 26 | 273 | 261 | 280 |
| Average | - | 21,9 | 82,7 | 25 | 271,9 | 258,4 | 276,1 |

When the planting distance was 4.5x2.0 m at Zarea variety, the studied index increased by 2.4%, but when the planting distance was 4.5x2.5 m with 5.6% compared with the planting distance 4.5x1.5 m. For Codreanca variety, the above numbers are valid too and the number of fruits increased respectively with 8.1 and 17.7%.

The average weight of a fruit is a direct relationship with the biological peculiarities of the variety and density of planting trees. The highest average weight of a fruits was recorded on Codreanca variety. In 2013, on Zarea variety the average weight of a fruit was 185-202 g, but on Codreanca variety the above index was 255-261g.

Starting with the fifth year of planting, it was noticeable an increase in the average weight of a fruit where the planting distance between rows was 4.0 and 4.5 m and between trees on the row 2.0 and 2.5 m. If in 2013, on Zarea variety where the distance of planting was 3.5x1.5 m, the average weight of a fruit was 185 g, then when the distance of planting was 4.5x2.5 m, the mentioned index increased by 9.2%. The same happened and on Codreanca variety where the average weight of a fruit was 255 and 261g.

Plantation productivity is directly influenced by biological particularities of the variety, planting density, number of fruit in tree and their average weight. The number of fruits on its crown and the fruits average weight directly determines tree productivity (tab. 2).

Table 2

Quince plantation productivity according to the planting distance

| Planting distance, m | Number of trees, pcs/ha | Yield | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | kg/tree | | | t/ha | | | |
| | | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 | Average |
| Zarea variety | | | | | | | | |
| 3.5 x 1.5 | 1904 | 5.32 | 22.20 | 6.63 | 10.12 | 43.31 | 12.62 | 20.52 |
| 3.5 x 2.0 | 1428 | 5.32 | 23.69 | 6.72 | 7.59 | 33.83 | 9.59 | 16.57 |
| 3.5 x 2.5 | 1142 | 6.01 | 24.76 | 7.26 | 6.86 | 28.20 | 8.29 | 14.23 |
| 4.0 x 1.5 | 1666 | 5.76 | 22.37 | 6.74 | 9.59 | 37.26 | 11.22 | 18.60 |
| 4.0 x 2.0 | 1250 | 6.40 | 24.89 | 7.62 | 8.00 | 31.11 | 9.52 | 14.86 |
| 4.0 x 2.5 | 1000 | 6.59 | 27.06 | 7.80 | 6.59 | 27.06 | 7.80 | 12.51 |
| 4.5 x 1.5 | 1481 | 5.95 | 23.43 | 7.05 | 8.81 | 34.70 | 10.44 | 17.13 |
| 4.5 x 2.0 | 1111 | 6.43 | 25.52 | 7.84 | 7.14 | 28.35 | 8.71 | 13.56 |
| 4.5 x 2.5 | 888 | 6.63 | 26.46 | 7.87 | 5.88 | 23.50 | 6.98 | 11.73 |
| Average | - | 6.04 | 24.48 | 7.28 | 7.84 | 31.86 | 8.68 | - |
| Codreanca variety | | | | | | | | |
| 3.5 x 1.5 | 1904 | 5.67 | 18.87 | 6.32 | 10.79 | 35.92 | 12.03 | 19.58 |
| 3.5 x 2.0 | 1428 | 5.67 | 20.30 | 6.35 | 8.69 | 29.99 | 9.38 | 16.02 |
| 3.5 x 2.5 | 1142 | 6.07 | 21.66 | 6.67 | 6.93 | 24.73 | 7.61 | 13.09 |
| 4.0 x 1.5 | 1666 | 5.87 | 19.63 | 6.85 | 9.78 | 32.70 | 11.41 | 17.96 |
| 4.0 x 2.0 | 1250 | 5.96 | 21.58 | 7.47 | 7.45 | 26.97 | 9.33 | 14.58 |
| 4.0 x 2.5 | 1000 | 6.03 | 23.67 | 7.42 | 6.03 | 23.67 | 7.42 | 12.37 |
| 4.5 x 1.5 | 1481 | 5.69 | 20.30 | 6.87 | 8.42 | 30.06 | 10.17 | 16.22 |
| 4.5 x 2.0 | 1111 | 6.32 | 22.38 | 6.65 | 7.02 | 24.85 | 7.38 | 13.08 |
| 4.5 x 2.5 | 888 | 6.28 | 24.27 | 7.28 | 5.57 | 21.55 | 6.46 | 11.19 |
| Average | - | 5.95 | 21.40 | 6.90 | 7.85 | 27.82 | 8.30 | - |

During the research, the lowest yield was obtained in 2012 and the highest value in 2013. Zarea variety registered a higher output than Codreanca variety. In

2013, on Zarea variety, the productivity of a tree was 22.20-26.46 kg/tree, while on Codreanca variety 18.87-24.27 kg/tree.

The planting distance influences directly over tree productivity. The variants with a higher planting distance registered lower value then the ones with the smaller planting distances

In 2013 on Zarea variety, the lowest yield of quince was registered where the planting distance was 3.5-1.5 m being 22.20 kb/tree. The differences in yield between the planting distances 3.5x1.5 m and 4.0x1.5 m was 0.17 kg/tree and between the planting distance 4.5x1.5 m -1.23 kg/tree. With increasing planting distance between trees up to 2.0 m, the difference between studied indexes is bigger, respectively 1.20 and 1.83 kg/tree. In the variants where the planting distance was 4.5x2.0 m and 4.5x2.5 m, the productivity for tree was respectively 27.06 and 26.46 kg/tree.

The yield of quince per unit area is calculated based on productivity per tree and density of planting. The highest yield was registered where the distance of planting was 3.5x1.5 m; 4.0x1.5 m; 4.5x1.5 m and 3.5x2.0 m comparing with the control variant. The yield of fruits in 2013 on the mentioned variants was respectively 42.31; 37.26; 34.20 and 33.53 t/ha. On the rest of the variants the yield of fruits was lower than in the control variant being 23.50 – 28.35 t/ha. The difference in produce between the densest variant (3.5x1.5 m) and the rarest variant (4.5x2.5 m) was 19.81 t/ha. Thus, planting distances 4.5x2.0 m and 4.5x2.5 m are great for Zarea variety grafted on BA29 rootstock.

A greater force growth and a lower level of productivity characterize Codreanca variety. Fruit production achieved per tree was 18.87-24.27 kg. The difference between the recorded production between the densest variant (3.5x1.5 m) and the rarest variant (4.5x2.5 m) was 28.6%. Because the productivity per tree decreased, the yield obtained per surface unit decreased too being 21.55-35.95 t/ha.

Investigations conducted demonstrates that as planting density per unit area increases fruit production increases, which means that the orchard land is exploited more effectively than planting distances greater. The reserved space per tree is occupied more rationally and records content yield after planting.

During that period, it is prematurely to recommend anything about the planting distance, but it is noticeable that once the density of planting is higher the production is high too.

## CONCLUSIONS

The number of fruits depends on the biological particularities of the variety and the density of planting. As the density of planting increases, the number of fruits in the trees crown decreases on both varieties.

The average weight of a fruit is in a direct relationship with the biological particularities of the variety and density of planting trees. The highest average weight of a fruit during the study was quince of Codreanca variety.

The planting distance influences directly on the productivity per tree and per surface unit. The variants with a larger planting distance register a higher productivity per tree, but lower productivity per unit area compared with the variants with a higher density.

## REFERENCES


Babuc V., 2000, Arhitectonica plantaţiei pomicole factor determinativ al productivităţii. Realizări, probleme şi perspective în pomicultură. Materialele conferinţei ştiinţifice ICP. Chişinău. 22-29.

Babuc V., 2012, Pomicultura. Edit. Tipografia Centrală, Chişinău, 662 pag.

Balan V., Cimpoieş Gh., Barbăroşie M., 2001, Pomicultura. Ed. Museum, Chişinău, 453 pag.

Balan V., 2003, Distanţa de plantare – factor determinativ al structurii plantaţiei pomicole. Luctări ştiinţifice UASM. 70 ani ai UASM, Horticultură, Silvicultură şi Protecţia Plantelor. Chişinău. 51-53.

Cepoiu N., Păun C., Spiţă V., 2005, Pomicultura practică. Edit. Cereş, Bucueşti, 195 pag.

Cimpoieş Gh., 2002, Pomicultura specială. Edit. Colograf-Com, Chişinău. 100-112.

Drăgănescu E., Mihuţ E., 2005, Cultura speciilor pomicole. Edit. Waldpress, Timişoara, 58-76.

Ghena N., Branişte N., 2003, Cultura specială a pomilor. Edit. Matrix Rom, Bucureşti, 171-178.

Grădinăriu G., 2002, Pomicultura specială. Edit. Ion Ionescu de la Brad, Iaşi, 107-1017.

Popescu M., Miliţiu I., Cireaşă V., Godeanu I., Cepoiu N., Drobotă Gh., Ropan G., Parnia P., 1993, Pomicultura (generală şi specială). Edit. Didactică şi pedagogică, Bucureşti, 422 pag.

Горин И.Т., 1961, Айва. Москва, 181 с.

Клименко С.В., 1993, Айва обыкновенная. Київ, 285 с.

Масюкова О., 1963, Култура гутуюлуй ын Молдова. Кишинэу, 72 р.

Мойсейченко В.Ф., Заверюха А. Х., Трифанова М. Ф., 1994, Основы научных исследований в плодоводстве, овощеводстве и виноградарстве. Из. Колос, Москва, 365 с.

Синягин И.И., 1970, Площади питания растений. Из. Россельхозиздат, Москва, 232 с.

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# STUDIES ON COMBATING PATHOGENS AND PESTS VINES IN THE VINEYARD CLIMATIC CONDITIONS DEALUL BUJORULUI VINEYARD

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## ABSTRACT

*Vines in plant protection is an important technological component for the production of quality grapes. At the moment it is more than necessary, finding integrated combat systems secure to avoid failures. Researches were conducted in 2015 respectively 2016 as SCDVV Bujoru where he was experienced the a program to combat major diseases and pests of grapes. Developed technology to aim at minimizing the impact of the use of plant protection products on the environment by optimizing the number of treatments and doses recommended by the manufacturer compliance. Agencies pathogens and pests controlled: grape mildew (Plasmopara viticola - Berk. et Curt.), powdery mildew vine (Uncinula necator - Schw.), red staining (Pseudopeziza trancheiphila), black rot (Guignardia bidwelli), gray rot of grapes (Botrytis cinerea Pers.). Pathogens were followed by field observations and notations to determine the frequency (F), intensity (I) and the attack (G.A%). The recorded data were correlated with specific climatic conditions Dealul Bujorului vineyard.*

## INTRODUCTION

After entry of pests and diseases in the Americas growing countries in Europe, growers, both practitioners and researchers alike have sought ways and means to prevent and fight as effectively.

Achieve high yields and good quality viticulture involves the correct and optimal timing of all technological links, where it occupies an important place against pathogens and pests.

The emergence and evolution of the main pathogens and pests of wine grapes in the vineyard area Dealu Bujorului is influenced by direct and indirect effects of technological and ecological factors specific area, affecting the quality and quantity of grape production.

## MATERIAL AND METHODS

The researches were conducted at the Research and Development Station for Viticulture and Winemaking Bujoru in a vineyard cultivated with Babeasca neagra and The Witness/ The Untreated Lot (Babeasca neagra ) aged 32 years.

Location experiment was conducted on land with a slope of 3-5% chernozem soil type with a humus content between 1.14 to 1.86% in the A horizon and a weak alkaline reaction (pH 7,44 to 8,30 ) and sandy loam; exhibition eastern

land, about 170-200 m altitude, orientation north-south rows, planting distance of 2,1 m x 1,2 m, density provided 3968 vines/hectare Berlandieri x Riparia rootstock selection Telecky Openheim SO4-4.

The organization experience of comprised the following varieties:

- Băbească neagră
- The Witness/ The Untreated Lot

In order to determine the gravity of the attack produced by the main vine diseases of (manna, powdery mildew, gray rot) have been commented on the intensity (I) the frequency (F) and degree of attack (AD/GA%) of their leaves and grapevine.

## RESULTS AND DISCUSSIONS

To characterize the specific vineyard microclimate conditions Dealu Bujorului were used weather data recorded at the meteorological station at S.C.D.V.V. Bujoru.

Climate conditions Spring 2015 season was unusual for that period by higher average temperatures than normal period, with average air temperature of 19.2 ° C/May, 22.1 ° C/June 25 8 ° C/July. Maxima April was 27.2 ° C. May and July of thermally topped multi values (Table 1).

Relative humidity was below normal period, with values below 50% throughout the growing season. During the active growing season began with low rainfall, so the rainfall records have not exceeded normal monthly average so in the months from May to June was recorded a deficit of 27.8 mm.

Rainfall during the growing season were deficient in April (15.5 mm to the multiannual), May (21.3 mm versus multi), June (6.5 mm to the multiannual) and in July a deficit 35.6 mm compared to normal. Note that water scarcity during April-July 2015 was 78.9 mm against the multiannual. Climatic elements highlight the fact that in 2015 the climatic conditions were unfavorable appearance and evolution of pathogens and pests.

Regarding the 2016 spring season Climate conditions manifested by lower average temperatures than normal period, with average air temperature of 15.3 ° C/May, 21.3 ° C/June, 22.9 ° C/22.3 ° C and in July/August. April of thermally topped both multi values, and 2015.

During the active growing season began with high rainfall, thus exceeded the average monthly rainfall records were normal, so in the months from April to June was recorded a surplus of 122.5 mm from the multiannual ..

Relative humidity has exceeded the normal period, with high values between 57% and 70%, throughout the growing season.

Analysis of climatic elements of 2016 highlights the fact that in May -June were favorable conditions for the development of developing and hand, and in July-August mildew.

Thus, in climatic conditions during the growing season of the years 2015, and 2016 they were alerted and executed 6-7 treatment plant to combat pathogens and pests of grapes.

Treatments were carried to the warning using MPSP drive 3-300, the products being complexed by pathogens which had combătuţi.Cantitatea solution used was 400 l/ha in the first two treatments, and treatments following 900-1000 l/Ha.

Meteorological station from SCDVV Bujoru

Table 1

Weather data from the period 2015-2016

| Month | Monthly average( t ºC) Average of the month | | | Precipitations (mm) | | | Hygroscopicity humidity (%) | | | Number of days with rain | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | the normal | 2015 | 2016 | the normal | 2015 | 2016 | the normal | 2015 | 2016 | 2015 | 2016 |
| IV | 11,2 | 11,6 | 13,0 | 40,4 | 24,9 | 66,2 | 49 | 49 | 68 | 10 | 7 |
| V | 18,6 | 19,2 | 15,3 | 31,2 | 9,9 | 63,4 | 53 | 49 | 70 | 8 | 15 |
| VI | 22,6 | 22,1 | 21,3 | 53,2 | 46,7 | 74,4 | 46 | 47 | 70 | 9 | 7 |
| VII | 24,9 | 25,8 | 22,9 | 54,9 | 19,3 | 12,4 | 41 | 48 | 57 | 10 | 4 |
| VIII | 23,2 | 25,4 | 22,3 | 61,7 | 69,7 | 38,0 | 66 | 47 | 58 | 7 | 8 |
| Sum | 100,5 | 104,1 | 94,8 | 241,4 | 170,5 | 254,4 | 255 | 240 | 323 | - | - |
| Average | 20,10 | 20,82 | 18,96 | - | - | - | 51,0 | 48,0 | 64,6 | - | - |

**The vines manna** (*Plasmopara viticola* - Berk. Et Curt.): 2015 climatic conditions were unfavorable to the emergence and the evolution vines manna. Thus, during the vegetation period the manna evolution was low. Regarding 2016, the favorable weather conditions (precipitation frequency, high atmospheric humidity etc.) favored the appearance and development of pathogen phenophase flourished, maintaining bunches to compaction. In this situation, the fungicides used were protected leaves and blossoms limiting the degree of attack (G.A%) to 1.15% Babeasca black leaf or 1.07% on grapes. Witness at (untreated) the degree of attack (G.A%) was 34.99% and 100% leaves inflorecente (Table 3).

**Grapevine powdery mildew** (*Uncinula necator* - Schw. Burr) pathogen recorded during the growing season a total of 15 generations. Among the factors favorable to the emergence and evolution meteorologicii vine mildew, air temperature plays a decisive role. The limits are broad, almost 10 ° C, the threshold biological vine up to 30 ° C, the optimum being around 22-26 ° C. During the years 2015 and 2016 was virulet pathogen, but was kept under control by applying the optimum time phytosanitary treatments with specific products (Shavit 72 WDG, 95 Sulfavit PP, Cosavet, Bumper 250 EC). At the end of blossoming, cultivar studied the degree of attack (G.A%) on leaves and grapes was 0.0% and the fruits of grapes 1.72% and 2.37% on the grape leaves (Table 4).

**Gray rot of grapes** (*Botrytis cinerea* - Pers.): Due to adverse climatic conditions in 2015 and 2016 (precipitations low from summer period, low atmospheric humidity) symptoms pathogen attacks not been signaled in experimental groups (Table 5).

Table 2

Scheme for combating pests and diseases of the grapevine 2015-2016

| Number treatment | Pheno-phases | Agents pathogenic combated | 2015 | | | Agents pathogenic combated | 2016 | | | Observations |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | The product | Date enforcement | Dose /ha | | The product | Date enforcement | Dose /ha | |
| I | 50% the shoots of 5-7 cm | The mites | Apollo 50 SC | 05 may | 0,4 | The mites | - | 11 mai | - | an row not give a row |
| | | Powdery mildew | Cosavet | | 3,0 | Powdery mildew | Sulfavit 95 PP | | 10,0 | |
| | | Red spot disease Gray rot | Folpan 80 WDG | | 1,5 | Red spot disease Gray rot | Folpan 80 WDG | | 1,5 | |
| II | Clearance the bunches | Red spot disease Gray rot Powdery mildew | Shavit 72 WDG + Sulfavit 95 PP | 20 may | 2,0 10,0 | Red spot disease Gray rot Powdery mildew | Shavit 72 WDG + Sulfavit 95 PP | 20 mai | 2,0 10,0 | an row not give a row |
| III | Before blooming | Powdery mildew | Orius 250 EW | 03 june | 0,4 | Powdery mildew | Orius 250 EW | 31 mai | 0,4 | row of after another |
| | | Vines manna | Vincare 51,7 WG | | 1,6 | Vines manna | Vincare 51,7 WG | | 2,0 | |
| IV | End blooming | Powdery mildew | Shavit 25 EC + Bumper 250 EC | 19 june | 0,1 0,2 | Powdery mildew | Bumper 250 EC | 16 iunie | 0,2 | row of after another |
| | | Vines manna | Sphix Extra | | 1,5 | Vines manna | Sphix Extra | | 2,0 | |
| | | Gray rot | Merpan 50 WP | | 2,0 | | | | | |
| V | The increase grains | Powdery mildew | Bumper 250 EC | 09 july | 0,2 | Powdery mildew | Orius 250 EW | 23 iunie | 0,4 | row of after another |
| | | Vines manna | Folpan 80 WDG | | 1,5 | Vines manna | Vincare 51,7 WG | | 2,0 | |
| VI | Compacting the bunches | Powdery mildew | Shavit 25 EC | 27 july | 0,2 | Powdery mildew | Bumper 250 EC | 05 iulie | 0,2 | row of after another |
| | | Vines manna | Vincare 51,7 WG | | 1,6 | Vines manna | Sphix Extra | | 2.0 | |
| VII | Firstfruits | Powdery mildew | - | - | - | Powdery mildew | Shavit 72 WDG | 25 iulie | 2,0 | row of after another |
| | | Vines manna | - | | - | Vines manna | Sulfavit 95 PP | | 20,0 | |

Table 3

The situation vines manna the attack *Plasmopara viticola* (Berk. et Curt.) at S.C.D.V.V Bujoru 2015-2016

| The year/cultivar | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | After blooming | | Berries growth | | Firstfruits grapes | | After blooming | | Berries growth | | Firstfruits grapes | |
| | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) |
| Babeasca neagra | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 1,15 | 1,07 | 2,17 | 4,10 |
| Witness/ Untreated Lot | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 34,99 | 100,00 | - | - |

Table 4

The situation attack oidium (*Uncinula necator* - Schw. Burr) to S.C.D.V.V Bujoru in 2015-2016

| The year/cultivar | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | After blooming | | Berries growth | | Firstfruits grapes | | After blooming | | Berries growth | | Firstfruits grapes | |
| | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) |
| Babeasca neagra | 0,0 | 0,0 | 0,0 | 0,0 | 0.12 | 0,24 | 0,0 | 0,0 | 1,40 | 0,03 | 1,72 | 2,37 |
| Witness/ Untreated Lot | 0,7 | 0,0 | 4,69 | 0,67 | 2,67 | 3,38 | 0,0 | 0,0 | 6,64 | - | - | - |

Table 5

The situation of grapevine gray rot to S.C.D.V.V Bujoru in 2015-2016

| The year/cultivar | 2015 | | | | | | 2016 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | After blooming | | Berries growth | | Firstfruits grapes | | After blooming | | Berries growth | | Firstfruits grapes | |
| | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | inflorescence (GA%) | leaves (GA%) | grapes (GA%) | leaves (GA%) | grapes (GA%) |
| Babeasca neagra | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Witness/ Untreated Lot | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | - | - | - | - |

## CONCLUSIONS

Climatic elements highlight the fact that in 2015 the climatic conditions were unfavorable appearance and evolution of pathogens and pests.

Analysis of the climatic elements of 2016 highlights the fact that in May - June were favorable conditions for the development of developing and hand, and in July-August mildew.

Technological scheme to combat pathogens and pests applied in specific climatic conditions of the years 2015 and 2016 point out that plant protection products applied at the recommended dose and the optimal time protects the vines.

## REFERENCES

Mirică I., Mirică A.,1976, Combaterea bolilor şi dăunătorilor la viţa de vie, Editura Ceres, 25-55.

Teodorescu G., Roman T., Mirică Sumedrea M., 2003, Entomologie horticolă, Editura Ceres, Bucureşti, 330-343.

Ulea E., 2003, Fitopatologie, Ed. "Ion Ionescu de la Brad", Iaşi, 205-229.

Tabaranu G., Simion C., 2005, Întrebări şi răspunsuri privind recunoaşterea şi combaterea dăunătorilor precum şi a celor mai frecvente boli în plantaţiile viticole din S-E Moldovei. Editura Pax Aura Mundi, Galaţi.

*** Ministerul Agriculturii şi Industriei Alimentare, 1980, Metodici de prognoză şi avertizare a tratamentelor împotriva bolilor şi dăunătorilor plantelor de cultură, Bucureşti.

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# EFFECT OF ORGANIC AND CHEMICAL FERTILIZERS ON DRAY DROGUE YIELD, ESSENTIAL OIL CONTENT AND OIL COMPOSITION OF *MATRICARIA CHAMOMILLA* L. IN CENTRAL GREECE

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***Keywords :*** *Matricaria chamomilla, essential oil, α-bisabolol, chamazulene, fertilization*

## ABSTRACT

*In this work, effect of fertilization levels organic and chemical fertilizers were studied on dry drogue yield, essential oil yield and oil composition of Matricaria chamomilla. In terms of dry drogue yield and flower essential oil content, the harvest revealed higher delivery quantities, in the crop applying organic fertilizer corresponding to 35 tons of manure per hectare. In the chemical composition of essential oils of M. chamomilla prevails the α-bisabolol in all treatments. Chamazulene and α-bisabolol content in the essential oil of M. chamomilla, was observed to have the higher values by adding 35 tons manure per hectare. Contrary, trans-β-farnesene and spiro ethers content in the essential oil of M. chamomilla was observed to have the lower values by adding of the same quantity manure.*

## INTRODUCTION

*Matricaria chamomilla* L. is an annual herbaceous plant belonging to the family *Asteraceae*. It is known from ancient times for aromatic and medicinal properties, with medicinal use in ancient Greece and Rome (Issac 1989).

The *Matricaria chamomilla* it has been found that contains terpenoids, flavonoids, commarins, spiroethers and other compounds, such as tannins (Newall et al. 1996).

Studies have shown that the plant has antioxidant, antimicrobial, inflammatory, anticonvulsant and anticancer properties (Carnat et al. 2004, Kroll et al. 2006, Weizman et al. 1993). Moreover, has sedative and antipyretic properties (Gardiner 2007, Roberts 1992).

Essential oils yield of *Matricaria chamomilla* flower ranges from (0.2 – 1.9)% (Bradley 1992). However, has been found that the essential oils yields of Chamomile flower vary according to the planting density, irrigation, fertilization, harvest stage, cultivation techniques and, environmental conditions (Franz et al. 1978, Galambosi et al. 1988, Nidagundi and Hegde 2007, Pirzad et al. 2006, Singh et al. 2011).

Essential oil of the *Matricaria chamomilla*, due to the strong antioxidant and antimicrobial properties, used in the pharmaceutical (Svoboda and Hampson 1999, Roby et al. 2013), as well as in food industry, cosmetics and perfumery (Franke and Schilcher 2005).

Therefore, the aim of this work was to examine the effect organic and conventional fertilization and fertilization level, in essential oil production, in essential oil composition and dry drogue yield of *Matricaria chamomilla*.

## MATERIALS AND METHODS

*Matricaria chamomilla* was cultivated in the Farm TEI of Thessaly on an area of 100 $m^2$, was sown in June 2014, applying 5 Kg seed mixed with 15 Kg sifted ash and 0.5 Kg of useful mycorrhiza per hectare. The experiment had a randomized block design with two fertilization types (organic fertilizer using sheep-goat manure and chemical fertilizer). Were applied three organic fertilization level with 560 Kg–N, 140Kg-$P_2O_5$, 455 Kg-$K_2O$, (O1) ; 280 Kg–N, 70 Kg-$P_2O_5$, 227.5 Kg-$K_2O$, (O2); 120 Kg–N, 30 Kg-$P_2O_5$, 97.5 Kg-$K_2O$, (O3), that correspond to 70, 35 and 15 tons manure per hectare respectively, and three chemical fertilization level with 255 Kg–N, 255 Kg- $P_2O_5$, 255 Kg-$K_2O$ (C1); 127.5 Kg–N, 127.5 Kg- $P_2O_5$, 127.5 Kg-$K_2O$ (C2); 63.75 Kg–N, 63.75 Kg- $P_2O_5$, 63.75 Kg- $K_2O$, (C3), per hectare respectively. There were four replicates per treatment combination. Plants were irrigated twice weekly with low salt irrigation water (0.48 $dS.m^{-1}$). Plants were harvest May 2015. Flowers were dried in the dark at room temperature, finely ground and kept at 4$^oC$.

**Soil samples were analysed** using the following methods which are referred by Page et al. 1982.

Organic matter was analyzed by chemical oxidation with 1 mol $L^{-1}$ $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol $L^{-1}$ $FeSO_4$.

Inorganic forms of nitrogen were extracted with 0.5 mol $L^{-1}$ $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) were extracted with 0.5 mol $L^{-1}$ $NaHCO_3$ and measured by spectroscopy. Exchangeable forms of potassium were extracted with 1 mol $L^{-1}$ $CH3COONH_4$ and measured by flame Photometer (Essex, UK).

**Extraction of the essential oil:** Oil was extracted by hydrodistillation using a Clevenger type apparatus. The duration of this procedure was 2 hours. The yield (v/w) of the obtained essential oil expressed as a percentage of absolute dry weight.

**GC/MSD analysis:** The chemical composition of the essential oils was analyzed using GC-MS techniques. The oil of *Matricaria chamomilla* was injected in an Agilent G1701EA GC/MSD ChemStation, fitted with an Agilent 5975 series MSD. The identification of the compounds was achieved by comparing the retention times and the mass spectra with those of the standards included in the GCMSD library.

**Statistical analysis**: Data analysis was made using the MINITAB (Ryan et al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

## RESULTS AND DISCUSSION

The soil used for the cultivation of *Matricaria chamomilla* L. had low content organic matter and low salinity (Table 1).

Table 1

Chemical properties of soil samples before the start of the crop

| Property | Soil depth (0-25) cm |
|---|---|
| Texture | Loam |
| pH, extract (1part soil:5parts H20) | 7.84 ± 0.16 |
| Electrical conductivity, extract (1:5), (dS $m^{-1}$) | 0.16 ± 0.03 |
| 0rganic matter (%) | 0.96 ± 0.04 |
| N-Inorganic (mg $kg^{-1}$) | 46.8 ± 4.02 |
| K-Exchangeable (mg $kg^{-1}$) | 353.3 ± 6.05 |
| P-0lsen (mg $kg^{-1}$) | 11.1 ± 1.95 |
| CaC03 (%) | 0.80 ± 0.10 |

Data represent average means and SE deviation. (n)=4

During the harvesting period (May), dry drogue yield per experimental piece was greater in fertilized plots with organic fertilizer (O2), corresponding to 35 tons manure per hectare (Table 2). Dry drogue production per experimental piece was higher using organic fertilizer than it using conventional fertilizer. Moreover, in the conventional fertilization dry drogue yield per experimental piece compared to fertilization levels did not show statistically significant differences. Plants were harvest with the opening of the flowers, when the concentration of essential oils maximized (Franz et al. 1978). Fresh to dry drogue ratio was 4.1/1.

Chemical fertilization level had no effect in plant flower essential oil production. However organic fertilization level significantly affected essential oil content. Maximum essential oil content was obtained, applying organic fertilizer (O2) corresponding to 35 tons of manure per hectare (Table 2).

Table 2

Dry drogue and essential oils yield of *Matricaria chamomilla* flowers

| Treatments | Dry mass (g) / experimental piece ($4m^2$) | Dry mass (Kg) / hectare | Essential oil (ml) / 100 g dry mass |
|---|---|---|---|
| Organic fertilizer (manure) | | | |
| O1 | 629.92b | 1574.8b | 0.55b |
| O2 | 667.40a | 1668.5a | 0.66a |
| O3 | 617.80b | 1544.5b | 0.50b |
| Chemical fertilizer (15% N - 15% $P_2O_5$ -15% $K_2O$) | | | |
| C1 | 458.40c | 1146.0c | 0.59ab |
| C2 | 456.60c | 1141.5c | 0.62ab |
| C3 | 456.00c | 1140.1c | 0.55b |

Columns with the same letter do not differ significantly according to the Tukey's test (P=0.01).

According to other authors, in *M. chamomilla* cultivation, organic and chemical fertilization, the fertilization levels, nutrient ratios during fertilization, and

irrigation regimes contribute to the change the yield and composition essential oils (Emongor et al. 1990, Gasic et al. 1989, Jeshni et al. 2015, Kariminejad et al. 2015, Pirzad et al. 2006).

The most important constituents of the essential oil of *Matricaria chamomilla* obtained at different fertilization levels, using biological and chemical fertilizers are shown in Table 3. In high concentration of chamazulene and a-bisabolol (terpenoids) is due the deep blue color of the essential oil of *M. chamomilla*. Chamazulene and α-bisabolol they have strong anti-inflammatory, antioxidant and fungistatic properties, α-bisabolol also is active against Staphylococcus aureus (Franke and Schilcher 2005, Petronilho et al. 2012).

Table 3

Composition (%)w/w of the essential oils of *Matricaria chamomilla* L.

| Compounds | Treatments | | | | |
|---|---|---|---|---|---|
| | Organic fertilization | | | Chemical fertilization | |
| | O1 | O2 | O3 | C1 | C2 |
| Chamazulene | 10.80b | 12.55a | 11.00b | 11.05b | 11.50b |
| α-bisabolol | 26.60b | 28.75a | 24.10c | 25.75b | 26.55b |
| α-bisabolol oxide B | 16.15b | 17.15a | 17.20a | 16.25b | 17.40a |
| α-bisabolol oxide A | 0.90a | 0.85a | 0.90a | 0.85a | 0.75a |
| Artemisia ketone | 0.80b | 1.20a | 1.30a | 1.15a | 1.35a |
| Trans-β-farnesene | 10.55c | 8.25d | 12.50a | 11.60b | 8.60d |
| Spathulenol | 1.40b | 1.85a | 1.45b | 1.35b | 1.70a |
| Spiro ethers | 17.70a | 13.60c | 15.65b | 16.60b | 12.65c |

Lines with the same letter do not differ significantly according to the Tukey's test (P=0.01).

The application of the organic fertilization (O2) compared to the organic fertilization (O1 and O3) and chemical fertilization in the cultivation of *M. chamomilla*, resulted production essential oil with higher content in α-bisabolol and chamazulene (Table 3).

Contrary, trans-β-farnesene and spiro ethers content in the essential oil of *M. chamomilla* was observed to have the lower values by adding of organic fertilization (O2). Moreover, the different chemical fertilization levels did not show statistically significant differences in composition of the essential oil of *M. chamomilla*, regarding content in chamazulene and a-bisabolol.

Also, the results showed that in the chemical composition of essential oils of *M. chamomilla* prevails the α-bisabolol in all treatments.

## CONCLUSIONS

Maximum quantity of dry drogue per experimental piece (4m$^2$) and higher essential oil yield of *Matricaria chamomilla* flowers where observed by adding organic fertilizer (O2) corresponding to 35 tons manure per hectare. Chamazulene and α-bisabolol content in the essential oil of *M. chamomilla*, was observed to have the higher values by adding 35 tons manure per hectare.

## REFERENCES


Bradley P.R., 1992, British herbal compendium. Volume 1. A handbook of scientific information on widely used plant drugs. Companion to Volume 1 of the British Herbal Pharmacopoeia. British Herbal Medicine Association.

Carnat A., Carnat A.P., Fraisse D., Ricoux L. and Lamaison J.L., 2004, The aromatic and polyphenolic composition of Roman camomile tea. Fitoterapia, 75(1): 32-38.

Emongor VE, Chweya JA, Keya SO, Munavu RM., 1990, The effect of nitrogen and phophorus on the essential oil yield. East African Agricultural and Forestry Journal, 55 (4): 191-194.

Franz C., Vömel A. and Hölzl J., 1978, Preliminary morphological and chemical characterization of some population and varieties of Matricaria chamomila L. , Acta Horticulturae, 73: 109-114.

Franke R. and Schilcher H.,(Eds.), 2005, Chamomile: industrial profiles. CRC press.

Galambosi B., Marczal G., Litkey K., Svab J. and Petri G., 1988, Comparative examination of chamomile varieties grown in Finland and Hungary. Herba Hungarica, 27(2-3): 45-55.

Gardiner P., 2007, Complementary, holistic, and integrative medicine: chamomile. Pediatrics in Review, 28(4): 16-18.

Gasic O., Lukic V., Adamovic R. and Durkovic R., 1989, Variability of content and composition of essential oil in various chamomile cultivators (Matricaria chamomilla L.). Herba Hungarica, 28: 21-28.

Issac O., 1989, Recent progress in chamomile research- medicines of plant origin in modern therapy. 1st ed. Czecho-Slovakia: Prague press.

Jeshni M.G., Mousavinik M., Khammari I. and Rahimi M., 2015, The changes of yield and essential oil components of German Chamomile (Matricaria recutita L.) under application of phosphorus and zinc fertilizers and drought stress conditions. Journal of the Saudi Society of Agricultural Sciences.

Kariminejad M. and Pazoki A., 2015, Effect of Biological and Chemical Nitrogen Fertilizers on Yield, Yield Components and essential oil content of German Chamomile (Matricaria chamomilla L.) in Shahr-e-Ray Region. In Biological Forum (Vol.7, No.1, p.1698). Research Trend.

Kroll U. and Cordes C., 2006, Pharmaceutical prerequisites for a multi-target therapy. Phytomedicine, 13: 12-19.

Newall C.A., Anderson L.A. and Phillipson J.D., 1996, Herbal medicines. A guide for health-care professionals. The pharmaceutical press.

Nidagundi R. and Hegde L., 2007, Cultivation prospects of German chamomile in South India. Indian Journal of Natural Products and Resources, 6: 135-137.

Page A.L., Miller R.H. and Keeney D.R., 1982, Methods of Soil Analysis Part 2: Chemical and Microbiological Properties, Agronomy, ASA and SSSA, Madison,Wisconsin, USA.

Petronilho S., Maraschin M., Coimbra M.A. and Rocha S.M., 2012, In vitro and in vivo studies of natural products: A challenge for their valuation. The case study of chamomile (Matricaria recutita L.). Industrial Crops and Products, 40: 1-12.

Pirzad A., Alyari H., Shakiba M.R., Zehtab-Salmasi S. and Mohammadi A., 2006, Essential oil content and composition of German chamomile (Matricaria chamomilla L.) at different irrigation regimes. Journal of Agronomy, 5(3): 451-455.

Roberts A, William JMG., 1992, The Effect of Olfactory Stimulation On Fluency Vividness of Imagery and Associated Mood a Preliminary Study. British Journal of Medical Psychology. 65:197-199.

Roby M.H.H. Sarhan M.A., Selim K.A.H. and Khalel K.I., 2013, Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.). Industrial crops and products, 44: 437-445.

Ryan B.F., Joiner B.L. and Cryer J.D., 2005, MINITAB Handbook: Updated for release 14, 5th edition.

Singh O., Khanam Z., Misra N. and Srivastava M.K., 2011, Chamomile (Matricaria chamomilla L.): an overview Pharmacognosy reviews, 5(9): 82-95.

Svoboda K.P. and Hampson J.B., 1999, Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, antiinflammatory and other related pharmacological activities. Plant Biology Department, SAC Auchincruive, Ayr, Scotland, UK., KA6 5HW, 1-17.

Weizman Z.V.I., Alkrinawi S., Goldfarb D.A.N. and Bitran C., 1993, Efficacy of herbal tea preparation in infantile colic. The Journal of pediatrics, 122(4): 650-652.

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# EFFECT OF NITROGEN FERTILIZATION LEVEL ON YIELD, ESSENTIAL OIL PRODUCTION, TOTAL PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF *ROSMARINUS OFFICINALIS* L. LEAF AND OF THE ESSENTIAL OIL IN CENTRAL GREECE

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**Keywords:** *rosemary, total phenolics, antioxidant activity FRAP, fertilization levels*

## ABSTRACT

*In this work, fertilization level effect were studied as to dry drogue yield, essential oil production, total phenolics content and antioxidant activity of Rosemary leaf and of the essential oil. Concerning the dry drogue yield and leaf essential oil content comparison between the two years harvests (March 2015 and January 2016), the second harvest revealed higher delivery quantities for both substances categories than first harvest. Analytically estimated concentration of total phenolics of the Rosmarinus officinalis leaf ranged of from (22.12 to 46.0) mg $g^{-1}$ and the antioxidant activity ranged from (10.84 to 26.12) µmols FRAP $g^{-1}$ dry weight. While the concentration of total phenolics of the essential oil ranged of from (512.4 to 2529 mg (GAE) $L^{-1}$ essential oil and the antioxidant activity ranged from (802 to 4130mM (AA) $L^{-1}$ essential oil.*

## INTRODUCTION

*Rosmarinus officinalis* L., it is a perennial plant, which reaches to 1.5 cm height, multibranched, with aromatic dark green leaves and blue blossoms, full production from the third year, belongs to the Family Labiatae. It is known from ancient times for aromatic and medicinal properties, ancient Greeks used rosemary to improve memory (Edwards et al. 2015, Holmes 1999).

The plant used as a condiment in cooking and as a food preservative (Peter 2004), while in the traditional medicine for anti-inflammatory and antimicrobial applications, for the diabetes treatment (Afonso et al. 2013, Arranz et al. 2015), for stimulate of the hair and against flatulence (Heinrich et al. 2006).

In the chemical composition of *Rosmarinus officinalis*, included substances with diverse biological and healing properties. Between them and phenolic compounds (Carnosic acid, Rosmarinic acid, Caffeic acid), carotenoids and volatiles (Arranz et al. 2015).

In addition, the rosemary extract exerts antimicrobial, antifungal, inflammatory, antioxidant, anti-cancer, antithrombotic and anti-diabetic activity

(Bakırel et al. 2008, Cui et al. 2012, Gougoulias 2012, Hussain et al. 2010, Naemura et al. 2008, Sanchez-Camargo et al. 2014).

Essential oil of Rosemary used in pharmaceutical, production of perfumes, of soaps and food industry (Celiktas et al. 2007, Zaouali et al. 2010, Hamedo et al. 2009, Okoh et al. 2010) However, variations in the chemical composition of rosemary essential oils related, to environmental conditions (Celiktas et al. 2007).

Therefore, the aim of this work was to examine the effect of different levels nitrogen fertilization, in dry drogue yield, in essential oil production, in total phenols content and in antioxidant activity of *Rosmarinus officinalis* leaves and of essential oils.

## MATERIALS AND METHODS

The experiment was conducted at the Technological Education Institute (TEI) of Thessaly. *Rosmarinus officinalis* was cultivated in the Farm on an area of 200 $m^2$, between May 2014 and January 2016, for two successive cultivation periods. The experiment had a randomized block design with three levels of nitrogen fertilization (0,100 and 200 Kg N/ha) for each growing period, two harvest times (March 2015 and January 2016) and each experimental piece had an area of 16 $m^2$ with 28 plants, while were irrigated twice weekly with low salt irrigation water (0.48 dS $m^{-1}$).

There were four replicates per treatment combination. Plants were harvest at the beginning of blossom period when essential oils concentration is maximized (Marquard et al. 2001).The leaves were dried in a dark place at room temperature, finely ground and kept at 4$^{o}$C.

**Preparation of the methanol extracts:** 500 mg of the finely ground sample were 2-fold treated by 20 ml 80% aqueous methanol. At first treatment the samples were incubated for 24 h in the extractant at stirring and the second one continued stirring for 2 h at ambient temperature. The extract was gathered after centrifugation/filtration and the volume was made up to 50 ml with aqueous methanol.

**Determination of Total polyphenols (TP):**Total polyphenolic content was determined with the Folin-Ciocalteu (F-C) reagent according to the method of Singleton and Rossi, 1965) using the microvariant proposed by (Baderschneider et al. 1999) and the results were expressed as gallic acid equivalent (GAE) in mg/g dry weight.

**Ferric reducing antioxidant power assay (FRAP reagent):** The ferric reducing antioxidant power (FRAP) was estimated according to the method of (Benzie and Strain, 1999) and was expressed as μmol FRAP reagent/g dry weight and ascorbic acid (AA) equivalent in mM/L essential oil.

**Soil samples were analysed** using the following methods which are referred by (Page et al. 1982).

Organic matter was analyzed by chemical oxidation with 1mol $L^{-1}$ $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol $L^{-1}$ $FeSO_4$.

Inorganic forms of nitrogen were extracted with 0.5 mol $L^{-1}$ $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) were extracted with 0.5 mol $L^{-1}$ $NaHCO_3$ and measured by spectroscopy. Exchangeable forms of potassium were extracted with 1 mol $L^{-1}$ $CH_3COONH_4$ and measured by flame Photometer (Essex, UK).

**Statistical analysis:** Data analysis was made using the MINITAB (Ryan et. al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

## RESULTS AND DISCUSSION

The soil used for the cultivation of *Rosmarinus officinalis* had low content organic matter and low salinity (Table 1). Concentrations of available forms of nitrogen in the soil for all levels of nitrogen fertilization at soil depth (0-25) cm are shown in Table 2.

Table 1

Chemical properties of soil samples of the experiment

| Property | Soil depth (0-25) cm | | |
|---|---|---|---|
| | Before beginning | After the first harvest | After the second harvest |
| Texture | Loam | - | - |
| pH, extract (1part soil : 5 parts $H_2O$) | 7.81 ± 0.16 | 7.82 ± 0.16 | 7.80 ± 0.18 |
| Electrical conductivity, extract (1:5), dS $m^{-1}$) | 0.11 ± 0.11 | 0.10 ± 0.01 | 0.13 ± 0.01 |
| Organic matter (%) | 0.93 ± 0.05 | 0.77 ± 0.04 | 0.97 ± 0.06 |
| K-exchangeable (mg kg-1) | 373.3 ± 7.45 | 314.5 ± 7.86 | 244 ± 6.16 |
| P-Olsen (mg $kg^{-1}$) | 13.10 ± 1.87 | 10.2 ± 1.46 | 7.0 ± 1.20 |
| CaCO3 (%) | 0.63 ± 0.07 | 1.04 ± 0.12 | 1.1 ± 0.17 |

Data represent average means and SE deviation. (n) = 4.

Table 2

Concentrations of available forms of nitrogen of the experiment for soil depth (0-25) cm

| Treatments (Fertilization) | N-inorganic (mg $kg^{-1}$) | | |
|---|---|---|---|
| | Beginning of experiment | After the first harvest | After the second harvest |
| Without fertilization | 44.8 ± 4.07 | 35.2 ± 4.10 | 29.4 ± 3.92 |
| 100 Kg N /ha | 44.8 ± 4.07 | 41.3 ± 3.44 | 39.9 ± 4.10 |
| 200 Kg N /ha | 44.8 ± 4.07 | 124.5 ± 7.20 | 210.2 ± 10.40 |

Data represent average means and SE deviation. (n) = 4.

In the first harvest year (March 2015) dry drogue yield per plant was greater in plots without fertilization, while in the second harvest year (January 2016) dry drogue yield per plant was greater in fertilized plots with adding 100 Kg nitrogen per hectare (Table 3). Plants were harvest at the beginning of blossom period when essential oils concentration is maximized (Marquard et al. 2001). Comparing yields of harvest periods, dry drogue production per plant was higher in the second harvest than in the first one for the fertilized plots, while in plots without fertilization dry drogue yield per plant in both harvest times did not show statistically significant differences.

In both harvest years (March 2015 and January 2016) essential oil yield of *Rosmarinus officinalis* leaves was greater in plots without fertilization compared

with the fertilized plots (Table 3). Harvesting year significantly affected essential oil content in fertilized plots, maximum essential oil content was obtained at second harvest year. However harvesting time had no effect in plant leaf essential oil production in plots without fertilization.

Table 3

Dry drogue and essential oils yield of *Rosmarinus officinalis* leaves

| Treatments (Fertilization) | Dry mass (g) / plant | | Essential oil (ml) / 100 g dry mass | |
|---|---|---|---|---|
| | In the first harvest year | In the second harvest year | In the first harvest year | In the second harvest year |
| Without fertilization | 158.3c | 147.9c | 0.70a | 0.65a |
| 100 Kg N/ha | 126.9d | 275.7a | 0.40d | 0.51c |
| 200 Kg N/ha | 61.5e | 187.5b | 0.50c | 0.56b |

Columns and lines with the same letter for dry drogue or essential oil yield do not differ significantly according to the Tukey's test (P=0.01).

Differences in the total phenolics content and antioxidant activity FRAP of *Rosmarinus officinalis* leaves were found according to fertilization level and harvest year (Table 4). The greatest total phenolic concentration as well antioxidant activity FRAP was measured at second harvest year in plants with fertilizer supply. However, the lowest total phenolic concentration as well antioxidant activity FRAP was measured at first harvest year in plants with fertilizers supply (Table 4).

Studies concerning the total content of phenols and antioxidant activity of Rosemary in differently extracts (aqueous and methanol), have shown that the Rosemary has a higher content of total phenols and antioxidant activity FRAP in aqueous extract (Tawaha et al. 2007).

Table 4

Total phenolic content and antioxidant activity FRAP of the Rosemary leaves

| Treatments (Fertilization) | Dry drogue of Rosemary | | | |
|---|---|---|---|---|
| | Total phenolic (TP) mg (GAE) /g dw | | Antioxidant activity FRAP µmols (FRAP) / g dw | |
| | First harvest year | Second harvest year | First harvest year | Second harvest year |
| Without fertilization | 23.78d | 32.9c | 12.44d | 19.08c |
| 100 Kg N/ha | 22.12e | 41.9b | 10.84e | 25.34b |
| 200 Kg N/ha | 22.35e | 46.0a | 11.02e | 26.12a |

Columns and lines with the same letter for TP content or FRAP activity do not differ significantly according to the Tukey's test (P=0.01).

Differences in the total phenolics content and antioxidant activity FRAP of essential oil were found according to fertilization level and harvest year (Table 4). The greatest total phenolic concentration as well antioxidant activity FRAP was measured, in the second year obtaining of the essential oil, where the growing of plants was performed with fertilizer supply (Table 5).

Table 5

Total phenolic content and antioxidant activity FRAP of Rosemary essential oil

| Treatments (Fertilization) | Essential oil of Rosemary | | | |
|---|---|---|---|---|
| | Total phenolic (TP) mg (GAE) /L essential oil | | Antioxidant activity FRAP mM (AA) /L essential oil | |
| | First harvest year | Second harvest year | First harvest year | Second harvest year |
| Without fertilization | 658.4c | 2280b | 1100c | 3879b |
| 100 Kg N/ha | 583.1d | 2503a | 982d | 4130a |
| 200 Kg N/ha | 512.4e | 2529a | 802e | 4071a |

Columns and lines with the same letter for TP content or FRAP activity do not differ significantly according to the Tukey's test (P=0.01).

## CONCLUSIONS

Maximum quantity of dry matter per plant of rosemary leave where observed by adding fertilizer 100 Kg N/ha at the second harvest year. While higher essential oil yield of *Rosmarinus officinalis* leaves where observed without adding fertilizers at the first harvest year. Total phenolics content and antioxidant activity was observed to have the higher values at the second harvest year in plants with fertilizers supply and in the essential oil respectively.

## REFERENCES


Afonso M.S., de O Silva A.M., Carvalho E.B., Rivelli D.P., Barros S.B., Rogero M.M., 2013, Phenolic compounds from rosemary (Rosmarinus officinalis L.) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats. Nutrition & Metabolism,10(1): 1.

Arranz E., Mes J., Wichers H.J., Jaime L., Mendiola J.A., Reglero G. and Santoyo S., 2015, Anti-inflammatory activity of the basolateral fraction of Caco-2 cells exposed to a rosemary supercritical extract. Journal of Functional Foods, 13: 384-390.

Baderschneider B., Luthria D., Waterhouse A.L. and Winterhalter P.,1999, Antioxidants in white wine (cv. Riesling): I. Comparison of different testing methods for antioxidant activity. Vitis, 38(3): 127-131.

Bakırel T., Bakırel U., Keleş O.Ü., Ülgen S.G. and Yardibi H., 2008, In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. Journal of ethnopharmacology, 116(1):64-73.

Benzie I.F. and Strain J.J., 1999, Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology, (299): 15-27.

Celiktas O.Y., Kocabas E.H., Bedir E., Sukan F.V., Ozek T. and Baser K.H.C., 2007, Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chemistry, 100(2): 553-559.

Cui L., Kim M.O., Seo J.H., Kim I.S., Kim N.Y., Lee S.H. and Lee H.S., 2012, Abietane diterpenoids of Rosmarinus officinalis and their diacylglycerol acyltransferase-inhibitory activity. Food Chemistry, 132(4): 1775-1780.

del Pilar Sánchez-Camargo A., Valdés A., Sullini G., García-Cañas V., Cifuentes A., Ibáñez E. and Herrero M., 2014, Two-step sequential supercritical fluid extracts from rosemary with enhanced anti-proliferative activity. Journal of Functional Foods, 11: 293-303.

Edwards S.E., da Costa Rocha I., Williamson E. M. and Heinrich M., 2015, Rosemary *Rosmarinus officinalis* L. Phytopharmacy: An Evidence-Based Guide to Herbal Medicinal Products, 328 pag.

Gougoulias N., 2012, Comparative Study on the Polyphenol Content and Antioxidant Activity of Some Medicinal Plants. Oxidation Communications, 35(4): 1001-1010.

Hamedo H.A. and Abdelmigid H.M., 2009, Use of antimicrobial and genotoxicity potentiality for evaluation of essential oils as food preservatives. Open Biotechnology Journal, 3:50-56.

Heinrich M., Kufer J., Leonti M. and Pardo-de-Santayana M., 2006, Ethnobotany and ethnopharmacology—Interdisciplinary links with the historical sciences. Journal of ethnopharmacology, 107(2): 157-160.

Holmes P., 1999, Rosemary oil The wisdom of the heart. International Journal of Aromatherapy, 9(2): 62-66.

Hussain A.I., Anwar F., Chatha S.A.S., Jabbar A., Mahboob S. and Nigam P.S., 2010, Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology, 41(4): 1070-1078.

Marquard R. and Kroth E., 2001, Anbau und Qualita tsanforderungen ausgewahlter Arzneipflanzen Bergen/Dunne: Agrimedia.

Naemura A., Ura M. Yamashita T., Arai R. and Yamamoto J., 2008, Long-term intake of rosemary and common thyme herbs inhibits experimental thrombosis without prolongation of bleeding time. Thrombosis research, 122(4): 517-522.

Okoh O.O., Sadimenko A.P. and Afolayan A.J., 2010, Comparative evaluation of the antibacterial activities of the essential oils of Rosmarinus officinalis L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chemistry, 120(1): 308-312.

Page A.L., Miller R.H. and Keeney D.R.,1982, Methods of Soil Analysis Part 2: Chemical and Microbiological Properties, Agronomy, ASA and SSSA, Madison, Wisconsin, USA.

Peter K.V., 2004, Introduction to herbs and species. In K. V. Peter (Ed.), Handbook of herbs and spices.

Ryan B.F., Joiner B.L. and Cryer J.D., 2005, MINITAB Handbook: Updated for release 14, 5th edition.

Singleton V.L. and Rossi J.A., 1965, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3):144-158.

Tawaha K., Alali F.Q., Gharaibeh M., Mohammad M. and El-Elimat T, 2007, Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry, 104(4), 1372-1378.

Zaouali Y., Bouzaine T. and Boussaid M., 2010, Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food and Chemical Toxicology, 48(11): 3144-3152.

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# THE DESIGNATION OF ORIGIN AND GEOGRAPHICAL INDICATION - PRIMARY LEGAL CONCEPTS IN 'EUROPE 2020'

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**Keywords**: designation of origin, geographical indication, specification book, opposition, implementing act

## ABSTRACT

*Creating an appropriate legislative framework designed to ensure the use of quality systems for manufactures of agricultural and food products represents a real gain for the European Economy, especially in the countryside, both in terms of rewarding producers for efforts to maintain the quality and originality products and in terms of protecting European consumers, entitled to require products whose quality is certified by the competent European and naţional institution in this regard.*

## INTRODUCTION

Pronounced development of competitive economic relations generated a much closer approach to the European strategy on agricultural product quality, strategy which aims to provide effective protection to producers which meet high quality standards, both in terms of the finished product, and regarding methods and technological processes leading to its performance.

The proclamation of legal rules designed to ensure fair competition among manufacturers, does nothing else but bring to the consumer quality products characterized by certain identifiable traits, especially in terms of their geographical origin.

Both agricultural and food producers and their beneficiaries, the consumers, are interested in creating a system of correct identification of products on the market.

If in respect of the producers, they are directly interested in the idea of profit, that cannot exist in the absence of a real competitive market, the predominant interest for the consumers is to purchase high quality products, quality given by processes and techniques acquired in time by certain producers in certain ways and in certain well-defined geographical areas.

European citizens' right to be correctly informed about the qualities of the products they purchase is as well an essential part of creating a fair competitive field that cannot subsist in the absence of European and national regulations aiming to regulate the "designations of origin" and" geographical indications".

Under the Regulation (EC) No. 1151/2012 (hereinafter referred to as Regulatory Framework) of 21st of November 2012 on agricultural products and food quality schemes, published in the Official Journal under number 343L dated 14th of December 2012, the political priorities in Europe in the regulated field, refers to *'aims to achieve a competitive economy based on knowledge and innovation and the purpose of promoting an economy with a high rate of employment, which ensures social and territorial cohesion"* (The Commission's Communication named "Europe 2020: A European strategy for smart, sustainable and favourable to the inclusion growth").

In the explanatory memorandum of the Regulation, the legislator sets as field that *"the policy of quality for agricultural products should provide producers with the appropriate tools to identify and promote those products thereof which have particular characteristics while protecting those producers against unfair practices"* (point 5 from the explanatory memorandum of the Regulatory Framework).

The rules for implementing this law were transcribed by the European Implementing Regulation (EC) No. 668/2014 of the European Parliament and of the Council on quality schemes for agricultural products and food stuffs published in the Official Journal under number L 179 from 19.06.2014.

## MATERIAL AND METHODS

In drafting the article was used the systematic method of interpreting european legal texts, method that consists in establishing the meaning of the legal norm by hiring its economy enactment of that form, or by reference to other laws economy.

## RESULTS AND DISCUSSIONS

According to European legislation, "the designation of origin" is the concept that aims to identify a particular product based on criteria which are designed to clearly determine whether it belongs to a strictly delimited geographical area, a product made in specific conditions of production.

To be the subject of a registration application of protection, the product must meet the following conditions (art. 5 paragraph 1 of the Regulatory Framework):

- To originate in a particular place, region or, in exceptional cases, country;
- Its qualities or characteristics to be essentially or exclusively due to a particular geographical environment with proper natural and human factors;
- All the stages of production to take place in a clearly defined geographical area.

We have to make clear that in the case of the products based on raw materials, living animals, the meat and the milk, certain terms may be treated as terms of origin, even if not all stages of the production (raw material) are conducted in the area clearly determined, if the following conditions are met:

- The production area of raw materials is clearly determined;
- The existence of specific conditions for the production of raw materials checked by special control entities;
- Those origin designations to have been recognized as such in their countries of origin before 1st of May 2004.

Pursuant to the Regulatory Framework, the Commission may issue delegated acts (under art. 56 of the Regulation) to determine, on the basis of

objective criteria based on quality, usage, natural factors, etc., certain exceptions regarding certain conditions imposed.

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Thus, in the case of animal products required to be registered as a designation of origin, given the specific nature of their production, there may be delegated acts issued by the Commission to establish restrictions or, conversely, exemptions regarding the sources of feeding in some grounded circumstances. Similarly, exemptions may be granted or impose restrictions in the slaughter of live animals or as regards the place of provenance of raw materials.

**'The Geographical indication'** is a name identifying a product whose quality, reputation or other characteristic can be attributed to the geographical origin of the product, provided that at least one of the stages of production take places in the designated geographical area.

To be the subject of a registration application of protection, the product must meet the following conditions (art. 5, paragraph 2, of the Regulatory Framework):

- To originate in a particular place, region or country;
- To hold a quality, reputation or other characteristic attributable to the geographical origin of the product;
- At least one of the stages of production to take place in the geographical area.

It cannot be subject of registration as designations of origin or geographical indications, generic mentions, names that are identical (or partially identical) with names for plant varieties and animal breeds, names that are intended to mislead the consumer about the identity of the product, given the existence of a trademark already registered regarding the product.

It can be noticed that the main difference between the two institutions aiming to provide protection to products is about the production process which in the case of designations of origin must take place wholly (with the exception of the derogation which the Commission may grant regarding certain products, as mentioned above) in the geographical area, unlike geographical indications, where it is sufficient that only a stage of the production process to be carried out in the geographical area.

The European regulatory document establishes that, for better product protection in the territories of EU Member States, designations of origin and geographical indications should be registered only at EU level, Member States accounting for them the obligation to create the legal framework for provisional protection until their final registration, conducted by the Commission.

The transfer into national law of the European provisions on how to acquire the protection of a geographical indication or designation of origin, was made by Order no. 1762/2015 issued by the Ministry of Agriculture and Rural Development, published in the Official Gazette, Part I no. 627 of 18/08/2015.

Prior to the existence of the Regulatory Framework, the national legislation consecrated the legal institution of geographical indication by Law no. 84/1998 on the trademarks and geographical indications published in the Official Gazette no. 161 / 23.04.20108.

According to this regulatory document, the geographical indication the designation which served to identify a product of a country, region or locality of a state, where a given quality, reputation or other determined characteristics may be essentially attributable to its geographical origin.

**Registration Procedure:**

**Temporary National Registration**

Interested people may submit to the Ministry of Agriculture and Rural Development (MARD) an application for registration which may cover the following protection programs:

- Acquiring protection for protected designation of origin (abbreviated name "PDO");
- Acquiring protection for geographical indication (abbreviated name "PGI");
- Acquiring protection for guaranteed traditional specialty (abbreviated name "GTS") [12]

The holders of the application for registration are the associations made by the producers, the processors interested in obtaining legal protection for the products (According to the Regulatory Framework, the applicant for registration is the "group", concept that defines any association, irrespective of its legal form, mainly composed of producers or processors of the same product).

It should be noted that the legislator allows within these associations the participation of organizations which only do actions to promote products made by members of the association concerned.

It should be noted that according to European (article 53 of the Regulatory Framework) and national legislation, it may be subject to registration and applications for approval of an amendment, that is not minor, of the specifications book of the product that has already been registered. In this case, the Commission will follow the procedure for standard recording applications.

If the changes are considered minor, the Commission will rule directly without having to resort to standard procedure. Minor changes cannot establish the essential characteristics of the product, the link between quality, features and geographic area (in the case of designations of origin) or to target link between a given quality, reputation, feature and geographic origin, (in the case of geographical indications).

**The procedure carried out by the specialized department of MARD**

The specialized department of the ministry will check if each application meets the conditions listed above, and if missing documents are discovered, it will communicate the applicant Group that they can complete the documentation within 30 days, under the sanction of rejection of the application in the case of noncompliance.

If the submitted documentation is complete, the specialized department will publish it on the website of MARD, time during which interested persons residing in the country can formulate "opposition" to the request for registration.

This opposition may be exercised within 60 days, the period in which electronic documents should be displayed under these conditions.

The opposition filed must contain the following elements:

1. Product name, as it is mentioned in the registration application;

2. The Official Reference, as it is published on the official website of MARD, with reference to its publication;

3. Identification data of the individuals / group / organization that formulates the opposition;

4. The reason that led to the formulation of national opposition, reasons particularly aiming infringement of the relevant legislation of the domain;

5. Details regarding the opposition that specifically target the legitimate interest justifying the formulation of this type of application;

In order for an opposition to be received, it must meet at least one of the following conditions:

a) The quality system sought to be registered is not falling within the scope of art. 5 paragraph. (1) or Article. 5 paragraph. (2) of the Regulatory Framework;

b) The specifications book does not include the minimum requirements prescribed by European and national legislature;

c) The registration request concerns a generic entry;

d) The request coincides with the name of a plant variety or an animal breed and may mislead the consumer about the true origin of the product;

e) he name proposed is wholly or partially homonymous with a name already registered in the register of protected designations of origin and protected geographical indications publicly available, in the case where it cannot be sufficiently distinguished from the conditions of local and traditional use and the presentation of the homonymous name registered subsequently, on the one hand, and the name already registered on the other hand, taking into account the interest of ensuring fair treatment of the producers in question and the interests of not misleading consumers;

f) When the proposed name is likely to mislead the consumer, given the prior existence of a mark characterized by reputation and distinction, which has been used for a long time;

g) The registration of the name proposed would prejudice either the existence of a wholly or partly homonymous name, or a factory or trademarks, or the existence of products which have been legally on the market for at least 5 years;

h) It is demonstrated that the name for which the registration is requested is generic in the sense of art. 41 of the Regulatory Framework.

Once the opposition received, the MARD specialized department must communicate the opponent the motivated decision of admissibility or rejection of the statement of opposition.

If the opposition is deemed to be admissible, the specialized department will communicate within 30 days the reached conclusions to the applicants and opponents, with the request that interested parties to initiate consultations within 30 days in order to be able to reach an agreement on the request for registration and complement the shortcomings of the application, to the extent that this procedure is possible.

If an agreement is reached, and the published documentation has not changed or it has undergone minor changes, it is considered eligible and it will be applied the procedure for communicating the outcome of the consultations to the MARD.

If the documentation has undergone major changes, the specialized department will repeat the examination of the documentation given by the new

changes. If the outcome of the conciliations is irreconcilable, or if the application does not meet the legal conditions, the application will be rejected.

The admission decision will be published and it will be the subject to review by the courts. Any person who has a legitimate interest may grant the admissibility decision. The decision can be challenged at the Court of Bucharest.

**European Registration**

If the national authority considers admissible a registration application, this one, together with the file, will be forwarded to the Commission.

The file will contain the nationally registered oppositions, including those filed by people who have used for 5 years prior to the registration of the opposition, continuously, those names, without being registered.

The verification of the documentation submitted by the national state cannot last more than 6 months.

During this period, the Commission will publish a monthly list of designations which are subject of an application and the date of their registration.

If the registration conditions are met, the Commission will publish the single document and send to publication of the specifications book in the Official Journal of the European Union.

Within 3 months from the publication, Member States, third countries or any natural or legal entity that has a legitimate interest may formulate an objection at the European level.

The people residing in the Member State which provided the documentation required to be registered cannot formulate an objection at the European level. Oppositions cannot aim, under the condition of being declared null, other grounds of illegality than those provided under the Regulatory Framework.

Within 2 months after the receipt of the opposition, the Commission invites the parties to undertake consultations on the registration application and the opposition one, consultations which must relate to the provisions of the European Regulatory Framework.

The negotiation period is 3 months. The outcome of the negotiations, whether embodied in an agreement or not, will be made available to the Commission.

In the case of failure to reach an agreement, or if the Commission considers that the application does not meet the legal requirements, the Commission shall convene the Committee on the policy for quality of agricultural products, consisting of representatives of the Member States to give their opinion for rejecting or to review the validity of the opposition filed in relation to the application for registration.

The Committee is chaired by a representative of the Commission which shall not vote. He/She will set no earlier than 14 days from the date of the meeting, the agenda and the draft implementing the act of registration. In well justified cased, the committee's opinion can be taken by written procedure, after the sending of the implementation project.

The vote will be taken by a qualified majority which shall be equal to at least 55% of members of the committee comprising at least 65% of their population.

The blocking minority must include at least the minimum number of members representing more than 35% of the population of the Member States they represent, plus one member, otherwise, the majority decision is taken.

In case of disagreement regarding the adoption of implementing the Commission's request, it will be initiated an appeals committee that within 14 days (as a rule) will decide on the Commission's request. And this appeal committee will be chaired by a member of the Commission.

The final vote will be taken based on the percentages expressed above. If the committee delivers no opinion, the Commission shall not adopt the draft implementing act if a simple majority of members opposes adoption.

Ex officio, or upon notification by the parties concerned, the Commission may adopt implementing documents to cancel the registration of a protected designation of origin or a protected geographical indication, following the procedure mentioned in the previous paragraph.If an agreement is reached between the parties or if no opposition is received, the Commission shall adopt implementing documents for the registration designation.

Registration documents and rejection decisions are published in the Official Journal of the European Union.

## CONCLUSIONS

Although the Romanian legislator has strove to implement European rules on the view, we believe that he was "confused" in terms of choice of the act by the means of which the legislating was carried out.

If the designations of origin are not to be talked about, regarding the geographical indications, on the other hand, we find ourselves in front of a Ministerial Order "trying" to amend existing regulations in Law 84/1998 on trademarks and geographical indications.

Given the legal force of the two regulatory documents, it is notorious that a Ministerial Order cannot modify the provisions of a law. We believe that, in this respect, the legislator must intervene in terms of the legislative technique entailing a higher-ranking rules (at least an order of urgency) for all geographical indications, taking into account the provisions of Law no. 84/1998 and unitary regulation envisaged by the European legislator and the proclamation of Regulation (EU) no. 1151/2012.

## REFERENCES

*** 2015, G.D. ¬ No. 152 establishing the institutional framework and measures for implementation of Regulation (EC) no. 1.151 / 2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs

*** 2015, Order no. 1762 issued by the Ministry of Agriculture and Rural Development, published in the Official Gazette, Part I no. 627 of 18.08.2015;

*** 2012, REGULATION (EU) NO. 1151 of 21 November 2012 on quality schemes for agricultural products and food law published in the Official Journal number 343L dated 14 December 2012;

*** REGULATION (EU). 182/2011 of the European Parliament and of the Council of 16 February 2011 published in the European Journal of the European Union of 28.02.2011;

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# CLIMATE CONDITIONS INFLUENCE ON VITICULTURAL YEAR ON GRAPE MATURATION IN THE SÂMBUREŞTI VINEYAR

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***Keywords***: *volatile compounds, higher alcohol, esters, distillations, maturating, aging*

## ABSTRACT

*Climate is a key consideration for winegrowers, so information regarding projected climate change and the relative global impacts are of great interest. Climate variation contributes to fluctuations in reproductive output, and spring temperature is thought to influence flower production in grapevines. Variations in early-season temperatures may alter substantially grapevine yield formation. The temperature effect may be a combination of direct effects on floral development and indirect effects arising from differences in shoot growth.For 2 years, it was studied the influence of maturation process on four different grapes varieties, two for red wines Cabernet Sauvignon and Merlot, other two for white wines Chardonnay and Sauvignon.*

## INTRODUCTION

Meteorological parameters have a crucial influence on grapevine (*Vitis vinifera* L.) production quantity and quality. Most of the commonly used bioclimatic indices are not appropriate to represent intravineyard micrometeorological variability, in particular the sub daily dynamics that are important in grape maturation processes (Matese et al. 2012, Beleniuc 2009).

Climate variation contributes to fluctuations in reproductive output, and spring temperature is thought to influence flower production in grapevines (Keller et al. 2010). There are several aspects of warming relevant for grape andwineproduction. First, thereis an increase in background average temperature that affects vines during the whole growing cycle, not with standing seasonal and daily asymmetries. Seasonal asymmetries include more intense warming during summer, during the course of berry ripening, than in winter (Webb et al. 2013).

The effects of climate, soil, and cultivar were found to be highly significant with regard to vine behavior and berry composition (an example being anthocyanin concentration). The impacts of climate and soil were greater than that of cultivar (Leeuwen et al. 2004). The strong link between climate and grapevine phenology suggests a potentially stronger impact of climate change on viticulture in climate-limited areas (Caffarra & Eccel 2011). The effect of temperature on grape berrycompositionandwineattributeshasbeenrecognisedhistorically,totheextent that cultivars are often classified in terms of their thermal requirements, and the

prevalent thermal regime is critical to characterise both wines and wine-producing regions world wide (Bonada & Sadras 2015).

## MATERIAL AND METHODS

This paper is based on research on the dynamics of grape maturation of two white wine grapes and two red wine grapes quality vineyard Sâmbureşti. White wine varieties are Chardonnay and Sauvignon and red wine varieties are Cabernet Sauvignon and Merlot. If the two white varieties were studied one variation for each of them at red wine varieties were studied two variants. Thus, Cabernet Sauvignon clones have studied 338 and 685, while the Merlot studied a single clone (181) but grafted on two different rootstocks: S04 and 1103P.
The study was conducted during wine years 2014 and 2015, during the ripening of the grapes from the 6 experimental variants.

## RESULTS AND DISCUSSIONS

The two years (2014 and 2015) they were very different from one another, causing varied developments. 2014 was a year too rainy, was the year with the largest amount of rainfall in recent decades in making determinations weather rigorous, with more than 1 200 mm rainfall until the end of the growing season, because until the end the last calendar year of 1 400 mm rainfall, which is a historical record. In contrast, 2015 was a year of excessive drought during the summer months but heavy rainfall in the autumn months, especially September and October (rainfall from November to December is no longer of interest to this study, being fallen off period vegetation and after harvesting the grapes, and therefore the end of its production).

Table 1 presents the evolution of ripening at 4 variants black grape and white grape 2 versions 2014

Due to difficult climatic conditions specific to the year 2014 with a very rainy and cooler summer compared to the average annual values, grape maturation it was delayed, so the prosecution began walking baking at oncerather late and 15 September respectively.

On parameter values observed for analysis of the dynamics of maturation, it appears that at this time, two white wine grapes (Chardonnay and Sauvignon) presents an advance of maturing to the two red wine grapes having the highest content in sugar. Instead, the two clones of Cabernet Sauvignon experienced significantly lower sugar content in.

Avanced maturity to white varieties compared to the red varieties is now highlighted by the values of total acidity

Thus, both white varieties had total acidity below 7 g/L $H_2SO_4$. At varieties for red wines, total acidity at the first measurement was greater in both varieties but while the Merlot values are lower, Cabernet Sauvignon values are much higher, with more than 2 g/L $H_2SO_4$, both clones.

Analyzing the composition together both parameters reveals that Sauvignon has the highest sugar content and the lowest total acidity, which means it has the most advanced maturity. It is followed very close, with very small differences by other variety for white wines (Chardonnay) and the two versions of Merlot. Instead, clones of Cabernet Sauvignon are delayed significantly, particularly clone 338 presenting almost 40 g/Lless sugar.

Table 1

The grapes maturation in 2014

| Variants | | 15.IX | 22.IX | 29.IX | 06.X | 13.X | 20.X |
|---|---|---|---|---|---|---|---|
| Merlot, 181/S04 | Weight of 100 grains(g) | 136 | 139 | 143 | 145 | 146 | 146 |
| | Sugar content (g/L) | 170 | 183 | 198 | 210 | 222 | 230 |
| | Total acidity (g/L $H_2SO_4$) | 6.64 | 6.64 | 6.10 | 5.78 | 5.40 | 5.16 |
| Merlot, 181/1103P | Weight of 100 grains(g) | 139 | 143 | 147 | 149 | 149 | 148 |
| | Sugar content (g/L) | 172 | 188 | 199 | 208 | 217 | 225 |
| | Total acidity(g/L$H_2SO_4$) | 7.02 | 6.54 | 6.22 | 5.69 | 5.55 | 5.04 |
| Cabernet Sauvignon, 338/R140 | Weight of 100 grains(g) | 124 | 127 | 130 | 132 | 134 | 133 |
| | Sugar content (g/L) | 137 | 155 | 178 | 190 | 202 | 210 |
| | Total acidity(g/L$H_2SO_4$) | 9.40 | 8.40 | 7.52 | 6.22 | 6.05 | 5.80 |
| Cabernet Sauvignon, 685/S04 | Masa a 100 de boabe (g) | 125 | 127 | 129 | 132 | 134 | 135 |
| | Sugar content (g/L) | 151 | 169 | 190 | 203 | 210 | 218 |
| | Total acidity(g/L$H_2SO_4$) | 8.38 | 8.16 | 7.10 | 5.87 | 5.72 | 5.62 |
| Chardonnay | Weight of 100 grains(g) | 152 | 155 | 160 | 162 | 163 | 163 |
| | Sugar content (g/L) | 174 | 190 | 202 | 212 | 220 | 225 |
| | Total acidity(g/L$H_2SO_4$) | 6.92 | 6.24 | 5.58 | 5.12 | 4.88 | 4.64 |
| Sauvignon | Weight of 100 grains(g) | 140 | 144 | 148 | 151 | 152 | 151 |
| | Sugar content (g/L) | 178 | 196 | 210 | 222 | 232 | 238 |
| | Total acidity(g/L$H_2SO_4$) | 6.62 | 6.08 | 5.84 | 5.40 | 5.02 | 4.54 |

The explanation for this delay ripening varieties for red wines (with special reference to the two clones of Cabernet Sauvignon) related to the specific climatic conditions of the wine. Being a year with more rain, it slowed ripening grapes Cabernet Sauvignon, which is one of the varieties very demanding conditions of heat and light from a vine area. White wine varieties are less demanding to light, better supports cooler and wetter summers and this is reflected in the difference ripening the same calendar date.

Last determination of ripeness evolution was done on October 20, a date rather late compared to previous years, when the harvest was usually over. In 2014, however, due to lower rainfall and heat during the year wine maturation has been much slower. At last measurement, it has been a slow down in the evolution of all parameters analyzed, which is explained by the fact that they reached half of

autumn, the end of the growing season, which makes the intensity of all phenomen a specific maturation to reduce greatly.

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Sugar content continued to grow but at lower rates compared to previous periods when accumulations were more intense, the day was longer, the weather was warmer. Under these conditions, the highest sugar content found in Sauvignon, Merlot followed, clone 181/S04, clone 181/1103P and Chardonnay, the last two places were the two clones of Cabernet Sauvignon. By comparison with the first measurement five weeks ago, it is noted that the variety which was at that time the highest sugar content (Sauvignon, 178 g/L) remained at first place in the end of ripening, with 8 g/L more than the variety of 2nd place, therefore a greater difference than at the beginning determinations.

During the 35 days of ripening, the sugar content up to the six variations of between 51 g/L and 73 g/L. The largest increase was recorded Cabernet Sauvignon clone 338, from 137 to 210 g/L, followed by Cabernet Sauvignon clone other (685) of 151-218 g/L. The small increase in sugar content was Chardonnay, from 174 g/L to 225 g/L.

Viticultural year 2015 was much different from the previous year. If 2014 was a year excessively rainy year 2015 has shown much lower rainfall and was warmly. Their distribution throughout the year shows a surplus of precipitation in spring and autumn. Instead, the summer months, particularly July and August, and the first half of September were particularly hot and dry. This has influenced the development of the ripening process of the grapes. Grapes have started earlier than the previous year maturing process. Therefore, pursuit the grapes maturation in 2015 began nearly a month earlier, the first determination being made on 19 August 2015 Data on ripening in 2015 are shown in Table 2.

At the first determinations it was found that all white varieties were the most advanced in the maturation having the highest sugar content and the lowest values of total acidity. Varieties of red wine, Merlot clones all had higher sugar content and lower in acidity than those of Cabernet Sauvignon, which this year were the least advanced in age at first determination. Regarding Cabernet Sauvignon clones should be noted that the previous year are more advanced in maturation, with higher sugar content and acidity with lower values earlier than the previous year. Another important finding from the 2015 first determination was that the beans are smaller than the previous year in all varieties and clones, which is a direct consequence of water scarcity caused by drought that over lapped with the period of grape maturation. Last determination walking baking took place on 14 October 2015 to 8 weeks after the first determination (August 19th 2015). After 8 weeks of follow gait baking largest sugar content plays Sauvignon, as in the first determination, which recorded an increase of 84 g/Lcompared to the first determination. No. 2 are two varieties: Chardonnay and Merlot clone 181/S04, but while the Chardonnay increase the sugar content compared to the first determination was 78 g/L, the clone of Merlot growth was 86 g/L.

Table 2

The grapes maturation in 2015

| Variants | | 13. VIII | 26. VIII | 02. IX | 09. IX | 16. IX | 23. IX | 30. IX | 07. X | 14. X |
|---|---|---|---|---|---|---|---|---|---|---|
| Merlot, 181/S04 | Weight of 100 grains(g) | 131 | 134 | 137 | 140 | 140 | 141 | 144 | 145 | 144 |
| | Sugar content (g/L) | 164 | 190 | 214 | 233 | 243 | 248 | 244 | 256 | 250 |
| | Total acidity (g/L $H_2SO_4$) | 6,62 | 5,94 | 5,82 | 5,30 | 4,70 | 4,24 | 3,52 | 3,36 | 3,18 |
| Merlot, 181/1103P | Weight of 100 grains(g) | 135 | 138 | 140 | 143 | 142 | 142 | 145 | 144 | 143 |
| | Sugar content (g/L) | 162 | 188 | 207 | 220 | 234 | 244 | 242 | 244 | 245 |
| | Total acidity (g/L $H_2SO_4$) | 6,80 | 6,22 | 5,90 | 5,42 | 5,02 | 4,44 | 3,60 | 3,42 | 3,20 |
| Cabernet Sauvignon, 338/R140 | Weight of 100 grains(g) | 108 | 110 | 112 | 115 | 116 | 117 | 120 | 120 | 119 |
| | Sugar content (g/L) | 154 | 178 | 196 | 212 | 224 | 234 | 232 | 234 | 238 |
| | Total acidity (g/L $H_2SO_4$) | 7,22 | 6,40 | 6,04 | 5,18 | 4,60 | 4,24 | 3,52 | 3,30 | 3,18 |
| Cabernet Sauvignon, 685/S04 | Masa a 100 de boabe (g) | 115 | 118 | 120 | 122 | 123 | 122 | 124 | 124 | 123 |
| | Sugar content (g/L) | 152 | 180 | 198 | 215 | 230 | 240 | 236 | 237 | 240 |
| | Total acidity (g/L $H_2SO_4$) | 7,54 | 6,74 | 6,22 | 5,36 | 4,78 | 4,44 | 3,60 | 3,36 | 3,22 |
| Chardonnay | Weight of 100 grains(g) | 138 | 141 | 144 | 146 | 147 | 147 | 150 | 159 | 148 |
| | Sugar content (g/L) | 168 | 190 | 210 | 228 | 238 | 250 | 245 | 246 | 250 |
| | Total acidity (g/L $H_2SO_4$) | 6,88 | 6,36 | 5,84 | 5,40 | 4,88 | 4,08 | 3,76 | 3,52 | 3,40 |
| Sauvignon | Weight of 100 grains(g) | 130 | 132 | 134 | 136 | 135 | 135 | 136 | 135 | 134 |
| | Sugar content (g/L) | 172 | 194 | 216 | 232 | 248 | 258 | 252 | 254 | 256 |
| | Total acidity (g/L $H_2SO_4$) | 6,12 | 5,60 | 5,16 | 4,72 | 4,34 | 4,04 | 3,52 | 3,30 | 3,18 |

No. 3 is Merlot clone 181 / 1103P, 83 g/L more than the first determination. The last two places were the two clones of Cabernet Sauvignon: 685, 88 g/Lmore

than the first determination (the highest growth of all variants) and 338, with 84 g/Lmore than the first determination.

## CONCLUSIONS

Following the ripening at wine grapes in the years 2014 and 2015 showed that the evolution Sâmbureşti vineyard grape maturation is highly dependent on the specific climatic conditions of the wine, which obviously influences the quality of the grapes used in wine-making raw material.As a result of the different conditions in terms of climate between seasons ripening grapes in the wine two years, all varieties and clones studied accumulated sugar amounts much higher in 2015 than in 2014. Comparing sugar content to date on the latest sugar determinations differences are found between 24 and 36 g/L in 2015 in addition to 2014. the slight difference is Sauvignon and Cabernet largest Sauvignon clone 338. Total acidity of the grapes in 2015 was much lower compared to 2014 due to more pronounced acids combustion under the hot summer of 2015. The biggest differences were the two clones of Cabernet Sauvignon, while the lowest the Chardonnay.

Different climatic conditions in the two years they have not only influenced wine grape quality and quantity of production but, as the weight of 100 grains. A comparison of this parameter in data last determinations of the 2 viticultural years shows that 2014 values were higher in all varieties and clones, the differences are between 2 and 17g. The smallest differences were in two Merlot clone 181 / S04 (2 g) and 181 / 1103P (5 g). Cabernet Sauvignon differences were much higher: 12 g to 14 g clone clone 685 and 338. The biggest differences were white varieties: Chardonnay (15 g) and Sauvignon (17 g).

## REFERENCES


Beleniuc G., 2009, Oenologie. Notiuni de vinificatie, biochimie, microbiologie, conditionare, stabilizare si imbuteliere a vinului. Editura Universitara, Bucuresti.

Bonada M., Sadras V.O., 2015, Review: critical appraisal of methods to investigate the effect of temperature on grape vine berry composition. Australian Journal of Grape and Wine Research, 21, 1, 1-17.

Caffarra A., Eccel E., 2011, Projecting the impacts of climate change on the phenology of grapevine in a mountain area. Australian Journal of Grape and Wine Research, 17, 1, 52-61.

Keller M., Tarara J.M., Mills L.J., 2010, Spring temperatures alter reproductive development in grapevines. Australian Journal of Grape and Wine Research, 16, 3, 445-454.

Leeuwen C., Friant Ph., Choné X., Tregoat O., Koundouras St., Dubourdieu D., 2004, Influence of Climate, Soil, and Cultivar on Terroir. American Journal of Enology and Viticulture, 55, 3, 207-217.

Matese A., Crisci A., Di Gennaro F.S., Fiorillo E., Primicerio J., Toscano P., Vaccari F., Di Blasi S., Genesio L., 2012, Influence of Canopy Management Practices on Vineyard Microclimate: Definition of New Microclimatic Indices. American Journal of Enology and Viticulture, 63, 3, 424-430.

Webb L.B., Watterson I., Bhend J., Whetton P.H., Barlow E.W.R., 2013,. Global climate analogues for winegrowing regions in future periods: projections of temperature and precipitation. Australian Journal of Grape and Wine Research, 19, 3, 331-334.

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# EXPERIMENTAL STUDY OF BAKERY PRODUCTS OBTAINED IN ELECTRICAL RESISTANCE HEATED OVENS AND OPPORTUNITIES TO IMPROVEMENT THE EFFICIENCY

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**Keywords:** *hardness, crust oven, thermal*

## ABSTRACT

*The quality of bakery products is a complex system because it encompasses several mandatory conditions: the amount of food harmlessness, the sensory quality and the presentation of the product. Due to continuous trend of increasing quality demands appeared and maintaining the demands in good features and economic performance appeared due to the continuous trend of increasing quality.*

## INTRODUCTION

The construction and operation of furnaces used in bakery have an important role in quality assurance in bakery. Electric heating has some advantages over heating with gas: the ability to adjust fast, precise and easy manual or automatic energy input and temperature of the heated area; the ability to develop heat in the desired location; direct heating of the heated area without contamination; energy high efficiency and constructive simplicity of ovens, etc. (Tisan 2009). The appearance and the consistency of bread crust, baking and oven regime used in the process, play a very important role. Due to the shape and construction of ovens, bread subjected to experiments can go through various stages in consistency of the crust: the half-soft crust to hard or very hard crust (Bâlc et al. 2013, Bâlc et al. 2016).

## MATERIAL AND METHODS

The tests were carried out on a sample of two loaves of bread, wheat flour, white baked in both the electric furnace and the traditional furnace, and measurements were made at every 60 minutes for 24 hours, using a hardness tester, Innovatest Impact TH-1100, a portable digital tester, operating in accordance with the method of rebound, standardized according to ASTM A956.

The following pictures show Innovatest Impact TH-1100 hardness testing device and its components.

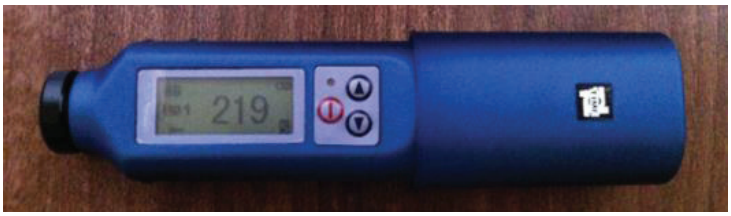


Figure 1. Tester Innovatest Impact TH-1100.

The characteristics of TH-1100 Hardness Impact Innovatest are shown in the following chart.

Table 1

The characteristics of Innovatest Impact TH-1100 hardness tester

| Nr. crt. | Characteristics | Values |
|---|---|---|
| 1 | Measuring range | 190~960 HLD |
| 2 | Measuring direction | 360º |
| 3 | Hardness scales | HL, HB, HRA, HRB, HRC, HV, HS |
| 4 | Display | 112x48 dot matrix LCD |
| 5 | The range of impact | 1~9 (optional) |
| 6 | Charger | 6V/400mA |
| 7 | Time of work | >8 h |
| 8 | Charging time | 2~3 h |
| 9 | Power | 3,7 V |
| 10 | Dimensions | 145x35x30 mm |
| 11 | Mass | 130 g. |

Its compact design enables easy testing on component surfaces which are difficult to transport or difficult to access by other hardness tester. This tester operates on different hardness scales such as Rockwell, Brinell, Vickers, Shore, Leebs and the test results appear directly on the screen. Also, testing may be performed at any angle, even in difficult positions. Other benefits include: LCD screen, battery capacity on the screen, Li-Ion rechargeable battery, simple handling without wires, reduced testing costs.

## RESULTS AND DISCUSSIONS

For testing, the hardness testing device has been set on Brinell hardness scale, one of the most common hardness testing methods used for testing materials with hardness below 350 HB. It was determined the hardness of the upper crust bread baked in a electrical oven and traditional oven too. Test results are shown in Table 2.

Table 2

The final experimantal results

| Time, τ, h | HB electric oven | HB traditional oven |
|---|---|---|
| 1 | 42 | 97 |
| 2 | 43 | 99.5 |
| 3 | 46 | 100 |
| 4 | 45.5 | 102 |
| 5 | 47.5 | 103.5 |

| | | |
|---|---|---|
| 6 | 48.5 | 107.5 |
| 7 | 50 | 108.5 |
| 8 | 51.5 | 110 |
| 9 | 52.5 | 110.5 |
| 10 | 55 | 114 |
| 11 | 56 | 117 |
| 12 | 58 | 117.5 |
| 13 | 59 | 120 |
| 14 | 61.5 | 120.5 |
| 15 | 62.5 | 122.5 |
| 16 | 63.5 | 123 |
| 17 | 69 | 125 |
| 18 | 69 | 126 |
| 19 | 71 | 126.5 |
| 20 | 74.5 | 127 |
| 21 | 74.5 | 127.5 |
| 22 | 75.5 | 128.5 |
| 23 | 77 | 133 |
| 24 | 77.5 | 133.5 |

Figure 2 shows the evolution of bread crust hardness make in electric oven and traditional oven.

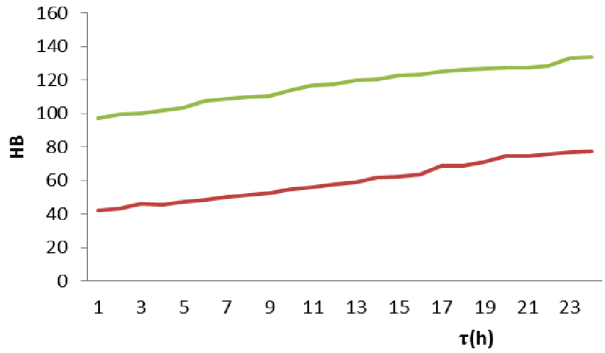


Figure 2. Hardness depending on time for electric oven (red curve) and traditional oven.

Consider a horizontal wall of infinite extension consisting of three layers of different thickness ($\delta_1 \neq \delta_2 \neq \delta_3$) made of materials with different thermal coefficients of conductivity ($\lambda_1 \neq \lambda_2 \neq \lambda_3$), for example different rocks (Figure 4).

The heat flow is unidirectional and temperature changes are modified only vertically. The temperatures on the faces of the layers are $t_1 > t_2 > t_3 > t_4$. If any parameter does not change with time, temperature field is stationary (Roşca et al. 2008, Şugar 2007).

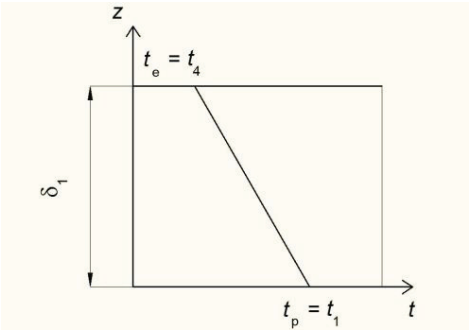


Figure 3.
Thermal conduction through
uniform wall plan.

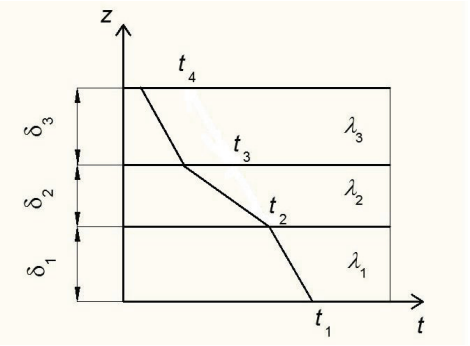


Figure 4.
Thermal conduction through
layered wall plan.

## CONCLUSIONS

As expected, the crust hardness of bakery products obtained in resistance heated electric stoves is lower than the products obtained in a traditional oven.

Ovens heated with electric resistance, due to the possibility of controlling the temperature and steam system, will lead to obtaining higher quality bakery products being substituted for other types of furnaces.

When using these layered wall ovens efficiency will increase depending on the number of layers and materials in construction.

## REFERENCES

Bâlc G., Şugar I.R., 2013, Maşini şi instalaţii pentru industria alimentară Vol.I, Editura Risoprint, Cluj-Napoca.

Bâlc G., Şugar I.R., Gaspar F., 2016, Maşini şi instalaţii pentru industria alimentară Vol.II, Editura Risoprint, Cluj-Napoca.

Burnete N., Băţaga N., Karamusantas D., 2001, Construcţia şi calculul motoarelor cu ardere internă. Editura Todesco, Cluj-Napoca.

Chiru A., Anca H.R., Cofaru C., Kuchar R., Stoica A., Ispas N., 1999, Materiale compozite, vol I. Universitatii Transilvania Publishing House, Brasov.

Roşca M., Blaga A.C., 2008, Termotehnică, Editura Universităţii din Oradea.

Şugar I.R., 2007, Utilizarea materialelor ceramice în arhitectura camerelor de ardere a motoarelor cu aprindere prin scânteie, Editura Risoprint, Cluj-Napoca.

Tisan V., 2009, Utilaje în Industria Alimentară, Editura Risoprint, Cluj-Napoca.

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# REDUCTION OR REPLACEMENT OF NITRITE IN PROCESSED MEAT PRODUCTS

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***Keywords***: *Cured meats, organic, natural, nitrite, nitrate*

## ABSTRACT

*This paper presents the problem of nitrite replacement in cured meat products. Increased global competition as well as changes in consumer demand of meat products is causing an unprecedented growth in processing and ingredient system developments within the meat manufacturing sector. Healthier meat products are demanded by consumers that are, in general, low in salt, fat, cholesterol, nitrites, calories and contain, in addition, health-promoting bioactive components. But, consumers also expect the taste, look and smell of these new meat products, with altered formulations, to stay the same way as their traditionally formulated and processed counterparts.*

## INTRODUCTION

Environmental factors associated with food, water and air were responsible for an estimated of 80% of human cancers (Walters, 1980). In addition, 40% of the human cancers may be directly or indirectly related to malnutrition, dietary habbits and lifestyle (Ologhobo, Adegede, & Maduagiwu, 1996). Based on epidemiology and clinical studies, high dietary intakes of nitrate and nitrite have been implicated in the etiology of human gastric cancer (Bartsch et al. 1990, Joossens et al. 1996).

Nitrite, in the form of sodium or potassium salt, is usually added to meat as a preservative, and nitrate is naturally present in leafy vegetables (Cammack et al. 1999). Additionally, in the oral cavity and in the stomach, nitrate can be reduced to nitrite (Duncanet al. 1997). Nitrite can form a group of carcinogens known as N-nitroso compounds once it enters the stomach and reacts with amines and amides, which are organics containing nitrogen such as amino acids (Archer, 1989). Since stomach acid catalyses nitrosation reactions, the stomach is most at risk from endogenous N-nitroso compound synthesis. In England, Colombia, Chile, Japan, Denmark, Hungary and Italy (Forman & Shuker 1997) increased risks of cancer of the stomach, oesophagus and bladder had been associated with exposure to endogenously formed N-nitroso compounds (Bartsch et al.1990).

Depending on factors such as farming practices, climate, soil quality, manufacturing processes and legislation the dietary intake of nitrates and nitrites in foods can vary greatly from region to region. Accurate and robust methods are necessary for long-term monitoring of nitrate and nitrite concentrations in foods for

susceptible populations due to the growing concern of N-nitroso compounds (James Hsu, Jayashree Arcot , N. Alice Lee 2009).

## PAST AND CURRENT SAFETY ISSUES ASSOCIATED WITH NITRITE

Chemical toxicity, formation of carcinogens in food or after ingestion, and reproductive and developmental toxicity are some of the issues that have been raised concerning the safety of using nitrate and nitrite for cured meat.

At the current regulated levels of use in processed meats, none of these issues represent relevant concerns for nitrate or nitrite. While nitrite is recognized as a potentially toxic compound, the normally controlled use of nitrite in processed meats represents no toxicity risk, but there have been cases where nitrite was mistakenly substituted for other compounds in food or drink at concentrations great enough to induce toxicity symptoms (Sebranek & Bacus, 2007).

In the 1970s, however, the issue of carcinogenic nitrosamines formed from nitrite in cured meat was a very serious concern. The problem of nitrosamine formation in cured meat was, fortunately, solved by reduced levels of nitrite used in curing and changes in manufacturing practices. In the 1990s, because of a lingering background concern about nitrite, a series of epidemiological studies reported that consumption of cured meat was related to childhood leukemia and brain cancer (Peters et al. 1994; Preston-Martin & Lijinsky, 1994; Preston-Martin et al. 1996; Sarasua & Savitz, 1994). Subsequent studies and careful scientific review have largely resolved both issues (nitrite as a carcinogen and as a developmental/reproductive toxicant) (Milkowski, 2006).

In the 1970s the issue of ingested nitrate and nitrite first arose when it was recognized that in the stomach, following ingestion, carcinogenic nitrosamines could be formed(Sebranek & Bacus, 2007).

Later studies have shown that less than 5% of the nitrite and nitrate typically ingested comes from cured meat; the rest is coming from vegetables and saliva (Archer, 2002; Cassens, 1997a; Milkowski, 2006). In 2006, in spite of that, the International Agency for Research on Cancer (IARC) concluded that ‘‘Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans’’ (Coughlin, 2006). Although the IARC report is still in progress, the conclusions are likely to ramp up concerns and questions about nitrite as a food additive. It is imperative, in light of the anticipated challenges to nitrite in cured meat, that as much information as possible is developed for all processed meat applications where nitrite and/or nitrate have a role.

## NITRITE REDUCTION OR REPLACEMENT IN MEAT PRODUCTS

In meat product manufacturing nitrite is one of the staple ingredients. For centuries rock salts, which naturally contain low levels of nitrites, have been known to be excellent preservative agents. In meat products nitrite inhibits the growth of Clostridium botulinum and thereby the formation of the neurotoxic proteins that are commonly known as botulinum toxin. In cured meat products nitrite also contributes to the development of flavor and in cured and smoked products is responsible for the formation of the characteristic pink/red color. Also, during storage, nitrite retards the development of rancidity and off odors and flavors. A reduction in the use of nitrites has become a key issue for the industry, regardless of the technological benefits (Weiss J., Gibis M., Schuh V. & Salminen H.,2010). Under certain circumstances (low pH and high temperature), nitrite can react with amines

to form nitrosamines, compounds that have shown in variety of animal studies to be carcinogenic (Jakszyn & Gonzalez, 2006). There is, nevertheless, pressure coming from the consumer side to further reduce or eliminate the use of nitrate in spite of currently approved levels in meat products that are deemed safe.

Different approaches to reduce the sodium/salt levels in various meat products using different mixtures have been taken by many scientists. Most notable of them are: the Na gluconate (22–33%), KCl (22–33%), NaCl (N 45%) mixture used in meat products to reduce the sodium/salt by 40-60% (Pfeiffer, Scholten and Oellers (2007), the NaCl (0.6–0.8%), tetra-potassium diphosphate, maltodextrin mixture used in Frankfurter Ground meat patties that reduced the sodium/salt used by 30-48% (Ruusunen, Vainionpaeae, Lyly, Laehteenmaeki, Niemistoe et al. 2004), the NaCl (0.5%), edible seaweeds (Sea spaghetti, Wakame, Nori) mixture that reduced the sodium/salt used by 75% (Lopez-Lopez, Cofrades, Ruiz-Capillas and Jimenez-Colmenero, 2009) and countless more.

## THE USE OF NOVEL ANTIOXIDANTS IN MEAT PRODUCTS

A key problem that reduces shelf life of frozen meats, fermented processed meat such as dry sausages, and cured raw ham is the oxidation of lipids in meat products. After refrigerated storage of precooked meats lipid oxidation leads to detrimental changes in the flavor of the reheated, precooked products, a phenomena known as “warmed-over flavor” (Weiss J., Gibis M., Schuh V. & Salminen H.,2010). Here, in a series of radical reactions that involve initiation, propagation, and termination steps with simultaneous formation of free radicals, both lipids and proteins may be oxidized (Ladikos & Lougovois, 1990). Hydro peroxides which are strong oxidizing agents (ROS — reactive oxygen species) are yielded by further reactions (Kubow, 1992). Ultimately, a rancid off-flavor is developed by these compounds. Mutagenic and carcinogenic potential have been observed in some of these oxidation products(Gao et al. 1987) making an extensive oxidation of meat and meat products a health problem.

Antioxidants that stabilize free radicals, delaying the propagation of lipid oxidation reactions, can result from additional enhancement of meat and meat product quality. For example, typical additives are propyl gallate, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), nitric oxide from sodium nitrite, and naturally occurring substances such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), spices and spice extracts such as rosemary (Rosmarinus officinalis L.), oregano (Origanum vulgare L.), or sage (Salvia officinalis L.) (Boon et al. 2008; Fernandez-Lopez et al. 2003; Hernandez-Hernandez, Ponce-Alquicira, Jaramillo-Flores, & Guerrero Legarreta, 2009). The use of natural antioxidants from spices, in contrast to synthetic antioxidants, is increasing since their application is less stringently regulated in most countries around the world. These compounds also often display antimicrobial activities making them useful to enhance food safety by inhibiting the growth of food pathogens (Weiss J., Gibis M., Schuh V. & Salminen H.,2010).

## INCREASED SHELF LIFE BY THE USE OF NITRITE SUBSTITUTES

The ability to inhibit the growth of food pathogens in meat products is one of the most important functionalities of nitrite. A variety of different mechanisms has been attributed to the inhibition of bacteria by nitrite, including the inhibition of oxygen uptake, oxidative phosphorylation and proton-dependent transport

(Davidson, Sofos, & Branen, 2004). A number of enzymes that are essential to the metabolism of bacteria such as aldolase were also inhibited by nitrite. Moreover, the breakdown of the proton gradient in bacteria needed to generate ATP is caused by nitrite in general. The key reason why the compound is so effective is because of the many different effects that addition of nitrite has on the metabolism of food pathogens. It is also the reason why nitrite is so difficult to replace as a preservative. It is very difficult for food pathogens or food spoilage organisms to adapt to its presence since nitrite acts on multiple sites simultaneously. Small concentrations of nitrite are sufficient to cause a broad spectrum inhibition of food pathogens(Weiss J., Gibis M., Schuh V. & Salminen H.,2010).

## CONCLUSIONS

Natural ingredients with high nitrate content can be added to meat as a method to avoid direct addition of nitrite. This method is used in the production of organic versions of cured meats (Sebranek & Bacus, 2007). All typical sensory properties (color, appearance, and shelf life stability) of nitrite-cured meat products are exhibited by organic “uncured” meat products. Unrefined sea salt, turbinado sugar (a raw sugar that is produced by first evaporating sugar cane juice followed by the removal of surface molasses by centrifugation), flavors and spices, celery, carrot, beet and spinach juice, are some of the ingredients that have been used to manufacture “nitrite-free” cured meat products. While it had been initially suggested that the technological effect of these ingredients may be due to their residual nitrite content, their nitrite level was either extremely low (e.g. 0.3–1.7 ppm for sea salt), or non-existent. High levels of nitrate are found in vegetable and spice matter that, during curing, can be converted by nitrate-reducing bacteria into nitrite (Weiss J., Gibis M., Schuh V. & Salminen H.,2010).

## REFERENCES


Archer M.C., 1989, Mechanisms of action of N-nitroso compounds. Cancer Surveys, 8: 241-250.

Archer D.L., 2002, Evidence that ingested nitrate and nitrite are beneficial to health. Journal of Food Protection 65: 872-875.

Bartsch H., Ohshima H., Shuker D., Pignatelli B., Calmels S., 1990, Exposure of humans to endogenous N-nitroso compounds: Implications in cancer etiology. Mutation Research, 238: 255-267.

Boon C.S., Xu Z., Yue X., McClements D.J., Weiss J., Decker E.A., 2008, Factors affecting lycopene oxidation in oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 56(4): 1408-1414.

Cammack R., Joannou C.L., Cui X.-Y., Martinez C.T., Maraj S.R., Hughes M.N., 1999), Nitrite and nitrosyl compounds in food preservation. Biochimica et Biophysica Acta, 1411: 475-488.

Cassens R.G., 1997, Composition and safety of cured meats in the USA. Food Chemistry, 59: 561-566.

Coughlin J.R., 2006, Update on International Agency for Research on Cancer monograph on ingested nitrite and nitrate. In Proceedings of the meat industry research conference. <http://www.meatscience.orgPubs/mircarchiv/2006/default.htm>

Davidson P.M., Sofos J.N., Branen A.L., 2004, Antimicrobials in food. Boca Raton: Taylor & Francis.

Duncan C., Li H., Dykhuizen R., Frazer R., Johnston P., MacKnight G., et al., 1997, Protection against oral and gastrointestinal diseases: Importance of dietary nitrate intake, oral nitrate reduction and enterosalivary nitrate circulation. Comparative Biochemistry and Physiology, 118A: 939-948.

Fernandez-Lopez J., Sevilla L., Sayas-Barbera E., Navarro C., Marin F., Perez-Alvarez J.A., 2003, Evaluation of the antioxidant potential of hyssop (*Hyssopus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) extracts in cooked pork meat. Journal of Food Science, 68(2): 660−664.

Forman D., Shuker D., 1997, Helicobacter pylori and gastric cancer - A case study in molecular epidemiology. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 379: S159.

Hernandez-Hernandez E., Ponce-Alquicira E., Jaramillo-Flores M.E., Guerrero Legarreta I., 2009, Antioxidant effect of rosemary (Rosmarinus officinalis L.) and oregano (Origanum vulgare L.) extracts on TBARS and colour of model raw pork batters. Meat Science, 81(2), 410−417.

Gao, Y. T., Blot, W. J., Zheng, W., Ershow, A. G., Hsu, C. W., Levin, L. I., et al. (1987). Lung cancer among Chinese women. International Journal of Cancer, 40(5), 604−609.

Jakszyn, P., & Gonzalez, C. A. (2006). Nitrosamine and related food intake and gastric and oesophageal cancer risk: A systematic review of the epidemiological evidence. World Journal of Gastroenterology, 12(27), 4296−4303.

James Hsu, Jayashree Arcot , N. Alice Lee (2009). Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. Food Chemistry, 115, 334-339.

Joossens, J. V., Hill, M. J., Elliott, P., Stamler, R., Stamler, J., Lesaffre, E., et al. (1996). Dietary salt, nitrate and stomach cancer mortality in 24 countries. International Journal of Epidemiology, 25, 494-504.

Kubow, S. (1992). Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radical Biology and Medicine, 12(1), 63−81.

Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: A review. Food Chemistry, 35(4), 295−314.

Milkowski, A. L. (2006). The controversy about sodium nitrite. In Proceedings of the meat industry research conference. <http://www.meatscience.org/Pubs/mircarchiv/2006/default.htm>

Ologhobo, A. D., Adegede, H. I., & Maduagiwu, E. N. (1996). Occurrence of nitrate, nitrite and volatile nitrosamines in certain feedstuffs and animal products. Nutrition and Health, 11, 109-114.

Peters, J. M., Preston-Martin, S., London, S. J., Bowman, J. D., Buckley, J. D., & Thomas, D. C. (1994). Processed meats and risk of childhood leukemia (California, USA). Cancer Causes and Control, 5, 195-202.

Preston-Martin, S., & Lijinsky, W. (1994). Cured meats and childhood cancer (Letters to the Editor). Cancer Causes and Control, 5, 484-485.

Preston-Martin, S., Pogoda, J. M., Mueller, B. A., Holly, E. A., Lijinsky, W., & Davis, R. L. (1996). Maternal consumption of cured meats and vitamins in relation to pediatric brain tumors. Cancer Epidemiology, 5, 599-605.

Sarasua S., Savitz D.A. (1994). Cured and broiled meat consumption in relation to childhood cancer: Denver, Colorado (United States). Cancer Causes and Control, 5, 141-148.

Sebranek J.G., Bacus J.N., 2007, Cured meat products without direct addition of nitrate and nitrite: What are the issues? Meat Science, 77, 136−147.

Walters C.L., 1980, The exposure of humans to nitrite. Oncology, 37, 289-296.

Weiss J., Gibis M., Schuh V., Salminen H., 2010, Advances in ingredient and processing systems for meat and meat products. Meat Science, 86: 196-213.

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# CHARACTERIZATION OF FRUIT BOTANICAL ORIGIN USING STABLE ISOTOPES

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***Keywords***: *botanical origin, fruits, stable isotopes, statistic methods*

## ABSTRACT

*Differentiation of fruits varietal origin is a topic of interest for both producers and consumers since the value of a fruit derivate product is often influenced by the individual perception and taste for a particular variety. Therefore, in this work, in an attempt to classify fruits according to their botanical origin, the stable isotopes ratios of the main bioelements (C, H and O) were investigated for 49 fruit samples produced in few selected regions of Romania, from 13 varieties during 2013 harvest year. Chemometric techniques were applied to the analytical results as a multi-criteria decision and the predictive abilities of different classification methods were evaluated. The observed differences between stable isotopes content of fruit juices are explained by the different embedding processes for various fruits constituents associated with each botanical origin. Therefore, isotopic fingerprinting may be an adequate approach to discriminate natural fruit juices.*

## INTRODUCTION

Fruit markets together with fruit juices production are important and evolving sectors within the food industry. In addition to orange and apple juice, which are highly recognized as the largest consumed fruits among the global market, other fruit juices, such as those made from cherries or various types of berries have become popular especially due to their antioxidant capacity, which is associated with the generation of positive effects on the human health. Like other highly valued food, economic value and natural juice production became the main object of falsifications. Due to this phenomenon the importance of ensuring control of the authenticity juices has increased. However, due to rapid and cheap means of transport, and due to the promulgation of various grant rules within the European Union it is necessary to implement a more effective monitoring system for these products. In addition to the conventional physical and chemical analysis, the use of stable isotopes analysis has proven to be particularly useful in this field of application. This was demonstrated for a variety of foods (Drivelos and Georgiou 2012) such as meat (Franke et al. 2008), dairy products (Fortunato et al. 2004; Rossmann et al. 2000), alcoholic beverages (Dinca et al. 2016; Larcher et al. 2003), rice (Kelly et al. 2002), honey (Dinca et al. 2014) and in this article will be used for fingerprinting the botanical origin of fruit juices. Traders, producers and

consumers are especially interested in the process of correct labeling the origin, and genuine products which are not adulterated (eg added sugar, water or acid). Determination of origin of fruit juice is generally applied in the control of those products with geographical origin labeled, especially by customs officers when applying for grants and for routine checks. When the botanical origin of the product is specified, different methods of adulteration can be identified more easily. The European Directive from 1993 for fruit juices requires a clearly description of the fruit juices and as well their authenticity. Developing methods to identify fraud using stable isotopes began about 20 years ago with the introduction of stable isotope analysis of light elements such as H, C, N and O. Depending on their origin and evolution before and after incorporation, these elements present a significant variation of the isotopic ratios. These variations are mainly due to the kinetic and thermodynamic isotope effects and, therefore, reflect the circumstances in which the physical or (bio) chemical processes were conducted. The isotopic ratio of light elements provides information regarding the climatic conditions, distance from the sea, altitude, latitude and agricultural practices. Stable isotope ratio of $\delta^{18}O$ and $\delta^{2}H$ are mainly correlated to the geographical origin since they are closely related with latitude. While water is the only source of hydrogen for the photosynthesis of plants, the oxygen is taken from many sources, from atmospheric oxygen and carbon dioxide, and mainly from the water in the soil. Isotopic ratios $(D/H)_I$ and $\delta^{13}C$ of sugar and ethanol extracted from fruit juices are influenced by the climatic factors, geographical and botanical origin, while the $(D/H)_{II}$ isotope ratio is important for the deuterium content of water juice and reflects the climatic conditions closely related to the geographical origin and year of harvest. Consequently, stable isotope values of fruit juices should reflect the botanical, geographical and product diversity.

Based on these considerations, we attempt to highlight in this work the importance that stable isotope analysis may have in establishing correlations between the specific environmental conditions and geographic region of the fruit growing area, essential in certifying the authenticity of fruit varieties. The paper presents preliminary results regarding the characterization of 13 fruits varieties grown in Romania, in different geographical regions.

## MATERIAL AND METHODS

*Fruit juices sampling*. Fruit samples were collected from different geographical regions of Romania, from trusted farmers within 2013 harvest year. A series of 49 samples of fruits from varieties as cherry (Prunus avium), sour cherries (Prunus cerasus), strawberry (Fragaria viridis), mirabelle (Prunus cerasifera) apple (Malus communis), plum (Prunus domestica), blueberry (Vaccinium myrtillus L.), apricot (Prunus armeniaca) blackberries (Rubus fruticosus L.), watermelon (Citrullus lanatus), pear (Pyrus communis), peaches (Prunus persica) and raspberry (Rubus idaeus) were analyzed for this study. Before the analysis, fresh fruit juice was obtained using an automatic squeezer. None of the samples did undergo any processes that might have changed their composition. Information on the region of production, harvesting dates, type and quality were recorded using the data provided by the producers.

*Sample preparation for the isotopic analysis*. Two methods of analysis were approached (Table 1), first by measuring $\delta^{18}O$ and $\delta^{2}H$ of fruit juice, and the second by determining deuterium and carbon-13 stable isotopes from the extracted

ethanol from the fruit juices after a fermentation process. For the first approach, the juice samples were subjected to a freeze-drying process, the water being extracted in the form of vapors using a high vacuum in order to determine $\delta^2H$ and $\delta^{18}O$ of water. For the second approach, the sugars from fruit juices were fermented for 7 days at 25 °C. A sample of about 800 ml fruit juice, with a sugar content between 10 and 25 Brix degrees was fermented by adding Saccharomyces cerevisiae yeast, and the conversion of sugars to ethanol was checked daily using a test for the quantitative determination of reducing sugars as well as a refractometer (Atago Pocket model). After the full conversion, in order to extract ethanol for isotopic analysis, the samples were distilled using automatic distillation system (ADCS) equipped with Cadiot columns with rotating bands.

Table 1

Analytical procedures used to analyze samples of fruit juices

| Method | Stable isotope ratio | Matrix | Aim | Official methods |
|---|---|---|---|---|
| IRMS | $^{13}C/^{12}C$ | Ethanol extracted from the fermented fruit juices | Geographical and botanical origin | OIV-MA-AS312-06 |
| | $^{18}O/^{16}O$ | Water extracted from the fresh fruit juices (using the lyophilization process) | Geographical origin | OIV-MA-AS2-12 |
| | $^{2}H/^{1}H$ | | | |
| SNIF-NMR | D/H | Ethanol extracted from the fermented fruit juices | Geographical and botanical origin | OIV-MA-AS311-05 |

*Statistical data analysis*. Data analysis was performed to test if significant differences can be highlighted between the botanical origins of fruit juice samples. The statistical analysis performed, conducted to the characterization of the authentic fruit juices samples using analysis of variance (ANOVA) and discriminant analysis (DA). The Statistical analysis of data was performed using Microsoft Excel 2010 and XLSTAT Addinsoft version 15.5.03.3707.

## RESULTS AND DISCUSSIONS

In order to establish the criteria for the classification and origin discrimination of the fruit juices statistical methods were applied using stable isotope content as physico-chemical descriptors. The average content of stable isotopes in a set of 49 samples of fruit juice from Romania are presented in Table 2, the results are expressed in ‰ for $\delta 1^3C$, $\delta^{18}O$ and $\delta^2H$ and in ppm for (D/H) ratios of ethanol resulted after the fermentation process.

The $\delta^{13}C$ value of the ethanol extracted from the fruit juice samples is specific to the C3 group of plants, ranging from -25.21‰ to -29.53‰. Was observed that the blueberry juice presents depleted $^{13}C$ isotope values comparing to other fruit juices (between -29.53‰ and -27.02‰), while the raspberry juice the most enriched $^{13}C$ isotope values, ranging from -25.86‰ to -25.21‰. In case of blueberries, a possible explanation for the obtained depleted $\delta^{13}C$ values could be the more intense microbiological activity in the provenance soil, phenomenon that lead to a $CO_2$ depleted $^{13}C$ isotope values. From Table 2 it is obvious that it is not possible to clearly differentiate the botanical origin of juices using $\delta^{13}C$ as a single

parameter, the measured values being relatively dispersed (standard deviation is 0.95). The $(D/H)_I$ value varies from 91.4 ppm (in this case strawberry juice) to 101.6 ppm (for mirabelle juice).

Table 2

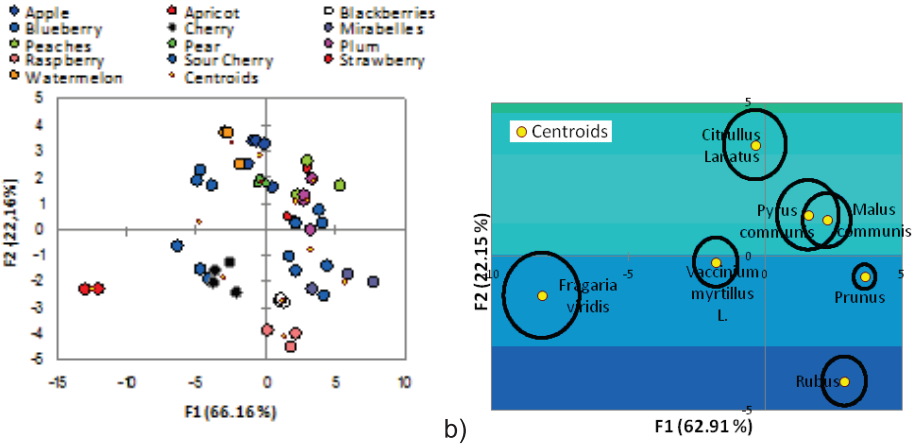
Means followed by different lowercase letters in the line differ significantly by ANOVA complemented by Tukey's test of multiple comparisons; significance level of 5%

| Samples | | $\delta^{13}C$ (‰) | $\delta^{18}O$ (‰) | $(D/H)_I$ (ppm) | $(D/H)_{II}$ (ppm) | R | $\delta^2H$ (‰) |
|---|---|---|---|---|---|---|---|
| Mirabelles | | -26,30$^{a,b}$ | +2,12$^{a}$ | 100,5$^{a}$ | 126,9$^{a,b}$ | 2,525$^{e}$ | -29,10$^{a,b,c}$ |
| Strawberry | | -26,70$^{a,b,c}$ | -0,49$^{a,b,c}$ | 91,6$^{e}$ | 126,3$^{a,b}$ | 2,758$^{a}$ | -14,2$^{a}$ |
| Cherry | | -27,48$^{b,c}$ | +1,21$^{a,b}$ | 96,7$^{b,c}$ | 127,6$^{a,b}$ | 2,639$^{b,c}$ | -18,71$^{a,b}$ |
| Blackberries | | -27,52$^{b,c}$ | +1,75$^{a}$ | 97,2$^{b,c}$ | 127,0$^{a,b}$ | 2,614$^{b,c,d}$ | -28,50$^{a,b,c}$ |
| Plums | | -26,90$^{a,b,c}$ | -1,34$^{b,c}$ | 99,3$^{a,b}$ | 126,8$^{a,b}$ | 2,527$^{e}$ | -40,56$^{c,d}$ |
| Sour Cherry | | -27,55$^{b,c}$ | +1,60$^{a}$ | 100,1$^{a}$ | 126,5$^{a,b}$ | 2,554$^{d,e}$ | -20,99$^{a,b}$ |
| Raspberry | | -25,51$^{a}$ | +1,40$^{a,b}$ | 95,8$^{c,d}$ | 124,8$^{b}$ | 2,606$^{b,c,d}$ | -36,56$^{b,c}$ |
| Watermelon | | -27,35$^{a,b,c}$ | -2,98$^{c,d}$ | 98,4$^{a,b,c}$ | 126,2$^{a,b}$ | 2,566$^{d,e}$ | -29,67$^{a,b,c}$ |
| Apples | | -27,85$^{b,c}$ | -4,06$^{d}$ | 97,1$^{b,c}$ | 129,2$^{a}$ | 2,661$^{b}$ | -55,29$^{d}$ |
| Apricots | | -27,06$^{a,b,c}$ | -1,35$^{b,c,d}$ | 98,6$^{a,b,c}$ | 124,4$^{b}$ | 2,523$^{e}$ | -35,32$^{a,b,c}$ |
| Pears | | -27,28$^{a,b,c}$ | -2,73$^{c,d}$ | 97,2$^{b,c}$ | 125,6$^{a,b}$ | 2,577$^{c,d,e}$ | -41,33$^{c,d}$ |
| Peaches | | -27,56$^{b,c}$ | -1,67$^{b,c,d}$ | 99,5$^{a,b}$ | 125,6$^{a,b}$ | 2,524$^{e}$ | -39,03$^{c,d}$ |
| Blueberry | | -28,46$^{c}$ | -1,86$^{c,d}$ | 94,2$^{d,e}$ | 124,8$^{b}$ | 2,650$^{b}$ | -31,13$^{a,b,c}$ |
| $R^2$ | | 0,569 | 0,830 | 0,858 | 0,484 | 0,893 | 0,782 |
| Significant | | Yes | Yes | Yes | Yes | Yes | Yes |
| 2013 harvest year | Average | -27,34 | -0,65 | 97,6 | 126,4 | 2,592 | -32,54 |
| | Min. | -29,53 | -4,74 | 91,4 | 121,9 | 2,49 | -57,69 |
| | Max. | -25,21 | +3,16 | 101,6 | 132,2 | 2,768 | -9,05 |
| | SD | 0,95 | 2,27 | 2,5 | 1,9 | 0,066 | 12,39 |

The $\delta^{13}C$ and $(D/H)_I$ ratio of the ethanol extracted from the fermented fruit juices are influenced by the climatic and geographical factors, being correlated to the botanical origin of the fermented sugars. The $(D/H)_{II}$ ratio is influenced by the deuterium content of the fruit juice water and reflects the climatic conditions related to geographical origin and harvest time, ranging from 121.9 ppm (apricot juice) to 132.2 ppm ( apple juice). For $\delta^{18}O$ we observed a wide range (the most depleted value of -4.74‰ was recorded for the apple juice samples - harvesting period with excessive rainfall, while the most enriched value +3.16 ‰ was recorded for cherry), variation dictated by the climatic conditions experienced by the fruits during the ripening and harvesting period. In the case of $\delta^{2}H$ values, was observed that the most depleted value was registered for the apple group (-57.69‰) and the most enriched for cherries (-9.05‰). The same pattern of depletion and enrichment in the stable isotope content for oxygen and hydrogen was noticed, which is correlation with the environmental conditions, especially rainfalls. The temporal and spatial differences observed through the correlation of $\delta^{18}O$ and $\delta^{2}H$ values are strongly related to the precipitation amount, temperature (seasonal effects) and altitude, the samples originating from different geographical regions.

By applying discriminant analysis using the isotopic data as independent variables and the variety as a dependent variable we obtained a total rate of correct classification of 88.32% and a cross-validation rate of 70.09% (Figure 1a).

The discriminant analysis managed to indicate the elements separating the juices depending on their botanical origin. Function 1 expressed 66.16% of variance and provides the main separation between strawberries, cherries and blueberries from the other varieties, while function 2 (22.16% of the total variance) separates raspberries, cherries and mirabelles. Among the six variables used for discrimination ($\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$, $(D/H)_I$, $(D/H)_{II}$ and R) the $(D/H)_I$ ratio was not statistically significant.




a) b)

Figure 1. Discriminant analysis of the fruits botanical origin based on the isotopic composition (a) and Discriminant analysis of fruits genus based on the isotopic composition (b).

However, an overlap of different types of fruit juices can be observed in Figure 1a and in trying to get an accurate classification of them according to variety, we conducted a discriminant analysis using as dependent variable the scientific classification like the genus (Figure 1b) because most types of fruit are part of the Rosaceae family, except watermelon (family Cucurbits) and blueberries (family Ericaceae). With this pattern for classification was obtained an excellent separation of the fruits samples depending on the type to which they belong. A successful classification according to variety was achieved confirming the existing relation between composition and specific conditions experienced by the fruits during ripening and harvesting period. Therefore, the proposed design could be considered relevant to discriminate among the 13 varieties during 2013 harvest year.

## CONCLUSIONS

The preliminary studies carried out in this article had an original approach of using stable isotopes ($^{2}H$, $^{18}O$ and $^{13}C$) in order to provide new insight into the fruit authentication in terms of their botanical origin. Simultaneously with the development of new analysis methods in order to enable fruit authenticity verification and identification of fraud, our research has achieved an improved performance in this preliminary study using multivariate statistical methods. The most important conclusion is related to the fact that fingerprinting techniques based

on stable isotopes and multivariate analysis provides a promising statistical model that may improve the classification of fruits according to their varietal origin.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## REFERENCES


Dincă OR, Ionete RE, Costinel D, Popescu R, Ștefănescu I şi Radu GL (2014) Geographical and Botanical Origin Discrimination of Romanian Honey Using Complex Stable Isotope Data and Chemometrics, Food Anal. Methods 8(2):401-412.

Dincă OR, Ionete RE, Geana EI, Costinel D, Popescu R, Ștefănescu I şi Radu GL (2016) Regional and Vintage Discrimination of Romanian Wines Based on Elemental and Isotopic Fingerprinting, Food Anal. Methods 9: 2406. doi:10.1007/s12161-016-0404-y

Drivelos SA and Georgiou CA (2012). Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union. Trends in Analytical Chemistry 40:38–51.

Fortunato G, Mumic K, Wunderli S, Pillonel L, Bosset JO, Gremaud G (2004). Application of strontium IR measured by MC-ICP-MS for food authentication. Journal of Analytical Atomic Spectrometry 19:227–234.

Kelly S, Baxter M, Chapman S, Rhodes C, Dennis J, Brereton P (2002). The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. European Food Research and Technology 214:72–78.

Larcher R, Nicolini G, & Pangrazzi P (2003). Isotope ratios of lead in Italian wines by inductively coupled plasma mass spectrometry. Journal of Agricultural and Food Chemistry 51 :5956–5961.

Rossmann A, Haberhauer G, Hölzl S, Horn P, Pichlmayer F & Voerkelius S (2000). The potential of multielement stable isotope analysis for regional assignment of butter. European Food Research and Technology 211:32–40.

***Oeno 17/2001 (2009) OIV method number MA-AS312-06.

***Oeno 353 (2009) OIV method number MA-EAS2-12-MOUO18.

***Oeno 426/2011 OIV method number MA-AS311-05 (2011).

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# STUDY ON THE FLAVOURING POTENTIAL AND ANTIOXIDANT ACTIVITY OF THE BASIL ON THE CLASSICAL COMPOSITION OF MAYONNAISE

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***Keywords***: *peroxide, mayonnaise, the essential basil oil*

## ABSTRACT

*The mayonnaise-type foods represent finely dispersed emulsions of O/W type, providing a significant degree of assimilation and biological and nutritional value very important for the product. This study aims to observe the flavouring potential and antioxidant activity of the basil on the classical composition of mayonnaise. Comparisons will be done between the simple mayonnaise and the basil flavoured mayonnaise, settling at certain periods of time if the basil has an antioxidant role in the composition of mayonnaise, by determining the peroxide value and shelf life of taste and smell of basil within the mayonnaise mass.*

## INTRODUCTION

A category of foods with a high quality potential in accordance with imposed domestic and international regulations consists of food emulsions.

The food emulsions are of several types, the most commonly used is the mayonnaise, consisting of vegetable oil which is dispersed. Firstly, the mayonnaise is characterized by high nutritional and taste qualities, which are determined by a structure specific for emulsions.

The mayonnaise-type foods represent finely dispersed emulsions of O/W type, providing a significant degree of assimilation and a biological and nutritional value important for the product. Emulsions are heterogeneous systems whose stability is limited in time (Leal-Calderon F., et al.2007, Dalgleish D.G. et al. 2006).

Lately, a particular attention was paid to researches on the oxidation of O/W emulsions (Chaiyasit W. et al. 2007, Hu M. 2002, Lethuaut L. et al. 2002). The reason lies in the variety of food emulsions on the world market and their availability as food used daily by the population.

The air contact with the oil in the emulsion during storage determines an increased acidity, resulting in a stench, uncharacteristically smell and a pungent taste (Căpruciu R. 2011).

These phenomena lead to the rancid of emulsions or of other fats with qualitative repercussions (the nutritional value is reduced, the price increases due to their change and in the worst case the products can be lost).

## MATERIALS AND METHODS

*Moisture determination of oven dried basil*

The moisture determination of the basil samples (flowers, foliage, leaves and flowers) freshly harvested was performed at 95 +/- 2°C in an oven under specified conditions.

As a result the arithmetic mean of two determinations is considered, if the conditions of repeatability are provided. The difference of the results of the two parallel determinations should not exceed 0.4% (in absolute value).

*Basil essential oil extraction with animal fat*

This process enables the extraction of essential oils without altering the natural composition, and enables also to perceive the clearest smell of the analyzed plants in this study (especially flowers). This procedure was applied as odorous substances belonging to these plants are very sensitive and in very small amount, so that the steam driving is excluded as an extraction process.

The method itself consists in immersing the plant material in the fat or oil heated to about 50-70°C. The used fats blend consists of 40% pork fat, 40% beef tallow and 20% sunflower oil. The plant material (1 kg of leaves and basil inflorescences) is held in the fat blend for 1-2 hours after which it is removed and replaced by a fresh portion. This is repeated 10-15 times until the extraction is complete due to the fat saturation with odorant. It is usually possible to extract a greater amount of 5-10 times of plant product from the same quantity of fats. Removing the flowers from pomade or oil is made by hot filtration and centrifugation. It forms a corpus. This one is combined with the synthetic solvents (ethanol) at 50°C. The fat retained by the plant is recovered by pressing. The oil separation is made by stirring with alcohol, freezing and decantation.

*Determination of peroxide value*

The peroxide value is used in addition to other quality parameters, for determining the oxidation degree of a product which is composed of fat.

This index is a measure unit for the oxygen content linked with peroxide in oils and fats and particularly for the hydroperoxides.

The peroxide value was measured by determining the amount of converted iodide to iodine under the action of the active oxygen of the peroxide. The result was expressed as the number of milligrams of active $O_2$/kg of fat.

There is only one principle for both methods of the peroxide value determination: the oxidation of iodides to iodine through the active oxygen of peroxide, and measuring the amount of free iodine by titration with 0.01 N sodium thiosulfate solution.

An index expressed in milligrams of active $O_2$ higher than 20, indicates that fats are rancid. If this index is very low, it has no meaning, in this case a simple heating at 130°C is sufficient to destroy the peroxides.

## RESULTS AND DISCUSSIONS

Following the conducted studies, there was found that the essential oil is concentrated in large quantities in flowers and at the top of the stem.

An organoleptic analysis is also needed because complete, healthy plants, reaching an early maturing, with diseases and pest free are necessary in the industry of essential oils.

Table 1 shows the organoleptic properties of samples formed of basil leaves and flowers.

Table 1

The organoleptic properties of basil leaves and flowers at harvest

| Characteristics | | Conditions for eligibility |
|---|---|---|
| Appearance | | Stem and flowers developed normally, without stains or other defects |
| Color | leaves | Skin deep green with turgescent |
| | flowers | Dark purple,specifically |
| Smell | | Characteristic flavor , slightly spicy, pleasantly, without foreign smell |
| Taste | | Strongly flavored, spicy, specifically |
| Infestation | | - |
| Yellow leaves (%) | | - |
| Leaves injured (%, max) | | 2% at based plant |
| Leaves undeveloped | | - |

As shown in the table, basil meets the conditions of admissibility in order to use it as raw material necessary to obtain essential oil for the flavouring of food.

Analyzing the data, there were observed admissibility features both physical and organoleptic according to STAS for the analyzed flowers and leaves.

If it is required the retention of flowers and leaves, the determined organoleptic properties lead to their preservation in good condition.

The organoleptic analysis also shows that there will be obtained a top quality essential oil, because both samples of flowers and leaves were fresh and within specific parameters of the standard in force for producing the essential oil of basil.

The determination of water content is an important analysis for the fresh aromatic plants, influencing the process of obtaining the essential oils.

The samples with basil leaves or flowers for chemical analyses must have a moisture content of at most 10 - 12%.

To determine the moisture content, samples were used as follows:

- Flowers which reached the technique maturation;
- Basil leaves from the top of the plant (located just below the flowers);
- Leaves and flowers of basil.

The samples above were formed in the laboratory on the same day of harvesting. The harvest was performed in the morning after the dew evaporated. The whole plants were handled carefully and brought to the laboratory for forming the samples.

After the visual examining in the field and laboratory, the samples were analyzed in terms of moisture according to the methodology described in the material and methods section.

Regarding the essential oil of basil, 1 kg of plant material was used for each sample, less the flowers (500 g), obtaining different values according to the parts of the analyzed plants (flowers, leaves from the top, flowers and leaves from the top).

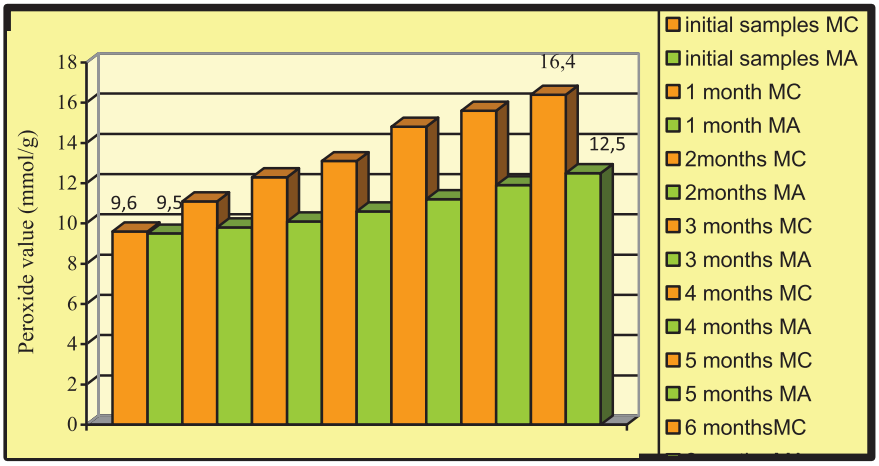
It appears also that the best composition of basil in terms of essential oil is in flowers (2.6%) followed by the top leaves that were cut together with the flowers (2.3%). There was obtained a satisfactory amount of essential oil (1.8%) from the top leaves analyzed separately.

Table 2

Determination of the content in water and essential oil from basil

| Elements analyzed | Basil plant material | | |
|---|---|---|---|
| | flowers | top leaves | flowers+ top leaves |
| Water (%) | 57 | 76 | 68 |
| Essential oil (%) | 2,6 | 1,8 | 2,3 |

By making a correlation between the water content and the essential oil content there can be seen that the top leaves of the analyzed plants, that are turgid, after the process of grinding in order to obtain the desired amount of essential oil, lead to easier movement of flavoured substances located in the alveoli of the cells, the obtained quantity being lower. There was analyzed how classical mayonnaise behaves in comparison with the basil flavoured mayonnaise in the storage process (the dynamics of the accumulation of oxidation products) by determining the peroxide value, a quality basic indicator in the food industry. During the storage of fats which also include the basil flavoured mayonnaise, they are subject to various actions, following an accumulation of oxidation products, namely, peroxides, hydroperoxides, ketones, aldehydes, etc. The dynamic of the accumulation of oxidation products was monitored during the storage of the two studied types of mayonnaise for a period of 6 months (Figure 1).




(MC- classical mayonnaise; MA- flavored mayonnaise)

Figure 1. Dynamics peroxide types of mayonnaise studied.

The evolution of the peroxide value at the types of mayonnaise in the study show that the classical mayonnaise sample after 6 months of storage has the highest value (16.4 mmol/ g of product) and differ considerably in terms of

organoleptic features (taste and pungent, rancid smell) from the samples of basil flavoured mayonnaise, which demonstrates the active formation of oxidation products.

There can be observed at the classical mayonnaise the dynamics of the accumulation of oxidation products which is higher than the one recorded by the basil essential oil flavoured mayonnaise. Oxidation compounds are formed in the case of flavoured mayonnaise, but the accumulation rate is slower.

The first 2 months of storage are characterized by a slow accumulation of oxidation compounds for both types of mayonnaise; the classic mayonnaise records a significant increase of peroxide values from the third month.

There are considerable differences of the values recorded by the two types of mayonnaise after 6-month storage (12.5 mmol/g for the flavoured mayonnaise and 16.4 mmol /g of product for the classical mayonnaise), showing the antioxidant effect of the basil oil.

## CONCLUSIONS

After the conducted studies there were found that the essential oil is concentrated in large quantities in the flowers and at the top of the stem (in the leaves that are next to the flowers).

For obtaining an essential oil rich in flavour chemicals, the harvest should be well chosen, an advanced ripening leading to wilting of the flowers and to a significant loss of aromatic substances. In this respect the qualitative determinations were conducted in the harvesting moment, the plants being analyzed in the same day.

The organoleptic analysis showed that the samples consisting of basil plant comply with the relevant standard for producing essential oil of basil, the latter meeting the conditions of admissibility in order to use it as raw material to obtain essential oil in order to flavour the classic mayonnaise.

The determination of the water content is an important analysis for fresh aromatic plants, influencing the process of obtaining the essential oils.

The samples with basil leaves or flowers for chemical analyses must have a moisture content of at least 10 - 12%.

From a quantitative perspective, the volatile oil is in flowers followed by the top leaves that were cut at the same time with the flowers. There was obtained a satisfactory amount of volatile oil from the top leaves that were analyzed separately.

Doing a correlation between water content and volatile oil there can be seen that the top leaves together with the basil flowers, with an average water content drives easily the flavour substances located in the alveoli of plant cells, the amount obtained being sufficient for flavouring the mayonnaise.

From the point of view of flavouring degree with basil essential oil, the mayonnaise performed very well, after the organoleptic determinations there was observed that after three months of storage the pleasant taste and smell, specifically for basil, was preserved, with soft non-persistent flavour on tongue.

After 4 months of storage, it loses its flavour becoming increasingly less noticeable, after the 5th month of analysis, it modifies in terms of aroma and taste, something that the classical mayonnaise recorded after the 3$^{rd}$ month of the study. In the 6$^{th}$ month there is a considerable degradation (more obvious for the classical

mayonnaise) which makes the product unfit for consumption. This was underlined also by the determination of the peroxide value, the recorded values being higher and with a strong dynamics in the case of the classical mayonnaise.

From measurements made on the two types of mayonnaise under study it can be said that both types of mayonnaise can be used within 2 months of preparation under low temperature (3-4°C temperature of the refrigerator), after that the classical mayonnaise quality drops significantly, so as after 4 months of storage to be unfit for consumption.

## REFERENCES


Căpruciu Ramona, 2011, Study of sunflower oil quality under different conditions of storage. Lucrare Ştiinţifică. Analele Universităţii din Craiova. pp.83-97;

Căpruciu Ramona, 2011, Tehnologii în industria uleiului. Editura Sitech. Craiova

Leal-Calderon F., Schmitt V., Bibette J. 2007, Emulsion science basic principles. New-York, USA: Springer, 227 p.

Dalgleish D.G., 2006, Food emulsions-their structures and structure–forming properties. FoodHydrocolloids, 20(4). 415-422 p.

Chaiyasit W., Elias J., Mc Clements D., 2007, Role of physical structures in bulk oils on lipid oxidation. Crit. Rev. Food Sci. Nutr. 47,. 299-317 p.

Hu M., Mcclements D.J., Decker E.A., 2002, Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. J. Agr. Food Chem. 51. 1435-1439p.

Lethuaut L., Metro F., Genot C., 2002, Effect of droplet size on lipid oxidation rates of oil in water emulsions stabilized by protein. Journal of the American Oil Chemist's Society 79, 425-430 p.

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# QUALITY CONTROL OF SOME RAW MATERIALS FOR STERILIZED CANNED VEGETABLES

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***Key words***: *sterilized cans, tomatoes, green peas, quality*

## ABSTRACT

*In this study we aimed to perform qualitative analysis of two raw materials of vegetables widely used in the food industry, namely: peas and tomatoes, with special importance both in terms of nutritional and availability throughout the year. In this respect, it was proceeded to the qualitative evaluation which consisted of sensory analysis of of peas and tomatoes as well as determination of some qualitative parameters of the canned content by jar (determination of moisture, salt content, soluble substance and titratable acidity).*

## INTRODUCTION

Peas and tomatoes sterilized canned are some of the most important food that can be found on store shelves over the year. Qualitative analysis of the two types of food by sterilization is necessary precisely because they are some of the most consumed food in any season. Tomato paste is an important source of energy that ensures normal daily activity and maintains health. Peas is one of the most popular vegetable raw materials, since besides nutritional qualities it is suitable for conservation in many forms and can be cooked in any season.

Nutritional quality is given by the chemical composition of these materials (Chira 2004). Other studies showed that tomatoes and products obtained from tomatoes may have a protective effect against various diseases like prostate cancer and cardiovascular disease (Sargeant et al. 2001, Barber and Barber 2002, Perkins-Veazie et al. 2007). Dinu et al. (2016) studies have shown large variability in the contents of bioactive components of tomato fruits that exist currently on the market. All stages followed in the cultivation technology and in the evaluation processes are of interest due to its nutraceutical and bioactive components as well as the growing interest that the consumer has regarding the relationship between food and health (Dinu et al. 2015).

## MATERIALS AND METHODS

Conducting organoleptic investigation implies the examination of the objective qualities like: smell, taste, exterior aspect or section (if appropriate) of the green peas and tomato fruits taken into consideration for this study.

Determination of moisture consists in the evaluation based on the weight loss of a sample of approximately 5 grams, by keeping it in the oven at 130 ± 30°C

for one hour. The expression of moisture is made by a digit with a decimal, by rounding up the the results of two parallel determinations among which there is no difference of more than 0.3%. In the case of a difference greater than 0.3% two more parallel determinations will be performed. If also in this case a bigger difference is obtain, the arithmetic mean of the four determinations will be made.

The determination of sodium chloride with Mohr's method consists of the chlorine ions titration from the aqueous extract of the sample to be analyzed, neutralized with silver nitrate, in the presence of potassium chlorate as an indicator.

The determination of the soluble substance (soluble dry extract) by the refractometric method is based on determining the refractive index at temperatures of 20 °C and from its value the soluble solids content expressed as sucrose will be deducted, using a conversion table.

The titratable acidity is the sum of acidic reaction substances (organic acids, acidic salts) which can be titrated with an alkaline solution.

The principle of this method is the titration of the sample to be analyzed, with sodium hydroxide in the presence of phenolphthalein as an indicator. The result is then multiplied by the respective acid milliequivalents, as follows: citric acid 0,070, malic acid 0,067, tartaric acid 0,075, lactic acid 0,090, acetic acid 0,060. As a result the arithmetic mean of the two determinations in parallel which do not differ by more than 2% (relative value) than the average is taken into consideration (by Ionică, 2014).

## RESULTS AND DISCUSSIONS

The organoleptic characteristics of the tomatoes and peas are properties that can be perceived through the senses and are important factors in determining the quality of vegetables in order to use them. Regarding the raw material used for the sensory analysis (Table 1, 2), the most consumed type of peas was chosen, with fine grain size within 8.2 and 8.75 mm, and the tomatoes being of medium size, with an uniform intense red color. The results of the organoleptic analysis shows that fine peas preserved by sterilization in a jar (Table 1) comply with the current standards regarding: color, smell and taste, general appearance of both the pea grains and the liquid.

Foreign particles were absent, this showing a good conditioning of the analyzed peas, prior to their packaging. Both dry beans and the remaining husks were in a small percentage that didn’t influence the sensory analysis from a qualitative point of view. The sensory evaluation was performed also on the tomato paste (Table 2) and it was found that it complies to the current standards regarding: the color, smell, taste, and general appearance.

The determination of the physicochemical properties (Table 3) of canned peas or tomatoes helps to establish a qualitative ranking from a nutritional perspective.

From the results of the laboratory determinantions it was found that:

- the moisture content was similar for the two types of cans;
- the total acidity expressed as citric acid on tomato dried weight basis, presents elevated values for the tomato paste compared with canned peas;
- peas in a jar has a lower content of sodium chloride, as compared to the tomato paste (0.6% versus 1.3%);
- a high total dry matter was recorded for the tomato paste, respectively, 27% versus 19% for peas sterilized by jar.

Table 1

Organoleptic properties of canned peas sterilized in a jar

| *Sensorial elements* | | *Canned peas* |
|---|---|---|
| | | Fine peas |
| Aspect | Peas jar | Whole, almost uniform size |
| | Liquid jar | Clear, without sediment |
| Color | Peas jar | Greenish |
| | Liquid jar | Yellowish green |
| Broken peas (relative to peas mass % max) | | 7 |
| Dry peas and husks (relative to peas mass % max) | | 1,5 |
| Yellow peas (based on the weight of the jar Pieces max) | | 5 |
| Brown peas (based on the weight of the jar Pieces max) | | 3 |
| Smell and taste | Jar peas | Pleasant, intense, specific to sterilized peas, without strange smell or taste, not sour, not fermented, no mold |
| | Jar liquid | Pleasant, medium intensity, specific to sterilized peas, without strange smell or taste, not sour, not fermented, no mold |
| Foreign particles | Jar peas | - |

Table 2

Organoleptic examination of the studied tomato paste

| Sensory elements | Characteristics |
|---|---|
| General appearance | Uniform mass, deep red, dense, without foreign material |
| Color | Intense red with crimson tint, uniform in all product mass. |
| Taste and smell | Specifically, sweet without foreign smell and taste, not: smoky, burnt, fermented, mold, no bitter or sour taste |

Table 3

The physicochemical characteristics of the studied cans

| Can type | Physicochemical characteristics | | | |
|---|---|---|---|---|
| | Dry substance (%) | Total acidity (%) | Water (%) | Sodium chloride (%) |
| Peas | 19 | 5,8 | 84,2 | 0,6 |
| Tomato paste | 27 | 8,3 | 83,9 | 1,3 |

## CONCLUSIONS

As a result of sterilized conservation there is a decrease of the organoleptic properties compared with peas as such (especially regarding the color).

Peas conserved in a jar showed good properties of taste, smell, the liquid appearance was clear, uniform, without sediment on the bottom of the jar.

The general appearance, color, taste and smell of the studied tomato paste were within the parameters required by the existing standard.

After analyzing the organoleptic properties it was found that both the studied raw materials and cans obtained by jar sterilization comply to the current standards, in terms of raw material, the method of packing making the difference from a qualitative point of view.

In terms of physical-chemical analyzes there were no quality deviations, which means that the steps of obtaining cans by sterilization were in accordance with the standards, starting from the reception of the raw materials to packaging and storage of the final products.

## REFERENCES

Chira A., Nicolae d., 2004, Calitatea produselor agricole și alimentare. Editura Ceres, București,

Barber N.J., Barber J., 2002, Lycopene and prostate cancer. Prostate Cancer and Prostatic Diseases, 5: 6–12.

Dinu M., Dumitru M.G., Soare R., 2015, The effect of some biofertilizers on the biochemical components of the tomato plants and fruits. Bulgarian Journal of Agricultural Science, 21(5): 998-1004.

Dinu M., Soare R., Gruia M., 2016, Bioactive compounds and antioxidant activity in commercial cultivars of tomato (*Lycopersicum esculentum* L.).16th International Multidisciplinary Scientific GeoConference SGEM Conference Proceedings, ISBN 978-619-7105-66-7 / ISSN 1314-2704, Book 5 Vol. 2, pp.149-156.

Ionică M.E., 2014, Metode de analiză şi control al calităţii fructelor şi legumelor proaspete şi divers prelucrate. Editura Universitaria Craiova.

Perkins-Veazie P., Roberts W., Colins J.K., 2007, Lycopene content among organically produced tomatoes. Journal of vegetable science, 12(4), 93-106.

Sargeant L.A., Khaw K.T., Bingham S., Day N.E., Luben R.N., Oakes S., Welch A., Wareham N.J., 2001, Fruit and vegetable intake and population glycosylated haemoglobin levels: the EPIC-Norfolk Study. Eur. J. Clin. Nutr. 55(5): 342-8.

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# THE ISOLATION OF CERTAIN STRAINS OF YEAST WITH OENOLOGICAL QUALITIES IN WINE PRODUCTION FROM THE "DEALU BUJORULUI" VINEYARD

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***Keywords***: *yeasts isolated, metabolic issues, wines*

## ABSTRACT

*At the vineyard "Dealu Bujorului" there were isolated 18 strains of yeast as follows: Saccharomyces genus belonging the sporogenous species, 10 strains belong to the species Saccharomyces cerevisiae var. ellipsoideus; 6 strains belonging Saccharomycea oviformis species and two species of stems Saccharimyces Rose. These strains were tested in terms of hydrogen sulphide production in normal conditions and induced. This is important in terms of achieving a correct fermentation, odorless sulfide. The production of acetaldehyde as the main component of the penultimate stages of alcoholic fermentation and cardioxide coupled, contribute to the increase of the administered dose of sulfur . After analysing all these strains we noted two strains of the first and second species and one species of Saccharomyces hydrogen sulfide redness that does not generate any terms or conditions naturally induced. These five strains we mentioned produce an acceptable amount of acetaldehyde without increasing the risk of overdose of sulfur dioxide.*

## INTRODUCTION

A lot of Romanian researchers wrote about the isolation, purification and clasification of yeasts from different vineyards. However, their work remained at research stage and with very few possibilities to be put into practice with local straines of yeasts. Further research regarding the use of yeasts in winemaking has been done by researchers like Danoaie (1989), Kontec (1978) and Beleniuc (1996) who studied extensively Târnave, Dealul Mare and Murfatlar vineyards.

In order to use yeasts in winemaking they have to fulfill certain biochemical qualities like: not to produce hydrogen sulfide naturally during the fermentation, not to produce large quantities of acetaldehyde which can mix with the sulf dioxide and create a stable and inactive compound which will increase the sulfiting dose of the wine and produce organic acids that have a low contribution to the total acidity of the wine (Ciubucă and Ailiesei 1998; Ciubucă 2011).

## MATERIAL AND METHODS

From the fermented wines with natural yeasts from the main varieties of grapes from the vineyard “Dealu Bujorului’’, we isolated, purified and tested 18 straines of yeasts with good possibilities in winemaking. We tested the level of

production of hydrogen sulfide, naturally produced and induced and the levels of acetaldehyde and organic acids.

The smell of hydrogen sulfide is more commonly found with new wines and can be explained by the excess sulfur which is used in plant treatment, the smoking of the barrels, the clarification of musts or during the fermentation. The smell can also be explained by a prolonged keeping of the wine on yeasts. In order to detect the hydrogen sulfide we used strips of paper impregnated with lead acetate which turns the paper black and can detect these compounds.

In the presence of yeasts, a considerable amount of acetaldehyde can be formed due to the enzyme predisposition of certain yeasts. During the alteration of the wine (it turns into vinegar), large quantities of acetaldehude are been made because the acetldehyde becomes an intermediar compound between alcohol and acetic acid. The acetaldehyde reacts very quickly with the sulphurous acid and creates aldehidosulphurous acid. The method used to determine the acetic aldehyde from food and other mediums was the UV method. The acetic aldehyde oxidizes quantitatively with the acetic acid in the presence of aldehyde dehydrogenase (Al-DH) and nicotinamide dinucleotide (NAD $^{+}$).

The deceleration of the production of organic acids by the yeasts was done through the impregnation of the culture medium with calcium carbonate and by cultivating the yeast in streaks. The transparent halo forming around the colonies is the proof of that the substance combined with the acids produced by the yeasts.

## RESULTS AND DISCUSSIONS

The morphological and physiological polymorphism of yeasts is highlighted by their difference in size, shape (Fig. 1), by their capability to produce spores (Fig,2), the capability to form a pseudomiceliu which is more or less developed (Fig.3), the capability to ferment and asimilate different sugars (Fig. 4) and the capability to produce other metabolites which are importnt in winemaking (glycerol, volatile acids, aroma compounds etc).

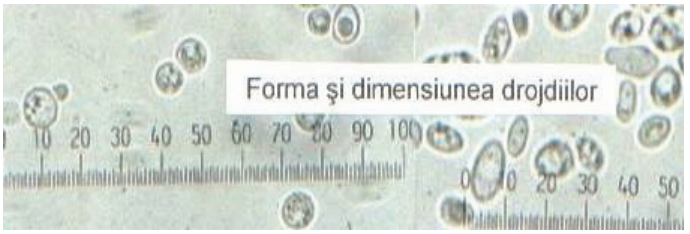

Figure 1. The shape and size of the cells.

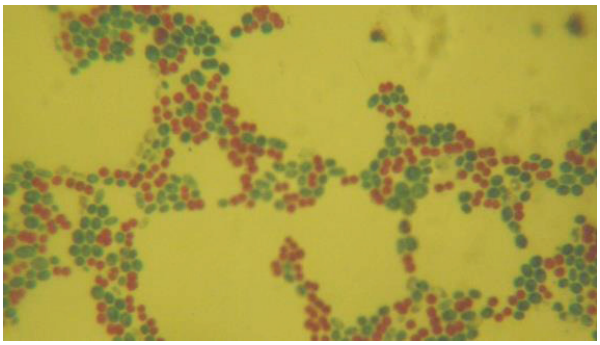

Figure 2. Spores (red).

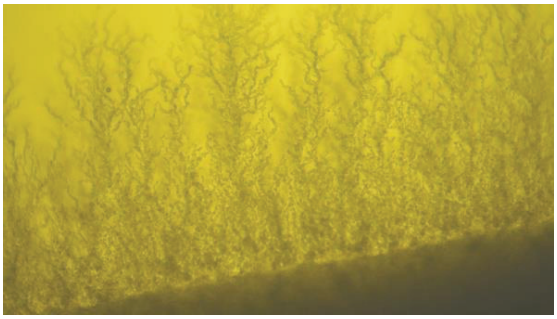

Figure 3. Pseudomycelium.


Figure 4. The fermentation and asimilation of sugars.

Analysing the data from table 1 regarding the production of metabolites by the straines of yeasts isolated from the Dealu Bujorului vineyard, we can observe that all the straines were tested for the naturally and non-induced production of hydrogen sulfide. They do not produce this unwanted compound except for strain 17 Saccharomyces rosa. By using substances which induce additional thiolic compounds (amino acid with the thiol group) we noticed an increase in the production of this compound in some of the strains of yeast with the exception of

*Saccharomyces ellipsoideus* staines 1, 2, 3, 4 and 5, from the *Saccharomyces oviformis* species straines 12 and 13 and from Saccharomyces rosa strain 18.

These strains which do not produce hydrogen sulfide not even by induction can be uded in winemaking because they can garantee wines without the smell of sulphur (Figure 5). As far as the production of acetaldehyde is concerned, we observed tht all the strains produce acetaldehyde during the alcoholic fermentation in acceptable quamtities with the exception of strain 12 Saccharomyces oviformis which produces 126mg/L (a considerable amount).

The testing of these straines of yeast regarding the production of organic compounds during the alcoholic fermentation reveals the fact that they all generate organic compunds in small quantities with the exception of strain 10 *Saccharomyces ellipsoideus* (Figure 6).


Figure 5. The production of $H_2S$.

Figure 6. The production of acids.

Table 1

The characteristics of isolated straines of yeasts from Dealu Bujorului vineyard regarding the production of metabolites

| Nr. crt. | Species/straine | The production of induced hydrogen sulfide | The production of natural hydrogen sulfide | Acetaldehide mg/L | The production of organic compounds |
|---|---|---|---|---|---|
| 1 | Saccharomyces ellipsoideus/1 | - | - | 78 | + |
| 2 | Saccharomyces ellipsoideus/2 | - | - | 65 | + |
| 3 | Saccharomyces ellipsoideus/3 | - | - | 48 | + |
| 4 | Saccharomyces ellipsoideus/4 | - | - | 52 | + |
| 5 | Saccharomyces ellipsoideus/5 | - | - | 42 | + |
| 6 | Saccharomyces, ellipsoideus/6 | +++ | - | 65 | + |
| 7 | Saccharomyces ellipsoideus/7 | +++ | - | 57 | + |
| 8 | Saccharomyces ellipsoideus/8 | ++ | - | 60 | + |
| 9 | Saccharomyces ellipsoideus/9 | +++ | - | 47 | + |
| 10 | Saccharomyces, ellipsoideus/10 | +++ | - | 52 | ++ |
| 11 | Saccharomyces oviformis/11 | +++ | - | 46 | + |
| 12 | Saccharomyces oviformis/12 | - | - | 126 | + |
| 13 | Saccharomyces oviformis/13 | - | - | 60 | + |
| 14 | Saccharomyces oviformis/14 | ++ | - | 57 | + |
| 15 | Saccharomyces oviformis/15 | +++ | - | 43 | + |
| 16 | Saccharomyces oviformis/16 | +++ | - | 23 | + |
| 17 | Saccharomyces rosei/17 | +++ | ++ | 94 | + |
| 18 | Saccharomyces rosei/18 | - | - | 45 | + |

* +++strongly positive test
\+ positive test;
\- negative test

## CONCLUSIONS

The straines of the Saccharomyces ellipsoideus species1,2,3,4 and 5, the Saccharomyces ovifromis, straine 13 and Saccharomyces rosa straine 18 do not produce suphide substance neither in induced conditions nor in natural conditions which indicates their potencial use for winemaking.

The considerable amount of acetaldehyde is produced by staine 12 Saccharomyces oviformis which indicates that cannot be used in winemaking although it does not produce sulphyde substance.

Most straines of yeasts produce organic compounds during alcoholic fermentation in moderation which is a good thing for the local musts which suffer from total lack of acidity.

## REFERENCES

Beleniuc G., 1996, Contribuţii la studiul drojdiilor izolate din podgoria Murfatlar, Teză de doctorat, Univ.” Al. I. Cuza” Iasi.

Ciubucă A., Ailiesei O., 1998, La production d'hydrogé sulfuré dans les vins. Lucrări ştiinţifice vol. 41, sr. Horticultură, Iaşi, Univ. Agronomică Ion Ionescu de la Brad.

Ciubucă A., 2011, Posibilitatea utilizarii germoplasmei levuriene autohtone in industria vinicolă, Oferta Cercetării Ştiinţifice pentru Transfer Tehnologic in Agricultura, Industria Alimentara si Silvicultura, Editura New Agris, Bucureşti.

Dănoaie F.I., 1989, Cercetări priivnd fiziologia drojdiilor izolate din podgoria Târnave. Teza de doctorat, Univ.’’ Babeş-Bolyai’’ Cluj Napoca.

Kontek A., 1978, Studiul factorilor care condiţionează formarea acidităţii volatile a vinurilor în vinificaţia primară. Teza de doctorat, Univ. Craiova.

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# RESEARCHES ON DEVELOPMENT OF PASTRY PRODUCTS WITH LOW CONTENT OF CARBOHYDRATES

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***Keywords***: *insoluble fibers, functional food, bran*

## ABSTRACT

*The purpose of this paper is to present a study on pastry made with added insoluble fibres (bran) and sweeteners (fructose) in different amounts.It was analyzed the rheological behavior of the samples with and without fibers n order to find the optimal amount of fibers to obtain pastry products. It was also analyzed the percent of protein and carbohydrates retrieved in the final product relatively to the initial supplementation. Sensorial tests were performed on the products containing bran and sweetener in and compared to a free control sample. It was calculated the energetically value and it was estimated the intake of the fibers in comparison with the recommended daily dose, Based on results that were obtained, it is recommended the use of 10% bran related to the flour content.*

## INTRODUCTION

Nowadays the food producers meet the demands of the consumers that want food products with an important role in health preservation. Thus, the bread producers focused their attention towards the researches of the functional food.

The scientific and technical development of the last years has significantly influenced and is still influencing the lifestyle of the present generation. These influences are reflected by the modern people's way of eating (Georgescu 2004). Food is being processed, refined, and concentrated to be prepared and consumed as easier as possible, to create gustative sensations and satisfy hungriness, without having in view the body's needs and long-term effects on health condition (Costin and Segal 2001). Higher and higher occurrence of nutritional diseases and costs required by their treatment has alerted nutritionists and authorities. The battle is being done now in reverse direction, towards rational, healthy food, by encouraging the individual to take up sports. The harder and more expensive the battle is, more serious and present the effects of food disequilibria are among children. A balanced diet means a diet consisting of adequate amounts of nutritive elements necessary to health of the human body. The general concern for the development of functional foods has led to the taking into consideration the use of food fibers in food manufacture and studies on their physiological role in the human body have been made (Segal 2003).

In the globalization conditions of the market of food products, consumers' attention is being drawn by new foods, different from those they are accustomed to

and terms insufficiently explained such as "dietetic food"', "light food", "fast food", "food supplements", "functional food". The tendency of the great corporation is to satisfy both, the consumer's demands, and also their desires to maintain health. Thus, the notion of functional foods had appeared, especially created for pretentious consumers, which want to maintain a healthy lifestyle (Georgescu 2001).

The overall concern for the development of functional foods has generated the need for studying and using new food ingredients with role in health and health condition maintaining and improvement. Thus, the use of food fibers in the manufacture of food products has been reconsidered and studies on their physiological role in the human body have been made (Guthrie and Morton 2010). A functional role it is attributed especially to the fibres and sweeteners, which help controlling the level of glucose and lipids in the blood (Giurea 2001). Therefore, the practical and theoretical researches were made in order to obtain pastry product with a low content of carbohydrates, and comply with legal requirements.

## MATERIAL AND METHODS

In order to obtain some available experimental data, pastry wheat flour obtained from DROPIA wheat variety ground in Chopin Laboratory Mill was used as control sample. The analytical flours' quality obtained (table 1) was determined according with the international standard methods (ash content - ICC104/1, wet gluten - ICC105/2, protein content - ICC106/2, hydration capacity with Pharinograph - ICC115/1). The moisture content of the wheat flour and bran were determined by oven drying at $130^0$C for 1 hour.

Table 1

Analytical parameters of Control flour

| Moisture, % | Ash, % | Wet gluten, % | Protein, % | Hydration capacity, % |
|---|---|---|---|---|
| 12.96 | 1.2274 | 27.2 | 12,3 | 78.3 |

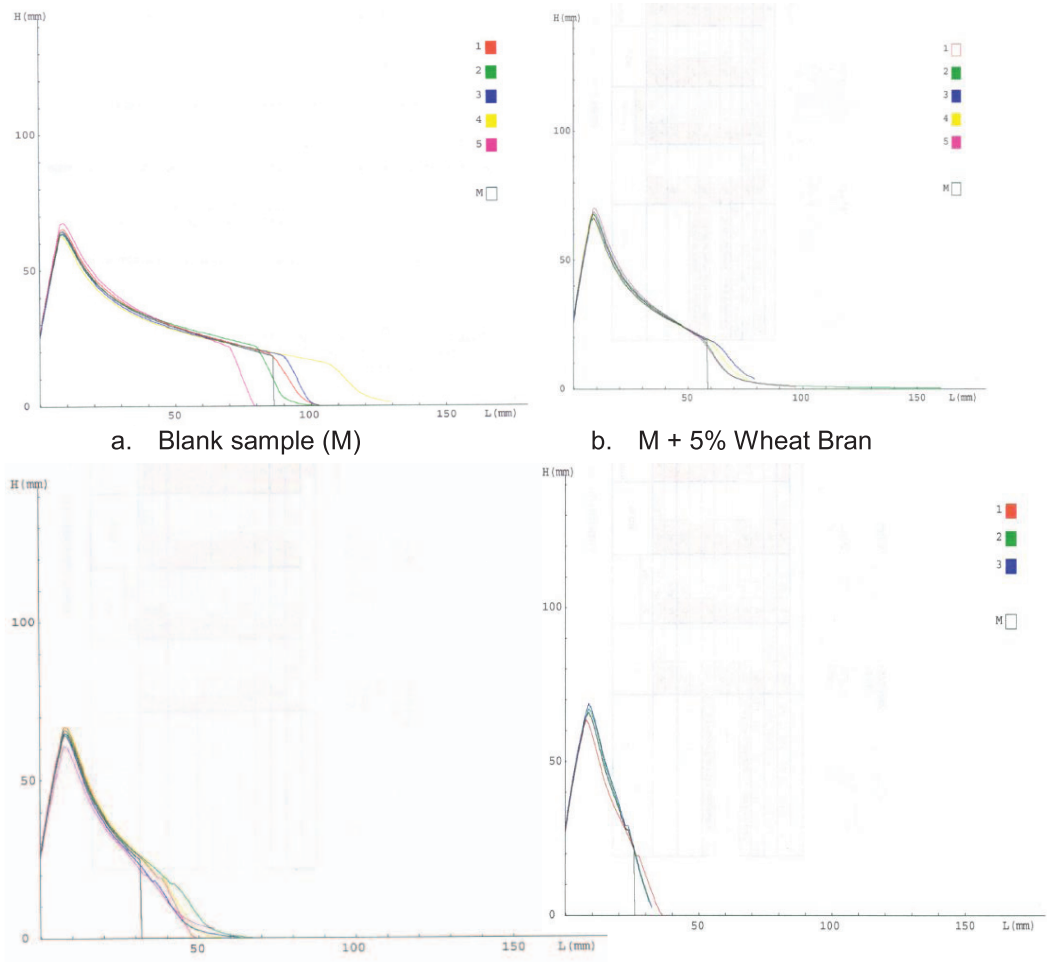
Compacted fresh yeast (*Saccharomyces cerevisiae)* provided by S.C. ROMPAK, Pascani, with 32.5% dry matter and 46.54% protein content (N x 6.25), was used as raw material.

A Chopin Alveoconsistograph was used to determine the deformation resistance (tenacity) P, dough extensibility, L, the value of P/L, and the mixing energy W according with the international standard SR ISO 5530 – 4. The bran samples were added to the baking formula during the mixing stage. The microbial charge was determinate after 24, 48 and 72 hours after cooling. After homogenizing lightly these ingredients, the composition was put in special silicone forms, halfway filled (about 45g of composition). They were baked at 180 Celsius degrees for about 30 minutes After baking, the samples were cooled for 6-8 hours in controlled atmosphere (UV lamps). The experiments were made in the research laboratory of the Faculty of Food Engineering, "Ştefan cel Mare" University of Suceava.

## RESULTS AND DISCUSSIONS

### Bran influence on rheological properties of dough

Alveograph curves for the flour with and without *insoluble fibers* addition were analyzed in order to establish the optimum percentage of *bran* both from the point of view of the protein intake and the rheological one.




a. Blank sample (M)

b. M + 5% Wheat Bran

c. M + 10% Wheat Bran

d. M + 15% Wheat Bran

Figure 1. Influence of *wheat bran* added on rheology of dough by Alveograph curves.

The analysis of the alveograms shows that the exogenous intake of wheat bran does influence significantly the rheological behavior of samples. As compared with the flour blank sample M, the 10% Wheat Bran addition decreases W energy by 21,5%, that of 2.5% addition by 22,86% and by 3,14% for 15% addition respectively. The ratio P/L increases by 29,4% in 15% Wheat Bran addition sample, *by* 40,4% in 10% addition sample and by 43,3% in 5% addition sample.

Research showed that a number of significant correlations, between physico-chemical parameters of flour with 10% wheat bran addition and dough alveographic parameters were established, suggesting that it is possible to achieve a predictive model of alveographic characteristics based on the physico-chemical properties of flour.

In order to define the profile for mixture obtaining by adding 10 % bran to flour it was make a mixograf analyze (figure 2).

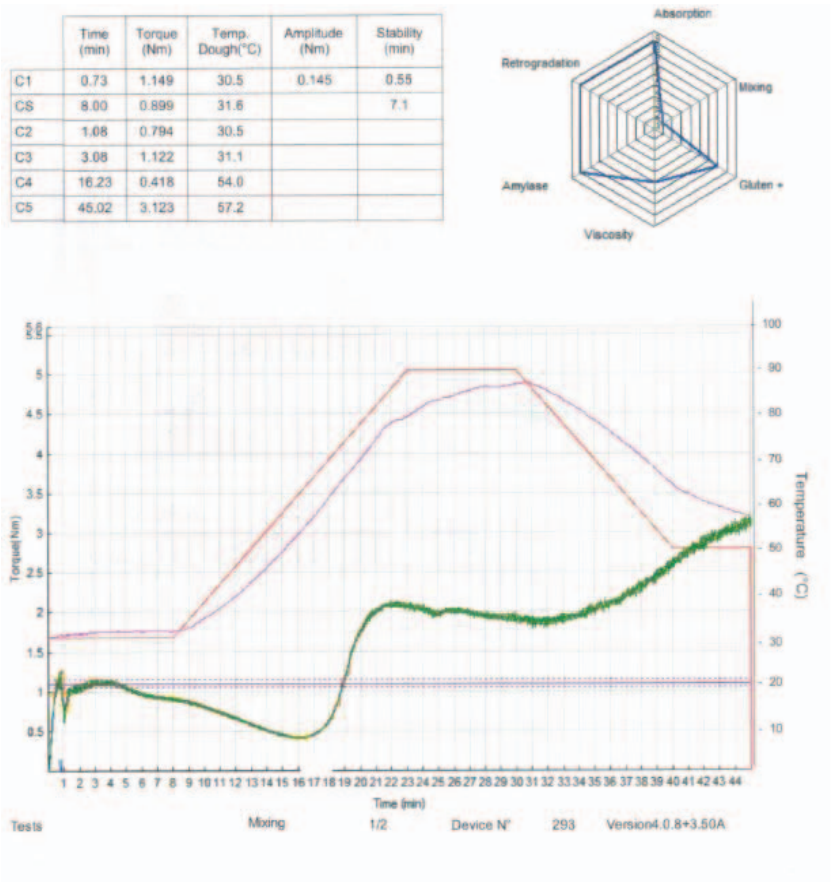



Figure 2. Technological profile of dough obtain with 10% wheat bran addition.

As a consequence of the results registered by Chopin Alveoconsistograph and Chopin Mixolab, in order to establish the optimum percentage of wheat bran, baking samples were also made.

**Check and validation of wheat bran percentage by sensorial analysis of baking samples**

Three baking samples for each variant were made in order to validate sensorial the optimum percentage of wheat bran. The products were organoleptically tested and their volumes were determined. The sensorial profile of muffins was established in order to choose the best wheat bran addition variant.

In the case of pastry products and not only, sensorial analysis plays a decisive role in carrying on experiments to establish the optimum percentage of wheat bran exogenous intake. Scores were taken into consideration and given for the attributes: color, core, smell, softness, uniformity, size, pores, basic taste (sweet), residual (small bran particles between teeth).

The sensorial attributes of the 3 samples with exogenous *wheat bran* intakes as against the blank sample were graphically represented in order to make a complex evaluation. We applied the scoring method, with a 5 points scale, 24 hour after baking. The selected peoples fills the analyze sheets, we pick out five fundamentally characteristics, which are graphic represented in figure 3.

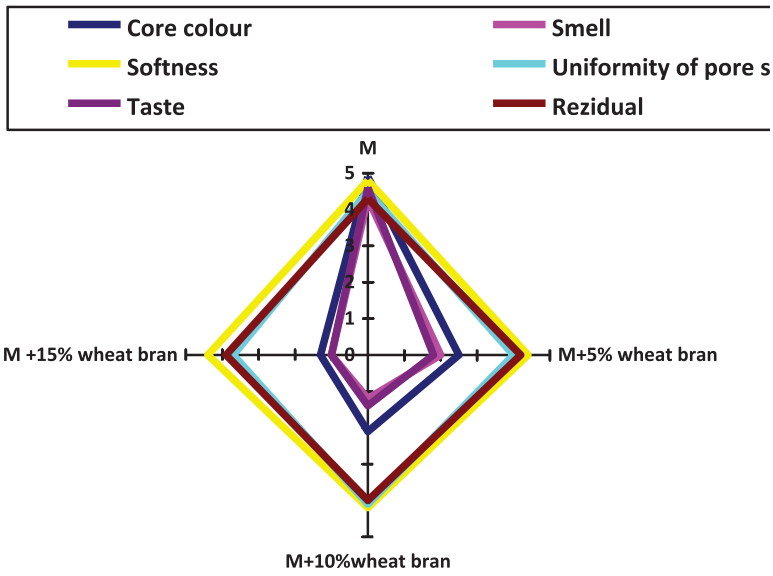



Figure 3. Graphic evaluation of samples' sensorial attributes.

As can be seen from the sensorial analysis made above, from the sensorial point of view, the attributes are relatively close in the samples with exogenous wheat bran intakes and generally in the inferior limit of acceptability.

## CONCLUSIONS

The aim of this research was to evaluate the possibilities to healthy pastry made with added insoluble fibres (bran) and sweeteners (fructose) in different amounts.It was analyzed the rheological behavior of the samples with and without fibers n order to find the optimal amount of fibers to obtain pastry products. It was

also analyzed the percent of protein and carbohydrates retrieved in the final product relatively to the initial supplementation.

Research showed that a number of significant correlations, between physical-chemical parameters of flour with 10% wheat bran addition and dough alveographic parameters were established, suggesting that it is possible to achieve a predictive model of alveographic characteristics based on the physical-chemical properties of flour.

Sensorial tests were performed on the products containing bran and sweetener in and compared to a free control sample. It was calculated the energetically value and it was estimated the intake of the fibers in comparison with the recommended daily dose, Based on results that were obtained, it is recommended the use of 10% bran related to the flour content.

## REFERENCES

Costin C.M, Segal R., 2001, Alimente pentru nutriţie specială. Editura Academica, Galaţi.

Georgescu A., 2004, Noi ingrediente functionale utilizabile in panificatie. Buletinul informativ pentru industria de morărit şi panificaţie – BIMP, 15(4): 24-30

Georgescu A., 2001, Fibrele alimentare. Studii biochimice si tehnologice. Buletinul informativ pentru industria de morărit şi panificaţie – BIMP, 12(1): 66-112.

Giurea A.M., 2001, Studiu privind fibrele alimentare: definitie, constituienti, implicatii nutritive si fiziologice. Buletinul informativ pentru industria de morărit şi panificaţie – BIMP, 12(3-4): 60-73.

Guthrie J., Morton J., 2010, Food Sources of Added Sweeteners in the Diets of Americans, Journal of the American Dietetic Association, Volume 100, Issue 1,

Segal R., 2003, Prebioticele – Ingrediente functionale in dezvoltarea produselor cerealiere. Buletinul informativ pentru industria de morărit şi panificaţie – BIMP, 14(2): 6-9.

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# VARIATION IN CHEMICAL COMPOSITION OF WHITE WINES CONTAMINATED WITH MOLDS

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***Keywords****: wine, contamination, molds, fermentation, mycotoxin, compounds*

## ABSTRACT

*Wine contamination start from the raw material, grapes, because it is possible to be contaminated with molds, and can continue throughout the technological process. If the wine is not contaminated from the beginning can be contaminated for various reasons during storage. The wine characteristics change from color, odor, bright, clarity and after all of these the wine it is proper for consumer. This study presents the variation in chemical composition from different samples of white wine infected with different type of molds.*

## INTRODUCTION

Grapes and grape-derived products have a significant worldwide importance. Most grapes are used for wine-making (71%), about 27% are consumed fresh, and only a minor portion (2%) are consumed as dried fruits. Grapes can be eaten fresh, pressed for making wine, squeezed to make grape juice or dried by sunlight for raisin or sweet wine production.

During maturation, the spoilage agents, *Aspergillus, Botrytis, Penicillium* and *Rhizopus,* increase their incidence. When the temperature is higher than 37°C, species in Aspergillus section Nigri, usually called “black Aspergilli”, predominate. At harvest time the conditions are optimal for fungal invasion, especially if physical damage has occurred on berries. After harvest, grapes are subjected to different processes, depending on the intended use.(Oliveri et al. 2016)

Grape berries, both for table consumption or wine making, are mainly contaminated in the field by *Aspergillus*, *Botrytis*, and *Penicillium* species, which often can be isolated from symptomless berries and successively by black *Aspergilli* and *Botrytis cinerea* in post-harvest cold storage. On dried fruits as well, *Aspergillus* and *Penicillium* species are often present; in particular the predominance of Aspergillus species on dried fruits is reported worldwide.

Determination of the mycoflora occurring on grapes at the different stages of growing and processing is important to establish an adequate program of treatments for the prevention of fungal contamination in the vineyard and in storage. Some of the fungal species occurring on grapes and grape products can produce mycotoxins, so species identification is critical to predict the potential mycotoxin contamination of grapes and wine. Certainly the *Aspergillus species* are present worldwide, in all the grape products and under all environmental conditions. (Tournas and Katsoudas 2005, Oliveri et al. 2016)

The initial environment that affects the microbial makeup of a wine fermentation is that of the vineyard. Although a drastically different environment than juice or wine, the types of microbes present on grapes will have an impact on the ensuing ecology in the wine fermentation, particularly in the early stages. Microorganisms appear to colonize around the grape stomata where small amounts of exudate are secreted. The apiculate yeasts, Hanseniaspora and Kloeckera, its asexual anamorph, are the most prevalent vineyard yeasts and typically represent over half the yeast flora on grape. Other yeast on berries include: Metschnikowia, Candida, Cryptococcus, Rhodotorula, Pichia, Zygosaccharomyces and Torulopsis. Also present in the vineyard are numerous other yeasts, some of which have an impact on wine: Sporobolomyces, Kluyveromyces, and Hansenula. Saccharomyces species are relatively scarce among healthy berries. On damaged berries, Saccharomyces is present at significant but low levels, compared to total microbial population levels of 107 to 108 CFU per berry. Mortimer suggested honey bees, wasps, and fruit flies as likely vectors for carrying and spreading Saccharomyces and other yeasts among damaged grapes. (Soma et al. 2012, Cocolin and Ercolini 2008). This study presents the influence of *Penicillium chrysogenum* and *Penicillium expansum* over the chemical composition of white wines origin from Romania and Greece.

## MATERIAL AND METHODS

6 samples of white wine from Romania and Greece was contaminated with *Penicillium chrysogenum* and *Penicillium expansum.* Fungi were isolated from samples obtained from contaminated cork (corkwood) stoppers. These fungi were isolated from damaged cork samples. After 4 days of incubation at 25 0C individual fungal colonies were subcultured. Further, pure fungi cultures were obtained from single spore's isolates using standard microbiological techniques. Each variant was contaminated with both of microorganisms.

Variants:

$V_1$ - Afuz Ali Sâmburești – 2015

$V_2$ – Sauvignon Sâmburești – 2015

$V_3$ – Sauvignon + Crâmpoșie + Tămâioasă românească Drăgășani – 2015

$V_4$ – Tămâioasă românească Cotnari – 2015

$V_5$ – Fetească regală Recaș – 2015

$V_6$ – Sabatavo Grecia – 2015

At the end of fungal incubation period wine samples were analyzed using the following methods of OIV

(i) Total acidity measured before and after contamination by titrimetric with NaOH 0,1N with bromothymol blue as indicator. Carbon dioxide is not included in the total acidity.

(ii) Volatile Acidity. Carbon dioxide was firstly removed from the wine and further volatile acids were separated from the wine by steam distillation and titrated using NaOH 0,1N and two drops of phenolphthalein solution.

(iii) Residual sugar was determined with portable refractometer

## RESULTS AND DISCUSSIONS

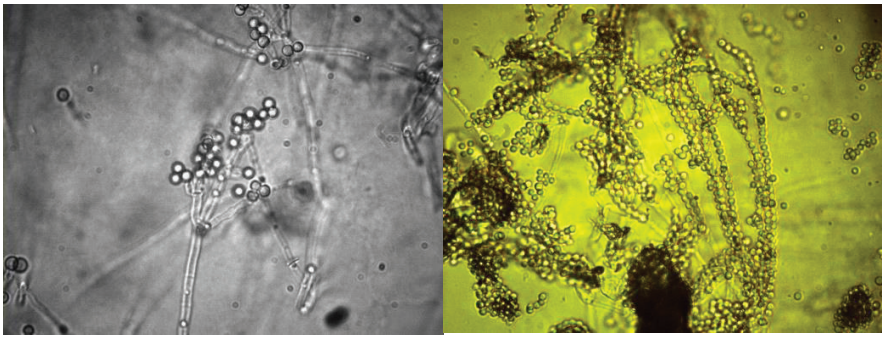

Figure 1. a) Micelia of *Penicillium chrysogenum* Ax145 b) Micelia of *Penicillium expansum* Ax122.

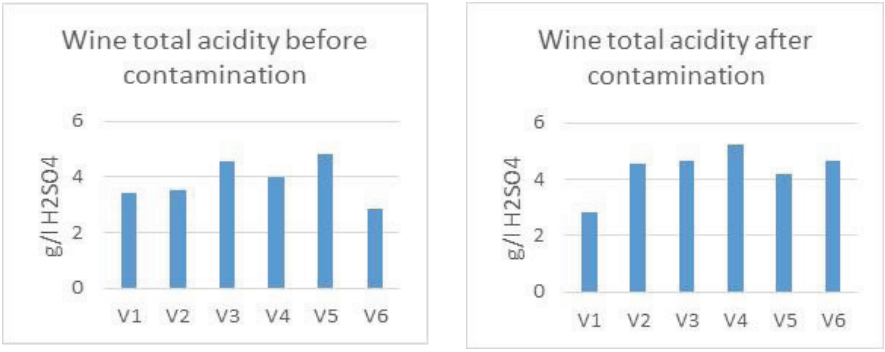


Figure 2. Total acidity of samples before and after mold contamination.

Contamination with Penicillium sp. determine a different variation of total acidity depend by each variant. Only variant $V_1$ record a decrease of total acidity with 16 %. All of the variants from $V_2$ to $V_6$ record an increase of total acidity after contamination with *Penicillium expansum* and *Penicillium chrysogenum.* The increase of total acidity was between 29% at variant $V_2$ and 63% at variant $V_6$. During the second fermentation process, molds produce in wine acids with short chain, acetic acid, propionic acid, butyric acid, isopropyl acid, etc. All of these substances increase the level of total acidity in contaminated wines.

In some cases, high levels of volatile acidity may result from growth of molds during storage period. Several intrinsic and extrinsic factors may affect formation of acetic acid by yeast, including the following: pH, sugar, available nitrogen, fermentation temperatures, interactive effects of other microorganisms

*Penicillium*, *Botrytis* and other fungi, pH impacts acetic acid production, with more acetic acid produced at low (<3.2) pH (Batt and Tortorello 2014).

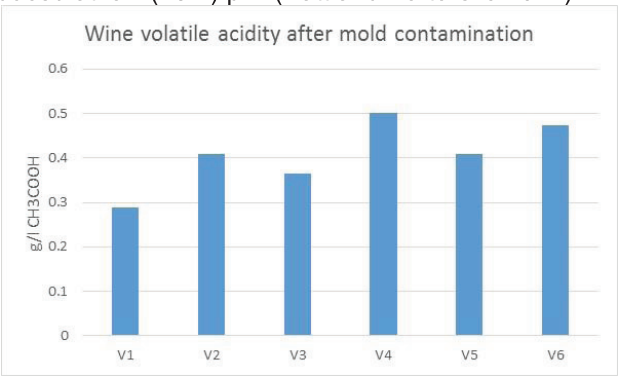


Figure 3. Wine volatile acidity after mold contamination.

All the samples record an increase of volatile acidity. The high level of volatile acidity obtains at variant $V_4$ wine with residual sugar. The low volatile acidity record at variant $V_1$ dry wine, low content in residual sugar. The variant $V_6$ Greek sweet wine presents the second level of volatile acidity. In general, molds produce in white wine volatile acidity if the wines contain in chemical composition residual sugars.

## CONCLUSIONS

Contamination with molds in white wines determine in most of cases an increase of total acidity. In one case obtain a decrease of total acidity, molds consume free acidity in metabolic processes. The level of total acidity is determinate by white wine content in residual sugars. The volatile acidity evolution is correlate with total acidity. All the wines with residual sugars content record an increase of volatile acidity. A special remark can make on sample $V_1$ where the total acidity decrease after molds contamination and volatile acidity increase. Molds transform free acidity by metabolic processes in volatile acids.

## REFERENCES

Batt A.C., Tortorello M.L. (2014), *Encyclopedia of food microbiology,* Second edition, Elsevier, pp. 805-811.

Cocolin L., Ercolini D., 2008, Molecular Techniques in the Microbial Ecology of Fermented Foods, Springer, 162-183.

Oliveri C., Bella P., Tessitori M., Catara V., Rosa R., 2016, Grape and environmental mycoflora monitoring in old, traditionally-cultivated vineyards on Mount Etna, southern Italy, J Sci Food Agric, 96(6): 1934-1944.

Somma S., Perrone G., Logrieco F.A., 2012, Diversity of black Aspergilli and mycotoxin risks in grape, wine and dried vine fruits, Phytopathologia Mediterranea, 51,1: 131-147.

Tournas V.H., Katspudas Eugenia, 2005, Mould and yeast flora in fresh berries, grapes and citrus fruits. International Journal of Food Microbiology, 105: 11-17.

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# NUTRITIONAL VALUE OF EUROPEAN BIRD CHERRY (*Prunus padus* L.) AND BLACKTHORN (*Prunus spinosa* L.) FRUITS

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***Keywords***: *blackthorn, European bird cherry, ascorbic acid, anthocyanins, phenolics, antioxidant activity*

## ABSTRACT

*Physical (diameter and weight) and chemical characteristics (moisture, soluble solids, total phenolic, total anthocyanins content and titratable acidity) as well as antioxidant activity were determined in blackthorn (Prunus spinosa L.) and european bird cherry (Prunus padus L.) fruits in order to assess their nutritional value and processing potential. The ascorbic acid content was found quite low (11.74 mg/100 g in blackthorn fruits and only 5.22 mg/100 g in European bird cherry fruits).The total anthocyanins content ranged between 65.54 mg CG/100 g in blackthorne fruits and 74.35 mg CG/100 g in European bird cherry fruits while the total phenolic content was higher in European bird cherries (640.16 mg GAE/100 g) than in blackthorns (189.83 mg GAE/100 g). Therefore, these fruits show high potential for use in the development of nutraceuticals or functional food ingredients.*

## INTRODUCTION

Blackthorn (*Prunus spinosa L.*) is a perennial shrub that grows in spontaneous flora on the slope-areas, creating a dense spiny mass. It can also be found near the roads, along the channels and in forest areas. Blackthorn is used in phytotherapy for the treatment of many diseases related to various forms of coughing. It is a mild laxative, diuretic, antispasmodic and anti-inflammatory agent. The fruits are used in the development of healthy juices, syrups, jellies, jams. Since the fruit contain various volatile compounds, blackthorn is suitable for flavoring of well-known alcoholic beverages such as liqueurs, brandy, blackthorn gin and wine.

Having beneficial effects in various diseases, including stroke, neuralgia and hepatitis, *Prunus padus* fruits (European bird cherry) have been widely used in the traditional medicine. Fruit mesocarp is juicy, fleshy and contains various biologically active compounds. Fruit taste is sweet-bitter and astringent due to tannins content. Usually people in northern Russia dry, crush and granulate the fruits then mix them with flour in order to bake them or use them as fillings for pies. The fruit added to alcoholic beverages and juices color the liquid in dark red. In the past, sauces, jams and juices were made from European bird cherry fruits in Europe. Fresh fruit extracts are an excellent source of phenolic compounds, free radical scavengers, which can reduce the negative effects of free radicals in the human body. Therefore, they play an important role in the prevention of neuro – degenerative and cardiovascular diseases, cancer as well (Velikovic et al. 2014).

Due to higher actual interest for unconventional food consumption, these fruits become even more important on an industrial scale.

The goal of this work was to determine the main physical (diameter and weight) and chemical characteristics (moisture, soluble solids, total phenolic, total anthocyanins content and titratable acidity) as well as antioxidant activity in blackthorn (*Prunus spinosa* L.) and European bird cherry (*Prunus padus* L.) fruits in order to determine the nutritional value and possibilities to exploit their potential for processing.

## MATERIAL AND METHODS

### *Plant material*

The plant material consisted in blackthorn (*Prunus spinosa* L.) and European bird cherry (*Prunus padus* L.) fruits randomly picked from wild shrubs growing in Oltenia region, Romania. Fruits (about 2.5 kg of fruit from each species) at full maturity were harvested in October and sorted to remove the damaged, shriveled and immature fruits. Within three hours following harvesting, the samples (whole fruit) were stored at -20 $^{o}$C for one month. For quality parameters analyses the fruits were stored at 4 $^{o}$C and used the day after.

### *Quality parameters*

Fresh fruits were ground to a homogeneous puree in a Waring blender for about 2 min. Moisture content (%) was determined gravimetrically by drying 5 g homogenate in a laboratory oven (Memmert, Germany) set at 100°C until constant weight was reached. Soluble solids content (%) was measured with a digital refractometer (Euromex, Arnhem, The Netherlands) after homogenate clarification by centrifugation (4000 rpm, 10 min). Titratable acidity (% as citric acid) was measured by titrating the water extract of fruit homogenate to pH=8.2 with 0.1 N NaOH.

### *Ascorbic acid content*

5 g of whole fruits were homogenized to puree in a porcelain mortar and diluted to 100 ml with 0.1 N HCl. After 30 minutes the extraction solution was centrifuged at 4200 rpm for 10 minutes. The supernatant was filtered through 0.45 $\mu$m pore size filter and injected on a Surveyor Thermo Electron system equipped with a reverse-phase C18 column (Hypersil Gold aQ) maintained at 10$^{o}$C. The mobile phase consisted of a phosphate buffer solution (50 mM $KH_2PO_4$ in water) adjusted to a pH value of 2.8. The absorbance was measured at 254 nm and the ascorbic acid peak area was quantified with the Chrom Quest 4.2 software on the basis of an external standard calibration curve (0–50 mg/L).

### *Total anthocyanins content*

Anthocyanins were extracted from fruit homogenate (2 g) with 20 mL of methanol containing 0.1% HCl (v/v) for 20 h at room temperature, in darkness. The quantification of total anthocyanins was performed using the pH differential spectrophotometric method (Giusti and Wrolstad 2001). Briefly, the extracts were separately diluted in potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). After incubation for 15 min at 23 $^{o}$C the absorbance was read against a blank both at 510 and 700 nm in a Varian Cary 50 UV spectrophotometer (Varian Co., USA). Total anthocyanins content was calculated using equation below and expressed in mg cyanidin 3-O-glucoside equivalents (CG) per 100 g of fresh weight.

Total anthocyanins (mg CG/L) = (A × MW × DF × 1,000)/(ε × l)

where $A = (A_{510\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{pH\ 4.5}$; MW = 449.2 g/mol for cyanidin-3-O-glucoside; DF (dilution factor of the samples); ε = 29,600 L/(mol·cm); l = cuvette path length in cm; and 1,000 = factor for conversion from g to mg.

***Extraction of phenolic compounds***

For the analysis of total phenolics and antioxidant activity, fruit samples (1.5 g) were extracted with 10 mL methanol in a Bandelin Sonorex Digital 10P ultrasonic bath (Bandelin Electronic GmbH, Germany) for 60 min at ambient temperature. After extraction, the samples were centrifuged for 5 min at 4200 rpm. Supernatants were collected, filtered through polyamide membranes with pore diameter of 0.45 µm and used for the assays.

***Total phenolic content***

Total phenolic content was assessed colorimetrically by using the Folin-Ciocalteu phenol reagent method according to the method of Singleton and Rossi (1965). Briefly, 1 mL of each methanolic extract (diluted 1:20 with methanol) was mixed with 1 mL distilled water and 500 µL Folin-Ciocalteau reagent and stirred for one minute. After 2 min, 4 mL of 7.5% sodium carbonate aqueous solution were added and the mixture was incubated for 2 hours at 25 °C. The same procedure was also applied to the standard solutions of gallic acid. Finally, the absorbance of the mixture was measured at 765 nm using an Evolution 600 UV/VIS spectrophotometer (Thermo Scientific, USA). Gallic acid was used for preparing the standard curve (0-100 mg/L) and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight.

***DPPH radical-scavenging activity***

The DPPH free radical scavenging capacity of the fruit extracts was measured according to the method described by Hatano et al. (1988). Briefly, each methanol diluted extract (50 µL) was mixed with 3 mL of a 0.004% (v/v) DPPH methanolic solution. The absorption at 517 nm was measured after 30 min against a blank using an Evolution 600 UV/VIS spectrophotometer (Thermo Scientific, USA). The inhibition of the DPPH radical by the samples was calculated according to the following formula:

DPPH scavenging activity (%) = [1 − Abs.sample/Abs.blank] × 100

The DPPH scavenging activity was subsequently calculated with respect to the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), which was used as a standard reference. Results were expressed as milimoles Trolox equivalents per 100 g of fresh weight.

***Statistical analysis***

Data were evaluated by using Statgraphics Centurion XVI Software (StatPoint Technologies, Warrenton, VA, USA). Data were presented as mean ± standard deviation (SD).

## RESULTS AND DISSCUSIONS

An individual fruit weight of 1.86 g was found for blackthorns and 0.5 g for European bird cherries while the diameter of the fruit has been 12.62 mm for blackthorns and 7.48 mm for European bird cherries. Ozcan (2005) reported for blackthorns a fruit weight of 1.28 g and a diameter of 12.59 mm.

The moisture content of blackthorn fresh fruit was 78.64%. Markoglu et al. (2005), in their studies on the blackthorn fruits in Turkey, found a moisture content

of 69.37%. Barros et al. (2010) reported a moisture content of 60.86% in whole blackthorn fruits from North-west of Spain. Also, Sikora et al. (2013) found in blackthorn fresh fruits from Poland a moisture content of 81.89%.

European bird cherries showed a lower moisture content than blackthorns (72.36%). Although this fruit is well known in the traditional medicine, it was not the subject of many studies so far, therefore we found only few data in the literature which can be compared whith our results.

Table 1

Weight, diameter, moisture, soluble solids and titratable acidity in blackthorn and European bird cherry

| Species | Weight (g) | Diameter (mm) | Moisture content (%) | Soluble solids (%) | Titratablle acidity (%, citric acid) |
|---|---|---|---|---|---|
| Blackthorn | 1.86 ± 0.08 | 12.62 ± 0.54 | 72,36 ± 0.80 | 17,50 ± 0.94 | 1.54 ± 0.06 |
| European bird cherry | 0.50 ± 0.02 | 7.48 ± 0.43 | 78,64 ± 1.40 | 11,57 ± 0.49 | 0,83 ± 0.04 |

A high content of soluble solids was found in blackthorn fresh fruits (17.5%) as compared to European bird cherry fruits that contained only 11.57%. By comparison, Erturk et al. (2009) reported in blackthorn fruits of different colors a soluble solids content between 11.98% and 14.78%.

The acidity of the fruit, expressed as citric acid, was significantly higher in blackthorn fruits (1.57%) than in European bird cherries (0.83%). However these values are much lower than those reported by Erturk et al. (2009) who found an acidity of 3.87% in the dark purple fresh blackthorn fruits.

The ascorbic acid content of the fruits (Table 2) was quite low, 11.74 mg/100 g in blachthorn fruits and only 5.22 mg/100 g in Eurpean bird cherry fruits. However, the values are similar to those reported in the literature e.g. Ozcan (2005) found in *Prunus spinosa* var. *Dasyphylla* an ascorbic acid content of 7.70 mg/100 g while Morales et al. (2013) found 7.73 mg/100 g fw. Higher values have been reported by Sikora et al. (2013) (23.84 mg/100 g) while Jabłońska-Ryś et al. (2009) found 21.94 mg/100 g.

Table 2

Ascorbic acid, total anthocyanins, total phenolics content and antioxidant activity in blackthorn and European bird cherry

| Species | Ascorbic acid (mg/100 g) | Total anthocyanins (mg EGC /100 g) | Total phenolics (mg GAE/100 g) | Antioxidant activity (mmol Trolox/100 g) |
|---|---|---|---|---|
| Blackthorn | 11.74 ± 0.42 | 65.54 ± 2.45 | 189.83 ± 9.37 | 2.95 ± 0.02 |
| European bird cherry | 5.22 ± 0.25 | 74.35 ± 2.88 | 640.16 ± 31.27 | 9.48 ± 0.15 |

Total anthocyanins content was 65.54 mg CG/100 g in blackthorns and 74.35 mg CG/100 g in European bird cherries, values that are in line with those obtained in previous studies. Thereby, Velickovic et al. (2014) found in dark purple colored fruits of *Prunus spinosa* a total anthocyanins content of 41.3 mg cyanidin-3-*O*-glucoside/100 g fw. Sikora et al. (2013) reported 71.75 mg/100 g in fresh fruits and 66.78 mg /100 in frozen ones. Similar values of total anthocyanins content in blackthorn fruits (66.9 mg/100 g) were found by Leja et al. (2007). Dragovic-Uzelac et al. (2007) reported slightly lower results, ie 30.5 mg/100 g in the fruits harvested in October and 49.74 mg/100 g in those harvested in November.

According to Ștefanut et al. (2011) anthocyanins represents a tenth of total phenolics present in the blackthorn fruits. Blackthorns had a total phenolics content of 189.83 mg GAE/100 g, values which are in good agreement with those presented by Velickovic et al. (2013) who reported total phenolics content ranging from 153.3 mg GAE/100 g to 209.4 mg GAE/100 g. Uzelac et al. (2007) reported far lower values of total phenolics content in blackthorn fruits that varied from 54.6 mg GAE/100 g to 86 mg GAE/100 g. Fraternale et al. (2009) found in their study a total phenolic content of 8350 mg/100 g dry matter that is significantly higher than our values due to the different way of expressing the results. Sikora et al. (2013) found a total phenolic content of 599.2 mg GAE/100 g fw while a similar content (523.48 mg GAE/100 g) was reported by Leja et al. (2007).

Significantly lower values were obtained by Dragovic - Uzelac et al. (2007). They analyzed blackthorn fruits harvested in October and November finding total phenolic contents of 54.65 mg GAE/100 g (fruits harvested in October) respectively 85.87 mg GAE/100 g (fruits harvested in November). These differences can occur due to the diversity of the biological material and of the analysis methodology.

The antioxidant activity was about 3.3 times higher in European bird cherries (9.48 mmol Trolox/100 g) compared to blackthorns (2.95 mmol Trolox/100 g). The obtained values (Table 2) are consistent with those reported in previous studies. Thereby, Dragović-Uzelac et al. (2007) reported in blackthorn fruits a level of the antioxidant activity (as determined by DPPH method) of 2.8 mmol Trolox/100 g fw while Sikora et al. (2013) found 4.36 mmol Trolox/100 g fw.

## CONCLUSIONS

The results obtained in this study are of particular interest to a better definition of the species and to confirm the importance of the genetic heritage for the availability of some specific compounds. These fruits can be a valuable source of antioxidants and nutrients, therefore they may have great potential to be used in the development of nutraceutical or functional food ingredients. Considering the high anthocyanins content and the intense color of their extracts, blackthorns and European bird cherries can be processed as juices, jams, jellies, liqueurs, or can be used as ingredients for ice cream, candy and pastries.

## REFERENCES

Barros L., Carvalho A.M., Morais J.S., Ferreira I.C.F.R., 2010, Strawberry-tree, blackthorn and rose fruits: detailed characterization in nutrients and phytochemicals with antioxidant properties. Food Chem., 120, 247-254.

Dragović-Uzelac V., Levaj B., Budisić D., Radojćić I., Biśko A., 2007, Total phenolics and antioxidant capacity assays of selected fruits. Agric. Consp. Sci., 72(4), 279-284.

Erturk Y., Ercisli S., Tosun M., 2009, Physico-chemical characteristics of wild plum fruits (*Prunus spinosa* L.). International Journal of Plant Production, 3(3), 89-92.

Fraternale D., Giamperia L., Bucchinia A., Sestili P., Paolillo M., 2009, Antioxidant activity of *Prunus spinosa* L. fruit juice. Ital. J. Food Sci. 12, 1665-1670.

Hatano T., Kagawa H., Yasuhara T., Okuda, T., 1988, Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin, 36, 2090-2097.

Jabłońska-Ryś E., Zalewska-Korona M., Kalbarczyk J., 2009, Antioxidant capacity, ascorbic acid and phenolic content in wild edible fruits. J. Fruit Ornam. Plant Res., 17 (2), 115-120.

Leja M., Mareczek A., Nanaszko B., 2007, Antyoksydacyjne właściwości owoców wybranych gatunków dziko rosnących drzew i krzewów [Antioxidant properties of selected species of wild grown trees and bushes], Rocz. AR Pozn., 383 (41), 327-331 [in Polish].

Marakoglu T., Arslan D., Ozcan M., Haciseferogullar H., 2005, Proximate composition and technological properties of fresh blackthorn (*Prunus spinosa* L. subsp dasyphylla (Schur.)) fruits. Journal of Food Engineering, 68, 137-142.

Morales P., Ferreira I.C.F.R., Carvalho A.M., Fernández-Ruiz V., Sánchez-Mata M.C., Cámara M., Morales R., Tardio J., 2013, Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. Eur. J. Lipid Sci. Technol., 115, 176-185.

Ozcan T., 2008, Some vitamin and organic acid contents in the fruits of *Prunus spinosa* L. subsp. *dasyphylla* (Schur) Domin from Europe-in-Turkey. IUFS J Biol, 67(2), 105–114.

Singleton V.L., Rossi J.A., 1965, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16.3, 144-158.

Sikora E., Bieniek M.I., Borczak B., 2013, Composition and antioxidant properties of fresh and frozen stored blackthorn fruits (*Prunus spinosa* L.). Acta Sci. Pol., Technol. Aliment., 12(4), 365-372.

Ştefănuţ M.N., Căta A., Pop R., Moşoarcă C., Zamfir A.D., 2011, Anthocyanins HPLC-DAD and MS characterization, total phenolic, and antioxidant activity of some berries extracts. Anal. Lett., 44, 2843-2855.

Uzelac V., Levaj B., Bursać D., Pedisić S., Radojčić I., Biško A., 2007, Total phenolics and antioxidant capacity assays of selected fruits. Agriculturae. Conspec. Sci., 72, 279-284.

Veličković J.M., Kostić D.A., Stojanović G.S., Mitić S.S., Mitić M.N., Ranđelović S.S., Đorđević A.S., 2014, Phenolic composition, antioxidant and antimicrobial activity of the extracts from *Prunus spinosa* L. fruit. Hem. Ind., 68 (3), 297-303.

Wrolstad R.E., Giusti M.M., Rodriguez-Saona L.E., Durst R.W., 2001, Anthocyanins from radishes and red-flesh potatoes, In: J.M. Ames and T. Hofinann (eds), Chemistry and Physiology of Selected Food Colorants. Amer. Chem. Soc., Washington, DC., 66-89.

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# THE COMPARATIVE EFFECTS OF FAR-RED LIGHT AND UV-C LIGHT ON SOME PHYSICAL-CHEMICAL-MICROBIOLOGICAL ATTRIBUTES IN THE RED BELL PEPPERS (*Capsicum annuum* L) DURING STORAGE

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***Keywords***: *bell peppers shelf-life, UV-C, Far Red light, phenolic compounds, catalase, chilling injury, yeasts and molds.*

## ABSTRACT

*Red bell pepper fruits were subject to six weeks storage in the darkness, Ultraviolet Light (UV-C) and Far Red light (FRL) at 8°C and 55% relative humidity. Fresh and stored fruits were sampled and analyzed in terms of chilling injury, catalase enzyme level, antioxidant activity, phenolic compounds level, total yeasts and molds charge and appearance. The storage in the UV-C and FRL seems to be more effective in the preservation of pepper fruits attributes as compared to the storage in the darkness. The bell peppers exposed in the FRL displayed lower chilling injuries, higher level of phenolic compounds and antioxidant capacity and improved appearance. In the bell peppers exposed in the UV-C, the catalase enzyme was accumulated to the largest extent while the proliferation of yeasts and molds occurred to the lowest rate.*

## INTRODUCTION

Postharvest quality of bell peppers decreased rapidly due to nutritional loss and bacterial spoilage. Their storage in the cold below 7°C induces chilling injuries while when they are stored above 13°C the ripening and bacterial soft rot are accelerated (Singh et al. 2014). Different treatments were proposed in the aim to prolong the shelf life of bell pepper fruits. Xing et al. (2011) suggests that chitosan–oil coating is a promising candidate for enhancing the keeping quality of sweet peppers due to the enzyme accumulation in the fruits and electrolyte leakage reducing. By immersion in water at 45°C for 3 minutes, the green and ripe peppers showed lower spoilage than the control, the soft rots shriveling, weight loss, color changes and fruits respiration were markedly reduced during storage (Rodoni et al. 2016). The shelf life of bell peppers was extended to 49 days in active packages (permeable polymeric films and sachets of silica gel crystals as moisture absorbents), 42 days in modified atmosphere packages (4.5% $O_2$, 7.8% $CO_2$ and 4.7% $O_2$, 7.5% $CO_2$) as compared to 21 days in the refrigeration temperature and 7 days in control sample (Singh et al. 2014). Pressurized argon (2-6 MPa) treatment for 1h prolongs the shelf life of fresh-cut green peppers by reducing the

proliferation of coliforms, yeasts and molds (Meng et al. 2012). The UV-C exposure prevents the water loss, chilling injury, microbial spoilage and stimulates the response mechanisms to stress by increasing the enzymes and phenolic compounds levels in fruits (Charles et al. 2008; Cuvi et al. 2011; Urban et al. 2016). The literature in the field of FRL application in the prolongation of postharvest shelf life of fruits is rather scarce. The red tomatoes stored in the FRL have longer shelf life as compared to those stored in the darkness (Mihaly Cozmuta et al. 2016).

In this end, the aim of this paper was to comparative assess the efficiency in storage of the darkness, UV-C and FRL, respectively in terms of some physical, chemical and microbiological attributes of red bell pepper fruits.

## MATERIAL AND METHODS

### 2.1. Biological material

Red bell pepper fruits (*Capsicum annuum* L. cv. Bogdan) were purchased from a local produced, inspected to remove the damaged and contaminated fruits and delivered to the laboratory no later than 1h after they were harvested. The fruits were washed in distilled water, air-dried and stored.

### 2.2. Storage and sampling

The bell peppers fruits were divided in three batches of thirtieth fruits each and stored for 6 weeks at 8$^{o}$C and relative humidity (RH) of 55±5% in three different designs. The bell peppers in the first batch, were exposed 12 hours/day to FRL (740 nm) to LumiBulb-Far Red LED lamp (LumiGrow, USA) at the light density of 5 Watts and another 12 hours/day to the darkness. The peppers in the second batch were subjected to UV-C irradiation in a dose of 10 kJ/m$^2$ provided by a germicidal lamp (TUV, 30W, Philips) under the same lighting cycle as above presented. The fruits in the third design were stored in the darkness. The lights doses were selected in the basis of previous attempts when the appearance of the stored peppers was screened and the doses corresponding to the best results were selected for further investigations. During storage, all fruits were manually rotated to assure an equally exposure. Weekly, samples of peppers were collected from each batch and analyzed. Similar investigation analyzes were conducted on the fresh fruits.

### 2.3. Chemical investigations

#### 2.3.1. Chilling injuries and enzymes catalase activity (CAT)

The method proposed by Cuvi et al. (2011) was used to assess the chilling injury index. According to it, twenty fresh and stored fruits were inspected weekly in terms of tissues discoloration, hardness, pitting, decays and surface shriveling. Their external CI symptoms were reported to a scale ranging from 1 to 4 where: 1 – no damage; 2 – low damage; 3 – moderate damage; 4 – severe damage. The CI was calculated according to the equation:

$$\text{CI index} = (\sum \text{injury level x number of samples inspected})/\text{Total number of fruits considered} \quad (1)$$

and expressed as mean±standard deviation.

The chilling injury is often associated with oxidative damage in the plant tissues and the presence of antioxidant enzymes contributes to adaptation of plants to the cold stress (Wang et al., 2012). Catalase enzyme was selected as marker of the fruits adaptation to the cold stress and its level in the fresh and

stored red bell peppers was measured according to the method proposed by Wang et al. (2012). According to it, 4 g of fresh pulp were mixed with 0.1 g PVPP (polyvinylpolypyrrolidone) and homogenized in 10 mL ice-cold phosphat buffered saline solution (25 mM) containing 1 mM EDTA (ethylenediaminetetraacetic acid). The mixture was centrifuged at 12,000 x g for 20 min at 4°C and the supernatant was analyzed for the catalase content (Xing et al. 2011). A mixture made of 2 mL sodium phosphate buffer (50 mM, pH 7.0) and 0.5 mL $H_2O_2$ (40 mM) was contacted with 0.5 mL enzyme extract. The decomposition of $H_2O_2$ was measured by the decline in absorbance at 240 nm (UV/VIS Lambda 35 Spectrometer, Perkin Elmer). CAT specific activity was expressed as U/kg of fresh sample, where U = $\Delta A_{240}$ nm/s.

**2.3.2. Antioxidant capacity against DPPH·**

Antioxidant activity in fresh and stored peppers was assessed according to the method proposed by Brand Williams et al. (1995) adapted to our study. Briefly, frozen pieces of fruits were milled and 1 g of powder was put in contact with 6 mL ethanol. After centrifugation at 9000 x g for 10 minutes, ethanol aliquots were mixed with 3.0 mL of fresh 0.025 g/L 2,2-diphenyl-1-picrylhydrazyl (DPPH•) in methanol. The absorbance at 515 nm was red at different moments until a plateau was reached. To express the antioxidant activity as $EC_{50}^{-1}$ ($Kg^{-1}$) the percentage of remaining DPPH• was plotted against the volume of extract required to decrease the initial DPPH• concentration by 50%.

**2.3.3. Phenolic compounds**

The method proposed by Vincente et al. (2005) was used. According to it, 3 mL of supernatant obtaining as above described were diluted up to 100 mL with distilled water. 200 µL was mixed with 1.11 mL of distilled water and 200 µL Folin-Ciocalteau 0.1 N. The mixture was incubated 3 minutes at 25°C and then 1.5 mL saturated solution of $Na_2CO_3$ was added and incubated for 1 hour. The absorbance was measured at 760 nm and the total phenolic compounds amount was expressed as mg/Kg by using gallic acid (GA) to build the calibration curve. The results are expressed as mg of GA per kg of fresh weight.

**2.3.4. Microbiological charge**

The efficiency of darkness, UV-C and FRL, respectively against microbial spoilage was investigated by screening the reduction of total molds and yeasts on red bell peppers surface (SR ISO 7954-2001). Bell peppers were previously rinsed with 1% sodium hypochlorite, washed in sterile distilled water, dried in ambient air and stored. Fresh and weekly sampled fruits were mixed with 9 ml of saline solution, homogenized for 1 h. Serial 10-fold dilutions were prepared in saline solution (9 mg/ml). Yeast and molds on tomatoes surface were assessed on Sabouraud Dextrose Agar W/Chloramphenicol (Scharlab, Germany) selective medium after incubation for 5 days in the darkness at $25^0$C. The colonies were counted and the results were expressed as log of colony forming unit per gram of pepper fruits (log CFU/g).

**2.4. Statistical analysis**

One-way ANOVA analysis (Tukey HSD Test $p < 0.5$) with Statistica 7.0 software (StatSoft, Inc., Tulsa, USA) was used to assess the significance differences between the experimental.

## RESULTS AND DISCUSSIONS

### 3.1. Chilling injuries and CAT enzyme level

After the first week of storage (Figure 1), in which all fruits were reported to have no damages (CI = 1), a significant rise in CI from 1 to 3 was reported in the case of darkness-stored peppers as compared to the rise from 1 to 2 in the UV-C fruits and no rise in the FRL fruits. In the middle of the storage range, the CI of darkness exposed samples achieved the value 4 corresponding to the severe damage. At the same moment, the CI raised to value 3 (moderate damage) and 2 (low damage) in the in UV-C- and FRL-, respectively stored fruits. After six weeks of storage, the most deteriorated fruits resulted from darkness storage (CI = 4), followed by the UV-C and FRL storage (CI = 3).

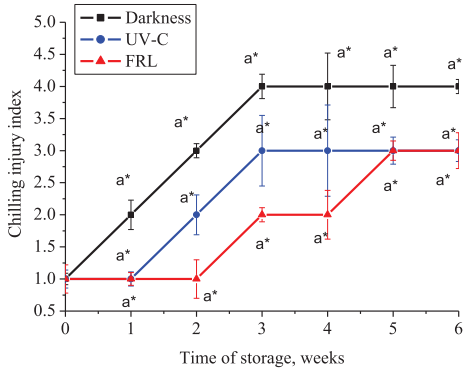


Figure 1. The evolution in the chilling index in the red bell pepper fruits during storage in darkness, UV-C and FRL

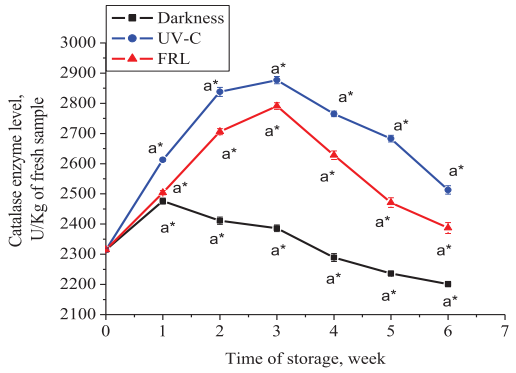


Figure 2. CAT enzyme level in the darkness, UV-C and FRL exposed pepper fruits

The results are expressed as mean ± standard deviation; a– significant difference ($p<0.05$) reported to the initial moment within the same storage design;
*- significant difference at $p<0.05$ reported to the corresponding value in the other storage designs; the error bars represent standard errors of the means (n=3)

Positive influence of UV-C in the reducing the chilling injuries in the stored peppers was also reported by Vicente et al. (2005) and Cuvi et al (2011) who found that UV-C treatments can reduce CI of peppers and did not cause negative effect in fruits surface color. Mihaly Cozmuta et al. (2016) reported that the injuries in the tomato fruits are significantly delayed when they are exposed for one month to FRL (740 nm) at 8$^{o}$C in relation to the tomatoes stored in the darkness. The CI evolution during the storage of pepper fruits is positively correlated with CAT enzyme levels in the fruits (Figure 2).

The same ascending trend in the CAT level was observed in the first week of storage in all stored samples, to a larger extent in the UV-C-stored fruits (1.12-fold), followed by the FRL (1.08-fold) and darkness (1.06-fold), respectively exposed fruits. Further, the CAT level in the darkness-exposed fruits continuously declines and at the end of storage became lower than in the fresh fruits. The lights seem to favor the CAT accumulation in the pepper fruits, the upward trend in UV-C and FRL continuing up to the third week of storage. In opposition to the other investigated parameters, UV-C light is more effective in enzyme accumulation than FRL. Thus, the maximum CAT level in the UV-C-exposed fruits is 2877 U/Kg, while in the FRL-exposed fruits it reaches the value of 2791 U/Kg. In the last three weeks, a downtrend in the CAT level in UV-C and FRL- stored peppers is noticed but it still remains above the initial CAT level. Our results are supported by the work of Cuvi et al. (2011) who reported an increase in the CAT level in the red bell peppers subjected to UV-C treatment. Accumulation of CAT enzyme in the tomatoes exposed to FRL was reported by Mihaly Cozmuta et al. (2016). Beside the CAT enzyme level, the wax composition also contributes to the fruits response to the cold stress. A higher hydrophobic wax results in a lower thermal conductivity and delaying the cold flow transfer from the environment to the fruit surface.

**3.2. Antioxidant capacity against DPPH·**

The antioxidant capacity against DPPH· radical increased in all stored fruits but the maximum peaks were reached at different moments (Figure 3). Thus, the fruits stored in the darkness achieved theirs maximum antioxidant capacity (7.41 Kg$^{-1}$) after two weeks at which point it begins to decline. At the end of storage, the antioxidant capacity in the darkness-stored peppers became 1.44-fold lower that in the fresh samples. In the cases of fruits stored in the UV-C and FRL, respectively the antioxidant capacity rises continuously over 4 weeks, to a larger extent in the FRL-exposed fruits (1.36-fold) and lower in the UV-C-exposed fruits (1.22-fold). The decrease in the antioxidant capacity in the UV-C and FRL-exposed peppers occurred at a slower trend as in the darkness-exposed peppers and at the end of storage did not fall bellow the level in the fresh peppers. The increase in the antioxidant capacity is the result of the increases in the levels of the antioxidant compounds (phenolic). The results of Rodoni et al. (2015) also reported the increase in the antioxidant capacity of pepper fruits stored in UV-C due to the ascending trend of ascorbic acid. Vicente et al. (2005) noticed an upward trend in pepper fruits stored at 10$^{o}$C for 12 days followed by a downward trend in the next 6 days but the antioxidant capacity still remained over the level in the fresh samples.

**3.3. Phenolic compounds**

The maximum phenolic compounds level in the darkness-fruits is reached in the second week of storage, 1.02-fold higher than in the fresh fruits (Figure 4). Until the end of storage, it decreases below the initial level 1.07-fold. UC-V and FRL induce the accumulation of phenolic compounds in the red bell pepper fruits a

week longer in comparison with accumulation in the darkness. In the second week of storage, the phenolic compounds level in UV-C fruits is 1.07-folder higher than in the fresh fruits and slightly higher in FRL fruits (1.08-folder). In opposition, their decrease trend in the FRL-exposed peppers is sharper than that in the UV-C-exposed peppers but at the end of storage the phenolics levels are upper than in the fresh peppers in both cases.

Accumulation of phenolic compounds in fruits induced by UV-C is also reported by Rodoni et al. (2015), Charles et al. (2008), Venditti and D'Hallewin (2014).The phenolics compounds play important role in the fruits structure. Lignin and suberin are involved in the formation of barrier against water loss and nutrients leakage, and pathogen penetration at wound site (Bostock and Stermer, 1989; Garrod et al. 1982).

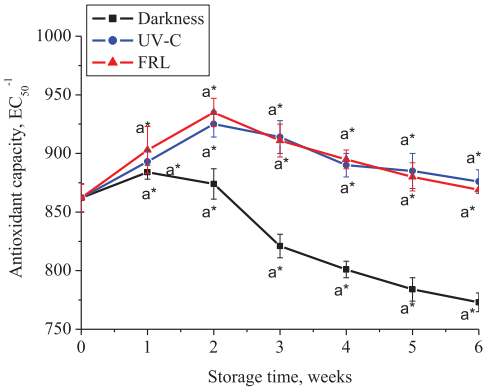


Figure 3. Evolution in the antioxidant capacity of peppers stored in the darkness, UV-C and FRL.

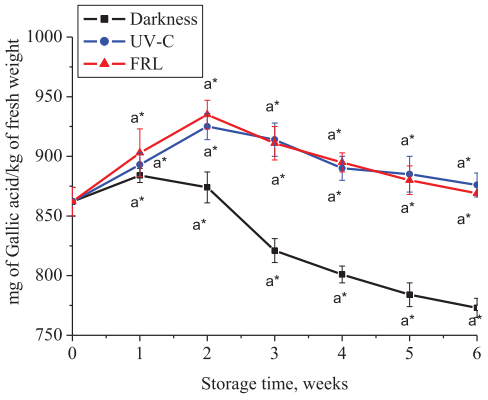


Figure 4. Phenolic compounds level in the darkness, UV-C and FRL exposed pepper fruits.

The results are expressed as mean ± standard deviation; a– significant difference ($p<0.05$) reported to the initial moment within the same storage design;

*- significant difference at $p<0.05$ reported to the corresponding value in the other storage designs; the error bars represent standard errors of the means (n=3)

### 3.4. Microbiology

Reduction in the yeasts and molds count in the pepper fruits during storage is presented in Figure 5. As observed, the multiplication increases during storage, the highest rate being noticed in the darkness-exposed fruits. At the end of storage, the total yeasts and molds charge in these samples was 19.5-fold higher then that in the sampled stored for 1 week.

As regard the light-exposed samples, the yeasts and molds proliferates to a larger extent in the FRL- as compared to UV-C-, respectively exposed fruits. After six weeks of storage, the total yeasts and molds count was 1.4-fold lower in the UV-C- exposed peppers and 1.5-fold lower in the FRL-exposed pepper, respectively as compared to darkness-exposed peppers. Defenses against microorganisms in the cases of UV-C and FRL exposure result from a combination of direct and indirect actions.

The direct action concerns the impact of lights on microorganisms integrity. Studies in this field reported that of all UV spectra, the UV-C portion in range of 250-260 nm is considered the most efficient in destroying the microorganisms, as results of damaging the cellular DNA pyrimidine dimer formation (Bintsis et al. 2000).

The FRL inhibits the germination and growth of some fungus by acting against membrane lipids, cytoplasmic enzymes and nucleic acids (Fuller et al. 2013). Indirect action refers to the morphological and physiological changes occurred in the fruits which result in reinforcement of existing defense mechanisms against microorganism attack or developing the new ones. Glazener (1982) and De Leeuw (1985) reported that the resistance against *B. cinerea* is linked to the accumulation of phenolics and the formation of lignin-like polymers and suberization and callose deposition in the cells surrounding the infection site.

Changes in the chemical nature in the cuticular wax into a more hydrophobic composition result in a better coverage in the fruit surface and subsequently in the closing of the possible open areas through which the microorganisms could have access to the substrate.

Moreover, a more hydrophobic wax has a lower compatibility with hydrolytic enzymes secreted by the yeast and molds and the fruits surface digestion slowed down.

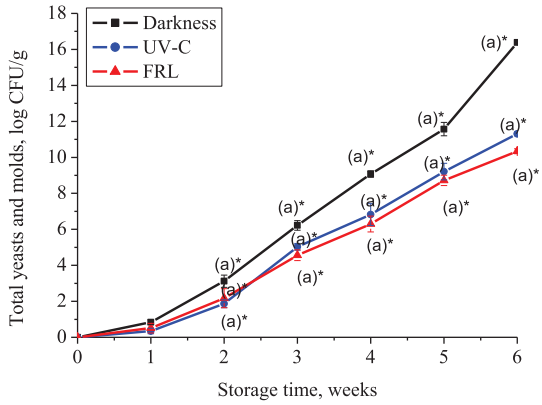


Figure 5. Evolution of total molds and yeasts on red bell pepper fruits during storage in the darkness, UV-C and FRL; results are expressed as mean ± standard deviation; (a)- significant difference at $p<0.05$ reported to the first week of storage within the same storage design; *- significant difference at $p<0.05$ reported to the corresponding value in the other storage designs; The error bars represent standard errors of the means (n = 3)

### 3.5. Appearance

Fruits appearance is a very important attribute in term of their marketability. As in Figure 6 can be seen, the pepper fruits exposed to FRL are less wilted and shriveled in comparison with those exposed to UV-C and darkness, respectively.

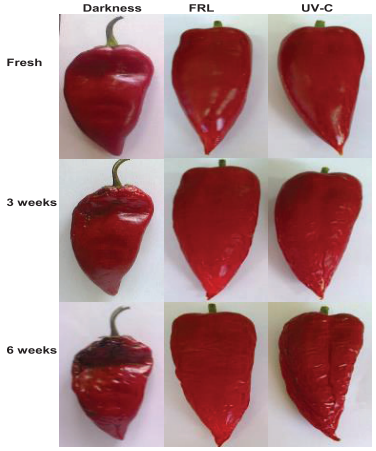


Figure 6. The appearance of fresh, 3 and 6 weeks respectively, stored red bell pepper fruits.

After six weeks, the most deteriorated fruits are those stored in the darkness. The numerous cuticular crakings, many being easily visible, favored the oxygen penetration and browning alteration. Moreover, bacterial black spots were observed on the surface of the darkness-stored peppers fruits from the second week of storage. They are missing in fruits stored in FRL and UV-C, the bactericidal effect of UV-C and FRL being well known (Santos et al. 2013; Charles et al. 2008; Fuller et al. 2013).

## CONCLUSIONS

The study discusses the influence of 6 weeks exposure to the darkness, UV-C and FRL on some physical, chemical and microbiological attributes in the red bell pepper fruits. Regardless the light nature, the storage in the UV-C and FRL is more effective in the prolongation the peppers shelf-life as compared to the storage in the darkness. The FRL enhances to a larger extent the attributes in the pepper fruits in relation to UV-C in terms of chilling injuries, antioxidant capacity, phenolic content and appearance, while the UV-C proved to be more effective in terms of catalase enzyme level and delaying of yeasts and molds multiplication. This ranking it is possible to be light dose-depending and further investigations are required to have a conclusion regarding the most effective light in the red bell pepper fruits storage.

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## REFERENCES

Brand Williams W., Cuvelier M.E., Berset C., 1995, Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 28: 25-30.

Bintsis T., Litopoulou-Tzanetaki E. and Robinson R.K., 2000, Existing and potential applications of ultraviolet light in the food industry- a critical review. J. Sci. Food Agric. 80: 637-645.

Bostock R. M. and Stermer B. A., 1989, Perspectives onwound healing in resistance to pathogens. Ann. Rev. Phytopathol. 27: 343-371.

Charles M. T., Makhlouf J. and Arul J., 2008, Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit II. Modification of fruit surface and changes in fungal colonization. Postharvest Biol. Tec. 47: 21-26.

Cuvi A., Vicente M.J., Concellón A.R., Chaves. A.R., 2011, Changes in red pepper antioxidants as affected by UV-C treatments and storage at chilling temperatures. LWT-Food Sci. Technol. 44: 1666-1671.

De Leeuw G.T.N., 1985, Deposition of lignin, suberin and callose in relation to the restriction of infection by *Botrytis cinerea* in ghost spots of tomato fruits. Phytopathol. Z. 112: 143-152.

Fuller K.K., Ringelberg C.S., Loros J.J., Dunlap J.C., 2013, Thee fungal pathogenAspergillus fumigatus regulates growth, metabolism, and stress resistance inresponse to light. mBio 4(2), http://dx.doi.org/10.1128/mBio. 00142-13(e00142-13).

Garrod B., Lewis B.G., Brittain M.J., Davies W.P., 1982, Studies on the contribution of lignin and suberin to the impedance ofwounded carrot root tissue to fungal invasion. New Phytol. 90: 99-108.

Glazener J.A., 1982, Accumulation of phenolic compounds in cells and formation of lignin-like polymers in cell walls of young tomato fruits after inoculation with *Botrytis cinerea*. Physiol. Plant Pathol. 20: 11-25.

Meng X., Zhang M., Adhikari B., 2012, Extending shelf-life of fresh-cut green peppers using pressurized argon treatment. Postharvest Biol. Tec. 71: 13-20.

Mihaly Cozmuta A., Mihaly Cozmuta L., Peter A., Nicula C., Vosgan Z., Giurgiulescu L., Vulpoi A., Baia M., 2016, Effect of monochromatic Far-Red light onphysical-nutritional-microbiological attributes of red tomatoesduring storage. Sci. Hortic. 211: 220-230.

Rodoni L. M., Zaro M. J., Hasperue J.H., Concellon A. And Vicente A.R., 2015, UV-C treatments extend the shelf life of fresh-cut peppers by delaying pectin solubilization and inducing local accumulation of phenolics. LWT-Food Sci. Technol. 63: 408-414.

Santos A.L., Oliveira V., Baptista I., Henriques I., Gomes N.C. M., Almeida A., Correia A., Cunha A., 2013, Wavelength dependence of biological damage induced by UV radiation on bacteria. Arch. Microbiol. 195(1): 63-74.

SR ISO 7954-2001- Analysis of yeasts and molds in food products.

Urban L., Charles F., Alcantara de Miranda M.R., Aarrouf J., 2016, Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. Plant Physiol. Biochem. 105: 1-11.

Venditti T., D'hallewin G., 2014, Use of ultraviolet radiation to increase the health-promoting properties of fruits and vegetables. Stewart Postharvest Review 10(3): 1-3(3).

Vicente A. R., Pineda C., Lemoine L., Civello P. M., Martinez G. A., Chaves A. R., 2005, UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biol. Tec., 35: 69-78.

Wang Q., Dinga T., Gao L., Pang J., Yang N., 2012, Effect of brassinolide on chilling injury of green bell pepper in storage. *Scientia Hortic.* 144: 195-200.

Xing Y., Li X., Xu Q., Yun J., Lu Y., Tang, Y., 2011, Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). Food Chem. 124: 1443-1450.

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# ESTABLISHING THE QUALITY INDICATORS OF THE RED WINES IN DRAGĂŞANI VINEYARD

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***Keywords***: *red wines, chemical composition, quality indicators, color parameters*

## ABSTRACT

*Alongside modern analysis for determining the quality of wines, it can be determined by analyzing the main parameters (alcohol, total acidity, extract, ash) and calculating the most important quality indicators (ratio alcohol / glycerol, alcohol / extract, extract / ash Halphen and Gautier). For this purpose were analyzed four red wines Cabernet Sauvignon, Merlot, Pinot noir cultivated in Dobrusa area from Dragasani vineyard.These quality indicators are used in particular to determining the naturalness of wine products and the balance of the composition.*

## INTRODUCTION

Wine is constituted as a polyphase system of substances, some of which come from the grapes, and most are formed during alcoholic and malolactic fermentation, also during the aging of wines (Burin et al. 2010).

The chemical composition of wine is affected by multiple factors, including production area, grape variety, soil type, climate (terroir) and viticole and oenological practices. These factors play an important role in differentiating the wines according to their geographical origin and year of harvest (Schlesie et al., 2009, Beleniuc 2009).

Basing on the fact that the term 'origin' is of considerable importance directly correlated with the quality of wines, ranking them in terms of geographical origin and variety became an issue of significant interest to the community producers and consumers (Banu et al, 2013, Stoica, 2015).

Knowledge of the chemical composition of wine and its association with the grape variety/cultivar is of paramount importance in oenology and a necessary tool for marketing (Muntean et al, 2015).

The colour and the volatile profile of red wines play a significant role in the perception of wine quality, having a big impact on the consumer preference for a certain wine (Gómez García-Carpintero et al, 2011, Antoce, 2012) .

An improvement of the extraction of both colour and volatile compounds from the grape skins during the winemaking can be achieved by addition of enzymes (Alvarez et al. 2006).

## MATERIAL AND METHODS

The study conducted, sought to determine the main constituents that define the quality and naturalness, of red wines obtained in 2015 in Drăgăşani vineyard, Dobruşa hill, Avincis Winery.

The wines considered for the study were obtained from grapes harvested at technological maturity or industrial maturity, when the composition was optimum for producing grape wine type desired. The wines were analyzed at 3-4 months after preparation after completion of malolactic fermentation, when they were determined: alcohol content, total acidity, volatile acidity, glycerol, mineral substances, extract and color parameters.

To complete the characterization of the wine, and calculated indices ecological namely:

Gauthier index = conc. alcohol.) + total acidity (g H2SO4 / l) lies between 13 ÷ 17;

Report Halphen = total acidity ($H_2SO_4$ g/L) / conc. alc. (% Vol.) with values between 0.2 ÷ 0.8;

The ratio R = total alcohol (g/L) / reduced extract, has the values for red wines between 2.5 to 4.5, and for white wines between 3.5 ÷ 6.5.

The rations between weight of dosed alcohol and glycerol

The rations between weight of extractl and ash

The rations between the yellow pigments (D0420 nm) and red pigments (D0520 nm) expressing coloration quality red wines through parameter called the color tone.

For determining the alcohol-glycerol ratio, alcohol degree is multiplied by ten to obtain the quantity of alcohol by volume. The quantity of alcohol by volume are then multiplied by 0.79 (the molecular weight) to obtain the amount of alcohol by weight. Then the calculation determining the ratio of the weights of the two elements.

For determining the ratio of extract - ash the extract is considered as 100%, and the ash is as:% of extract.

For determining the color tone by spectrophotometric analysis extinctions were measured at wave lengths 420 nm and 520 nm.

These ratios are considered especially to determine the naturalness of wines and their compositional balance.

It is noted that the level of naturalness of wines and balance physical - chemical normally are made when the proportion of glycerol to alcohol and ash (mineral) to extract are closer to 10%.

When the ratio glycerol / alcohol is below 6.5%, it means that the wine was alcoholized to 10% higher if the suspicion arises that the wine was glycerinated. So by considering this report and can detect both alcoholozation and glycerination.

The determinations were performed, some in Enology laboratory and some at Winery Avincis using official methods OIV. In determining the phenolic constituents of red wines obtained and for defining their chromatic structures were used spectrophotometric methods.

## RESULTS AND DISCUSSIONS

The main composition parameters of red wines under study are enrolled in Table 1.

Considering that the hill Dobrusa has favorable climatic conditions produce red wines with long sunshine, the degree alcoholic registered was in any case less than 13 vol% corresponding to a content in sugars of 221 g/L Negru de Dragasani and 13.5 vol% for Cabernet Sauvignon wine.

Table 1

Te main characteristics of composition of red wines of Dobrusa hill

| Variety | Alcohol %vol. | Total acidity g/L $H_2SO_4$ | Volatile acidity g/L $H_2SO_4$ | Glycerol g/L | Reduced extract g/L | Ash g/L |
|---|---|---|---|---|---|---|
| CABERNET SAUVIGNON | 13,5 | 4,10 | 0,41 | 10,92 | 26,50 | 2,52 |
| MERLOT | 13,2 | 3,95 | 0,39 | 10,90 | 26,15 | 2,60 |
| PINOT NOIR | 13,4 | 3,65 | 0,38 | 10,78 | 26,25 | 2,59 |
| NEGRU DE DRĂGĂŞANI | 13,0 | 4,15 | 0,41 | 10,28 | 26,45 | 2,65 |

Total acidity recorded values above 4 g/L, the varieties Cabernet Sauvignon and Black Dragasani, except varieties Pinot Noir and Merlot, which are slightly deficient in this parameter.

Glycerol, component with essential role to red wines in modeling roughness taste imprinted of tannin, which prints the wines finesse and softness, has values over 10 g/L to all wines, being consistent with the degree alcoholic components formed in parallel by the fermentation, both fermentable carbohydrates depending on the contents of the must. The proportions of glycerol registered in all 4 wines analyzed, it is a valuable factor in the composition and organoleptic nature.

Extractivity, over 26 g/L in all analyzed wines, is able to confer attributes in order to be classified in categories of high quality.

The ash content, of more than 2.5 g/L, keeping the specific proportions, aims proportion in the extract contents.

Table 2

Enological indicators of red wines - Dobruşa hill

| Variety | Glycerol × 100 / alcohol | Ash × 100 / unreduced extract | Gauthier index | Halphen ratio | R ratio |
|---|---|---|---|---|---|
| CABERNET SAUVIGNON | 9,76 | 9,50 | 17,6 | 0,30 | 4,02 |
| MERLOT | 9,56 | 9,94 | 17,15 | 0,29 | 3,98 |
| PINOT NOIR | 9,82 | 9,86 | 17,05 | 0,27 | 4,04 |
| NEGRU DE DRĂGĂŞANI | 9,79 | 10,01 | 17,15 | 0,31 | 3,91 |

Analyzing oenological indices calculated values (tabel2) shows that the ratio alcohol - glycerol recorded values between 9.56 and 9.82, which excludes glyceration or alcoholization wines and ratios ash - extract between 9.5 and 10.01 indicates a high degree of naturalness of wine and compositional balance.

Gauthier index (alcohol concentration (% vol.) + Total acidity g/L H2SO4) to the wines analyzed is between 17.5 and 17.6.

It is estimated that the value of this index is 17 when wines have a high alcohol concentration, which corresponds to a minimum acidity. The wines are diluted with water if the index value is less than 13.

Halphen ratio - the ratio between total acidity and alcohol, to wines analyzed present values between 0.27 and 0.31. This index has significant value in terms of geography and degree alcoholic wine.

The ratio R - degree alcohol / extract is between 3.91 and 4.04 is framed above the minimum and below the maximum set for this index. Extract content increases with increasing alcohol concentration.

Value of the report alcohol / extract restricted varies with different sizes for white wines to reds. The mean ratio Rup to a alcohol concentrations between from 8.5 to 15% vol. Is 3.6 for red wines, with the upper limit of 6.5. Exceeding this value indicates the addition of alcohol.

The anthocyanins of red wine are constituents which distinguish of these products from other types of wine. Their importance is considerable, both on compositional and hygienic food aspect. They obviously rich composition that contains wines.

Optical density values to wave lengths of 420 nm, 520 nm and 620 nm specific to different types of pigments used to calculate attributes chromatic wines, listed in Table 3 reveals important differences that exist in the utmost nature of genetic the variety and less than primary wine-making technology.

Table 3

The optical density of red wines - Dobruşa hill

| Variety | DO 420 nm Yellow pigments | DO 520 nm Red pigments | DO 620 nm Blue pigments |
|---|---|---|---|
| CABERNET SAUVIGNON | 0,371 | 0,898 | 0,193 |
| MERLOT | 0,359 | 0,796 | 0,269 |
| PINOT NOIR | 0,258 | 0,544 | 0,175 |
| NEGRUDE DRĂGĂŞANI | 0,435 | 0,758 | 0,276 |

The yellow component to the lowest value recorded at wine Pinot noir, and the greatest at wine produced from the variety Negru de Drăgăşani. Component red tops the wine Cabernet Sauvignon, and the last wine Pinot noir. The blue component ranked first wine Negru de Drăgăşani, and the last wine Pinot noir.

An accurate imagine of the quantity and quality of material coloring the wine is obtained from: content absolute anthocyanins, the participation percentage of different types of pigments and values characteristics color for their definition being considered optical density values (Table 4).

The content of anthocyanins has the highest value to wine Cabernet Sauvignon, 815 mg/L, and the lowest Pinot Noir at 415 mg/L.

Data on participation of various categories of pigment composition of coloring matter and levels characteristics color of the complex anthocyanins, show structures chromatic very advantageous at all wines and proportions of different types of pigments are able to provide levels of color totally appropriate in the claims current.

Table 4

The chromatic composition of red wines - Dobruşa hill

| Variety | Anthocya-nins mg/l | Participation pigments % | | | Ic | Tc | dA% |
|---|---|---|---|---|---|---|---|
| | | Yellow pigments DO 420 nm | Red pigments DO 520 nm | Blue pigments DO 620 nm | | | |
| CABERNET SAUVIGNON | 815 | 32,2 | 54,6 | 13,2 | 1,462 | 0,590 | 58,39 |
| MERLOT | 695 | 25,2 | 56,05 | 18,75 | 1,424 | 0,451 | 56,53 |
| PINOT NOIR | 415 | 26,4 | 56,08 | 17,52 | 0,977 | 0,474 | 54,05 |
| NEGRU DE DRĂGĂŞANI | 740 | 29,7 | 51,91 | 18,39 | 1,469 | 0,573 | 57,92 |

Corresponding content in anthocyanins and participation of various categories of pigments, presents and characteristics chromatic intensity of the color (Ic), tone color (Tc) and proportions of cations flavilium (dA%) that the quantities recorded offers peace of stains pleasant and balanced under visual report.

Phenol compounds in quantities much higher in red wines compared to white ones are those which confer specificity, general deportment, firmness and "robustness".

Along with anthocyanins in red wine plays a significant role polyphenols and tannins. In addition to complex chemical composition of red wines, polyphenolic constituents and essentially influence the organoleptic characteristics. Polyphenolic composition is shown in Table 5.

Table 5

The polyphenols composition of red wines - Dobruşa hill

| Variety | Total polyphenols g/l | Tanin g/l |
|---|---|---|
| | | |
| MERLOT | 3,41 | 2,69 |
| PINOT NOIR | 2,87 | 2,45 |
| NEGRU DE DRĂGĂŞANI | 3,56 | 2,96 |

Analyzing the data obtained, it appears that at polyphenols content was between 2.87 g/L of Pinot noir wine and 3.56 g/L at Negru de Drăgăşani (which explains the astringent taste of wine). The tannin contents were quite close at all wines, ranging from 2.45 g/L Pinot Noir and 2.96 g/L at Negru de Drăgăşani.

## CONCLUSIONS

Dobruşa Hill from the Drăgăşani vineyard has heliothermic and hydric resources, harmoniously combined, which favors quality red wines. Exceptional environmental conditions, allow perfect adjustment and red wine varieties of high quality, as are the famous varieties, aimed at the study.

Red wines, meet the main characteristics in terms of compositional attributes set for top quality wines with denomination of origin:

- The alcohol content between 13.0% vol. and 13.5% vol .;
- The contents of acidity of between 3.65 g/L and 4.10 g/L (in H2SO4);
- Content in the extract with values of around 26 g/L;
- The proportions of ash between 2.52 g/L and 2.60 g/L,
- Ratios Glycerol × 100/100 × ash and alcohol / extract closer than the 10% threshold.
- Index Gauthier, Halphen report, R report, within the limits established for these indices oenological.

Physico-chemical components of red wines are accompanied by chromatic qualities lie in the similar products obtained in the most reputable vineyards in the country and abroad. Anthocyanin contents between 415 mg/L (Pinot noir) and 815 mg/L (Cabernet Sauvignon), within which occupies yellow pigments proportions between 32 and 34.9%, red pigments varies between 53% and 55%, pigments blue values between 10% and 13.6%. Firmness and general attire of red wines are supported by the polyphenol content (between 2.97 g/L and 3.96 g/L) and tannins (ranging between 2.55 g/L and 3.07 g / it).

## REFERENCES


Antoce A.O., 2012, Influence of maceration enzyme treatment on the colour and volatile profile of two red Romanian wines. Revista de Chimie, vol 63 (9), 859-864.

Banu C., Bulancea M., Ianiţchi D., Bărăscu E., Stoica A, 2013, Industria alimentară între adevăr şi fraudă. Editura ASAB, p. 359-392.

Beleniuc G., 2009, Oenologie. Notiuni de vinificatie, biochimie, microbiologie, conditionare, stabilizare si imbuteliere a vinului. Editura Universitara, Bucuresti.

Burin V.M., Falcão L.D., Chaves S.E., Fortes Gris E., Preti L.F., Bordignon-Luiz T.M., 2010. Phenolic composition. colour. antioxidant activity and mineral profile of Cabernet Sauvignon wines. International Journal of Food Science & Technology 45 (7), 1505-1512.

Gómez García-Carpintero E., Sánchez-Palomo E., Gómez Gallego M.A., González-Viñas M.A., 2011, Volatile and sensory characterization of red wines from cv. Moravia Agria minority grape variety cultivated in La Mancha region over five consecutive vintages. Food Res. Intl., 44, 5, 1549-1560.

Muntean C., Stoica F., Băducă C., 2015, The main constituents of red wines that define the level of quality and naturalness. Annals of University of Craiova, XX (LVI), 59-64.

Stoica F., 2015, The main parameters and indicators that define the quality and authenticity of white wines from vineyard Drăgăşani – Romania. Carpathian Journal of Food Scence and Technology, 7(4), 139-144.

.*** 2012, Recueil international des méthodes d'analyses – OIV, Caractéristiques Chromatiques, Méthode OIV-MA-AS2-11

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# OPTIMUM EXTRACTION YIELD OF TANNING SUBSTANCES IN GRAPE BUNCHES OF FETEASCA REGALA AND FETEASCA ALBA

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***Keywords****: ethanol, Folin-Ciocalteu, grape bunches, tannin*

## ABSTRACT

*This paper aims to evaluate the optimum possibility for extracting tanning substances from grape bunches in order to subsequently value them. The selected varieties were from indigenous harvests: Feteasca Regala (FR) and Feteasca Alba (FA). Grape bunches were dried and then grinded so as to obtain a powder. Extraction methods focused on different ethanol concentrations (50%, 65%, 80%) under variable temperature and time conditions. The most significant results were obtained when by using ethanol 50% for to 10 hours extraction time, at a temperature of 30 $^0$ C; under these conditions, there were detected values between 6.05 g/L and 7.45 g/L tannins, these values being 28-30% higher than by using a higher concentration of alcohol.*

## INTRODUCTION

Tannins, especially oenological ones, are important compounds on the wine making market, playing a role in appreciating wine quality, conservation and development. Tannins confer wines a pleasant, slightly astringent and bitter character; however, a too high level of tannins is not desirable, as this causes a coarse taste, because it favours wine browning. They protect wine from oxidation and the unwanted effect of some micro-organisms. Insufficient tannin causes wine to be disharmonious, vulnerable to micro-organisms and less resistant to preservation (Pomohaci et al. 2001, Augustin–Salazar et al. 2014). From the chemical point of view, tannins are phenolic compounds, which may occur as large molecule polymers. Tannins can be hydrolysable, as natural esters of carbohydrates, or condensed, as polymers of catechins. In plants, tannins are found in the leaves and stem, playing a role in the oxidation and preservation processes; in grapes, they can be found both in the bunch stem, skin and seeds (Țârdea 2007, Cotea et al. 2010). Tannin induced astringency is directly proportional to their amount, structure and condensation degree; non-hydrolysable and condensed tannins are specific for grapevine (Tița 2004, Pomohaci et al. 2000, Târdea et al. 2010). Tannins are soluble in alcohol and less soluble in water. Due to this, they are extracted from bunch stems, grape berry seeds and skins

(Spigno and De Faveri 2007). Their solubility in alcohol represents an essential basis for evaluating their extraction yield from various wine sub-products (Spigno et al. 2007).

The purpose of this study was to evaluate the optimum possibility for extracting tanning substances from grape bunches in order to subsequently value them.

## MATERIALS AND METHODS

There were used white grape bunches from indigenous harvests: Feteasca Regala (FR) and Fetească Alba (FA). Grape bunches were dried and then grinded so as to obtain a powder. Three alcoholic solutions were prepared – 50%, 60% and 80% (powder/solvent ratio: 1/1). There were used a BUCHI R-100 Rotavapor (Buchi Labortechnik AG, Essen, Germany) for ethanol recovery, and a High Energy Ball Mill Emax (Retsch GmbH, Haan, Germany) for the preparation of the powder. The extraction temperatures were $20^0$ C, $30^0$ C, $40^0$ C and the time 4, 7 and 10 hours. The tannins were identified and quantified through the Folin-Ciocâlteu method: 1 mL extract was introduced in a 100 mL volumetric flask, 50 mL distilled water were added, the mixture was homogenized, then 5 mL Folin-Ciocâlteu solution and 20 mL Sodium carbonate 20% were added. The flask was filled with distilled water and left to rest for 30 minutes. Absorbance was read at a wavelength of 750 nm with a SPECORD 200 PLUS Spectrophotometer (Analytik Jena, Jena, Germany). The control sample contained the same ingredients, except the extract. In order to determine the concentration in the sample, a standard curve was achieved, using successive concentrations of oenological tannins.

## RESULTS AND DISCUSSIONS

The extracts from Feteasca Regala grape bunches resulted in different amounts of tannins, depending on the extraction conditions. Figure 2 shows that, in the case of 50% ethyl alcohol extraction, temperature is an influencing factor, and it is proportional to the time. Values between 3.22 g/100g and 5.36 g/100g tannin were determined at $20^0$ C. Minimum values were obtained after 4 hours, while, for 7 and 10 hours, tannin values increase with 66.45%. By using an extraction temperature of $30^0$ C, an increase in the amount of tannin amount was observed, as it reached a minimum value of 5.27 g/100g after 4 hours and a maximum of 6.25 g/100g after 10 hours. The amount of extracted tannins reached values that fell between 3.97 g/100g and 5.45 g/100g at $40^0$ C; on average, these values were 30% lower than in the case of extraction at $30^0$ C. Minimum tannin amounts extracted from FR bunches were determined around 3.22 g/100g; the extraction was made using 50% alcohol at $20^0$ C, for 4 hours. Maximum tannin amounts reached 6.25 g/100g, the extraction conditions being 50% alcohol, $30^0$ C and 10 hours.

When solvent concentration was 65%, the values determined ranged between 3.18 g/100g and 5.19 g/100g tannin, depending on the extraction time and temperature. Therefore, at $20^0$ C, the values reached 3.18 g/100g tannin when the extraction time was 4 hours. At $30^0$ C, linear values that did not exceed 4.95 g/100 g were recorded. Procedurally, the differences found were low, ranging between 1.8% and 7%. At $40^0$ C, the values recorded ranged between 4.21 g/100 g tannin after 4 hours and 5.14 g/100 g after 10 hours. When the extraction solution used was 80% ethyl alcohol, tannin amounts were generally low. Thus, at $20^0$ C, tannin amounts extracted ranged between 1.93 g/100 g after

4 hours and 2.32 g/100 g after 10 hours. At $30^0$ C, it was observed an increase of tannin concentration from 2.91 g/100 g after 7 hours to 3.57 g/100 g after 4 hours extraction time. This proves that a prolonged extraction leads to important tannin losses of about 22.68%. At $40^0$ C extraction temperature did not lead to spectacular tannin accumulations, being on 10.3% higher than those recorded at $30^0$ C extraction temperature.

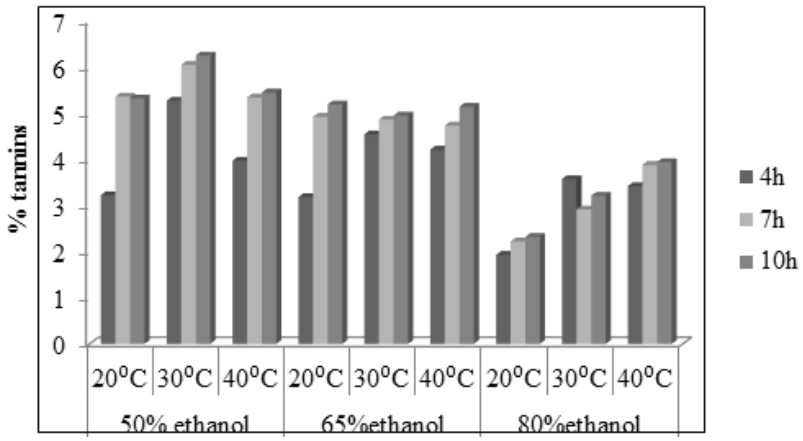


Figure 1. Evolution of tannin amounts extracted from Feteasca Regala bunches.

When solvent concentration was 65%, the values determined ranged between 3.18 g/100g and 5.19 g/100g tannin, depending on the extraction time and temperature. Therefore, at $20^0$ C, the values reached 3.18 g/100g tannin when the extraction time was 4 hours; they increased to 4.86 g/100 g when the extraction time was 7 hours and 5.19 g/100 g when it was 10 hours.

At $30^0$ C, linear values that did not exceed 4.95 g/100 g were recorded, and the extraction time was no longer an absolute yardstick.
Even after 4 hours, the values recorded were 4.54 g/100 g, respectively 4.86 g/100 g after 7 hours.

Procedurally, the differences found were low, ranging between 1.8% and 7%. At $40^0$ C, the values recorded ranged between 4.21 g/100 g tannin after 4 hours and 5.14 g/100 g after 10 hours. Prolonged extraction time of 7 hours lad to an increase by 12.3% in tannin concentration, and a 10-hour time to an increase of tannin concentration by 22%.

When the extraction solution used was 80% ethyl alcohol, tannin amounts were generally low. Thus, at $20^0$ C, tannin amounts extracted ranged between 1.93 g/100 g after 4 hours and 2.32 g/100 g after 10 hours.

At this temperature, the tannin concentrations determined were up by 4.5% to 15%. At $30^0$ C, it was observed an increase of tannin concentration from 2.91 g/100 g after 7 hours to 3.57 g/100 g after 4 hours extraction time. This proves that a prolonged extraction leads to important tannin losses of about 22.68%.

At $40^0$ C extraction temperature did not lead to spectacular tannin accumulations, as the values recorded ranged between 3.42 g/100 g tannin after 4 hours and 3.94 g/100 g after 10 hours extraction time. These values are on average 10.3% higher than those recorded at $30^0$ C extraction temperature.

Figure 2 shows the evolution of tannins extracted from Feteasca Alba bunches in relation to extraction temperature, time and solvent alcoholic concentration Thus, when the ethyl alcohol concentration was 50%, tannins reached values between a minimum of 4.22 g/100 g and a maximum of 7.25 g / 100 g.

At $20^0$ C, there were determined values between 4.22 g/100 g and 5.07 g/100 g tannin. Minimum values were recorded after 4 hours, while, after 7, respectively 10 hours extraction time, tannin values increased by 20.1%.

At $30^0$ C, an increase of the tannin amount was ascertained, as it reached a minimum value of 6.22 g/100 g after 4 hours, 7.05 g/100 g after 7 hours and a maximum of 7.25 g/100 g after 10 hours. At $40^0$ C, the amount of tannin extracted reached values that fell between 4.97 g/100 g and 5.35 g/100 g; on average, these values were 27% lower than in the case of extraction made at $30^0$ C and 0.5% higher than at $20^0$ C.

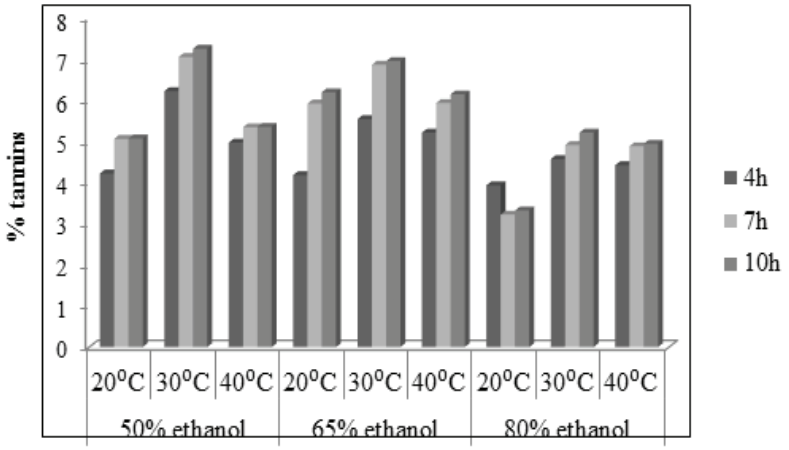


Figure 2. Evolution of tannin amounts extracted from Feteasca Alba bunches.

When solvent concentration was 65%, the values determined ranged between 4.18 g/100 g tannin and 6.95 g/100 g, depending on extraction time and temperature. Therefore, at an extraction temperature of $20^0$ C, the values recorded reached 4.18 g/100 g tannin after 4 hours extraction time, increasing to 5.92 g/100 g after 7 hours extraction time and 6.19 g/100 g after 10 hours extraction time. At $30^0$ C extraction temperature, the minimum value recorded was 5.54 g/100 g tannin after 4 hours and 6.95 g/100 g after 10 hours extraction time. At $40^0$ C extraction temperature, the values recorded ranged between 5.21 g/100 g tannin after 4 hours, 5.93 g/100 g at 7 hours and 6.14 g/100 g at10 hours extraction time. Seven hours extraction time led to an increase in tannin concentration by 13.8%, and 10-hour time to an increase of tannin concentration by 17.8%.

When using 80% ethyl alcohol solution, the tannin values recorded were generally lower compared to a 65% ethyl alcohol extraction solution. Thus, at $20^0$ C, the amount of tannin extracted reached values that ranged between 3.93 g/100 g at 4 hours and 3.32 g/100 g at 10 hours. At $30^0$ C, an increase of the tannin concentration was observed, as it went up from 4.57 g/100 g at 4 hours to 4.91 g/100 g at 7 hours and 5.21 g/100 g at 10 hours extraction time. A $40^0$ C

extraction temperature led to tannin amounts that ranged between 4.42 g/100 g at 4 hours and 4.94 g/100 g at 10 hours extraction time.

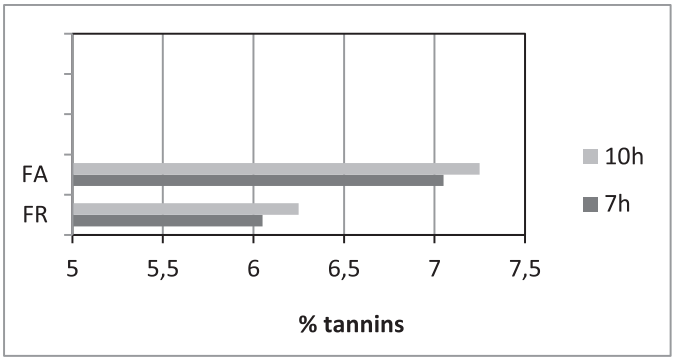


Figure 3. Determining optimum extraction yield from FA and FR.

Figure 3 shows the evaluation of optimum extraction yield. It can be observed that the most efficient extractions took place at $30^0$ C and 50% alcohol concentration. The minimum value was 6.05 g/100 g for FR. In the case of an extraction time over than 10 hours, maximum values did not exceed 7.25 g/100 g in the case of FA.

## CONCLUSIONS

This study showed that the extraction of tannins from grape bunches depends a lot on solvent concentration; in this case, 50% ethyl alcohol delivered the best results. Extraction temperature resulted as an important parameter for good tannins extraction, the optimum determined temperature being $30^0$ C. Higher temperatures resulted in lower tannins extraction, as it is more than obvious that some volatile compounds evaporated. The extraction time represented an important factor and allowed the quantification of tannin compounds; a 4-hour short extraction time is not always enough, while a 10-hour extraction time did not lead to accumulations that could justify it from an economic point of view.

## ACKNOWLEDGMENT

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## REFERENCES

Agustin-Salazar S., Medina-Juárez L.A., Soto-Valdez H., Manzanares-López F., Gámez-Meza N., 2014, (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research, vol. 20 (2), 208–213.

Cotea V. D., Zănoagă C.Z., Cotea V.V., 2010, Tratat de oenochimie, vol.II. Ed. Academiei Române, București.

Pomohaci N., Stoian V., Gheorghiță M., Sîrghi C., Cotea V.V., Năməloșanu I., 2000, Oenologie vol.I, Ed. Ceres, București.

Pomohaci N. , Stoian V., Gheorghiță M., Sîrghi C., Cotea V.V., Năməloșanu I., 2001, Oenologie, vol.II, Ed. Ceres, București.

Spigno G., De Faveri D.M., 2007, Journal of Food Engineering, vol. 78 (3), 793–801.

Spigno G., Tramelli L., De Faveri D.M., 2007, Journal of Food Engineering, vol. 81 (1), 200-208.

Tiţa O., 2004, Tehnologii de obţinere a vinurilor, Ed. Universităţii Lucian Blaga din Sibiu.

Țârdea Constantin, 2007, Chimia și analiza vinului, Ed. Ion Ionescu de la Brad, Iași.

Țârdea C., Sârbu Ghe., Țârdea A., 2010, Tratat de vinificație, Ed. Ion Ionescu de la Brad, Iași.

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# THE EFFECT OF STORAGE CONDITIONS ON WINE QUALITY

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***Keywords***: *FRAP, Folin Ciocalteu, PCA, CIELab.*

## ABSTRACT

*This paper presents the influence of temperature and light on the polyphenol content, on the antioxidant activity and on the color of some homemade wines. Measurements were performed at temperatures of 10 and 18 degrees Celsius under darkness and light conditions, for two months. The antioxidant capacity was determined by a spectrophotometric analysis, using the Folin Ciocalteau and Frap method, and the color characteristics were determined with a chroma meter. In order to determine in what measure do the FRAP ($Fe^{2+}$ concentration), TPC (total polyphenol content) and color parameters contribute to the characteristics of the wine, the PCA (principal components analysis) was used. The study shows major differences in terms of parameter values measured under the mentioned conditions.*

## INTRODUCTION

According to the “French paradox”, a moderate consumption of wine during meals justifies the minimisation of the mortality index, by coronary diseases. This cardiovascular protection is attributed to polyphenolic compounds present in red wines (Cioroi and Muşat 2007, Paixao et al. 2007).

Wine polyphenols include phenolic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acid), trihydroxy-stilbenes (resveratrol and polydatin), and flavonoids (catechin, epicatechin, and quercetin) (Damianak, et al., 2000, Busuricu et al., 2008, Campanellaa et al. 2004). The polyphenolic compounds protect the biologic systems against free radicals, having the capacity to chelate metals such as iron and copper (Cioroi and Muşat 2007).

The resveratrol (3,5,49-trihydroxy-stilbene) is a polyphenol present in red wine, which has been thought to be responsible for the cardiovascular benefits associated with moderate wine consumption (Hung et al., 2000). Resveratrol suppresses proliferation of a wide variety of tumor cells, including lymphoid, myeloid, breast, prostate, stomach, colon, pancreas, thyroid, skin, head and neck, ovarian, and cervical.

A high fat diet is known to cause high levels of oxidative damage to plasma lipoproteins, which is counteracted by antioxidants present in wine. Oxidative stress is also associated with chronic diseases, including atherosclerosis, heart failure, cancer, and neurological degeneration (Guilford and Pezzuto 2011).

These compounds are one of the most important quality parameters of wines, since they contribute to the wine is organoleptic properties such as colour, astringency, bitterness (Gris 2013, Plavsa et al. 2012, Büyüktuncel et al 2014).

The colour intensity and hue of the wine depend on the amount and chemical state of the pigments that are present, and correspondingly to the quantity and quality of light reflected (Jackson 2009).

Exposure of wine to light damages the colour, flavours and the aromas which are produced by the initiation of chemical reactions, resulting in the formation of sulphurous compounds with an unpleasant smell and taste (Hartley 2008).

If wines are stored at temperatures cooler than recommended - below 10°C - they may not develop their full potential regarding aroma and flavour, and if they are stored at an elevated temperature – above 16°C, this may cause an excessive extraction of odours from the corks and it increased loss of protective sulphur dioxide or certain wine aromas (Butzke 2010).

The primary aim of this paper is thus to evaluate the antioxidant capacity and colour of wines under different conditions of storage during two months, in darkness, exposed to light, at warm (18°C), and cold (10°C).

## MATERIAL AND METHODS

***Chemical Reagents.*** Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate, gallic acid, sodium acetate, glacial acetic acid, hydrochloric acid, TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine), anhydrous ferric chloride, ferrous sulphate were purchased from Sigma - Aldrich.

***Samples of wines.*** Ten homemade wine were analysed: 2 white (W1-White Feteasca, W2- Muscat Ottonel), 7 red (W3-Cabernet, W4–Black Feteasca, W5-Pinot Noir, W6-Merlot, W7-mixture of Merlot and Black Feteasca, W8-mixture of Merlot and Cabernet, W9-mixture of Cabernet and Black Feteasca) and 1 rose (W10-Busuioaca of Bohotin).

***FRAP determination.*** This assay measures the change in the absorbance at 593 nm due to the formation of a blue-coloured Fe(II)-tripyridyltriazine compound from the colourless oxidized Fe(III) form by the action of electron-donating antioxidants (Azevedo et al. 2014). FRAP reagent was prepared by mixing acetic buffer, TPTZ and $FeCl_3 \cdot 6H_2O$ (20 mM water solution) at a ratio of 10:1:1. Briefly, to a volume of 200 µL of wine, 3.8 mL of FRAP reagent were added. After 4 min, the absorbance was measured (Plavsa et al. 2012).

***Folin Ciocalteu determination.*** The Folin Ciocalteu index is the result obtained by applying the method described in Compendium (2015), using gallic acid as standard. The phenolic compounds contained in wine are oxidized by the Folin Ciocalteu reagent. The blue coloration produced had a maximum absorption in the region of 750 nm. There was introduced into a 100 mL volumetric flask, in the following order: 1 mL of wine, previously diluted 1/5; 50 mL of distilled water, 5 mL of Folin Ciocalteu reagent; 20 mL of sodium carbonate solution and brought to 100 mL using distilled water, then mixed to dissolve. It was left for 30 minutes for the reaction to stabilize, then it was measured the absorbance.

***Chromatic parameters.*** These ones was carried out precisely using CIELab colour coordinates.

***Equipment.*** Spectrophotometer measurements for antioxidant capacity were recorded by using an UV-VIS-NIR, Shimadzu 3600, and for the colorimetric measurements a Chroma meter CR-400/410, Konica Minolta was used.

***Statistical analysis.*** In order to determine in what measure do the FRAP, TPC and color parameters contribute to the characteristics of the wine, the PCA (principal components analysis) was used. The variables was conducted using the Varimax rotation of the PC (Hernández-Martínez et al. 2016). SPSS Ver. 16.0 software was used to provide the PCA analysis.

## RESULTS AND DISCUSSIONS

The total phenol concentration in the 10 wine samples was determined spectrophotometrically according to the Folin Ciocalteu colorimetric method, by using the calibration curve of gallic acid and expressing the results as GAE.

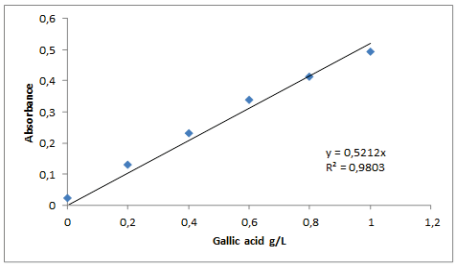


Figure 1.
Gallic Acid calibration curve.

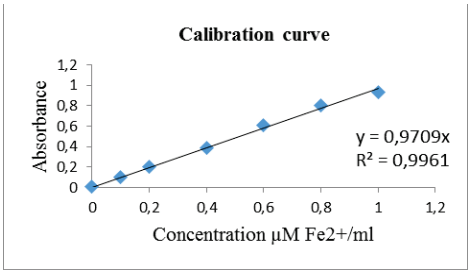


Figure 2.
Calibration curve expressed in µM Fe2+/mL.

The total phenolic content is represented in figure 3, by Folin Ciocalteu Index. The result expressed in the form of an index is obtained by multiplying the absorbance by 100 for red wines diluted 1/5 and by 20 for white wines.

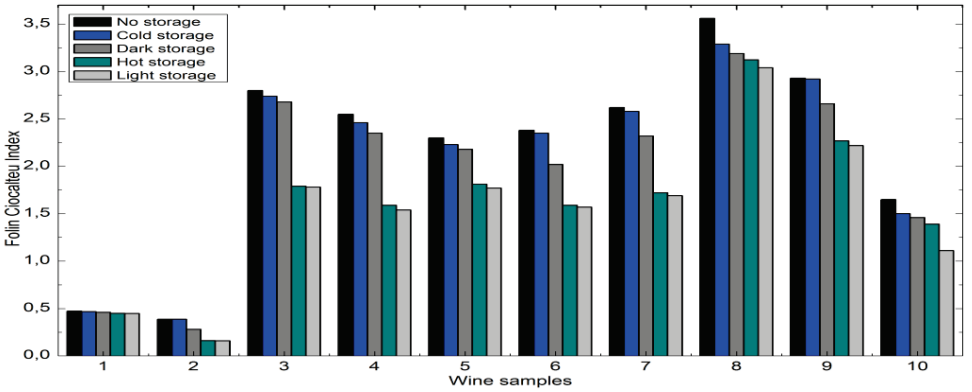


Figure 3. Folin Ciocalteu Index of wine samples under different conditions of storage.

From the graph it can be seen that the samples were stored at cold have an index value greater than the hot and samples stored in darkness retains its antioxidant capacity better than those exposed to light.

Total polyphenol content measured by the Folin method is higher for red wine and lower for white wine, while rosé wine is intermediate between red and

white wines. A calibration curve was used for the FRAP method and the concentration of antioxidant capacity was calculated and expressed in μM $Fe^{2+}$/ml.

All analysed wines demonstrate significant antioxidant capacity with the FRAP test, (fig. 4). Wines stored at 10º C had stronger reducing power than wines which were exposed to 18ºC, and the samples from the dark place had stronger reducing power than the samples exposed to light.

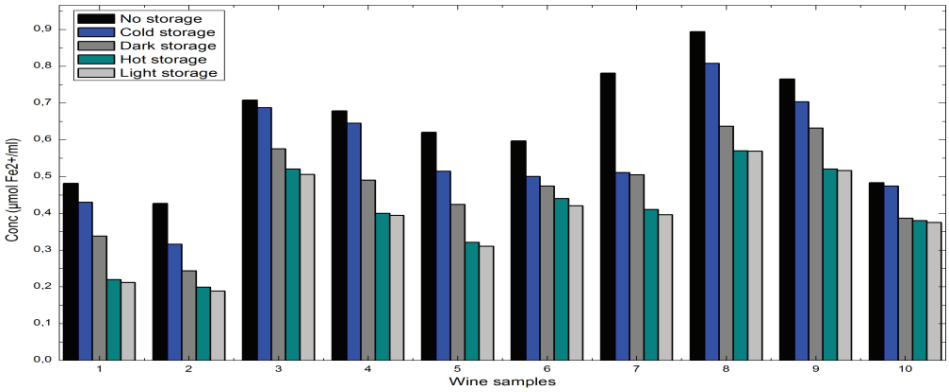


Figure 4. FRAP (μM $Fe^{2+}$/ml)

The colour of wine is very important because it can indicate an appropriate pH (2.8 ÷ 4.0), low in $SO_2$, and at an adequate ethanol concentration (10-16%), and suggest high flavour. The values of CIELab method are in the table 1.

Table 1

CIELab parameters

| | Condition | W1 | W2 | W3 | W4 | W5 | W6 | W7 | W8 | W9 | W10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **L*** | No storage | 25,9 | 28,1 | 17,4 | 17,9 | 20,5 | 23,1 | 24,4 | 17 | 17,5 | 18,8 |
| | Dark | 26,0 | 28,1 | 17,5 | 18,2 | 20,8 | 23,4 | 24,9 | 17,0 | 17,7 | 19,1 |
| | Cold | 28,3 | 29,1 | 17,7 | 18,7 | 21,3 | 24,8 | 25,0 | 17,3 | 18,1 | 19,4 |
| | Hot | 30,4 | 29,8 | 17,8 | 18,6 | 22,1 | 24,9 | 26,0 | 17,7 | 18,3 | 21,2 |
| | Light | 31,8 | 30,1 | 17,8 | 18,9 | 22,9 | 25,0 | 26,8 | 18,4 | 18,4 | 21,5 |
| **a*** | No storage | 0,71 | 3,28 | 2,58 | 6,85 | 3,43 | 10,8 | 7,24 | 3,12 | 5,86 | 5,61 |
| | Dark | 0,62 | 3,11 | 2,72 | 3,39 | 3,91 | 10,7 | 6,2 | 3,19 | 5,58 | 2,3 |
| | Cold | 3,66 | 2,38 | 5,94 | 6,43 | 3,03 | 8,71 | 7,59 | 5,94 | 5,79 | 5,82 |
| | Hot | 1,41 | 1,26 | 6,44 | 4,33 | 5,74 | 9,50 | 5,47 | 3,36 | 7,13 | 3,79 |
| | Light | 1,57 | 1,3 | 6,59 | 4,46 | 5,92 | 9,63 | 5,58 | 3,5 | 7,26 | 3,99 |
| **b*** | No storage | 6,59 | 9,72 | 1,08 | 2,17 | 4,23 | 6,37 | 7,65 | 1,09 | 1,88 | 1,81 |
| | Dark | 6,92 | 9,78 | 1,26 | 2,31 | 4,34 | 6,49 | 7,88 | 1,22 | 2,08 | 1,86 |
| | Cold | 7,82 | 9,92 | 1,82 | 3,2 | 4,04 | 6,46 | 7,82 | 1,82 | 2,3 | 3,19 |
| | Hot | 9,20 | 9,67 | 2,11 | 3,23 | 6,29 | 6,89 | 8,68 | 1,08 | 2,77 | 4,75 |
| | Light | 9,34 | 9,86 | 2,18 | 3,31 | 6,11 | 7,01 | 8,83 | 1,12 | 2,82 | 4,87 |

W1-W10- Wines sample

With CieLab, colour is described in a uniform tree dimensional space: L*, a*, b* (Birse 2007). Thus, L* is a measure of luminosity. An L* value of 0 represents pure black whereas L*=100 represents pure white. a* is a measure of the redness or greenness of the color. Positive values of a* represent a redder value, negative values represent more green. The b* value is a yellow-blue measurement. Positive values of b* represent a more yellow sample, negative values a more blue sample (Bain 2009).

Color analysis characterized by cartesian coordinates proves that the wine brightness does not show significant changes at darkness and at 10°C storage, but increase with exposure to light and to 18℃ so that the color change can be easily observed.

A PCA was done taking into account CA and TP values, together with chromatic parameters belonging to all analysed samples. Statistical program restrains all data to two principal components covering the 85,75% of the variance (PC1 captured 64,97% and PC2 20,78% of the variance).

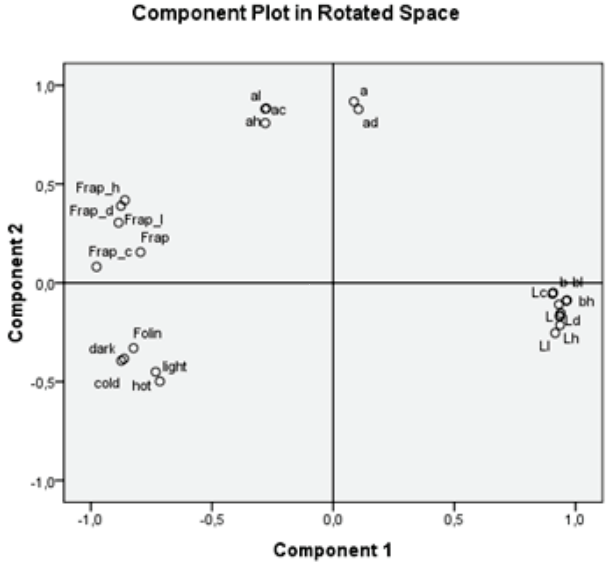


Figure 5. Principal Component Analysis.

Frap_c (Frap-cold), bl (b*- light), Ld (L* - dark), L (L*- no storage), Lc (L*-cold), bc, (b*- cold), Ll (L*- light), b (b* - no storage), bd (b* - dark), Frap_d (Frap-dark), Frap_h (Frap_hot), ah (a* - hot), hot (Folin-hot), Frap_l (Frap – light), Dark (Folin – dark), Cold (Folin – cold), Folin, (Folin – no storage), Frap (Frap – no storage), Light (Folin – light), a (a* – no storage), ad, (a* - dark), al ( a* -light), ac (a* - cold), Lh (L*- hot), bh (b* - hot).

The variables b, bd, bl, bh, bc, L, Ld, Lc, Lh Ll, Frap, Frap_d, Frap_l, Frap_h, Frap_c and Folin, dark, hot, light, cold which is near the first PC (principal components) of variables clearly differentiate the direction a, ad, ac, ah, al which is on the second PC. Among the group of variables b*, L*, variable FRAP and Folin, and the group a* has no correlative relationship. It can be seen that in plotting points b-bd and L-Ld and dark-cold are very close this warning that b-bd variables and L-Ld correlates strongly and positively in the direction of PC1 and variable cold-dark is correlate strongly powerful and negative in the direction PC1.

## CONCLUSION

The total antioxidant capacity decrease due to reduction of total polyphenols content through the ageing of wine. The study results confirm that the improper storage conditions (light and heat) had a negative impact on total antioxidant capacity, polyphenol content and color, so that the wines must be stored in cool and dark places in order to preserve the quality.

The PCA statistical analysis describe a strong correlation between the total antioxidant capacity, the content of polyphenols and b *, L * colour parameters.

## REFERENCES


Azevedo J. et al., 2014, Migration of phenolic compounds from different cork stoppers to wine model solutions: antioxidant and biological relevance. Eur. Food Res. Technol. 239, 951-960.

Busuricu F. et al., 2008, The evaluation of the wines antioxidant activity. Innovative Romanian Food Biotechnology, Vol. 2 No. 2.

Butzke C., 2010, Wine storage guidelines. Commercial Winemaking Production Series, Purdue University.

Büyüktuncel E., et al., 2014, Comparison of total phenolic content and total antioxidant activity in local red wines determined by spectrophotometric methods. Food and Nutrition Sciences, 5, 1660-1667.

Campanellaa L. et al., 2004, Biosensors for determination of total and natural antioxidant capacity of red and white wines: comparison with other spectro-photometric and fluorimetric methods, Biosensors and Bioelectronics 19, 641-651.

Cioroi M., Muşat C.L., 2007, Investigations on the correlations between polyphenol content from red wines and their antioxidant capacity, polyphenol content from red wines and antioxidant capacity, Cercetări Agronomice în Moldova, 4, 132.

Compendium of International Methods of Wine and Must Analysis, International Organisation Of Vine And Wine, Edition 2015, Volume 1.

Damianaki A., et al. (2000). Potent inhibitory action of red wine polyphenols on human breast cancer cells. Journal of Cellular Biochemistry 78, 429-441.

Gris E.F., 2013, Phenolic profile and effect of regular consumption of Brazilian red wines on in vivo antioxidant activity. J. Food. Comp. Anal., 31-40.

Guilford J.M., Pezzuto J.M., 2011, Wine and Health: A Review. Am. J. Enol. Vitic. 62:4.

Hartley A., 2008, The effect of ultraviolet light on wine quality, Wrap, Material change for a better environment.

Hernández-Martínez V., et al., 2016, Color, phenolic composition and antioxidant activity of blue tortillas from Mexican maize races. CyTA - Journal of Food, 14, 3, 473-481.

Jackson R.S., 2009, Wine Tasting: A Professional Handbook, Second Edition, Elsevier Inc.

Hung L.-M., et al., 2000, Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovascular Research 47, 549-555.

Paixao N. et al., 2007, Relationship between antioxidant capacity and total phenolic content of red, rose and white wines. Food Chemistry 105, 204-214.

Plavsa T. et al., 2012, The influence of skin maceration time on the phenolic composition and antioxidant activity of red wine Teran (*Vitis vinifera* L.). Food Technol. Biotechnol. 50 (2), 152-158.

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# INFLUENCE OF LACTIC ACID ADDITION ON COLOR AND CHEMICAL PROPERTIES OF FRESH PREPARED ORANGE JUICE

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***Keywords***: *fresh prepared orange juice, lactic acid, organolpetical characteristics, chemical properties*

## ABSTRACT

*The aim of this study was to establish the influence of lactic acid (LA) in preservation of fresh prepared orange juice (FPOJ) at $22^0$C and $4^0$C, respectively. The lactic acid was added in two different mass percentages (0.02 wt% and 3 wt%, respectively). After 6 days of storage at $22^0$C, the organoleptical parameters of FPOJ modified with lactic acid 3 wt% were unchanged. In contrast, the unmodified FPOJ and that modified with LA 0.02 wt% were deeply altered in terms of flavour, color and texture. The most reduced mass loss was measured in FPOJ modified with LA 3 wt% after 6 days of storage at $4^0$C and $22^0$C, respectively. As we expected, the highest acidity was measured in FPOJ modified with LA 3 wt% stored in the two mentioned conditions. The content of ascorbic acid decreased during storage, but the most reduced variation was established in FPOJ modified with LA 3 wt%, at $4^0$C. The browning index increased during storage for 6 days, but the most reduced increase was observed for FPOJ modified with 3 wt% during storage at $4^0$C. Chemical treatment with 3 wt% LA is an alternative to diminish the fresh prepared orange juice alteration during storage.*

## INTRODUCTION

Organic acids are added in food products as preservatives and antioxidants. The concentration of these acids is subjected to regulation, since in great amounts they are harmful for the people health. Especially, lactic acid is used in a wide range of food applications such as bakery products, beverages, meat and dairy products, salads, dressings, ready meals, etc. Lactic acid in food products usually serves as either as a pH regulator or as a preservative. It is also used as a flavoring agent (Amelin et al. 2012). In classification of food additives, lactic acid is known as (E-270). Because of its mild taste, lactic acid is used as an acidity regulator in beverages such as soft drinks and fruit juices. Lactic acid is effective in preventing the spoilage of olives. Lactic acid may be also used as a preservative in salads and dressings, resulting in products with a milder flavor while maintaining microbial stability and safety. Formulating hard-boiled candy, fruit gums and other confectionery products with lactic acid results in a mild acid taste, improved quality, reduced stickiness and longer shelf life (Stiles, 1996). Lactic acid in combination

with nisin was used to improve the microbiological quality of cold-smoked rainbow trout (Nykänen et al. 1999)

Fresh prepared orange juice is a wide consumed fruit product (Emamifar et al. 2010, Meléndez-Martínez et al. 2007). The research studies are focused in developing of innovative techniques for prolonging the shelf life of orange juice, such as: non-thermal processing techniques (Baxter et al. 2005; Han et al. 2007), techniques for decontaminating of orange juice (Gajjar et al. 2009) and nanotechnology that can potentially provide solutions to food packaging challenges (Peter et al. 2014, Emamifar et al. 2010).

The aim of this study was to establish the influence of lactic acid (LA) in preservation of fresh prepared orange juice (FPOJ) at $22^0$C and $4^0$C, respectively, in order to establish the preservation activity of lactic acid.

## MATERIALS AND METHODS

**Samples preparation.** The oranges were purchased from a local market in Baia Mare, Romania. The fresh prepared orange juice (FPOJ) contains pulp and was obtained by manually squeeze of the washed oranges. A volume of 60 mL of FPOJ modified with lactic acid (LA) 0.02 wt% and 3 wt%, respectively, was introduced in a plastic flask. The two concentrations of LA were selected by consulting the literature data (Huang et al. 2008). For reference, unmodified samples were also prepared. The samples were kept for 6 days at $22^0$C in a vegetation room and at $4^0$C in a refrigerator.

**Organoleptical analysis.** The organoleptical analysis consisted in monitoring of color, aspect, texture, flavour and presence of microorganism colonies.

**Mass loss.** Mass loss consisted in measuring the mass of the flask at begining and ending of the experiment. It was determined with the formula:

$$Mass\ loss(\%) = \frac{mass_{initial} - mass_{final}}{mass_{initial}} x100 \quad (1)$$

**Acidity.** Acidity was determined according the method decribed in our previous study (Peter et al. 2015).

**Ascorbic acid content and browning index.** Ascorbic acid content and browning index were measured according to Emamifar et al. (2010).

The analyses were performed in triplicate and the level of confidence did not exceeded 95%.

## RESULTS AND DISCUSSIONS

**Organoleptical analysis.** At the start of the experiments, FPOJ (noted as blank) has a orange color, homogeneous, with flavor and taste of oranges. The macroscopic views of the samples during storage are illustrated in Figure 1a-f. The samples kept for 2 days at $22^0$C (Figure 1a) and at $4^0$C (Figure 1b) were identical with the blank sample, in terms of organoleptical aspects. No differences between the reference and samples modified with LA were observed. After storage for 4 days at $22^0$C (Figure 1c), reference (sample 7) and sample with LA 0.02 wt% (sample 8) are deeply altered and discolored and the pulp is at the botom of the flask. The flavor is altered. Colonies of moulds appeared onto the surface of

samples 7 and 8. Sample modified with LA 3 wt% was almost identical with blank. After 4 days of storage at $4^0$C (Figure 1d), some changes appeared to reference (sample 10) and sample with LA 0.02 wt% (sample 11) consisting in deposition of the pulp at the botom of the flask, light discoloring and slight fermented odor. Sample modified with LA 3 wt% was similar with blank, homogeneous, with orange color and agreable flavour. Reference (sample 13) and sample modified with LA 0.02 wt% (sample 14) stored at $22^0$C (Figure 1e) for 6 days are deeply altered in terms of texture, color and flavour. The organoleptical characteristics of these samples are: heterogeneity, deeply discoloring, unpleasant falvour, presence of mould colonies onto the whole surface of the juice. Sample modified with LA 3 wt% was little affected, meaning that is homogeneous, the color remained orange and the falvour was agreable. The samples kept for 6 days at $4^0$C (Figure 1f) have suffered some changes during storage.The pulp is deposited at the botom of the flask in all samples, the color has suffered slighlty discoloring and the flavour does`nt recorded significantly changes. Sample modified with LA 3wt% looks best as the others in terms of color and flavour.

**Mass loss.** The variation of mass loss after 6 days of storage is presented in Figure 2. The mass loss decreases in order: reference, sample with LA 0.02 wt% and with LA 3 wt%, for the samples stored at $22^0$C as well as at $4^0$C. The mass loss is significanlty lower for the samples stored at $4^0$C. The mass loss for the sample with LA 3 wt% at $22^0$C is lower by 10% than that of the reference at $22^0$C and in the case of the samples stored at $4^0$C, the percentage is ~ 24%. By comparing the mass loss of the samples at two temperatures, it can be observed that the mass loss at $4^0$C is lower by ~ 70% than that at $22^0$C.

**Acidity.** Acidity increased in all investigated samples during 6 days of storage (Figure 3). The most accentuated increase was observed for the samples modified with LA 3 wt% at $22^0$C as well as at $4^0$C, as a result of the lactic acid presence in orange juice. The acidity of the reference at $22^0$C increased by 48% during storage as compared with that at $4^0$C who increased by remained almost unchanged. The increase in the acidity of reference at $22^0$C is explained by the fermentation reaction occuring in the sample during storage that generates acidic compounds (Peter et al. 2014).

**Ascorbic acid content and browning index.** The variation of ascorbic acid content is illustrated in Figure 4. The content of ascorbic acid dropped in all samples durring storage as a result of degradation and fermentation processes (Peter et al. 2014; Emamifar et al. 2010) and due to the non-barrier properties of packaging against oxygen (Fellers, 1988; Sadler et al. 1992). The most accentuated decrease was observed for reference at $22^0$C, followed by sample with LA 0.02 wt%, reference at $4^0$C, sample with LA 0.02 wt% at $4^0$C. The most reduced drop was achieved for the samples modified with LA 3 wt%. Browning index is a parameter that monitors the antioxidant activity.High values of browning index demonstrates a high concentration of toxic compounds like cetones, aldehydes, acids, quinones, generated by non-enzimatic oxidation of phenols, stimulated by light, oxygen, heat (Plaza et al. 2006).

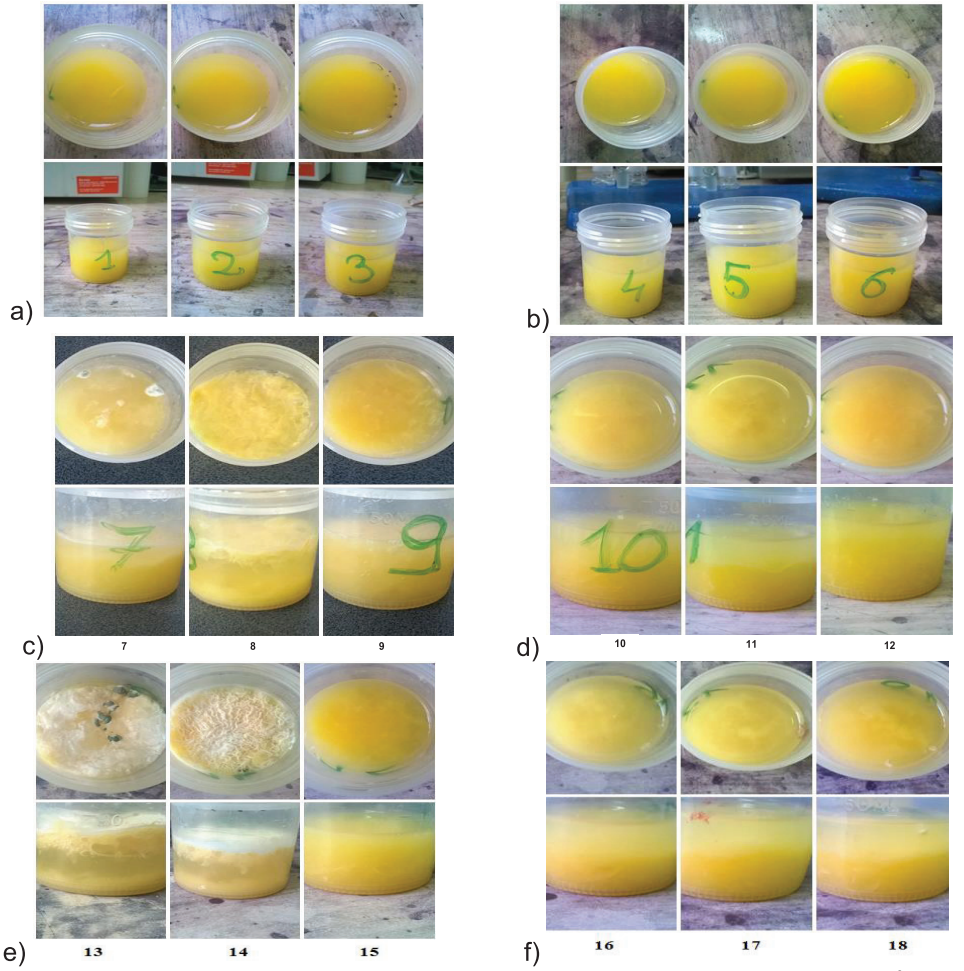


Figure 1. Macroscopic view of flasks with FPOJ after 2 days of storage at $22^0$C a) and $4^0$C b), after 4 days of storage at $22^0$C c) and $4^0$C d) and after 6 days of storage at $22^0$C e) and $4^0$C f) (1, 4, 7, 10, 13, 16 - reference sample; 2, 5, 8, 11, 14, 17 - sample with LA 0.02 wt%; 3, 6, 9, 12, 15, 18 - sample with LA 3 wt%)

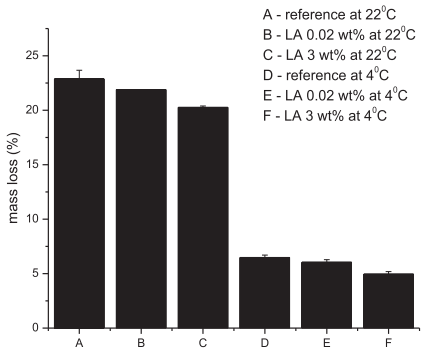


Figure 2. Variation of mass loss after 6 days of storage.

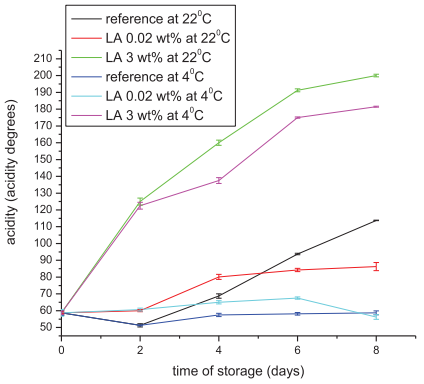


Figure 3. Profiles of acidity variation.

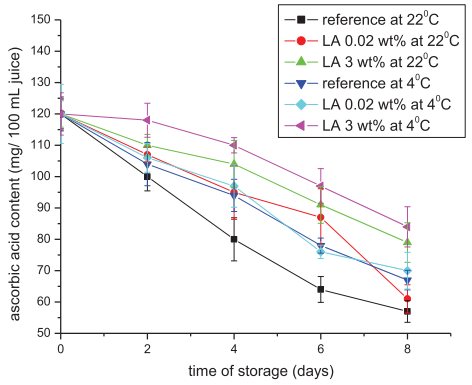


Figure 4. Profiles of ascorbic acid content variation.

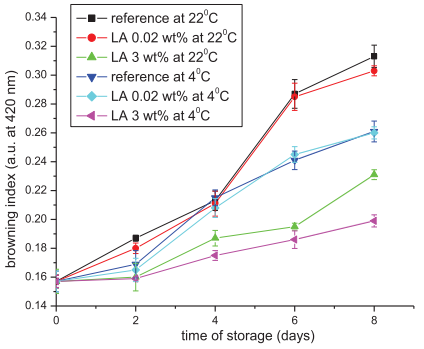


Figure 5. Profiles of browning index variation.

The variation of browning index is presented in Figure 5. The values peaked in all samples as a result of non-enzimatic oxidation occuring during storage. The most accentuated increase was observed for reference and sample with LA 0.02 wt% at 22$^0$C, followed by reference and sample with LA 0.02 wt% at 4$^0$C and the most diminished increase was assigned for samples modified with LA 3 wt%.

## CONCLUSIONS

The aim of this study was to establish the influence of lactic acid (LA) in preservation of fresh prepared orange juice (FPOJ) at 22$^0$C and 4$^0$C, respectively. The results of this study have demonstrated that the fresh prepared orange juice can be treated with lactic acid 3 wt% in order to preserve their organoleptical properties, ascorbic acid level, browning index and mass loss even at 22$^0$C.

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## REFERENCES

Amelin V.G. et al., 2012, Determination of Organic Acids in Alcoholic and Nonalcoholic Beverages by Reversed-Phase High-Performance Liquid Chromatography, J. Analyt. Chem., 67(3): 262–268.

Baxter I.A., Easton K., Schneebeli K., Whitfield F.B., 2005, High pressure processing of Australian navel orange juices: Sensory analysis and volatile flavor profiling. Innovative Food Science and Emerging Technologies, 6: 372−387.

Emamifar A. et al., 2010, Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice, Innov. Food Sci. Emerg. Technol. 11: 742–748.

Fellers P.J., 1988, Shelf life and quality of freshly squeezed, unpasteurized, polyethylene-bottled citrus juice. J. Food Sci., 53: 1699−1702.

Gajjar P. et al., 2009, Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, Pseudomonas putida KT2440. J. Biol. Eng., 3(9): 1183-1189.

Han J.H., 2007, Packaging for nonthermally processed foods. In J.H. Han (Ed.), Packaging for nonthermal processing of food (pp. 213−225).

Huang R.H. et al., 2008, Effect of salicylic acid on the antioxidant system in the pulp of 'Cara cara' navel orange (*Citrus sinensis* L. Osbeck) at different storage temperatures. Postharvest Biol. Technol. 47: 168-175.

Meléndez-Martínez A.J. et al., 2007, Review: Analysis of carotenoids in orange juice. J. Food Comp. Anal. 20: 638-649.

Nykänen A. et al., 1999, Applicability of lactic acid and nisin to improve the microbiological quality of cold-smoked rainbow trout, Z Lebensm Unters Forsch A, 208: 116–120.

Peter A. et al., 2015, Testing the preservation activity of Ag-TiO2-Fe and TiO2 composites included in the polyethylene during orange juice. J. Food Proc. Eng. 37: 596-608.

Plaza L. et al., 2006, Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization, European Food Res. Technol., 223: 487-493.

Sadler G.D. et al., 1992, Microbial, enzymatic, and chemical challenges during storage of fresh and processed orange juice. J. Food Sci., 57(5): 1187-1192.

Stiles M.E., 1996, Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek, 70: 331-345.

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# THE DESIGN OF HACCP SYSTEM IN MIXTURE OF SEAFOOD CHOPPED TECHNOLOGY

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**Keywords**: food savety, HACCP system, mixture of seafood chopped

## ABSTRACT

*This paper presents the designing and developing a system for monitoring the quality of a mixture of seafood chopped by drawing HACCP plan corresponding obtaining product technology and operational control programs. The biological, chemical, and physical hazards were analyzed and critical control points were identified. Such was studied the probability that potential hazard relevant to consumer health, under certain conditions, exceed a threshold limit and depending on severity and frequency of the event to turn into a potential risk to human health at consumption time. The HACCP system has been studied as a preventive control method of food safety, moving the central pole of the finished product to control a modern method, proactive and control potential hazards.*

## INTRODUCTION

Even if seafood do not have tradition of growing, harvesting, processing and consumption in Romania, they started to become more popular and present in consumer food in our country. Both fish and seafood, were represented in time as one of the basic foods.

The mussels are widely used as bio-indicators to monitor health status of aquatic environments, both freshwater and marine. Population, structure, physiology, behavior or the contamination level of mussels with elements or compounds, can indicate the status of the ecosystem Mussels and other seafood consumed phytoplankton containing nutrients such as nitrogen (N) and phosphorus (P). A living mussels contains an average of 1.0% N and 0.1% P (Lindahl et al. 2005).

Omega-3 fatty acids are essential fatty acids found primarily in fish and seafood, nuts and some plants. Consumption of omega-3 fatty acids may help reduce the risk of disease cancer, heart disease and rheumatoid arthritis. A portion of 100 grams of squid contain 630 milligrams of omega-3 fatty acids. The squid is one of the best sources of omega-3 fatty acids (http://www.livestrong.com/article /538120-omega-3-in-squid/).

The overall quality of seafood (the characteristic properties which determines degree of excellence) includes both the healthiness and sensory

acceptability of products by consumers. Healthiness is affected by the chemical composition (content valuable nutritional components) in particular proteins, lipids, vitamins and minerals and the presence of compounds or come from the environment, such as toxins and pollutants, but also through bacterial contamination and parasites (Sikorski et al. 1988).

Food safety is related to the presence of hazards in food origin foods when consumed. Since the introduction of food hazards can occur at any stage of the food chain, adequate control throughout the food chain is essential so food safety is ensured through the combined efforts of all parties participating in the food chain, from feed manufacturers and to retail stores (Rotaru et al. 2005).

HACCP is a world-recognized, effective, and preventive food hygiene management system. At present, the HACCP system has been widely adopted by many countries such as the United States, Japan, the United Kingdom, and member states of the European Union, as well as international organizations such as the World Health Organization, Food and Agriculture Organization, and Codex Alimentarius Commission (CAC) (Meng et al. 2011; Junchao Lu et al. 2014).

HACCP is a management system efficacious of food safety in production, distribution and preparation, more effective control of operations, because the role of inspectors is centered on compliance HACCP plan, on confirming its effectiveness (Rotaru and Moraru 1997).

HACCP is defined as "a system designed to logically hazard identification and/or critical situations to establish a structured plan their control. " "HACCP is an activity developed to identify and control potential hazards that may be critical to the health of the consumer. " "HACCP is a method of systematic approach to ensuring food innocuousness, based on the identification, evaluation and control of all hazards that could interfere in the manufacturing, handling and distribution processes "(Rotaru and Moraru 1997; http:// www.codexalimentarius.com).

Application of HACCP system involves certain foods produced and consumed safely by performing control in all processes: from farm to table (Mencinicopschi and Raba 2005).

The Hazard Analysis System in the Critical Control Points is a systematic approach of the desire to food safety and consists the application of 7 basic principles: the risks associated evaluation with obtaining raw materials and ingredients, processing, handling, storage, distribution, preparation and consumption of food cooking; determination of the critical control points which can keep under control the identified risks; the establishment critical limits which must be respected in each critical control point; the establishment of the procedures for monitoring of critical control points; the establishment corrective actions to be applied when, in after monitoring critical control points, it detected a deviation from the critical limits; the establishment an efficient system of keeping records, which is HACCP system documentation; the establishment procedures that will verify if system HACCP is working correctly (Mencinicopschi and Raba 2005; Ștețca et al. 2012).

## MATERIAL AND METHODS

Current systems HACCP are based on 7 principles defined by FAO (Food and Agriculture Organisation)/ WHO (World Health Organisation) and The Codex Alimentarius Commission (CAC), which introduced the HACCP principles in a document provides guidelines for establishing and maintaining HACCP plans in all

department food industry -CAC/RCP 1-1969, which was adopted in our country as standard SR 13462-2: Agricultural and food hygiene. Hazard Analysis and Critical Control Point (HACCP) and its application guide (Rogoz 2005).

## RESULTS AND DISCUSSIONS

The flow technological of the mixture of seafood chopped is shown in Fig.1, including raw material reception, raw material storage, raw material processing, chopping, mixing, homogenization, seasoning, packing, labeling, storage and delivery. The results of the study published in this paper are shown in tables 1, 2 and 3. In table 1 were identified critical control points from the reception of materials to the delivery of the final products also hazard analysis was performed at every technical stage to define type risk (biological, chemical and physical) and risk degree (1, 2, or 3) that may affect food safety.

Table 2 consists of HACCP plan prepared for obtaining technology mixture of seafood chopped and table 3 represent operational control programs specific for each stage of the technological process with reference to establishing monitoring procedure, corrective measures, responsible and records.

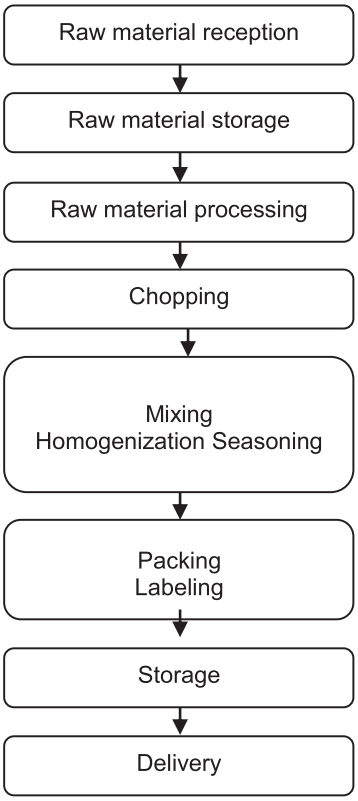


Figure 1. The mixture of seafood chopped technological process.

## CONCLUSIONS

A HACCP study will not always result in eliminating all risk, but allows the most efficient decisions to reduce to an acceptable level of those identified. Following the study, the findings have outlined some defining, namely, the food industry operator is primarily responsible for food safety, the food industry operator responsibility is to apply procedures based on HACCP principles, namely the use of good hygiene practices.

Food safety must be ensured across the food chain, starting from primary production to finished product. It was performed to identify any hazards that must be prevented, eliminated or reduced to acceptable level and to identify the critical points where checks are indispensable. It was also implemented some effective monitoring procedures at critical points and the necessary corrective measures in cases where monitoring shows that a critical point is not under control.

These principles applied contain a number of requirements that must be met during the entire cycle of production, processing and distribution to enable, based on hazard analysis, identification of critical points that must be taken under control to ensure food safety.

## REFERENCES


Junchao L., Xiao-Hui P., Chi-Te L., Che-Lang C., Kuan-Chen C., 2014, The implementation of HACCP management system in a chocolate ice cream plant. Journal of Food and Drug Analysis, 22, 391-398.

Lindahl O., Hernroth R., Kollberg S., Loo L.-O, Olrog L., Rehnstam-Holm A.-S., Svensson J., Svensson S., Syversen U., 2005, Improving marine water quality by mussel farming: A profitable solution for Swedish society, Ambio 34 (2), 131-138.

Mencinicopschi Gh., Raba D.N., 2005, Siguranța alimentară. Autenticitate și trasabilitate, Edit. Mirton, Timișoara.

Meng L., Yang Q., Cheng J., et al., 2011, Study on the application of HACCP system in ice-cream premix powder processing. J Anhui Agric Sci., 39, 6531-6533.

Rogoz M.S., 2005, Igiena și securitatea produselor alimentare în contextul integrării României în Uniunea Europeană, Actualitatea românească. Standardizarea.

Rotaru G., Borda D., Sava N., Stanciu S., 2005, Managementul calității în industria alimentară, Edit. Academica, Galați

Rotaru G., Moraru C., 1997, HACCP – Analiza Riscurilor. Punctele critice de control, Edit. Academica, Galați.

Sikorski Z.E., Sawyer B. Sun Pan et al., 1988, Preservation of seafood quality, 168-195.

Ștețca Gh., Pop A., Mocuța N., 2012, Strategii de management privind calitatea alimentelor. Edit. Risoprint, Cluj-Napoca.

*** http://www.livestrong.com/article/538120-omega-3-in-squid/

*** http://www.codexalimentarius.com

Table 1

Identifying critical control points

| The stage | Type risk | Risk degree | 1. There is at this stage preventive measures identified hazard? | 2. Delete the hazard at this stage or decreases its probability of repetition to a acceptable level? | 3. Contamination with the identified hazard could exceed acceptable levels? | 4. The hazard may be eliminated or its probability may be reduced in a subsequent stage? | **NR. CCP** |
|---|---|---|---|---|---|---|---|
| | | | If **YES**, go to the following questions | If **NOT**, go to the next question | If **NOT –** stop, this is not a CCP | If **NOT**, this is a CCP | |
| | | | If **NOT**, required the control in this stage for safety? | If **YES**, this is a CCP | If **YES**, go to the next question | If **YES –** stop, this is not a CCP | |
| | | | If **YES**, change the stage or process changes and return to question 1; | | | | |
| | | | If **NOT**,, stop –this is a PCC establishes how and where will be controlled this risk factor | | | | |
| Raw material reception | B | 3 | Yes | Not | Yes | Not | |
| | C | 2 | Yes | Not | Yes | Not | |
| | F | 2 | Yes | Not | Yes | Not | |
| Auxiliary materials reception | B | 3 | Yes | Not | Not | | **CCP 1** |
| | C | 2 | Yes | Not | Yes | Not | |
| | F | 2 | Yes | Not | Not | | |
| Drinking water reception | B | 3 | Yes | Not | Not | | |
| | C | 2 | Yes | Not | Not | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Raw materials storage | B | 3 | Yes | Not | Not | | **CP 1** |
| | C | 2 | Yes | Not | Not | | |
| | F | 2 | Yes | Not | Not | | |
| Auxiliary materials storage | B | 3 | Yes | Not | Not | | |
| | C | 2 | Yes | Not | Not | | |
| | F | 2 | Yes | Not | Not | | |
| Raw materials processing | B | 3 | Yes | Not | Not | | **CP 2** |
| | C | 2 | Yes | Not | Not | | |
| | F | 2 | Yes | Not | Not | | |
| Chopping | B | 3 | Yes | Yes | Not | Not | **CCP 2** |
| | C | 3 | Yes | Yes | Not | Not | |
| | F | 3 | Yes | Yes | Not | Not | |
| Mixing Homogenization Seasoning | B | 3 | Yes | Not | Not | Not | **CCP 3** |
| | C | 3 | Yes | Not | Not | Not | |
| | F | 3 | Yes | Not | Not | Not | |
| | F | 2 | Yes | Not | Not | | |
| Packing Labeling | F | 2 | Yes | Yes | Not | | **CP 5** |
| Storage | B | 3 | Yes | Yes | Not | | **CP 6** |
| Delivery | B | 3 | Yes | Not | Not | | **CP 7** |
| | F | 2 | Yes | Not | Not | | |

B – biological, C – chemical, F – physical, CCP – critical control point, CP – critical point

Table 2

HACCP plan appropriate technologies for mixture of seafood chopped

| The stage | Hazards | Monitoring | | | | | Correction | Responsible | Records |
|---|---|---|---|---|---|---|---|---|---|
| | | what | critical limits | how | when | who | corrective measures | | |
| Raw material reception **CCP 1** | **Biological:** microorganisms specific. **Insects rodents** **Chemical**: heavy metals: Cadmium, Lead, Copper **Physical:** sand, gravel, algae, metal chips, cardboard pieces | **Sensory exam:** appearance, odor, color, the cleanliness. Coliforms, *E. colli*, Staphylo-coccus coagulate-positive, *Bacillus cereus* | According to technical specification and quality certificate issued by the supplier | Through sensory analysis, physico-chemical, micro-biological<br><br>Quality certificates verification accompanying the delivered good | Every receiving | Operator | Back to vendor Notification vendor Exclusion LAV (list of assumed vendors) Not be received without quality certificates from vendor | Raw material administrator, Acquisitions manager | Data inspection at reception, Internal analysis report |
| Chopping **CCP 2** | **Biological:** contamination by pathogenic bacteria **Chemical:** traces of detergents left after sanitation. **Physical:** chopped pieces of fish, scraps of bones | The cleanliness of the equipment chopped and rinsing his appropriate<br><br>Paste appearance | Equipment sanitized after each use<br><br>Paste appearance according to technical specification product | Sanitation monitoring sheet<br><br>Visual verification | After each use of chopper In each batch | Operator | Isolating non-conforming products Sanitation chopper Relocation paste | Technologist engineer | Monitoring sheet half-finished |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mixing Seasoning **CCP 3** | **Biological:** contamination by pathogenic bacteria **Chemical**: traces of detergents **Phycal:** mineral and organic impurities | The cleanliness of the agitator and rinsing his appropriate<br><br>Paste appearance | Equipment sanitized after each use<br><br>Paste appearance according to technical specification product | Sanitation monitoring sheet<br><br>Visual verification | Lastingly | Operator | Confiscation, destruction | Technologist engineer | Monitoring sheet half-finished |

CCP – critical control point; LAV - list of assumed vendors

Table 3

Operational control programs

| The stage | Monitoring | | | | Correction Corrective measures | Responsible | Records |
|---|---|---|---|---|---|---|---|
| | what | how | when | who | | | |
| Raw material reception | The cleanliness and maintenance of a the room | Visual according Procedure System | Before reception | Quality inspector preoperational | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | Origin according with the technical specification and quality certificate | Inspection at reception | Every reception | Administrator | Back to vendor Exclusion LAV (list of assumed vendors) | Supply manager | Register Entries |
| Water reception | Potability water | Tests laboratory | According to the plan of self-control | External laboratory | Retest Notification vendor | Quality supervisor | Analysis bulletin |
| Auxiliary materials reception | The cleanliness and maintenance of a the room | Visual according Procedure System | Before reception | Quality inspector preoperational | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | Origin according with the technical specification and quality certificate | Inspection at reception | Every reception | Administrator | Back to vendor Exclusion LAV (list of assumed vendors) | Supply manager | Register Entries |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Raw material storage | The cleanliness and maintenance of a the room | Visual | Every day | Quality inspector preoperational | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | | | | | Maintenance work | Supervisor administrativ | Evidence Preoperational control |
| | Respecting of rules storage | Visual | Every day | Quality inspector | Corresponding storage Personal training | Quality engineer | Monitoring sheet temperatures |
| | Temperature and humidity room | By two or a day | 24h/24h | Administrator | Remedial defects | Responsible automation | Monitoring sheet temperatures |
| Auxiliary materials storage | The cleanliness and maintenance of a the room | Visual | Every day | Quality inspector preoperational | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | Respecting of rules storage | Visual | Every day | Quality inspector | Corresponding storage Personal training | Handler administrator | Evidence Preoperational control |
| Raw material processing | The cleanliness and maintenance of a the room | Visual | Every day | Quality inspector | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | Respecting of good hygiene practices and food safety | Visual | Every day | Quality inspector Technologist engineer | Personal training | Quality engineer | Register samples half-finished |
| | | Of ten in ten minutes at each stage | Every day | Quality inspector Responsible automation | Personal training | Quality engineer | Register samples half-finished |
| Chopping | Respecting of good hygiene practices and food safety | Visual | Permanent | Quality inspector | Personal training | Quality engineer | Evidence Preoperational control |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Homogenization | Respecting of good hygiene practices and food safety | Visual | Every day | Quality inspector preoperational | Personal training | Responsible for sanitation | Evidence Preoperational control |
| | The cleanliness and maintenance of a the room | Visual | Every day | Quality inspector | Personal training Resanitation, repairs | Quality engineer | Evidence Preoperational control |
| Packing labeling | Maintenance of a the room | Visual | Every day | Quality engineer | Remedial defects | Responsible for sanitation | Evidence Preoperational control |
| | Metal detector | Permanent | Every day | Technologist engineer | Product confiscation | Technologist engineer | Sheet metal detector |
| | Respecting of good hygiene practices and food safety | Visual | Every day | Supervisor automation Quality engineer | Personal training | Logistics supervisor Quality engineer | Register samples |
| Storage | The cleanliness and maintenance of a the room | Visual | Every day | Quality inspector preoperational | Resanitation, repairs Personal training | Responsible for sanitation | Evidence Preoperational control |
| | Temperature and humidity cold store | By two or a day | 24h/24h | Technologist engineer Supervisor automation | Remedial defects Correction program | Supervisor automation | Monitoring sheet temperatures |
| | Respecting of good hygiene practices and food safety | Visual | Every day | Quality engineer | Personal training | Quality engineer | Register samples |
| Delivery | The cleanliness transport means goods | Visual | Before loading | Quality inspector | Resanitation, Personal training | Responsible for sanitation | Car diary |

LAV - list of assumed vendors

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# BIODEGRADABLE AND EDIBLE MATERIALS FOR FOOD PACKAGING

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***Keywords***: *biopolymer, biodegradable, food packaging*

## ABSTRACT

*This paper presents the production of films that can replace the conventional plastics used for food packaging. Seven films were made from biodegradable materials, such as agar, carrageenan, sodium alginate, chitosan, starch, glycerol, with different characteristics and applicability in a wide range of food products. The appearance, thickness, color, microstructure, and swelling ratio were evaluated. The films obtained from agar and chitosan can be used for foods with high moisture content, in contrast with those made from carrageenan, sodium alginate and starch which, due to their high solubility, as evidenced by the higher values of the swelling capacity, can be used for the packaging of low humidity products. The results showed that these biofilms can successfully replace plastics, being entirely made from natural materials, biodegradable and environmentally friendly.*

## INTRODUCTION

Lately, terms such as “biodegradable”, “biocompatible”, “eco-friendly”, “compostable”, “renewable”, “biopolymers”, “sustainability”, “green” have been intensively used, often becoming keywords in literature. This certainty points to the population's concern to the environmental pollution caused by the non/degradable solid waste and depletion of natural resources. According to the European Bioplastic, the production of bioplastic would experience an increase of 700000 tons (2010) to 1.7 Mtons (2020). (Soroudi and Jakubowics, 2013). The environmental problems have come into consumers’ view, so that they sought the use of bio packaging as an alternative to the synthetic, conventional ones, obtained from non-renewable resources.

Food packaging has to protect the product against the agency of the environment and maintain its quality through shelf life; it has to be a good commercial and communication instrument, while respecting the law. (Wikstrom, F., et al. 2014) In addition, bio packaging is non toxic and is obtained from the by-products of various sectors (agricultural, forestry), being less influenced by oil price. (Mostafa et al. 2015)

A valuable alternative for conventional food packaging is the edible films and coatings; they are actually entirely made from edible ingredients. Edible films and coatings possesses the ability to improve the barrier and the mechanical properties, sensory perception, ensuring antimicrobial protection and the possibility of extending the shelf life of the product it protects. (Pascall and Lin, 2012)

Polysaccharides, proteins, and lipids are the most widely used ingredients for the biofilms production. Polysaccharides- based films have a hydrophilic character and many hydrogen bonds in the structure, fact that promotes the addition of additives (flavors, colors, antioxidants, and micronutrients); it also ensures protection against oxidation, but low moisture protection. Same properties are found in protein-based films, due to their hydrophilic nature. (Siah and Aminah 2015, Elsabee and Abdou 2013, Falguera et al. 2011) The lipid-based films presented a good moisture barrier, but low mechanical properties due to their hydrophobic nature. The inconvenience due to individual component films can be reduced by the use of composite films. (Hernandez- Izquierdo and Krochta 2008)

The study aimed at the development of edible and biodegradable films using natural ingredients (agar, chitosan, carrageenan, starch, sodium alginate) that can be used for the production of food packaging materials.

## MATERIAL AND METHODS

### Materials

Agar (A), chitosan (CH), starch (S), carrageenan (CR), sodium alginate (SA), glycerol were purchased from Sigma-Aldrich; all substances are edible, and their ingestion is safe.

### Film preparation

To obtain the film-forming solution was pursued the modified method presented by Rhim, J.W. 2013. Thus, the development of edible films was carried out according to the Table 1. The solution was cast on a silicone support and maintained at ambient temperature until complete dryness.

Table 1

Ingredients used for film- forming solutions

| Assay | Film | A mass (g) | SA mass (g) | CR mass (g) | CH mass (g) | S mass (g) | Glycerol mass (g) | Volume of water (ml) |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | A | 1 | - | - | - | - | 0.5 | 100 |
| $P_2$ | A-CR | 0.5 | - | 0.5 | - | - | 0.5 | |
| $P_3$ | CR | - | - | 1 | - | - | 0.5 | |
| $P_4$ | A-CR-SA | 0.5 | 0.25 | 0.25 | - | - | 0.5 | |
| $P_5$ | A-SA | 0.5 | 0.5 | - | - | - | 0.5 | |
| $P_6$ | A-CH* | 0.5 | | | 0.5 | - | 0.5 | |
| $P_7$ | A-S | 0.5 | | | | 0.5 | 0.5 | |

*To obtain these films 1 ml of glacial acetic acid necessary for solubilization of chitosan was used; the final solution was obtained by homogenizing (400 rpm- 30 minutes) the two distinct solutions (agar/glycerol and chitosan/acetic acid/glycerol).

### Films characterization

The physical properties (appearance, color, odor, taste, thickness) were inspected visually and by touch. The thickness was performed by the electronic micrometer, at least five distinct zones of the surface of the films, noting the average thereof.

### Film microstructure

Was performed using stereo microscope Motic Microscope. Films have been observed in initial form and after immersion in water.

**The color of film surface**

It was performed using Chroma-Meter CR400 colorimeter (Konika Minolta); white standard was used as background (L*= 94.4, a* = -5.4, b*=8.82). For L*, a*, b* values three readings on different areas of the sample surface were performed.

**Transmittance determination**

It was performed using the method described by Sing, T.P. et al. 2015: film strips (3 cm x 1cm) were placed in a cuvette with distilled water and the percentage of transmittance at a fixed wavelength (660 nm) was read, using the Ocean Optics HR 4000 CG-UV-NIR spectrophotometer.

**The determination of solubility**

For this step, strips of biofilms were cut and immersed in distilled water for different periods of time. They were weighted before and after water immersion, the result being calculated with the formula (Atef et al. 2015):

$SR\ (\%) = (W_s - W_u)/ W_u \times 100$

where $W_s$, $W_u$ represent the swollen/ unswollen film strip, in grams.

**Moisture content determination**

The method used was based on the one described by Kumar Reddy et al. 2015: 3 cm x 3 cm film strips were weighted to the analytical balance and dried for 24 hours in hot air oven, reweighted after the completion of this period, the results being obtained according to the formula (Kumar T. et al. 2015):

$MC\ (\%) = (W_0 - W_1)/W_0 \times 100$

where $W_0/W_1$ is the initial/after drying mass, in grams.

## RESULTS AND DISCUSSIONS

**Film characterization**

**$P_1$** shows low adherence and could be easily removed from the drying surface; the film presents the uniform distribution of the agar granules in the matrix, it is fine, smooth, transparent, without taste or smell, with light yellow colour, and a drying time of 48 hours. And **$P_2$** could be easily removed from the silicon material; it was smooth, pleasant to the touch, without odour and taste, with dark yellow colour, dried after 60 hours. **$P_3$** was fine, smooth, yellow colour (lighter than **$P_1$** and **$P_2$**), the granules tend to agglomerate in the middle of the matrix film, breaking tendentious, dried after 96 hours. **$P_4$** is a film with fine surface, pleasant to the touch, without taste or smell, dark yellow colour; drying time: 72 hours. **$P_5$** was fine, smooth, and matte, with dark yellow colour, without taste or smell, dried after 36 hours. **$P_6$** assay presented strong adherence to the silicone surface, was very thin, glossy, transparent, with strong smell of acid acetic; drying time: 48 hours. **$P_7$** presented a rough surface, less glossy, without taste or smell, with yellow colour, relatively transparent (less than **$P_6$** and **$P_1$**), dried after 48 hours.

**The colour of the film surface**

Food packaging appearance still presents a great interest in the product selection. Therefore, the colour of film surface is an important parameter for the creation of such material. The results obtained are listed in table 2. Except **$P_4$** assay, which presents lower value for the brightness and higher value for b* (yellow), apparent with the naked eye; the other samples do not present special changes in L*, a*, b* values. The agar/chitosan – based film presents the highest values of L* and a*, aspect met in literature, as well. (Sing et al. 2015)

**The determination of transmittance**

The transmittance is the film ability to present barrier properties against light. It is an important parameter, especially when films are used as food packaging material, preventing lipid oxidation caused by UV rays. The results are noted in Table 2. The highest value is highlighted in $\mathbf{P_1}$ assay, and the lowest for the film made from agar and chitosan. This fact could indicate the chitosan capacity not to allow the UV rays to pass through the material. Same results were noted by Pal Singh et al. 2015 and Lopez- Mata et al. 2013 – chitosan sample showed the lowest values of transmittance. We can conclude that, in terms of transmittance, the films obtained from agar and chitosan or sodium alginate, plasticized with glycerol, may represent a suitable material for food packaging, by preventing UV transfer in the food they protect.

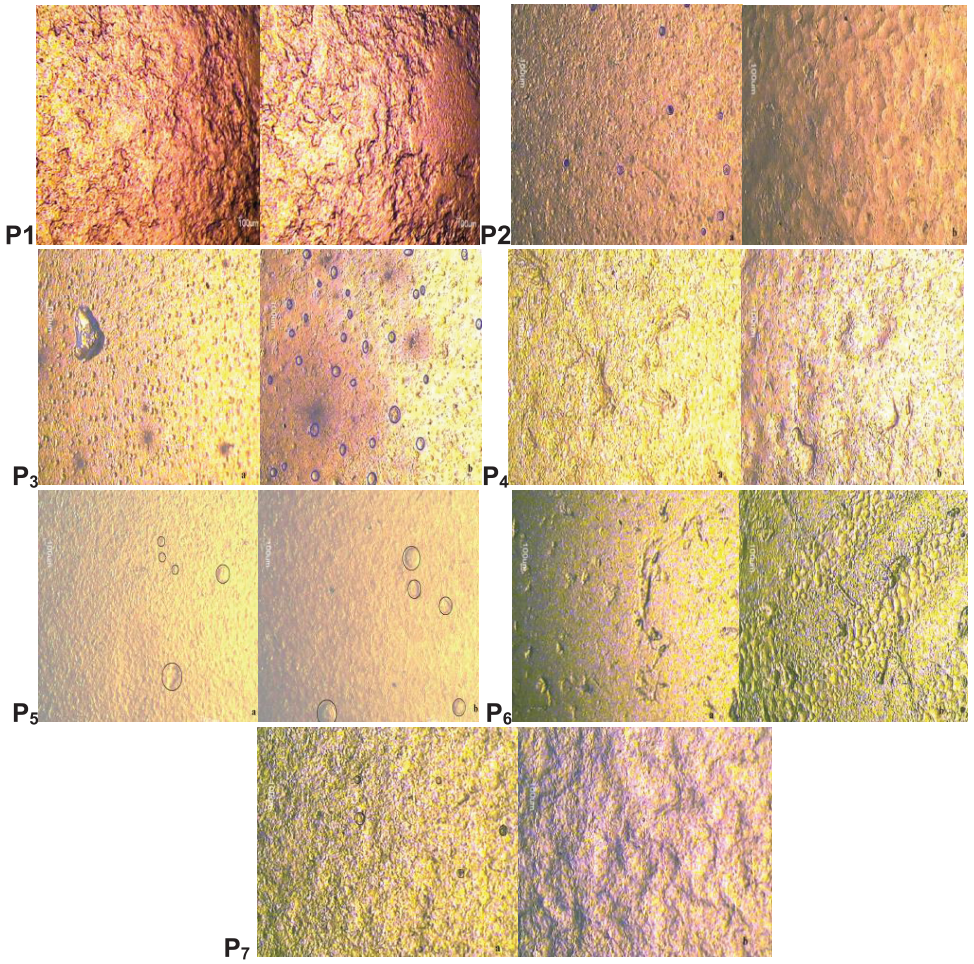


Figure 1. The figures of film microstructure: dried form (a), wet form (b).

**Determination of moisture content**

The moisture content is the amount of water from a material, emphasizing its hydrophilic character. This property is important in the choice of packaging material, since it influences the shelf life of the product which it protects (impropriety can cause damage to the product). The results are shown in Table 2; the presented values highlight the hydrophilic character of films, as evidenced by the results shown in fig 2.

Table 2

Physical characteristics of samples

| Assay | Colour | | | Thickness (μm) | Transmittance (%) | MC (%) |
|---|---|---|---|---|---|---|
| | L* | a* | b* | | | |
| $P_1$ | 90.28 | -4.84 | 15.91 | 55 | 5.308 | 16.769 |
| $P_2$ | 90.82 | -5.08 | 15.59 | 78 | 5.083 | 31.21 |
| $P_3$ | 90.90 | -5.20 | 16.01 | 80 | 4.582 | 32.72 |
| $P_4$ | 91.47 | -5.39 | 14.96 | 70 | 4.855 | 28.23 |
| $P_5$ | 92.03 | -6.22 | 15.74 | 72 | 4.035 | 15.38 |
| $P_6$ | 88.74 | -4.91 | 20.45 | 58 | 4.335 | 16.4 |
| $P_7$ | 90.29 | -5.13 | 16.15 | 78 | 4.716 | 9.94 |

**Solubility determination**

Solubility is an important characteristic of a material used for packaging; it can be appreciated as film capacity to maintain its integrity after liquid immersion. [Rhim et al. 2000] Soluble edible packaging is advantageous for ready-to-eat products, since it is dissolved in mouth or hot water. This is extremely important in ecosystem protection because in the end there will be zero waste.

All samples were solubilised; **$P_3$** was totally disintegrated after 60 minutes immersion. **$P_1$** and **$P_2$** had the lowest values; these films can be used for packaging high moisture content products, as opposed to samples with carrageenan sand sodium alginate, which were eluted and swelled fairly quickly. This is the reason why carrageenan is extensively used in pharmaceutical industry for the dragées and drugs (Liu et al. 2015, Li et al. 2014). All obtained films can be used as packaging material for ready-to-eat food, which solubilised in mouth or water.

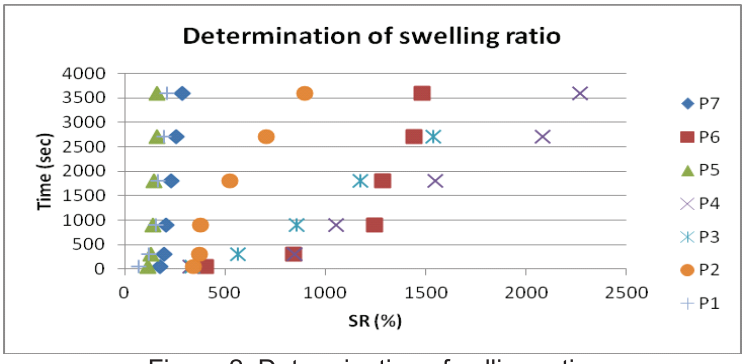


Figure 2. Determination of selling ratio.

## CONCLUSIONS

This study was aimed to design biodegradable edible films that can be used as packaging material for food. Nowadays, their use has become a necessity, representing a viable alternative to synthetic packaging materials which affect the ecosystem and environment. Although the costs exceed those of convention packaging, the investment is amortized over time, especially when they are produced at an industrial level. Edible packaging represents the packaging of the future; they may be improved by addition of flavors, colours, antioxidants or micronutrients; they produce zero waste, are 100% biodegradable, and can harness by-products of the food industry.

## REFERENCES


Atef M. et al., 2015, Characterization of physical, mechanical and antibacterial properties of agar-cellulose nanocomposite films incorporated with savory essential oil. Food Hydrocolloids, 45, 150-157.

Elsabee M.Z. and Abdou, E.S., 2013, Chitosan based edible films and coatings: a review. Material Science and Engineering C, 33, 1819-1841.

Falguera V. et al., 2011, Edible films and coatings: structures, active functions and trends in their use. Trends in Food Sci. and Technol., 22, 292-303.

Hernandez- Izquierdo V.M. and Krochta, J.M., 2008, Thermoplastic processing of proteins for film formation: a review. J. Food Science, 73, R30-R39.

Kumar Reddy T.R. et al. 2015, Bio-nanocomposite properties and its food packaging applications. Polymer Science, 77-88.

Li L. et al., 2014, Carrageenan and its application in drug delivery. Carbohydrate Polymers, 103, 1-11.

Liu J. et al., 2015, Review for carrageenan - based pharmaceutical biomaterials: Favorable physical features versus adverse biological effects. Carbohydrate Polymers, 121, 27-36.

Lopez-Mata M.A. et al., 2013, Physicochemical, antimicrobial and antioxidant properties of chitosan films incorporated with carvacrol. Molecules, 18, 13735-13753.

Mostafa N.A. et al., 2015, Production of biodegradable plastic from agricultural waste. Arabian Journal of Chemistry, 1-8.

Pascall A.M. and Lin, S.H., 2012, The application of edible polymeric films and coatings in the food industry. J. Food Process Technol. 4:2, 1-2.

Rhim J.V., 2012, Mechanical and water barrier properties of multicomponent (agar/carrageenan/konjac) hydrogel films. Dept. Food Engin, Mokpo University, 112-116.

Rhim J.W., et al., 2000, Solubility, tensile and colour properties of modifies soy protein films. J. of Agric. and Food Chem., 48, 4937-4941.

Siah, M.W. et al., 2015, Edible films from seaweed. International Food Research Journal, 22(6), 2230-2236.

Sing T.P. et al., 2015, Development of chitosan based edible films: process optimization using response surface methodology. Journal Food Science Technol, 52(5), 2530-2543.

Soroudi A. and Jakubowics I., 2013, Recycling of bioplastics, their blends and biocomposites: a review. European Polymer Journal 49, 2893-2858.

Wikstrom F., et al., 2014, The influence of packaging attributes on consumer behavior in food-packaging life cycle assessment studies – a neglected topic. Journal of Cleaner Production 73, 100-108.

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# THE INFLUENCE OF PRESSURE AND VACUUM CYCLIC PROCESS IN ORDER TO IMPROVE *BEEF PASTRAMI* TENDERNESS

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***Keywords***: *pressure and vacuum cyclic process, beef meat tenderizing, Warner - Bratzler method*

## ABSTRACT

*The paper presents the mechanical characteristics of raw meat's and cured-smoked final product tenderized by using successive pressuring and vacuuming process in order to produce Romanian traditional product "Beef Pastrami" type. The tenderizing process performed to decrease the duration of curing / marinating period too, consists in successive pressuring and de - pressuring cyclic process (0…10 bar), followed by vacuuming and de - vacuuming cyclic process (0…- 0,92 bar). The mechanical characteristics are based on the shear force diagrams obtained by using Warner - Bratzler method of the final product, before and after the tenderizing obtained by using pressuring and vacuuming cyclic process. The paper presents characteristic diagrams obtained by using Warner - Bratzler testing method for the tenderized raw meat, and cured-smoked final product, respectively, in comparison with the initial no tenderized meat sample.*

## INTRODUCTION

The word *pastramă* is etymologically rooted in the Romanian *a păstra* which means "to keep" or "to preserve". But the word is maybe more ancient and come from the Latin *pastor* who means *sheperd*; so *Pastramă* is *sheperd's meat* of lamb or mutton. *Pastrama* is a popular delicatessen meat traditionally in Romania made from lamb, sheep, mutton, goat and also from pork and beef. *Pastramă* was originally created as a way to preserve meat before modern refrigeration. For pastrami, the raw meat is brined, partly dried, seasoned with various herbs and spices, then smoked and steamed (http://en.wikipedia.org/wiki/Pastrami).

Traditionally *Beef Pastrami* is made from the brisket (which comes from the lower chest of the steer), or from the navel (a small piece cut from the muscle known as the plate). Traditional made *Pastrami* is a cured meat, meaning that it has been quickly injected with brine usually containing preservation additives (in industrial process) or otherwise infused for long time with brine (in homemade or small enterprise process) (http://en.wikipedia.org/wiki/Pastrami).

Both beef brisket and beef navel are tenderless parts of the animal's carcass. Therefore in the industrial process the meat is tenderized for 4 - 8 hours in massaging vacuum equipment (maximum relative vacuum - 0,65…- 0,75 bar). For the same reason, in homemade or small enterprise process, the meat is tenderized for

1 - 3 weeks in high concentration brine containing additional flavors added. Then, traditional *pastrami* is cold smoked, and finally dried in ventilated cold air (McGee 2004, Shewfelt 2000).

Tenderizing is a process that breaks down collagens in meat to make it more palatable for consumption. There are several ways to tenderize meat: mechanical tenderization, such as or piercing; the tenderization that occurs through cooking, such as braising; tenderizers in the form of naturally occurring enzymes, which can be added to food before cooking (examples of enzymes used for tenderizing: papain from papaya, bromelain from pineapple and actinid in from kiwifruit; marinating the meat with vinegar, wine, lemon juice, buttermilk or yogurt; brining the meat in a salt solution; dry aging of meat at 0 to 2°C (Institute of Food Technologists 1991, Larousse 2000, Shewfelt 2000, Tyszkiewicz & Klossowska 1996, Xargayó et al. 2011, Xianzhong & Shaofang 2011, http://en.wikipedia.org/wiki/Tenderizing).

Mechanical tenderization actions produce multiple cuts in the meat muscle in order to increase the surface area and thereby facilitate extraction and solubilization during the massaging phase. Softening of the muscle is also obtained, making the meat more adaptable to the cooking moulds. Tenderization, pre-massage and massage are closely inter-related, and not all products require the same mechanical action. Thus the mechanical action must be intensified and adapted in order to compensate for some of the negative consequences that may result in the product's quality. This will depend on the rest of the process and, above all, on the presentation and final quality of the product itself.

In low-injection products where meat content represents more than 80% of the final composition, meat quality is a determining factor in mastication, while in more highly injected products, this is not as important as the process and technology used (Shewfelt R. L. 2000, Tyszkiewicz & Klossowska 1996, Xargayó et al. 2011, http://en.wikipedia.org/wiki/Tenderizing).

This paper presents a novel tenderizing method based on *successive pressuring and vacuuming process* in order to reduce the time tenderizing process as much is possible, and to improve the final product tenderness', too.

## PROCESSING METHOD AND EQUIPMENT

In order to produce *Beef Pastrami*, commercial beef brisket (12 boneless pieces; 0,8…1 kg/piece;) was used; according Animal Slaughter Certificate: cow, 12 years, small farm. Two of these pieces were used to produce *Beef Pastrami* respecting traditional home-made or small enterprise process: the meat was pierced for 4 times (Figure 1), then infused for 14 days in 10% concentration brine, then cold smoked in several steps during 2 days, and finally, dried in cold free ventilated air for 16 hours.

The piercing step was realized by using the *Multi - needle piercing device*, that in principle consists in 120 needles (ø5; $20^0$ edge conical sharp) disposed in the same shape and reciprocity distance as into the industrial brine injection equipment (Figure 2) (Roşca & Roşca 2011, Roşca 2016, Roşca & Roşca 2014, UTEFIL Data Base 2005 - 2016).

In actual massaging vacuum processing equipment the vacuum level do not exceed - 0,7 bar. Recent American and West - European meat tenderizing research papers recommend increasing the vacuum level up to - 0,95 bar (Xargayó et al. 2011, Xianzhong & Shaofang 2011).

The tenderizing method proposed in this paper consists in several cyclic pressuring and de - pressuring step, followed by cyclic vacuuming and de - vacuuming step of the raw meat and the brine, too, into a pressure vessel. During the pressuring process, the pressure level is 3 - 4 times higher than during brine injection in industrial equipment, and 2 - 3 times higher than the dynamic pressing during the massaging industrial process.

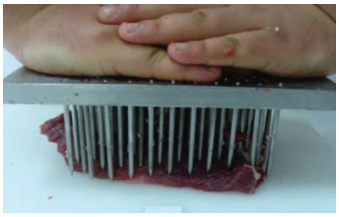

Figure 1. Beef brisket piercing.

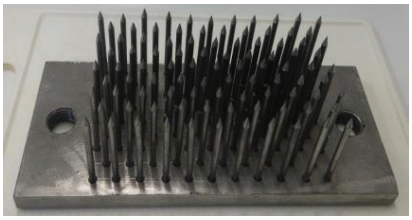

Figure 2. Multi - needle piercing device.

In order to put in evidence the influence of pressuring cyclic and vacuuming cyclic process (PC.VC.P) on meat tenderization, *Experimental Equipment* (EE - PC.VC.P) was used. *Experimental Equipment for PC.VC.P* and *Multi-needle piercing device* were designed and made by Unconventional Technologies and Equipment for Agro - Food Industry Laboratory (UTEFIL) within Faculty of Horticulture, in collabo-ration with Laboratory for Environmental Protection in Industry (EPIL) within Faculty of Electrical Engineering, within the University of Craiova.

In principle, EE - PC.VC.P is composed in a pressuring and vacuuming process hydraulic cylinder (PV-HC) consisting in a cylindrical vessel (inner ø80; length 180 mm) made in stainless steel W1.4571 and a piston made in food grade Teflon. PV-HC is provided with a 12 bar manometer gauge, and -1…1,5 bar manovacuumeter gauge when vacuuming process is actuated (Figure 3). In order to evacuate the liquid / gas excess before and after PV-CP, the piston is provided with G1/4" tap connected to ø8 Rilsan tube (UTEFIL Data Base 2005 - 2016).

In order to actuate into EE - PC.VC.P the pressuring process, or the vacuum-ing process, respectively, testing machine *LBG 10* (EPIL within Faculty of Electrical Engineering), was used.

Figure 3. Experimental Equipment for pressuring cyclic and vacuuming cyclic process for meat tenderization (EE - PC.VC.P).

The pressuring and vacuuming cyclic method consists in the following processing steps:

- The beef brisket 4 times manually pierced (as was presented above) is

introduced into the PV-HC of the EE-PC.VC.P that contains 10% salted concentration brine (proportion 1:1 for beef brisket, and salt brine, respectively).

- Each pressuring and vacuuming cycle lasts 12 min consists in 2 successive steps, each step lasting 6 min (Figure 4):

- pressuring cycle (3 minutes): slow pressuring (during 1 min) up to 10 bar; maintaining for 1 min at 10 bar, followed by fast de-pressuring up to the ambient atmosphere; maintaining for 1 min at the ambient atmosphere pressure;

- vacuuming cycle (3 minutes): slow vacuuming (during 1 min) up to - 0,92 bar; maintaining for 1 min at - 0,92 bar, followed by fast de-vacuuming up to the ambient atmosphere; maintaining for 1 min at the ambient atmosphere pressure.

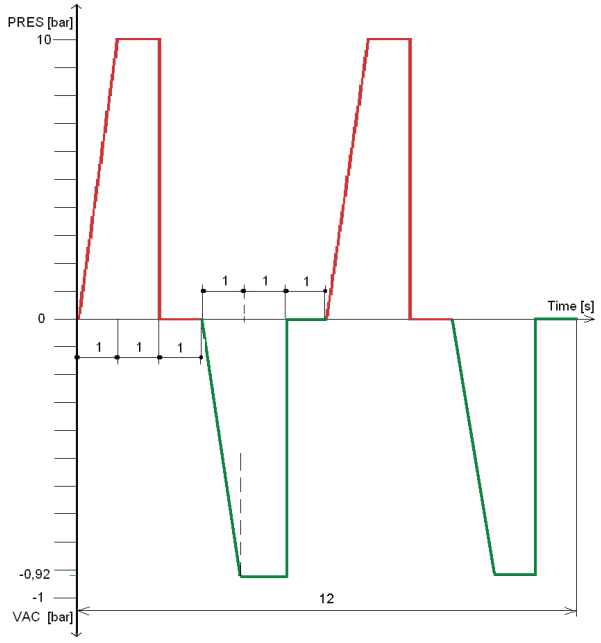


Figure 4. Diagram for 12 min pressuring and vacuuming cyclic process.

For this paper were used such pressuring and vacuuming cycles that last 48 min (PC.VC.P - 48), 60 min (PC.VC.P - 60), 72 min (PC.VC.P - 72), 84 min (PC.VC.P - 84), and 96 min (PC.VC.P - 96), respectively. For each pressuring and vacuuming cycle process lasting 48 min, 60 min, 72 min, 84 min and 96 min, respectively, two beef brisket were used.

All the 10 beef brisket tenderized by using pressuring and vacuuming cyclic process were smoked and dried in the same time and the same technological conditions with the pieces used to obtain *Beef Pastrami* by using traditional method (Figure 5).

Figure 5. *Beef Pastrami* obtained by using beef brisket tenderized after PC.VC.P – 84.

## WARNER - BRATZLER METHOD TO EVALUATE THE TENDERNESS

Instrumental - objective methods are based on fundamental tests measure properties that are familiar to mechanical engineers (strength, Poisson's ratio, Young's modulus, shear modulus). Mechanical tests cover a wide range of simple and rapid tests, including puncture, compression, extrusion, shear, and others, which measure one or more textural properties and are commonly used in quality control applications. Instruments tend to be more sensitive to small differences between samples and may be able to detect trends in quality loss before they can be detected by humans (Roşca & Roşca 2011, Shewfelt 2000, Xargayó et al. 2011).

The most relevant and utilized destructive texture and tenderness tests are puncture/ penetration test, compression test, and Warner - Bratzler shear test. In Warner - Bratzler test a blade cuts through a specimen, thus the shear force behavior gives information about tenderness, as well as the bite characteristic products (www.lloyd-instruments.co.uk).

To perform interdisciplinary researches concerning general texture and tenderness analysis, universal testing machines *Lloyd Instruments LRXPlus 5* (0,5 accuracy class for force and extension) within UTEFIL, is used since several years ago to perform comparative texture measurements for fruits and vegetable firmness's (ripeness' degree, springiness, skin strength), and for the tenderness of row-meat and cooked-meat (Figure 6). The mechanical characteristics of the specimens as a function of force - deformation are recorded controlled, measured and displayed by using specialized software NEXYGEN *Plus* (www.lloyd-instruments.co.uk).

Figure 6. Universal testing machines Lloyd Instruments LRXPlus 5 and Warner - Bratzler experimental equipment

Figure 7. *Beef Pastrami* made by PC.VC.P - 72 during Warner - Bratzler shear force test

Figure 8. *Beef Pastrami* made by PC.VC.P - 96 during Warner - Bratzler shear force test

Due to collaboration between UTEFIL and EPIL, an *experimental equipment Warner - Bratzler* was made: special rigid frame (made in food-grade Teflon) supporting a shear bar that permits interchangeable Warner - Bratzler shear blades (V and square plate cut blade made in stain-less steel DIN W1.4571) to slide fit into the frame (Figure 6) (Roşca & Roşca 2011, Roşca 2016, Roşca & Roşca 2014). During this experiment, 100 mm/min cutting speed was used.

*Beef Pastrami* obtained by using beef brisket tenderized after PC.VC.P - 72, and after PC.VC.P - 96, respectively, during Warner - Bratzler testing shear force, are presented in Figure 7, and Figure 8.

## RESULTS AND DISCUSSIONS

In order to determine the influence of tenderizing process on the final product tenderness', *Beef Pastrami* pieces made by using traditional homemade method, and tenderized by using PC.VC.P, respectively, were tested by using Warner - Bratzler shear force method. During the Warner - Bratzler shear force tests, each of all 12 pieces of *Beef Pastrami* were sliced in 8 parts. Representative Warner - Bratzler shear force diagrams are presented in Figures 9 and 10.

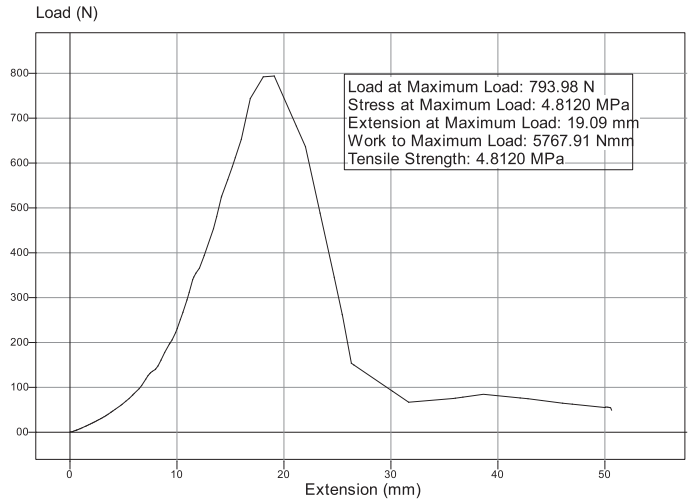


Figure 9. Warner - Bratzler shear force test for *Beef Pastrami* obtained by traditional homemade method

In Table 1 are presented: the maximum shear force amount and the shear force average for each of the five of *Beef Pastrami* types; the decrease of percentage average shear force (in comparison with traditional homemade *Beef Pastrami*'s tenderness) by using each process method, that demonstrate the tenderness' increase of the final product, that was tenderized by using pressuring and vacuuming cyclic process.

Table 1 presents a synthesis of the influence of pressuring and vacuuming cyclic process on final product *Beef Pastrami* tenderness':

- in comparison with traditional homemade *Beef Pastrami*'s tenderness, PC. VC.P - 48 determines a small increase of the final product tenderness' (13,11 %);

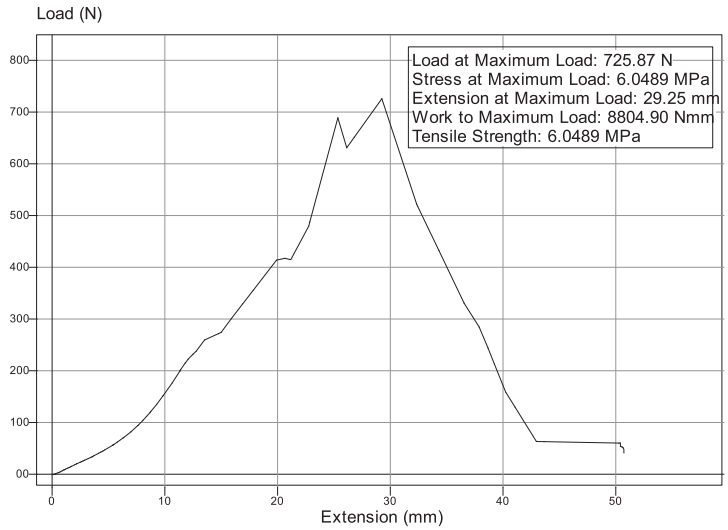


Figure 10. Warner - Bratzler shear force test diagram
for *Beef Pastrami* made by using PC.VC.P - 48 method.

- in comparison with traditional homemade *Beef Pastrami*, an important fast increasing (from 18,94 % to 25,98 %) of the final product tenderness' is observed when PC.VC.P - 60 and PC.VC.P - 72 were used;

- instead, large increase (from 31 % to 35,89 %) of the final product *Beef Pastrami* tenderness' is observed when PC.VC.P - 84 and PC.VC.P - 96, in comparison with traditional home-made *Beef Pastrami*'s tenderness.

Table 1

Warner - Bratzler shear force for *Beef Pastrami* types

| Sample code | Maximum shear force min…max amount, N | Shear force average, N | Decrease of shear force average, % |
|---|---|---|---|
| TRAD | 745,39…851,21 | 813,27 | - |
| PC.VC.P - 48 | 672,33…747,54 | 706,63 | 13,11 |
| PC.VC.P - 60 | 634,59…683,74 | 659,23 | 18,94 |
| PC.VC.P - 72 | 573,71…644,36 | 602,67 | 25,98 |
| PC.VC.P - 84 | 536,45…598,17 | 561,11 | 31 |
| PC.VC.P - 96 | 502,74…554,86 | 521,32 | 35,89 |

## CONCLUSIONS

Cyclic pressuring and vacuuming process represents a novel method to obtain increasing of *Beef Pastrami* tenderness'. Due to pressure and vacuuming amount levels, and fast pressuring and de - pressuring steps, and vacuuming and de-vacuuming, too, cyclic pressuring and vacuuming process effect's concerning much faster osmosis phenomena that determine the brine infusion into the meat' tissues, and no other wet salting / brining is necessary.

Cyclic vacuum process determines the smallest brine marinating / tenderizing period, only 84 - 96 min, in comparison with 4 - 8 hours in massaging vacuum

equipment in industrial processing, or 1 - 3 weeks marinating in homemade or small enterprise processing.

The method presented in this paper is similar in efficiency term with vacuum cyclic processing and represents a much more intensively tenderizing method than massaging vacuum equipment in industrial processing (Roşca & Roşca 2014).

As one of the most recommended analyze method, the Warner - Bratzler shear force test offers objective results concerning the influence of cyclic pressuring and vacuuming process on meat final products tenderness'.

This paper opens further experimental researches concerning the influence of similar cyclic pressuring and vacuuming process (higher pressure level, shorter or longer pressuring and de-pressuring, vacuuming and de-vacuuming, too) to produce *Beef Pastrami*, by using other much more tenderless parts of animal's carcass.

## REFERENCES


Institute of Food Technologies, 1991, Sensory evaluation guide for testing food and beverage products, Food Technol., 35 (11), pp. 50-59.

LAROUSSE, 2000, *Gastronomique*. Hamlyn, pp. 1204, ISBN 0-600-60235-4.

McGee H., 2004, *On food and cooking, the science and lore of the kitchen*. Scribner, pp. 155.

Roşca D., 2011, Instrumental texture evaluation - An objective measuring method for quality assurance in food industry. Annals of the University of Craiova, Biology, Horticulture, Food produce processing technology, Environmental engineering Series, vol. XLI (LII), 2011, Universitaria Publishing House Craiova, pp. 361-367.

Roşca A., Roşca D., 2013, Instrumental texture evaluation - An objective method to evaluate fresh vegetables quality. Journal Progress of Cryogenics and Isotopes Separation, ICSI - ICIT Rm. Valcea, Vol. 16, issue 1/ 2013.

Roşca A., 2016, Food industry equipments. Course Support, Faculty of Horticulture, University of Craiova.

Roşca A., Roşca D., 2014, The influence of vacuum cyclic process on tenderizing Beef Pastrami, Proceedings of the 20-th ICSI - ICIT National Conference "Progress in Cryogenics and Isotopes Separation", Calimanesti, Romania, October 25.

Shewfelt R. L., 2000, Consumer-friendly specifications to meet the demands of a global market, Food Australia, 52, pp. 311-314.

Tyszkiewicz I., Klossowska B.M., 1996, Mechanical Tenderization of Pork Meat: Protein and Water Release due to Tissue Damage. Journal of the Science of Food and Agriculture, Vol. 73(2), pp. 179 -185.

Xargayó M., Lagares J., Fernández E., Borrell D., Sanz D., 2011, The impact of tenderization on increased slicing yield, http://en.metalquimia.com/upload/document.

Xianzhong Xu, Shaofang Yuan, 2011, An examination of the force generated from incisor penetration into foods with different textural properties part I: Experimental observation, Journal of Texture Studies, Special Issue, Vol. 42, Issue 3, pp. 228-235.

Data Base. Unconventional Technologies and Equipment for Agro-Food Industry Laboratory, 2005 - 2016.

http://en.wikipedia.org/wiki/Pastrami

http://en.wikipedia.org/wiki/Tenderizing

www.lloyd-instruments.co.uk

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# ESTERS COMPOUNDS IN ROMANIAN WINES

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**Keywords:** *white wine, esters, flavor, GC-FID*

## ABSTRACT

*The aims of this study is to evaluate the flavor compounds (esters) presents in three types of wines derived from intra and extra- Charpatian areas (Sauvignon blanc, Chardonnay, Riesling Italian). GC-FID methods led to the identification and quantification of the esters (ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl lactate, isoamyl acetate, ethyl acetate, ethyl formate), compounds which participate in the formation of wine bouquet.The results obtained by this study leads to the conclusion that esters can bring pleasant fruity flavors to the selected wines-depending on the area and specific environmental factors.The difference of the values is leading to customize and personalize this types of wines, even if they are from the same variety.*

## INTRODUCTION

On a very competitive market the consumers are aware of the impact of foods or certain constituents of food on their health.The best possibility of honest producers is to protect their products, especially that one already branded by verification/confirmation of their authenticity and quality of raw materials that underpin their products is a real advantage. Currently there is a clear trend of implementing on the market certain varieties of more or less hybridized wines. This is the reason that is leadin to their standardization and globalization, to the detriment of indigenous, authentic varieties. The wine market is currently faceking some practices that lead to consumer's deception regarding the origin of the wine, its authenticity but also to his fraud.The aroma of the wine is the result of a harmonious blend of many chemicals of different origins and structure. The investigation is in this case an extremely complex process due to a lot of factors that are influencing their formation.The most responsible eitems of the sensorial properties of the wine is determined by the biological and technological parameters. By detecting the volatile compounds profile determined the differences of the wines regarding their geographic region.

In 1980, 1986 it was successfully determinate for the very first time the difference between Pinot noir French wines the ones based on 1-hexanol and cyclohexanone content (Garcia-Jarez et al. 1995) managed to identify the

geographic area of some French red wines and Spanish white wines because of the higher content of volatile compounds such as ethyl esters, isoamyl esters, aldehydes, acetals.

The biosynthesis of varietal odorous substances is determined by the biological characteristics of each variety, which manifests itself more strongly at aromatics varieties (Falcao et al. 2007, Lengyel et al. 2012, Lengyel 2012). Therefore knowing the content and structure and determination the different correlations between volatile compounds represents a viable way to authentificate its variety (Loscos et al. 2010, Luisier et al. 2008, Ferreira et al. 2002). As a result of various biochemical and physicochemical processes during preparin and storagin the wine, the wine's volatile profile is having somechanges, especially during the development of the bouquet of maturation and aging (Ferreira et al. 1997, Ferreira et al. 2007). Volatile flavor compounds are therefore perceived by the nose olfactory receptors. It has been estimated that humans beings are receptive to thousands of aromatics flavors. The flavor compounds concentrations is low in wine products; the lowest detection compound (0,02ng / l) is 1-p-menthene-8-thiol (Bolens 1993), witch gives a grapefruit aroma. Hundreds of active aroma compounds can be isolated from a wine product. However, the characteristic aroma is mostly provided by several substances-compounds of impact flavor. Many flavors are secondary metabolites. Other flavors are generated by chemical reactions or they are enzyme-catalyzed during the grapes processing (Fisher 1997, King et al. 2011). The wine flavor is a very complex one. His development is influenced by a lot of biological biochemical and technological parameters. The originality of the flavor compounds can be in grapes beans or may be developpad during alcoholic fermentation under other technological interactions also during ripening (Bayonove et al. 1998).

## MATERIAL AND METHODS

The varieties of white wine: Sauvignon Blanc, Chardonnay, Riesling Italian wine years 2011, 2012 and 2013 from Oltenia: Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin; Dobrogea: Murfatlar; Muntenia: Halewood Cellars / Ploiesti; Transylvania: Jidvei and Sebes-Apold-Apold.

The work samples used in the study were analyzed with GC/FID system (gas chromatography coupled with flame ionization detector by previously using the method Head-Space (Stegăruș 2015). The included gas chromatograph Varian 450 GC coupled with Varian 240 MS model mass spectrometer (Varian Inc - California, USA) equipped with a capillary column Thermo Scientific TG-WAXMS (Waltham, MA USA) (60m x 0.32 x 0.25 pm).

The final amount of the sample was 1 ml and was injected with a one ml syringe into the injection system 01: 10splittered.

The injector temperature of the volatile compounds was 150°C;

The column temperature is increased from the initial temperature of 30°C to 3°C/minute until it reaches $120^0$ C, $175^0$ C to 10°C/min and then increased to $220^0$ C where it is maintained for 10 min.The carrier gas was at a flow rate of 1.2 ml / min. The identification of the flavor compounds was made by comparing the spectrum with the injected in advance standards.The standards used were provided by Sigma Aldrich.

## RESULTS AND DISCUSSIONS

The esters represent the largest and most important group of aromatic compounds produced during fermentation. They are present in all types of wine and significantly affect wine flavor and quality by providing that “fruity” taste (Etievant 1991).

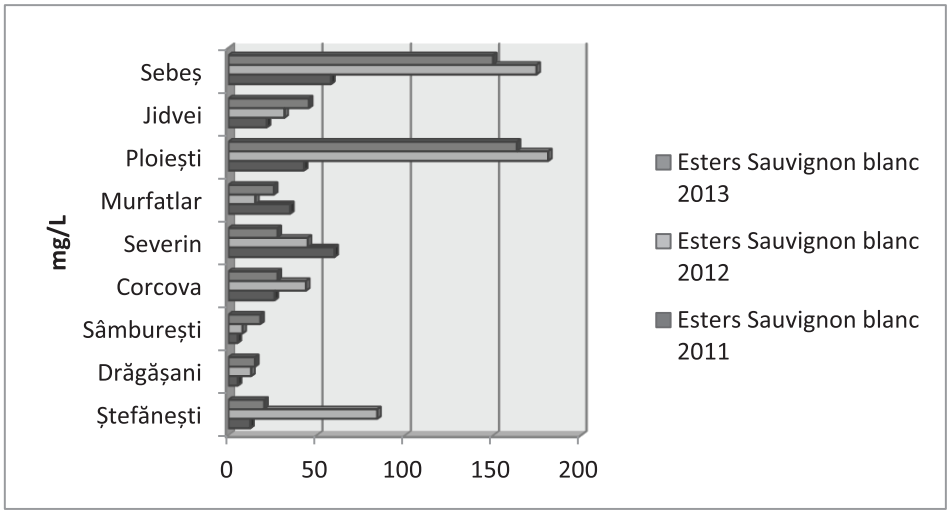


Figure 1. Evaluation of esters content in white wines Sauvignon blanc from Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars / Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

The two main types of products are esters, ethyl esters and alcohol esters of fatty acids. There is a number of esters of alcohol to organic acid in the wine.

The Sauvignon blanc concentration of ester was (2011): minimum of 5.2674 mg / L at Sâmbureşti and 5.3742 mg / L at Drăgăşani and maximum of 60.1200 mg / L at Severin and 58.0836 mg / L at Sebes-Apold.The average ester concentrations in Ploiesti and Murfatlar (2011) was-42.7523 mg/L respectively 34.9382 mg/L. At Corcova the esters value was half of maximum value. For the same year the average concentration of ester in Ploiesti and Murfatlar where 42.7523 mg/L respectively 34.9382 mg/L. At Corcova esters values were situated at half maximum, and at Ştefăneşti they exceeded 12.4050 mg/L. In 2012 the Sauvignon blanc wines had a maximum accumulation of 181.3085 mg/L at Halewood Cellars/Ploiesti and a minimum value of 8,0000 mg/L at Sâmburești. In Sebes-Apold the ester was determined 4.15% under the maximum and in Ştefăneşti-half of the values. At Corcova and Severin the value of 2012’ ester is 43.8809 mg/L respectiv 44,8870 mg/L In 2012 the Sauvignon blanc.

In 2013 there is a maximum value of 163.3815 mg/L at Halewood Cellars/Ploiesti and a minimum value at Drăgăşani (15.0751 mg/L). Significant values are having the wines from Sebes-Apold where esters showed 149.8088 mg/L (Figure 1).

At Jidvei, this values did not exceed more than 45.5504 mg/L; at Ştefăneşti and Sâmbureşti -approximate 20.3270 mg/L and 18.1288 mg/L. At Murfatlar, Corcova and Severin we can detect a decrease of the ester’s concentration. That

for the obtained values do not exceed 25.7730 mg/L-27.8326 mg/L.The Chardonnay white wine is characterized by a maximum concentrations of esters in 2013 146.8661 mg/L at Halewood's Cellars/Ploiesti, 147.0841 mg/L at Sebes-Apold and a minimum value of 0.0317 mg/L Ştefăneşti in 2011 (Figure 2).

The Italian Riesling white wines have oscillating values of esters, startding from a minimum of 0.0317 mg/L to a maximum of 241.8069 mg/L in 2011.

In the same year the average was between 18 mg / L and 26 mg/L to Sâmbureşti (18.2886 mg/L), Corcova (19.5269 mg/L), Severin (20.5492 mg/L) and Murfatlar 26.6827 mg/L. At Halewood's Cellars/Ploiesti and at Sebes-Apold we have significant values that reach up to 69.2638 mg/L respectively 84.4388 mg/L.

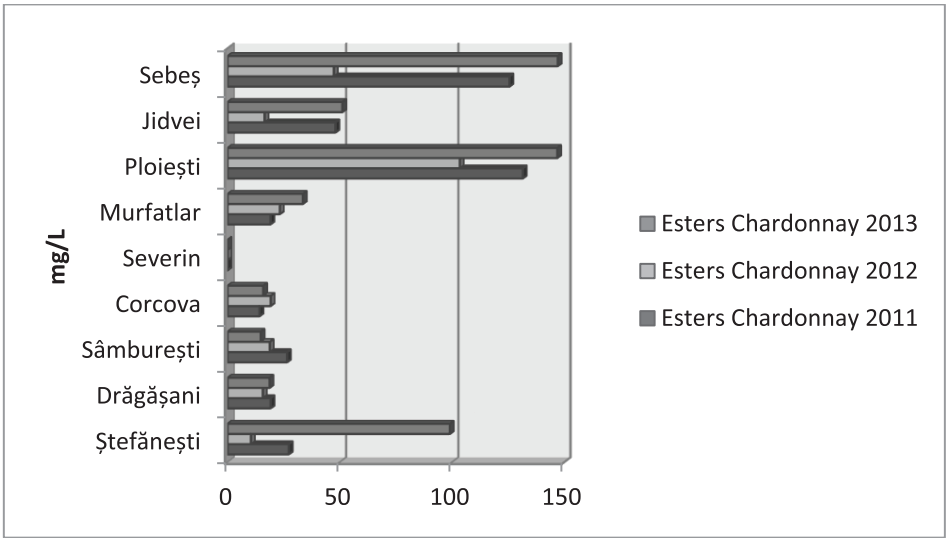


Figure 2. Evaluation of ester content in white wines Chardonnay from Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars / Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

The Wines monitored in 2011 is having the maximum of ester at Halewood Cellars/Ploiesti of 131.4553 mg/L and at Sebes-Apold 125.5131 mg/L. At Drăgăşani and Murfatlar were registered low values - 19.1700 mg/L and 19.1046 mg/L. A value up to 3.1% higher was detected at Sâmbureşti - an esters accumulations of 26.4349 mg/L. In 2012 most of the results were situated between 10.3976 mg/L and 47.2181 mg/L that's the reason why the maximum has been detected only in Halewood's Cellars/Ploiesti where there were identified esters accumulations of 103.3089 mg/L. At Drăgăşani, Jidvei, Sâmbureşti and Corcova the values increased progressiveely in a percentage of 8-10% each. In Murfatlar this increase was up to 16%. In 2013 we can notice a higher accumulation of esters - 147.0841 mg/L in Sebes-Apold. 48% lower is the results on Ştefăneşti wines.The values of 51.0564 mg/L is on Jidvei. At Sâmbureşti, Corcova and Drăgăşani esters values is minimum 14.7551 mg/L, 15.8802 mg/L and 18.6872 mg/L.In 2012 the esters values determined in Italian Riesling white wines is

between 12.5093 mg/L at Ştefăneşti and a maximum of 107.9153 mg/L Sebes-Apold (Figure 3).

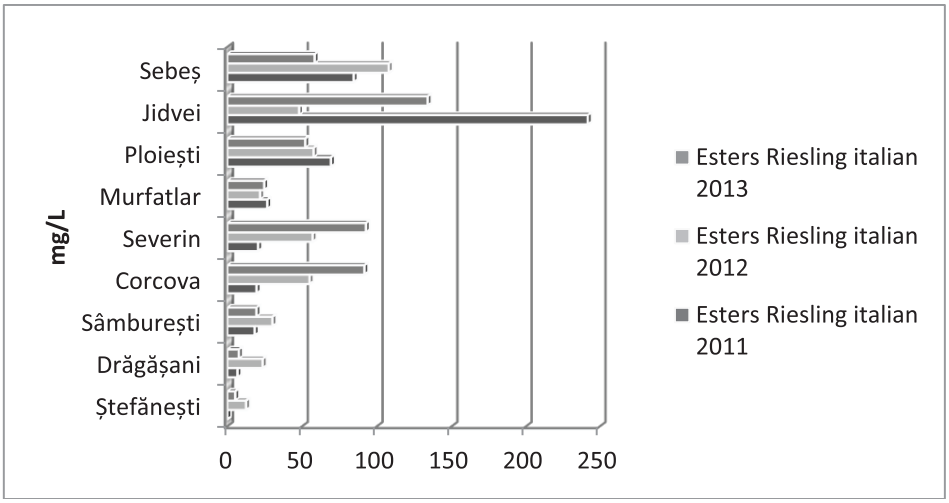


Figure 3. Evaluation of the content of esters in Riesling Italian white wines from Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars / Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

Half of the maximum values can be found at Cricova Cellars Halewood/Ploiesti and Jidvei 55.0036 mg/L, 57.6508 mg/L and 47.9749 mg/L. At Drăgăşani and Sâmbureşti the values do not exceed 23.6466 mg/L, respectively 30.1311 mg/L. 2013 revealed two lows values of 5.2612 mg/L at Ştefăneşti and 7.7235 mg/L at Drăgăşani and values of 133.9155 mg/L to Jidvei. 30% less is the value in Corcova and Severin (91.6243 mg/L, 92.6268 mg/L), while Sebes-Apold and Halewood ‘s Cellars/Ploiesti do not exceed 58.3989 mg/L and 52.0630 mg/L.

## CONCLUSIONS

The esters give that very substantial flavors to the wines; they are detected in the white wines from Wallachia and Transylvania, followed by Oltenia and Dobrogea.It can be said that depending on the region/area of these wines although it is the same variety their aromatic structure sometimes is very different. The esters quantified by this study demonstrates that the very low fractions can contribute to the formation of aromas in wines.The values of butanoate ethyl gives to the wine a ripe fruit flavors, pears. Decanoate acetate gives to the wine a dried fruity flavor. Diethyl succinate brings to the wines earthy, spicy cheese values in most samples were below par. The amyl acetate ester brings to the wine that banana flavor present in almost all samples.

## ACKNOWLEDGMENT

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of food origin for implementing innovative fingerprinting methodologies - Emphasis on fruit-based products.

## REFERENCES


Bayonove C., Baumes R., Crouzet I., Gunata Z., 1998, Arômes. In: Flanzy, C. (Ed.)Oenologie, fondements scientifiques et technologiques. Lavoisier, Tec & Doc. London, Paris, New York, 163-235.

Boelens M.H., 1993, Volatile character-impact sulfur compounds and their sensory properties. Perfumer & Flavorist 18: 29-39.

Falcao L. D., De Revel G., Perello M. C., Moutsiou A., Zanus M. C., Bourdignon-Luiz M. T., 2007 A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C13-norisporenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. J.of Agri. and Food Chem. 55: 3605-3612.

Ferreira V., Escudero A., Campo E., Cacho J., 2007, The chemical foundations of wine aroma-a role game aiming at wine quality, personality and varietal expression. Proceedings of the Thirteenth Australian Wine Industry Technical Conference, Adelaide, Austalia, ( Australian Wine Industry Technical Conference, Inc.) 142-150.

Ferreira V., Escudero A., Fernandez P. and Cacho J.F., 1997, Changes in the profile of volatile compounds in wines stored under oxygen and their relationship with the browning process. Zeitschrift fur Lebensmittel-Untersuchung und - Forschung A 205: 392–396.

Ferreira V., Lopez R., Aznar M., 2002, Olfactometry and aroma extract dilution analysis of wines. In: Jackson, J. F., Linkens, H. F. (Eds.) Molecular methods of plant analysis, Analysis of Taste and Aroma. Springer Verlag, 89-122.

Garcia-Jares, C. M., Garcia-Martin M. S., Marino N., Torrijos C., 1995, GC-MS identification of volatile components of Galician (northwestern Spain) white wine, Application of differentiate rias baixas wines from wines produced in nearby geographical regions J. Sci. Food Agri. 69: 175-184.

King E.S., Osidacz P., Curtin C., Bastian S.E.P., Francis I.L., 2011, Assessing desirable levels of sensory properties in Sauvignon Blanc wines- consumer preferences and contribution of key aroma compounds, Australian Journal of Grape and Wine Research, vol. 17, 2: 169-180.

Lengyel E., Oprean L., Ketney O., Iancu R., Bociu D., Tita O., 2012, Physical and chemical characterization of flavored and demy flavored wines from Recas vineyard, Progress of cryogenics and isotopes separation, vol.15, 1: 87-92.

Lengyel, E., Primary aromatic character of wines, 2012, Acta Universitatis Cibiniensis, Series E : Food Technology , vol.16 1: 3-18.

Loscos N., Hernandez-Orte P., Cacho J. and Ferreira V., 2010, Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varietals during accelerated wine ageing. Food Chemistry 120: 205-216.

Luisier J.-L., Buettner H., Völker S., Rausis T. And Frey U.,2008, Quantification of cysteine S-analysis. Journal of Agricultural and Food Chemistry 56: 2883-2887.

Stegarus D., Niculescu V., Georgescu C., Iancu R., Lengyel E., Tita O., 2015, Techniques for extraction and enhancing flavour substances in Chardonnay and Sauvignon blanc grapes by enzyme substrate, Rev. Roum. Chim., 60 5-6: 507-513.

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# QUANTIFICATION OF HIGHER ALCOHOLS IN ROMANIAN WINE BY GC-FID METHOD

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**Keywords:** *romanian wine, higth alcohols, GC-FID*

## ABSTRACT

*This study presents the accumulation of superior alcohols in Romanian wines, Sauvignon blanc, Chardonnay, Riesling Italian). The method was used is GC-FID led to the identification and quantification of the superior alcohol (1-propanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-octanol, benzyl alcohol) The results obtained by this study leads to the conclusion that superior alcohols gives wine pleasant and authentic aroma.*

## INTRODUCTION

The alcoholic fermentation is very important for the wine flavor; the most of the volatiles compounds is formed in this stage. Non-volatile precursors are converted to active flavor compounds by the yeast enzymes during alcoholic fermentation (Wust 2003). The yeast strain used can influence the flavor of the wine, especially the *high alcohols*. In addition, the malolactic fermentation may influence the flavor of the wine and the accumulation of alcohols (Garcia Jarez et al. 1995, King et al. 2011). These compounds are bringing butter or milk.The volatile compounds composition is continuously changing during ripening process (Lengyel 2012).

In both pre-fermentation and alcoholic fermentation stages there are a lot of chemical reactions caused by yeasts and lactic bacteria's action which ultimately lead to the creation of new wine aromatic profile (Ferreira et al. 2002). Some of the primary flavors of fragrant wine can also pass to the wine but many of them are subsequently formed. The wine fresh fruity floral, spicy or herbal flavors is given by Hexanol-1, 2-feniletanol, p-hidroxifenil-etanol (tirosol) (Lengyel 2012). Higher alcohols have in their molecule more than two carbon atoms (Parr et al. 2007). The wine were dosed isobutyl alcohol, amyl alcohol. Higher alcohol content in wine varies between 0.10 g/L and 0.75 g/L. The highest share has isobutyl alcohol content of which can be reached in coming to 0.2 g/L ie 35-50% of the total higher alcohols (Romano et al. 1992). Both acetic acid and glycerol are important in the composing and flavoring the wine-buying responsible on the yeast's way and possibility to assimilate azoth.

## MATERIAL AND METHODS

Romanian white wine: Sauvignon Blanc, Chardonnay, Riesling Italian wine years 2011, 2012 and 2013 from Oltenia: Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin; Dobrogea: Murfatlar; Muntenia: Halewood Cellars / Ploiesti; Transylvania: Jidvei and Sebes-Apold-Apold.

The wine samples were analyzed with GC/FID system (gas chromatography coupled with flame ionization detector by previously using the method Head-Space (Stegăruș 2015).

The measurements identified some compounds that bring flavor to the wine -like superior alcohols (1-propanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-octanol, benzyl alcohol). The amount of the sample was 1 ml. The samples was injected into the injection system 01:10 splittered.

The temperature of the injector for the *higth alcohols* was 150°C;

The column temperature is increased from the initial temperature of 30°C to 3°C/minute until it reaches $120^0$ C, $175^0$ C to 10°C/min and then increased to $220^0$ C where it is maintained for 10 min.

The identification of the highes alcohols was comparing with the standard spectrum (Sigma Aldrich).

## RESULTS AND DISCUSSIONS

Higher alcohols or fusel alcohols are produced by the yeast during alcoholic fermentation in order to give fruity character or "fruit" at optimal levels.

The excessive concentrations of higher alcohols is leadin to a strong smell and a “prickly" taste. The figure below (Figure 1) shows that the Sauvignon blanc wines superior alcohol concentration values is between 600 mg/L and 800 mg/L. The maximum values were recorded in 2013 in Drăgăşani - these compounds have reached the value of 1375.196 mg/L and the minimum values were recorded at Ştefăneşti in 2012 with a rate of 408.4014 mg/L. By following the evolution of higher alcohols compounds on this three years under study we can see that the values are close- with few exceptions. In Ştefăneşti these values reach an average of 543.7036 mg/L comparable to that in Drăgăşani in 2011 and 2012. Jidvei stands at constant values, the same as Sebes-Apold where the amount of higher alcohols keeps an average of 720 234 mg/L respectively 623.0012 mg/L. At Corcova and Severin the values are maintaining an average of 697.133 mg/L respectively 700.2349 mg/L, mean values that are close to those obtained at Jidvei and Sebes-Apold. At Murfatlar in 2012 it can be observed an accumulation of superior alcohols 1177.614 mg/L and in the same year at Halewood Cellars/Ploiesti value 1043.0910 mg/L. The values of superior alcohols in Sâmbureşti - 573.6254 mg/L in 2011 and 816.5617 mg/L in 2013 with an average over three years in the study of 670.6800 mg/L. Maximum values for 2011- at Murfatlar 892.1729 mg/L. Minimum values for 2011- at Halewood Cellars/Ploiesti 454.9635 mg/L. In 2012 the maximum values were identified in Murfatlar 1177.614 mg/L and the minimum values-532 664 mg/L at Drăgăşani. In 2013 the maximum values were 1375.196 mg/L at Drăgăşani and the minimum value 543.7036 mg/L at Ştefăneşti.

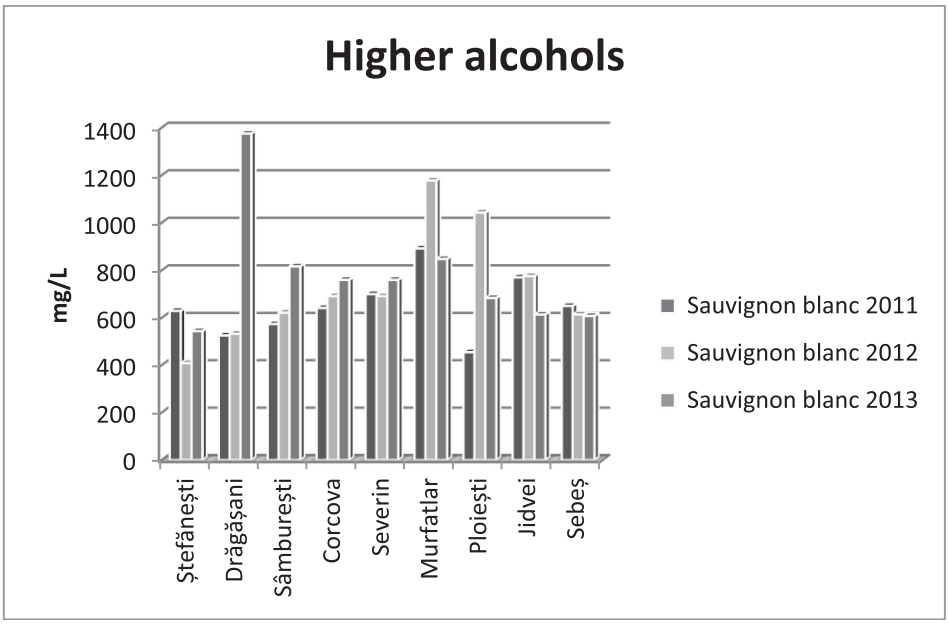


Figure 1. Assessment of higher alcohol content in white wines Sauvignon blanc from Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars / Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

The Chardonnay wine (Figure 2) is an accumulation of higher alcohols reaching a maximum of 1358.215 mg/L in 2012 at Drăgăşani and a minimum of 269.9855 mg/L Sâmbureşti in same year. Ştefăneşti notice a minimum amount of higher alcohols 494.4403 mg/L in 2011 and a maximum of 972.7056 mg/L in 2013 with an average of 804.03 mg/L.

At Drăgăşani the average values are at a rate of 1026.282 mg/L with minimum 750.8789 mg/L in 2011 and maximum 1358.215 mg/L in 2012.

At Sâmbureşti the minimum values were determined in 2012 with a rate of 269.9855 mg/L and a maximum of 735.7884 mg/L in 2011.

The average values of higher alcohols in Corcova were located at a rate of 697.6837 mg/L, exceeding by 15% the average values determined at Halewood Cellars/Ploiesti (591.8816 mg/L) and Sebes-Apold (589.7734 mg/L).

The Italian Riesling white wine presents the most substantial amounts of higher alcohols until now (Figure 3).These values can be seen clearly in 2013, when in the most of the regions the exceed was over 700 mg/L. That for the values of higher alcohols in Ştefăneşti were framed between 834 078 mg/L in 2012 and 899 095 mg/L in 2013 with an average of 874.1178 mg/L. At Drăgăşani these values had an average of 570.6362 mg/L in 2011, a minimum of 448.1076 mg/L and a maximum of 768.2623 mg/L in 2013.At Sâmbureşti the maximum accumulation of higher alcohols is noticed in 2013 when it reaches a value of 1076.7800 mg/L, while the average is 791.2148 mg/L.

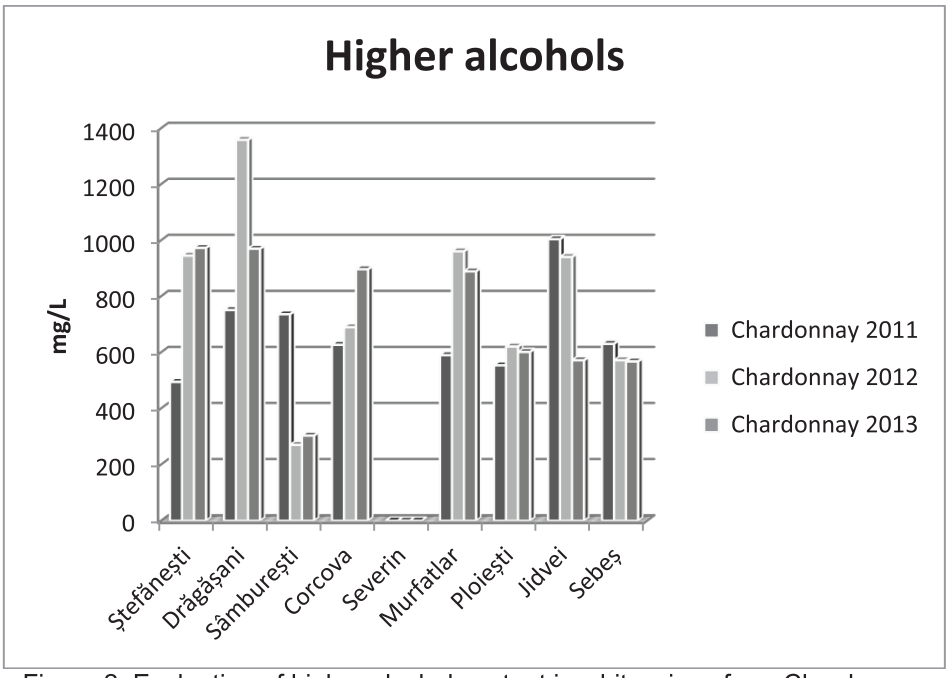


Figure 2. Evaluation of higher alcohol content in white wines from Chardonnay Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars / Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

At Severin and Corcova the average values are 864.8636 mg/L and 857.6004 mg/L, values close to those achieved in Ştefăneşti.

Maximum values of higher alcohols were obtained in Murfatlar where in 2012 it was achieved a rate of 1039.915 mg/L on average- 30% stronger than in 2011 (744.2782 mg/L), respectively 2013 (708.5933 mg/L).

In Halewood Cellars / Ploiesti we can observe the same difference of 30% between call reference only that the maximum higher alcohols determined reached in 2013 at a value of 1096.341 mg/L.The minimum values were established in 2011(562,4312 mg/L) and the average on this three years of research is 811.0731 mg/L.

Jidvei has reached a maximum of superior alcohols 831 564 mg/L in 2012 and a minimum 663.2156 mg/L in 2013.The average in this three years study has value 433.8807 mg/L, with a minimum of 404 999 2013 mg/L and a maximum of 450.6634 mg/L 2012.

By following this results we find that prevails averages is reaching 700 mg/L depending on the area of origin of the grapes.

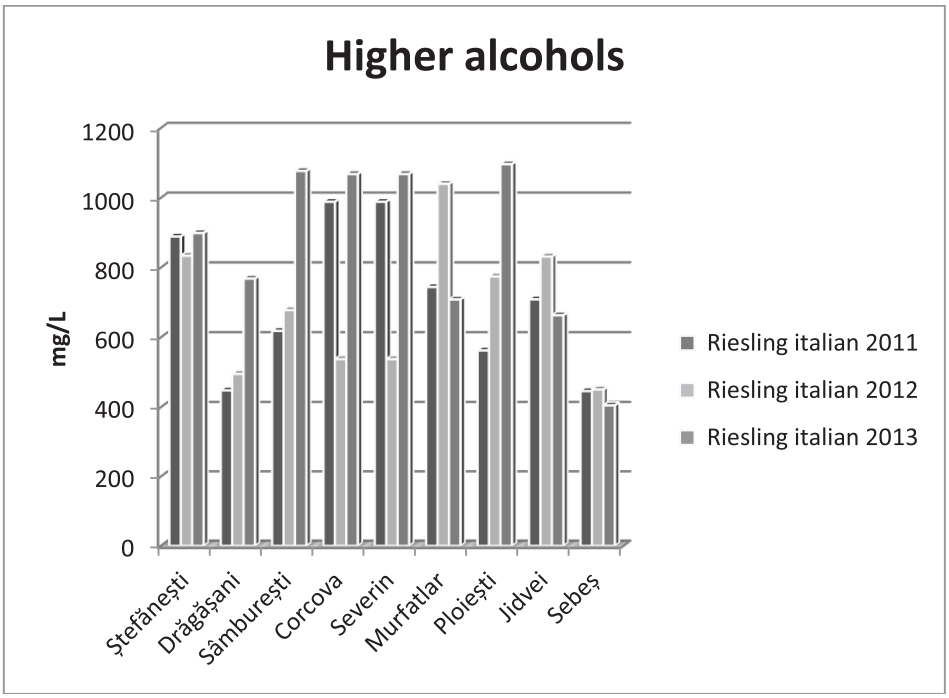


Figure 3. Evaluation of higher alcohol content in Riesling italian white wines from Ştefăneşti, Drăgăşani, Sâmbureşti, Corcova, Severin, Napa, Halewood Cellars/Ploiesti, Jidvei and Sebes-Apold, the harvest of 2011, 2012, 2013

## CONCLUSIONS

By comparing the results we can say that the Dobrogea's wine is having the highest level of superior alcohols. This are followed by the wines from Muntenia and Oltenia. The lowest level is detected in Transylvania's wines. The maximum values of superior alcohols from the Sauvignon blanc wines were recorded in 2013 in Drăgăşani and the minimum values were recorded at Ştefăneşti.

The Chardonnay wine is an accumulation of higher alcohols reaching a maximum in year 2012 in Dragasani, and minimum value at 2011.

Maximum values of higher alcohols were obtained in Murfatlar where in 2012 for the Riesling italian variety of romanian wine.

## ACKNOWLEDGMENT

The authors thank to Romanian Ministry of Education and Research, National Authority for Scientific Research for the financial support, under the NUCLEU Program, project PN16360402 Advanced research on the authentication of food origin for implementing innovative fingerprinting methodologies - Emphasis on fruit-based products.

## REFERENCES

Ferreira V., Lopez R., Aznar M., 2002, Olfactometry and aroma extract dilution analysis of wines. In: Jackson, J. F., Linkens, H. F. (Eds.) Molecular methods of

plant analysis, Analysis of Taste and Aroma. Springer Verlag, Berlin, Heidelberg, New York. 89-122.

Garcia-Jares C.M., Garcia-Martin M.S., Marino N., Torrijos C., 1995, GC-MS identification of volatile components of Galician (northwestern Spain) white wine, Application of differentiate rias baixas wines from wines produced in nearby geographical regions J. Sci. Food Agri. 69: 175-184.

King E.S., Osidacz P., Curtin C., Bastian S.E.P., Francis I.L., 2011, Assessing desirable levels of sensory properties in Sauvignon Blanc wines- consumer preferences and contribution of key aroma compounds, Australian Journal of Grape and Wine Research, vol. 17, 2: 169-180.

Lengyel E., Oprean L., Ketney O., Iancu R., Bociu D., Tita O., 2012, Physical and chemical characterization of flavored and demy flavored wines from Recas vineyard, Progress of cryogenics and isotopes separation, vol.15, 1: 87-92.

Lengyel E., Primary aromatic character of wines, 2012, Acta Universitatis Cibiniensis, Series E : Food Technology , vol.16 1: 3-18.

Parr W.V., Green J.A., White K.G., Sherlock R.R., 2007, The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterization by wine professionals. Food Quality and Preference 18: 849–861.

Romano P., Suzzi G., Comi G., Zironi R., 1992, Higher alcohol and acetic acid production by apiculate wine yeasts, Journal of Applied Bacteriology, Vol. 73, 2:126–130.

Stegarus D.,Niculescu V., Georgescu C., Iancu R., Lengyel E., Tita O., 2015, Techniques for extraction and enhancing flavour substances in Chardonnay and Sauvignon blanc grapes by enzyme substrate, Rev. Roum. Chim., 60 5-6: 507-513.

Wüst, M., 2003, Zur Biochemie des sortentypischen Weinaromas, Chem.Unserer Zeit 1/37, 8-17.

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# STUDIES ON CHEMICAL COMPOSITION AND SENSORY EVALUATION OF CRUDE AND AGING APPLES DISTILLATES

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***Keywords***: *volatile compounds, higher alcohol, esters, distillations, maturating, aging*

## ABSTRACT

*The aim of this study was to determinate the main quality volatile compounds (higher alcohols and esters) in some beverages obtained from apples, cider and also calvados. Calvados could be called Normans cognac, because it is a drink too refined to be regarded as just an ordinary apple brandy (sometimes pear). The technique for obtaining Calvados are the five essential elements of the process: cider making, double distillation, aging, choosing wood blend. The volatiles were determined by gas chromatography. The results showed that the apples and cider are a good substrate for fermentation and respectively distillation, and the sensory analysis performed revealed that the produced beverages and aging had good acceptance by the tasters.*

## INTRODUCTION

Apples are appreciated as fruit throughout the world. The high productivity of apples generates post-harvest losses. An alternative to disposing of the fruit to reduce waste and increase income to farmers is the sale of processed fruit to generate industrial products such as jams, juices, cider and spirits. The use of the fruit as a substrate for producing high added value products has been accomplished; an example is spirits obtained by the fermentation and distillation of fruit (González et al. 2010).

Fruit spirits are produced all over the world using various fruits, according to the availability in different countries and seasons. In this way, the current commercialization of known alcoholic beverages obtained from fruit could facilitate the market penetration of such spirits (González et al. 2010). Some fruits that have been used to produce distillates are melons (Hernández-Gómez et al. 2008), mulberries (Soufleros et al. 2004) plums and cherries (Schehl et al. 2005),) jabuticaba (Duarte et al. 2011), black mulberries and blackcurrants (González et al. 2010) pears (García-Llobodanin et al. 2008) and oranges (Santos et al. 2013).

The process needed to produce fruit spirit is complex and involves various factors that influence the quality of the final product. However, the main physico-chemical and sensorial differences among spirits are due to the particular composition of their corresponding raw materials, the fermentation process and the distillation process (Stoica et al. 2015).

## MATERIAL AND METHODS

### *Fruits*

One hundred kilograms of apples (mixed varieties) were collected in October 2014 in Dragasani, Valcea. The fruit is washed with bleach (10 g / l) and cut longitudinally. Then crushed and fermented leaves forming the apples marc that will be distilled.

### *Apples juice*

The apples juice was prepared according to the methods of Dias et al. (2007) with minor modifications. In the apples juice, the initial sugar concentration was approximately 11ºBrix, and the pH was 4.5. The apples juice was mixed with a sugar solution (1:1 v/v) to adjust the sugar concentration to 16° Brix. The juice was transferred to three stainless steel fermentation vats. Sulphur dioxide, in the form of potassium metabisulphite ($K_2S_2O_5$), was added at a concentration of 100 mg/L to inhibit bacterial growth (Duarte et al. 2010).

### *Fermentation*

Fruit marc and juice fermentation were carried out in vessels of 40 liter capacity filled with 30 l of substrate. The temperature was adjusted to 20 ° C. The substrates were inoculated with a commercial yeast (Saccharomyces cerevisiae UCLM 325) to a concentration of about $10^6$ cells / ml. The process was monitored daily by measuring residual sugars and the end of fermentation was determined based on sugar consumption.

### *Distillation*

After fermentation, the distillation process was performed in a copper alembic equipped with a condenser and gas heater and with a working capacity of 30 L. The distillate was separated into three fractions. The first fraction (head fraction) and the last fraction (tail fraction) was collected separately and standardized to a volume corresponding to approximately 10% each of the total volume of spirit. The intermediate fraction (heart fraction) was then collected was stored in glass bottles and maintained at 20°C for physico-chemical and sensory analysis.The major volatile compounds were analyzed of heart fraction by gas chromatography. Three distillates were obtained and analyzed heart fraction of each variant.

Distilled from cider was subjected maturing and aging process in oak vessels for a period of 2 years and analyzed every 6 months.

### *Sensory evaluation*

Sensory evaluations were conducted to select the best of the three spirits products.

The sensory analysis was performed with trained tasters. Ten-millilitre samples were served in clear glasses with a capacity of 25 mL. The evaluation sessions took place between 9:00 and 10:00 a.m. at room temperature (20-22°C) under white light. The samples were evaluated for taste, aroma, appearance and overall impression.

Some analyzes were performed in the laboratory of Food Technology Faculty of Agriculture and Horticulture and the analysis of volatile compounds were carried out at ICIC Rm. Valcea (EU 2000).

## RESULTS AND DISCUSSIONS

In Table 1 are presented the results of the comparative analysis of the main chemical components - higher alcohols and esters in apple and cider natural distillates.

Table 1

The main chemical compounds in crude distillates analyzed

| Analyzed compound | Apple distillate | Cider distillate |
|---|---|---|
| Etylic alcohol, %vol. | 32.0 | 40.0 |
| pH | 3.55 | 3.55 |
| 1 - Propanol, mg/l | 556.0 | 732.4 |
| 1 - Hexanol, mg/l | 53.4 | 90.7 |
| Isobutyl alcohol, mg/l | 136.5 | 152.5 |
| Benzil alcohol, mg/l | 2.1 | 1.9 |
| Ethyl acetat, mg/l | 50.5 | 64.6 |
| Isaomil acetat, mg/l | 5.0 | 5.1 |
| Esters +Superior alcohols | 195.2 | 225.3 |
| Furfurol mg/100 ml alcoool | 0.3 | 0.1 |
| Acetaldehyde mg/100 ml alcohol | 3.8 | 5.4 |
| Extract, mg/l | 420 | 114 |
| Ash, mg/l | 55 | 22 |

As can be seen, the alcohol content is consistent with the sugar content in the raw material from which the distillate. Distillate with small alcohol content is apple distillate with an alcoholic concentration of 32% vol. And cider distillate has the highest alcohol concentration of 40% vol.

Higher alcohols are responsible for the accentuation of the complex sensory attributes of spirit drinks. 1-propanol has a pleasant smell, sweet but excessive concentrations can enter notes of solvent which can mask all the positive notes distillates. The highest content of 1-propanol was recorded in cider distillates; instead, it was lower in distillate apple.

Hexanol content varies between 53.4 mg/L at apple distillate and is almost double 90.7 mg/L to cider distillate. 1-hexanol and 3-methyl-1-butanol herbaceous, strong flavors share.

Benzyl alcohol is related to the amount of benzaldehyde, the latter is important because it gives a flavor of bitter almonds at levels 2-3 g/L. In this study, concentrations were far from the threshold of perception.

Acetaldehyde is 90% of the total aldehydes. More than 1.200 mg/ of ethanol is proof ethanol oxidation during alcoholic fermentation or enzymatic decarboxylation of pyruvic acid (Cantagrel et all, 1993, Stoica et al. 2015).

Furfurol may be formed as a result of oxidation of ascorbic acid. Slightly higher furfural content was observed in apple distillate.

Esters are associated with pleasant odors (Steger & Lambrechts, 2000). This is especially true in the case of ethyl acetate, which contributes to the complexity of the flavoring and has a positive impact at very low levels (50-80 mg/L). The ethyl acetate was higher in the cider distillate, followed by the apple.

Amyl acetate, judging by its nature, has a pleasant flavor, banana, concluded that it is more the result of a side effect (higher concentration of acetic acid precursors and isoamyl alcohol) than an actual sensory impact.

Along with the main chemical constituents of distilled spirits, higher alcohols and esters were determined and the main flavor compounds, especially specific fruit from which they originated.

Total content of higher alcohols and esters is higher in distillate cider. Extract and ash content values are reversed, as higher to distillate apple.

For obtaining calvados, distilled from cider was subjected maturing and aging process in oak vessels for a period of 2 years and analyzed every 6 months (Table 2).

Table 2

The main chemical compounds in maturing distillates analyzed

| Analyzed compound | The period of maturing and aging | | | |
|---|---|---|---|---|
| | Initial | 1 year | 1+½ years | 2 years |
| Alcohol, %vol | 40.0 | 39.8 | 39.2 | 39.1 |
| pH | 3.55 | 3.70 | 3.73 | 3.90 |
| Total acidity, mg/L acetic acid | 35.2 | 69.15 | 70.2 | 72.6 |
| Ethyl acetat, mg/100 ml alcohol | 75,6 | 95.3 | 97.1 | 101.9 |
| Isobutyl alcohol, mg/100 ml alcohol | 148.0 | 186.3 | 190.0 | 193.1 |
| Acetaldehyde, mg/100 ml alcohol | 6.5 | 7.5 | 8.0 | 8.5 |
| Furfurol, mg/100 ml alcohol | 0.12 | 0.50 | 0.68 | 0.71 |
| Esters +Superior alcohols, mg/L | 226.7 | 275.9 | 286.1 | 295,2 |
| Extract, mg/l | 115.2 | 485.0 | 599.9 | 630.1 |
| Ash, mg/l | 24.2 | 55.1 | 56.2 | 58.7 |

During the 2 years of distillate cider aging in oak vessel entire a chemical composition amplified, especially fixed and volatile substances.

Thus, the alcohol content decreases in the 2 years to 39.1% vol. PH values go from 3.55 to 3.90. The extract of the distillate increases from 115.2 mg / l to 630.1 mg / L and the content of ash increase to 24.2 mg / l 58.7 mg / l.

Also, the total acidity increases from 35.2 mg / L to 75.2 mg / l and furfural also increases from 0.12 mg / L 0.71 mg / L.

As a consequence of the evolution acidity, aldehydes, esters, higher alcohols and furfural is noticed a significant improvement in sensory qualities.

If at the beginning of aging, the distillate was tough, rough, with a bunch insignificant, as the aging of 2 years, it becomes softer, velvety, with a bunch more delicate. These changes go distillate natural raw spirits among highly valued by informed consumers.

Regarding sensory, all crude distillates are colorless, with typical brilliance, clear, no sediment or slurry. Following a gyratory movement of the liquid in the glass, it has tears slowly leaking showing relevant content in alcohol. The flavor was different. Thus, the distillate apple flavor is intense, raw apple fresh fruit but not very persistent. The taste is pleasant, agreeable, easy burner, supple.

Regarding cider distillate obsolete, its color is bright yellow with amber hue, with brilliance and clear. The flavor in transformed in aging bunch more delicate and softer. The taste is pleasant, very balanced, agreeable, burner, round, slightly sweet.

In Figure 1 is shown top three spirits obtained by sensory analysis.

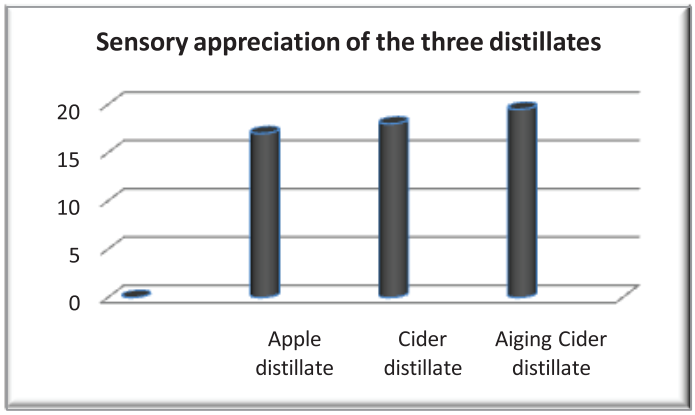


Figure 1. Sensory appreciation of three distillates.

## CONCLUSIONS

The study made the following conclusions:

In terms of organoleptic distillates analyzed all substandard and wishes of consumers, is of exceptional quality.

As regards the determination of the composition, consisting in higher alcohols, esters and aroma main constituents of all three distilled beverages meet established requirements.

Cider and Calvados distillates contain similar amounts approximately the same chemical Comps 1-propanol, 3-methyl 1-butanol, ethyl acetate, difference is given to the compound of flavor.

Calvados is consumed traditionally, after a meal, partly thanks to its digestive.

## ACKNOWLEDGMENT

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## REFERENCES

Cantagrel R., Lablanquie O., Snakker G, Vidal J. P., 1993, Caracteristiques analytiques d'eaux de vie nouvelles de Cognac de différentes récoltes. Relation analyse chimique-analyse sensorielle. Conaissance aromatique des cépages et qualité des vins. Revue Francaise d'Oenologie, 5, 383-391.

Duarte W.F., Dragone G., Dias D.R., Oliveira J.M., Silva J.B.A., Teixeira J.A., Schwan R.F., 2010, Fermentative behavior of Saccharomyces strains during

microvinification of raspberry (Rubus idaeus L.). International Journal of Food Microbiology 143, 173-182.

García-Llobodanin L., Ferrando L.M., Güell C., López F., 2008, Pear distillates: influence of the raw material used on final quality. European Food Research and Technology 228, 75-82.

González E.A., Agrasar A.T., Castro L.M.P., Fernández I.O., Guerra N.P., 2010, Production and characterization of distilled alcoholic beverages obtained by solid-state fermentation of black mulberry (Morus nigra L.) and black currant (Ribes nigrum L.). Journal of Agricultural and Food Chemistry 58, 2529-2535.

Hernández-Gómez L.F., Úbeda-Iranzo J., Briones A., 2008, Characterisation of wines and distilled spirits from melon (Cucumis melo L.). International Journal of Food Science and Technology 43, 644-650.

Santos C.C.A.A., Duarte F.W, Carreiro S.C., Schwan F.R., 2013, Inoculated fermentation of orange juice (Citrus sinensis L.) for production of a citric fruit spirit. Institute of Brewing & Distilling, 119, 280-287

Schehl B., Lachenmeier D., Senn T., Heinisch J.J., 2005, Effect of the stone content on the quality of plum and cherry spirits produced from mash fermentations with commercial and laboratory yeast strains. Journal of Agricultural and Food Chemistry 53, 8230-8238.

Soufleros E.H., Mygdalia A.S., Natskoulis P., 2004, Characterization and safety evaluation of the traditional Greek fruit distillate 'Mouro' by flavor compounds and mineral analysis. Food Chemistry 86, 625-636.

Steger C., Lambrechts M.G., 2000, The selection of yeast strains for the production of premium quality South African brandy base products. Journal of Industrial Microbiology and Biotechnology 24, 431-440.

Stoica F., Muntean C., Băducă C., Popescu Mitroi I., 2014, Influence of must processing ofn quality natural distillates, Analele Universităţii din Craiova, seria Biologie, Horticulltură, Tehnologia prelucrării produselor agricole, Ingineria mediului, vol. XIX (LV), p. 359-362

Stoica F., Băducă C., Muntean C., Giurgiulescu L., 2015, Researches on chemical composition and sensorial attributes of natural fruit distillates, Analele Universităţii din Craiova, seria Biologie, Horticulltură, Tehnologia prelucrării produselor agricole, Ingineria mediului, vol. XX (LVI), 285-291.

*** European Union (2000), Commission Regulation (EC) No. 2870/00 laying down Community reference methods for the analysis of spirit drinks.

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# VARIATIONS IN THE CHEMICAL COMPOSITION OF BISCUITS OBTAINED THROUGH TRADITIONAL METHODS AND INDUSTRIAL PROCESSING

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**Keywords:** *biscuits, acidity, ash, soaking capacity, alkalinity*

## ABSTRACT

*Biscuits are favorite treats for a wide category of consumers. The survey shows a comparison between the chemical composition of biscuits produced through the traditional method and those produced through industrial method.*

## INTRODUCTION

Biscuits are an important product in the bakery industry. It became a staple food consumed and enjoyed by all social categories. Along with tea or coffee, biscuits are a nutritious and tasty snack. As food, they have outstanding qualities in terms of taste and nutritional value, are easy to digest and can be preserved for a long period. Caloric power of superior varieties can be up to 4900 kcal / kg; they represent an important source of energy for the human body. (Davidson 2016)

In order to produce biscuits, the ingredients used are added to the dough made from the basic raw materials processed in advance in the form of creams, emulsions, diluted and as such, in a specific order. After kneading the dough made according to recipes and specific technologies, it is put to rest and compaction operations to better meet the needs for further processing. The dough is subjected to modeling by stamping and drawing (Niculescu 1965).

## MATERIAL AND METHODS

In order to make this the study there were used 5 types of biscuits, two types were prepared in laboratory and three varieties have been commercially available. The two varieties in laboratory dishes were marked with FA for the biscuits made of white flour and FN for the biscuits made of dark flour. The manufacturing recipe: 300 g flour (white / black), 150g butter, 100g caster sugar, 1 egg, 100 ml milk, one teaspoon baking powder, ½ tsp vanilla essence.

Preparation: Mix the butter and the powdered sugar until it becomes a foam. Add the egg, the milk, vanilla, the flour and the baking powder.

Homogenize until the composition becomes creamy. Allow to rest for one hour at 8 to 10 degrees Celsius. Spread a sheet of dough with the thickness of 0.5 to 0.8 mm and give the desired shapes of biscuits. The biscuits are baked in the oven for 10-15 minutes at a temperature of 1800 degrees Celsius.

*Analysis Methods*

i) **Alkalinity**. The method is based on the titration of the alkali extracted from the product solution of HCl in the presence of bromo-thymol blue. Weigh 25 g of pastry (gingerbread, cookies, etc.)at the technical balance are introduced quantitatively into a jar with glass stopper and mix with 250 ml water. The contents were stirred three times for 1 minute at intervals of 10 minutes, and then allowed to soak for 30 minutes. It was filtered through cotton wool. 100 ml of filtrate is taken and we add three drops of bromothymol blue and titrate with 0.1N HCl to transfer the color from blue to yellow.

ii) **Acidity**. The method is based on the extraction of the dough acids and their titration with NaOH solution up to complete neutralization. Weigh 5 g of sample and place them in a 250 ml conical flask. Add 50 ml of water and mix well by shaking. Add 3 drops of phenolphthalein and titrate with 0.1 N NaOH until the appearance of pink coloration, persisting for 30seconds.

iii) **Humidity**. The method is based on drying a sample of known initial weight of the product to constant weight. Take 300 g of sample and mix. The mixed sample is spread on a plate or sheet of paper of square shape in a uniform layer. Divide into 4 quarters the layer by diagonals. Pour into the drying stove and maintained for 40 minutes at 130 degrees Celsius. Cool the sample in a desiccator for about 30 minutes and weight the product.

iv)**The ashes.** In a porcelain melting pot put about 10 g of purified sand. The sand will be previously washed with 10% HCl and water, then dried, calcined and sifted through a 1 mm mesh sieve. During the calcination, the ash is stirred once to dislodge it from the coal agglomerate. Cool the capped melting pot in a desiccator and weigh it after.

## RESULTS AND DISCUSSIONS

In this study we analyzed the follow variants:

Traditional biscuits white flour: white flour, sugar, eggs, milk, vanilla essence, baking powder; Traditional biscuits black flour: black flour, sugar, eggs, milk, vanilla essence, baking powder; Industrial biscuits white flour: white flour, sugar, inverted sugar, palm oil, water, baking powder, iodized salt, emulsifier, metabisulphite of sodium, acidifying, vanilla aroma; Industrial biscuits white flour and honey: white flour, honey, sugar, inverted sugar, palm oil, water, backing powder, iodized salt, emulsifier, metabisulphite of sodium, acidifying, vanilla aroma. Industrial biscuits wholemeal: wholemeal flour 56%, sugar, inverted sugar, sodium carbonate, sodium bicarbonate, ammonium carbonate, magnesium carbonate, malt syrup, powdered milk, salt. Biscuits acidity content is dependent on the flour, sugar, butter, milk and eggs. Acidity influences the type of flour products in a higher weight. Acidity gives indications about biscuits: the quality of flour used, type of baking process, technological process, degree of freshness, flour alteration grade in storage rooms. Biscuits acidity results from the degradation of fats from germs (embryo) during the storage period. Flours black with high extraction content, lead a higher acidity in raw materials. Biscuits obtain from black flour or wholemeal flour present a higher acidity content than biscuits obtain from white flour.

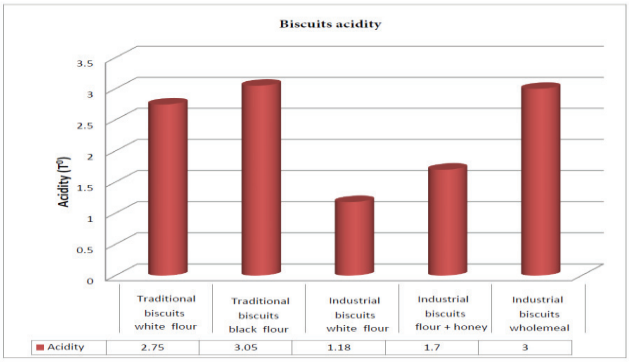


Figure 1. Biscuits acidity at analyzed samples.

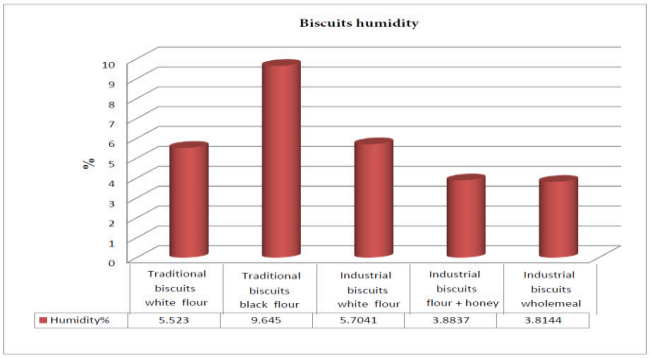


Figure 2. Biscuits humidity at analyzed samples.

The biscuits humidity represent water quantity used in baking process. Humidity of biscuits obtained from black flour are higher than other samples. The biscuits obtained by traditional method present a higher humidity than biscuits obtain by industrial methods. Addition of vegetable fats in preparation recipes determine decrease of humidity. Traditional and industrial biscuits obtained from white flour have a similar humidity. In baking process in all the recipes were used backing powder. Baking powder contain: sodium carbonate, sodium bicarbonate, ammonium carbonate. All of these substances determine an increase of biscuits alkalinity. (Nasar et al. 2008) On the other hand, biscuits obtain from white flavor by traditional method record double alkalinity value than other samples. Industrial biscuits obtained from wholemeal present higher content in alkalinity compare with samples prepare from white flour. Ash depend the flour sort. The ash content increased in function the ratio of small endosperm cells and peripheral cells. Free starch granules increase the ash content. If the number of covered cells are higher than open cells, the ash content decrease. (Saari et al. 2016). In our experiment biscuits obtained from black flour and wholemeal flour present a higher content in ash then biscuits obtained from white flour. Compare the baking process, biscuits obtained by industrial method increase the ash level towards biscuits prepare by traditional method.

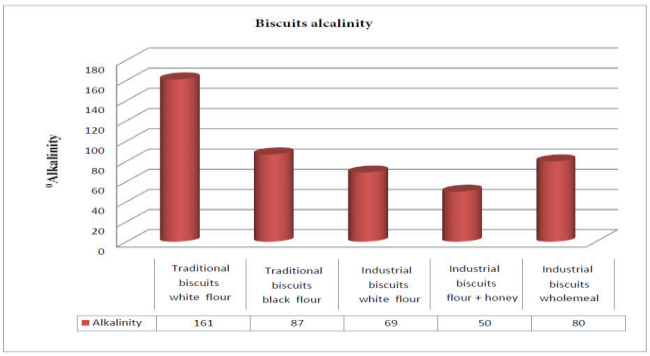


Figure 3. Biscuits alkalinity at analyzed samples.

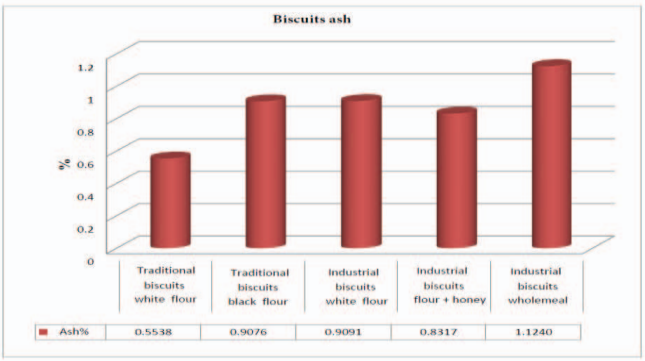


Figure 4. Biscuits ash at analyzed samples.

## CONCLUSIONS

Different types of fat added in baking process positively influence biscuits characteristics. The fat contained in the biscuit recipes increase the products acidify. The flour used in the preparation of biscuits influences the humidity and ash content. Thus, biscuits containing black flour differs significantly from other samples of biscuits, if they have increased the amount of humidity. Low levels of ash were obtained for the composition of the biscuits obtained from wholemeal. Biscuits obtained from black and wholemeal flour increase the ash content. In addition, higher capacity water retention have biscuits prepared with black flour.

## REFERENCES

Saari H., Heravifar K. et al., 2016, Preparation and characterization of starch particles for use in pickering emulsions, Cereal Chemistry, 93(2): 116-118.

Davidson, I., 2016, Biscuit Baking Technology - Processing and Engineering Manual. Academic Press, London, 32-127.

Nassar A.G., AbdEl-Hamied A.A., El-Naggar E.A., 2008, Effect of Citrus by-Products Flour Incorporation on Chemical, Rheological and Organolepic Characteristics of Biscuits, World Journal of Agricultural Sciences 4 (5): 612-616.

Niculescu N.I., Bejenaru V., 1965, Tehnologia produselor făinoase și de patiserie, Editura Tehnică, București, pp. 298-367.

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# EVOLUTION PROCESS MATURATION SEMI-HARD CHEESE DEPENDING ON THE TEMPERATURE

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***Keywords***: *maturation, glutamic acid, temperature, physical and chemical parameters*

## ABSTRACT

*In this study we aimed to analyze the evolution of physical and chemical parameters that depend on the temperature of maturation and have a direct influence during maturation on the characteristics of semi-hard cheese, as a finished product, particulary textural characteristics. Findings the degree of maturation of cheese consists of the determination of degradation products of the proteins is carried out by several methods. Also for determining the degree of maturation of cheese we used a modern method namely determining enzyme glutamic acid using tests that can be used as an indicator for the proteolysis of cheese.*
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## INTRODUCTION

In order to obtain high quality cheese, an important role in the maturation process has operation of maturation (Sousa et al. 2001). This stage the maturation leads to finalize textural characteristics (Hanaei et.al. 2015), characterizing any cheese. Cheeses reach their own characteristics through many changes chemical, microbiological and biochemical (Bergamini et al. 2010), changes that protein, fats and residual lactose is broken down for primary products which are further degraded by secondary products (Kheadr et al. 2003). Dynamic characteristic of amino acids (Yvon et. al. 2001) during cheese ripening is that some proportion increases progressively until the end of the maturation process. These variations reflect changes continue into other compounds of amino acids (Costin et al. 2003). Glutamic acid is considered by some researchers as one of the most important amino acids.

Glutamic acid is a product of degradation of proteins and can be used as an indicator for the proteolysis of cheese. Our department processing the cheese is new, and we must find the most optimal conditions for maturation process. In maturation cellars, must secure a certain temperature (Sihufe et al. 2010) the relative humidity and air, specific to each assortment of cheeses. Temperature has a very important role in regulating the maturation of cheese: high temperatures gives favors multiplication and activity of microorganisms and a decrease in temperature, on the contrary, slows their development, through this delaying aging (Banu et al. 1998). Generally aging is done at temperatures between 10-20ºC. Our department processing the cheese is new, and we must find the most optimal

conditions for the maturation process. As the maturation at room temperature was highly variable both in summer and winter, I used a special type of thermometer EBI 20 TH1. With this thermometer we achieved an optimal temperature of maturation.

## MATERIAL AND METHODS

In this study we analyzed semi-hard cheese paste, processed in a processing center for cow's milk, in Sibiu County, which has been matured in different temperature conditions. For this study we used the following analysis, (Tita 2002) highlighting the maturation process of this cheese:

- Determination of the cheese acidity, expressed in Thorner degrees;
- Determination of the content of sodium chloride - Argentometry titration method, expressed in g /100 g product;
- Determination of dry matter - Moisture Analyzer AND ML- 50 Moisture analyzer AND ML-50 is based on the principle of thermo-gravimetric analysis, drying samples using halogen lamp and obtain moisture content in %;
- Determination of glutamic acid by enzymatic analysis, clinical biochemistry analyzer semiautomatic Stat Fax 1904, expressed in grams /100 grams product.

## RESULTS AND DISCUSSIONS

In this study we analyzed semi-hard cheese paste "Albota" a maturation period of 60 days, of maturation in different conditions, namely the temperature at which this operation was conducted important in finalizing the specific identifying characteristics of this cheese. To better track the evolution of the main physico-chemical characteristics, we made the following notation:

-BAV- Cheese "Albota" matured during the summer, which has not been done too of maturation temperature of 14-16$^0$C ,specific assortments so that the temperature reached 22 to 24$^0$C;

- BAI- "Albota" matured during the summer, which has not been done too of maturation temperature of 14-16$^0$C, specific so the temperature reached 8-11$^0$C;

-BAT "Albota" matured during summer and winter, thanks to the use of the thermometer EBI 20 TH1, as well as an isolation room, proper maturation temperature was obtained of specific assortment maturation of 14-16$^0$C.Following the analysis performed we obtained the following results:

Determination of acidity cheese maturation process subject:

Lactic acid influences the structure and consistency of the paste, but it also plays an important role in the formation of compounds of cheese flavor that gives a specific taste. Lactic acid is a component of flavor, directly or substances that may arise from the transformation of lactates .Following the analyzes conducted over a period of 60 days, respectively in the 1st day, 30 day and 60 days, the results are shown in the following figure:

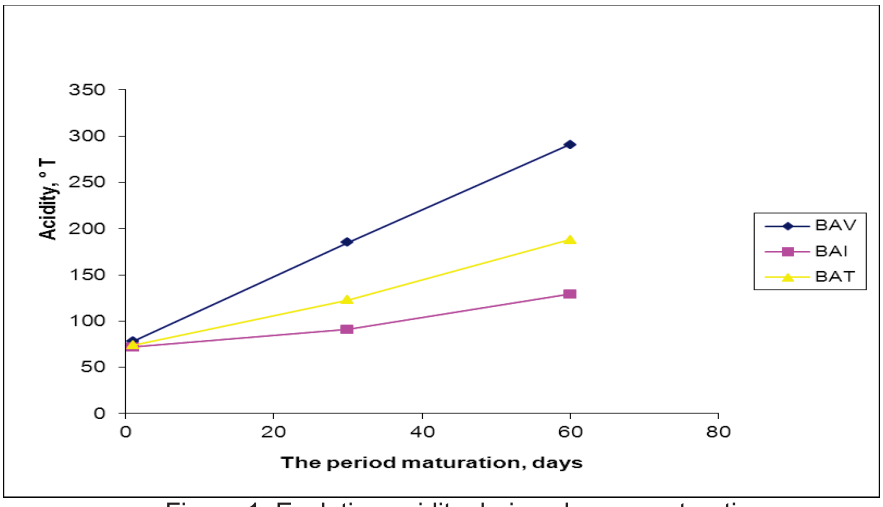

Figure 1. Evolution acidity during cheese maturation.

From the graph shows that for BAV were obtained elevated acidity caused by the high temperature which was achieved maturation, acidity for BAI is low for the period. It is noted that for monitoring the temperature with a thermometer, acidity values correspond to BAT standards and obtain a high quality cheese paste is smooth and soft. Also cheeses with high acidity are crumbly and cheese with not enough acid content is hard and rubber and white.

Determination of salt

Following the analysis performed salt content development for the period under review is shown in the following figure 2.

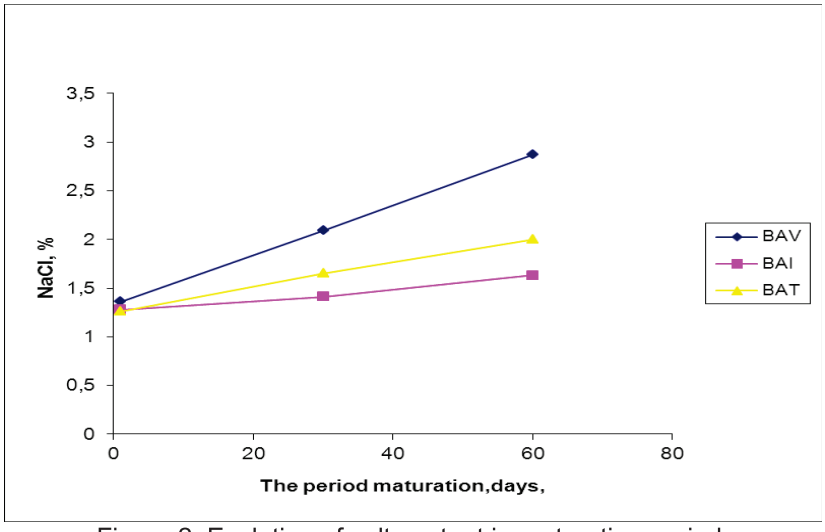

Figure 2. Evolution of salt content in maturation period.

It is noted that at a high temperature of 22-24$^0$C, increases the concentration of salt in the cheese composition, reaching a value of 2,87%, a value that is higher than that provided in the standards of quality 2- 2,5%.

BAI matured cheese at a temperature of 8-11$^0$C, has little salt content of 1,63%, below the quality standards set in, which leads to a cheese tasting vapid, because salt serves to provide a taste to liked them. Salt also has contribution in accelerating the formation and strengthening rind cheeses. The optimal salt content in cheese can be found in matured at 14-16$^0$C, it is at the end maturation process of 2%.

Determination of dry matter in cheese

Following the analysis performed dry for the period under review is shown in figure 3.

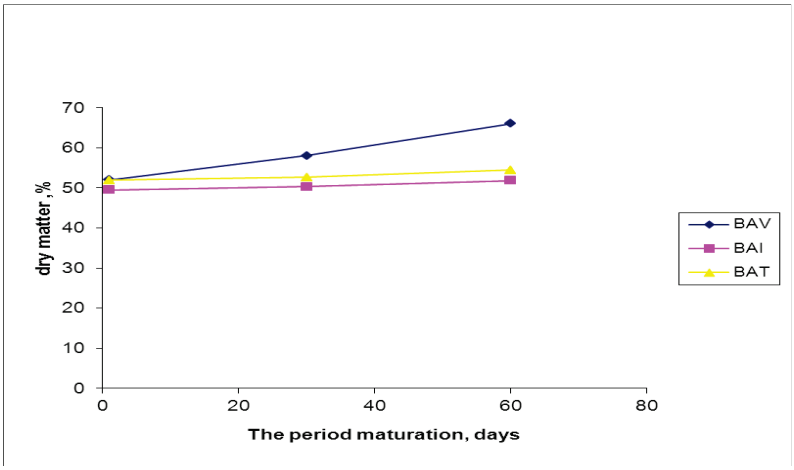


Figure 3. Evolution of dry matter content in the maturation period.

Our results show the matured cheese at higher temperatures than recommended, leading to an increase in the dry, which leads to cracked rind and cheese matured at low temperatures has a paste with a taste definite and rind mildew.

Determination of glutamic acid by enzymatic analysis

Following the analysis performed for the period under review developments glutamic acid is shown in figure 4.

From the results it is observed that if BAV cheese, proteolysis is more advanced due to higher temperature, finding that in addition to the high content of glutamic acid at smelling they felt a slight odor of ammonia. This odor of ammonia, is not specific to this cheese.

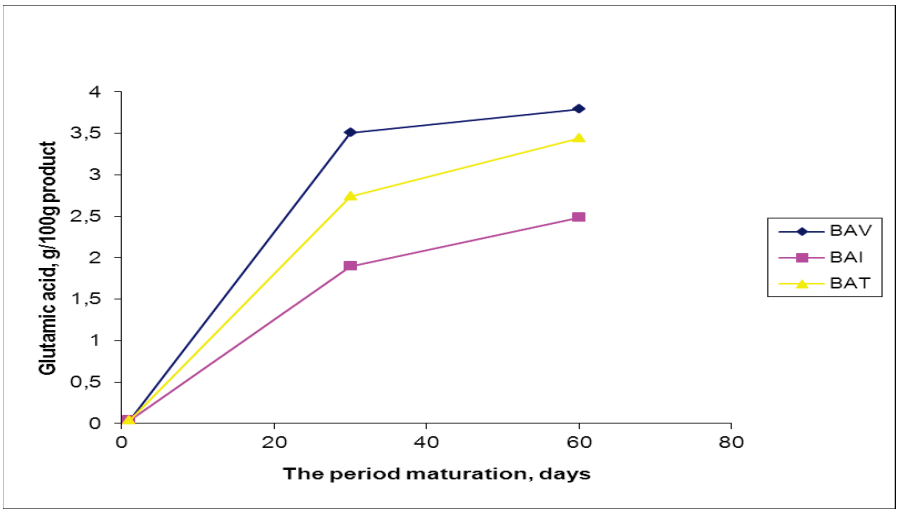

Figure 4. Evolution of glutamic acid on maturation period.

Glutamic acid content of BAI cheese, show lower value which suggests that proteolysis was not conducted under optimal temperature. For BAT cheese, the content of glutamic acid is optimum, which means that proteolysis was carried out under optimal conditions. The process of proteolysis, change fluid consistence of cheese, which is soft, unctuous giving the impression that it is more fat.

## CONCLUSIONS

The topic of dissertation thesis was chosen so that on the basis of laboratory results to highlight the role of the maturation process and the conditions into it shall be conducted in order to obtain high quality cheeses.

Based on the results conclusions can be drawn with both theoretical and practical nature. The conclusions stemming from this study are:

- matured cheese at a temperature of 22-24$^0$C, have higher acidity, salt content, dry matter and high glutamic acid, which leads to a crumbly cheese with texture, taste and slight odor of ammonia salt;
- matured cheese at 8-11$^0$C, have little acidity, salt content, dry matter and low glutamic acid, which leads to a rubbery consistency and cheese with a surface moldy taste without a definite taste;
- matured cheese at 14-16$^0$C exhibits the physic and chemical quality standards, consistency of cheese paste is soft, unctuous giving the impression that it is more fat. Glutamic acid and also fit into the values that exist in specialty literature.

## REFERENCES

Bergamini C.V., Wolf I.V., Perotti M.C., Zalazar C.A., 2010, Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. Small Rumin. Res. 94, 79-89.

Banu C., Vizireanu C., 1998, Procesarea industrială a laptelui. Editura Tehnică,Bucureşti;

Costin Gh.M., 2003, Ştiinţa şi ingineria fabricării brânzeturilor. Editura Academica, Galaţi.

Hanaei F., Cuvelier G., Sieffermann J.M., 2015, Consumer texture descriptions of a set of processed cheese. Food quality and preference, 40, 316-325.

Kheadr E.E., Vuillemard J.C., El-Deeb S.A., 2003, Impact of liposome-encapsulated enzyme cocktails on cheddar cheese ripening. Food Res. Int. 36, 241-252.

Sihufe G.A., Zorrilla S.E., Rubiolo A.C., 2010, The influence of ripening temperature and sampling site on the proteolysis in the Reggianito Argentino cheese, LWT-Food Science Technology 43 (2), 247-253.

Sousa M.J., Ardo Y., McSweeney P.L.H., 2001, Advances in the study of proteolysis during cheese ripening. International Dairy Journal 11 (4), 327-345.

Tiţa M., 2002, Manual de analiză şi controlul calităţii în industria laptelui. Editura Universităţii „Lucian Blaga" din Sibiu, Sibiu.

Yvon M., Rijnen L., 2001, Cheese flavour formation by amino acid catabolism, International Dairy Journal 11(4-7), 185-201.

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# THE INFLUENCE OF THE TEMEPERATURE OVER FERMENTATION ACTIVITY OF AN *SCCHAROMYCES CEREVISIAE* (*OVIFORMIS*) STRAINS ISOLATED FROM SPONTANEOS MICROFLORA

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***Keywords***: *fermentation activity, Saccharomyces cerevisiae (oviformis)*

## ABSTRACT

*Knowing yeast's from a viticol center is important because it is the source from which can be selected yeast's stalks with oenological characteristics favorable for conduced fermentation of the grape juice. The study of oenological aptitudes was made for more than one isolated stalk from the spontaneous microflora placated taxonomically in the Saccharonyces genre, cerevisiae specie, var. oviformis. In this project being presented the characteristics of the stalk codified as S.O.10. Research conducted were right about comparing the effect of the temperature on alcohol production and reduce phase before fermentation, so if selected yeast fermentation induced and if natural fermentation.*

## INTRODUCTION

It has been know that temperature in excess or minimal adversely affect yeast cell growth. Are considered to be thermotolerant the yeast's care grow at 40°C. The high temperature induced respiratory defiency in yeasts (Ribereau-Gayon P., 1998). Effect of temperature upon activity of yeast's was suject of study for many researchers (Hacking 1984; Gacto 2003; Van Uden 1984, Noé Arroyo-López 2009).

## MATERIAL AND METHODS

The biological material retained in the study was represented by the yeast stalk codified S.O.10, which belongs to the *Saccharonyces cerevisiae var.oviformis* species. Colonial isolates tested were standard identification (Anghel et al. 1991; Barnett et al. 2000).

Growing medium on which was maintained this culture is YMA. The testing of oenological aptitudes was made in sterilized musts liquid medium with different concentrations of sugar (175 g/L, 250 g/L and 350 g/L). For stalk testing in different conditions of temperature, the inseminated medium were maintained at four termical thresholds (2-4°C, 18°C, 25°C, 35°C). The insemination of the liquid medium was realized with $nx10^8$ cells/ml. Density of cells was determinated with nephelometer. The witness was represented by the musts medium, which ferments naturally. The concentration of sugar was monitored daily by refractometry.

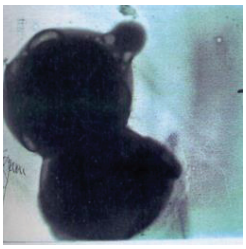

Photo 1. Cells of *Saccharonyces cerevisiae var.oviformis* (x 14000) (original) .

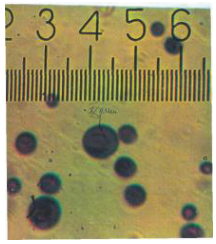

Photo 2. *Saccharonyces cerevisiae var.oviformis*- cells in liquid medium (original).

## RESULTS AND DISCUSSIONS

At the temperature of 2-4$^{o}$C, the stalk S.O.10 metabolized very slowly small quantities of sugar, but when after 45 days of maintaining at low temperature, the stalk was maintained at 25$^{o}$C, the fermentation process was resumed in 24 hours, this demonstrates that this low temperature realized just a inhibition of the stalk metabolism.

The figure 1 presents fermentation curves of the S.O.10 stalk on the three temperatures which permits growing and at a sugar concentration of 175 g/L.

At temperatures between 18 and 25ºC, natural fermentation starts in the same time with induced by the selected stalk. The temperature of 35$^{o}$C and a concentration of sugar of 175 g/L permitted to the selected stalk to start the fermentation more rapidly, the duration of the prefermentation phase being reduced to a few hours. Maximum duration of fermentation is being recorded in the selected stalk case at temperatures between 18 and 25ºC.

When the quantity of sugar in the medium is 250 g/L, at all the temperature thresholds is recorded a delay between the natural fermentation and the fermentation realized with the S.O.10 stalk. (Figure 2).

The fermentation realized with the S.O. 10 stalk at a sugar concentration of 250 g/L is starting with 6 days earlier more rapidly than natural fermentation at the temperature of 18$^{o}$C while at the temperature of 35$^{o}$C the delay between the two types of fermentation is 4 days.

The maximum level of metabolized sugar in 24 hours is recorded in the S.O.10 stalk case on the three temperature thresholds, at only three days from insemination while in the natural fermentation case, the maximum level of metabolized sugar is recorded after 4-9 days from the time when the fermentation process was started.

When the sugar concentration is exceeding 300 g/L and the fermentation temperature is between 18 and 35ºC, the S.O. 10 stalk starts the fermentation process in just a few hours at a temperature of 25-35$^{o}$C while at 18$^{o}$C the fermentation process is started after 2 days from insemination of the cells in the new medium (Figure 3).

Fermentation duration is between 8 and 29 days depending of the incubation temperature. The characteristics of the medium are presented in the table 1 and 2, with the two types of fermentation.

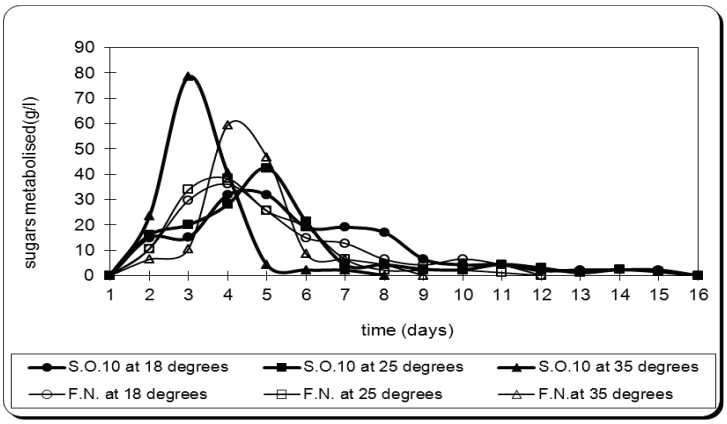


Figure 1. The fermentative curves of natural fermentation and with S.O.10 at three temperatures and 175 g/L sugars.

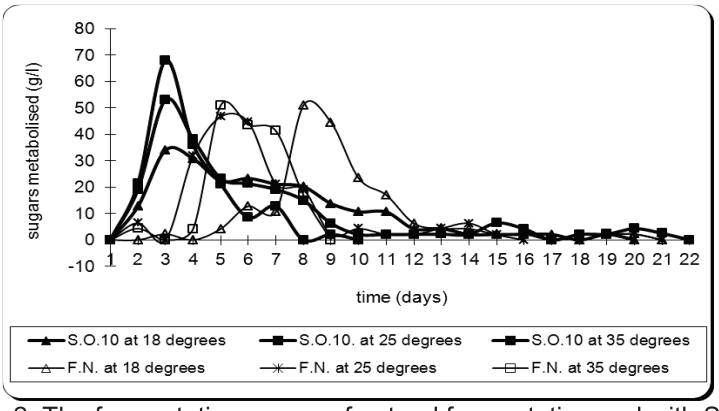


Figure 2. The fermentative curves of natural fermentation and with S.O.10 at three temperatures and 250 g/L sugars.

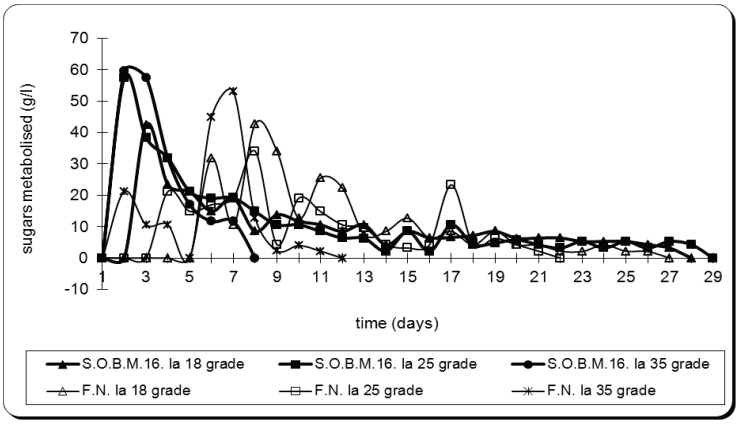


Figure 3. The fermentative curves of natural fermentation and with S.O.10 at three temperaratures and 350 g/L sugars.

Table 1

The characteristics of the medium for the natural fermentation

| | T1= 18ºC | | | T2= 25ºC | | | T3= 35ºC | | |
|---|---|---|---|---|---|---|---|---|---|
| | C1 175 | C2 250 | C3 350 | C1 175 | C2 250 | C3 350 | C1 175 | C2 250 | C3 350 |
| Alcohool (vol.%) | 9.0 | 11.5 | 14.0 | 8.0 | 11.5 | 13.0 | 8.5 | 9.0 | 9.0 |
| Residual sugar (g/L) | 16 | 47 | 87 | 25 | 45 | 113 | 27 | 75 | 182 |
| Time of prefermentative phase (hours) | 22 | 70 | 115 | 22 | 25 | 70 | 11 | 13 | 13 |

Table 2

The characteristics of the medium for the fermentation with S.O.10

| | T1= 18ºC | | | T2= 25ºC | | | T3= 35ºC | | |
|---|---|---|---|---|---|---|---|---|---|
| | C1 175 | C2 250 | C3 350 | C1 175 | C2 250 | C3 350 | C1 175 | C2 250 | C3 350 |
| Alcohool(vol.%) | 9.0 | 11.5 | 14.0 | 8.0 | 11.5 | 13.0 | 8.5 | 9.0 | 9.0 |
| Residual sugar (g/L) | 16 | 47 | 87 | 25 | 45 | 113 | 27 | 75 | 182 |
| Time of prefermentative phase (hours) | 22 | 70 | 115 | 22 | 25 | 70 | 11 | 13 | 13 |

## CONCLUSIONS

S.O. 10 stalk metabolizes intensely sugars at the temperature of 35ºC when the duration of the fermentation is reduced at only 8 days in case of sugar concentration equals 175 g/L. At a sugar concentration of 250 g/L, the fermentation duration varies between 9 and 21 days while the duration of the prefermentation phase is 6 to 12 hours. When the growing medium is very rich in sugar, the fermentation realized with the S.O.10 stalk recorded the lowest duration at the temperature of 35ºC, alcoholic degree maxim being registered at 18-25ºC. S.O.10 stalk isolated from spontaneous microflora responds to conduct at temperatures of fermentation 18-25ºC and 170-350 g/L concentration of sugar.

## REFERENCES


Anghel et al., 1991, Biologia si tehnologia drojdiilor. Vol.II, Ed. Tehnică, Bucuresti.

Barnett J.A. et al., 2000, Yeast's. Characteristic and identification. 3rd Edition. Cambrige Univ. Press.

Hacking A.J., Taylor T.W.F., Hanas C.M..1984. Selection of yeast able to produce ethanol from glucose at 40°C. Appl. Microbiol. Biotechnol. 19: 361.

Gacto M., Soto T., Vicente-Soler J,, Villa G, Cansado J.,2003, Learning from yeasts: intracellular sensing of stress conditions. International Microbiology; 6:211219

Noé Arroyo-López F., Orlić S., Querol A., Barrio E., 2009, Effects of temperature, pH and sugar concentration on the growth parameters of Saccharomyces cerevisiae,S. kudriavzevii and their interspecific hybrid, International Journal of Food Microbiology 131 (2009) 120-127

Ribereau-Gayon P. Et al.,1998, Traite d,Oenologie, vol.I.Microbiologie du vin.Vinification. Dunod, Paris.

Van Uden, N..1984. Effects of ethanol on the temperature relations of viability and growth in yeast. CRC Crit.Rev. Biotechnol. 1: 263.

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# OSMOTIC PRESSURE EFFECT ON A STRAIN OF *SACCHAROMYCES CEREVISIAE* (*ELLIPSOIDEUS*) ISOLATED FROM WILD MICROFLORA

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***Keywords***: osmotical pressure, *Saccharomyces cerevisiae* (*ellipsoideus*)

## ABSTRACT

*The accumulation of sugar in grapes id conditioned of many factors which are on the one hand are pedoclimated conditions of the viticultural center and on the other hand are the genetic characteristics of the breed. Then, when in must the sugar concentration is very large, because of the high osmotic pressure, one part of yeast's, which realize the natural fermentation, dies because of plasmolisa process. In these conditions, residual sugar can provide the carbon source for the yeast resistant to osmotic pressure, where natural fermentationIncreasing the concentration of sugar in the grape from 240 g/L to 320 g/L caused an inhibition of fermentative activity of the yeast responsible for natural fermentation and a slowdown if selected yeast*

## INTRODUCTION

The yeast's *Saccharomyces cerevisiae* is still the primary chois for musts fermentation. These produced fermentation in medium with high concentration of sugars (> 150 g/L) (Ribereau-Gayon et al. 1998). The effects of sugars concentrations on cell membranes and membrane protein have been studied (Banbalov and Tzvetanov 1995, Tzvetanov et al. 1994, D'Amore et al. 1988, Hohmann 2002; Punchal and Stewart 1980, Stefan 2002, Owades 1981, Safri 2011) but the effect of osmotic pressure on ethanol production by yeast's is less studied (Younis 1998). Was determined that the growth rate and fermentation rate decrease when the osmotic pressure increased, the yeast viability and fermentative ability decreased (Laurent et al. 2000, Morris et al. 1986, Patricia et al. 2003, Belloch et al. 2008, Charoenchai et al. 1998, D'Amato et al. 2006, Nagodawithana et al. 1974).

## MATERIAL AND METHODS

A strains of *Saccharomyces cerevisiae* (*ellipsoideus*) represented the biological material; strains isolated from spontaneous microflora and codified S.E.7. The isolation of the strains was made from the musts of Riesling italian in the fermentation. Identification and systematic classification was based on standard tests (Anghel et al. 1991; Barnett J.A. et al. 2000). The fermentation environment

was the must of grapes sterilized before the insemination of the selected strain, wanting through this insemination to have a number of nx$10^8$ cell/ml.

To determinate the influence of sugar concentration in strain S.E.7 metabolism where made insemination in sterilized musts with sugar concentration of 180, 240 and 320 g/L. The temperature of the fermentation environment was at 16-35ºC. The witness probe was represented by an sample of grape juice with the same concentration of sugar and termostated at the same temperatures.

The fermentation curves were stated from daily determination made on metabolized sugars at 24 hours. The experiment was in laboratory conditions. Cells were grown in 100 ml medium distributed in shake flasks whith 500 ml capacity. Metabolized sugars determination was made by refractometry, every 24 hours.

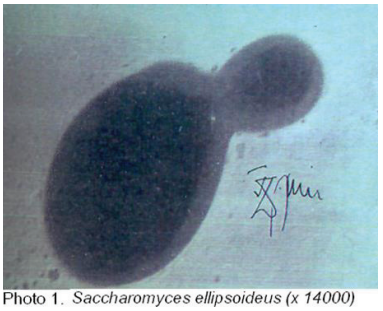

Photo 1. *Saccharomyces ellipsoideus (x 14000)*

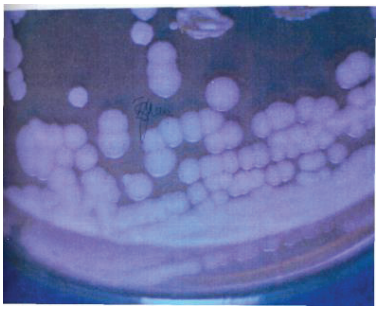

Photo 2. Saccharomyces cerevisiae (ellipsoideus) – colonies (original)

## RESULTS AND DISCUSSIONS

From daily determinations made in experimental variant, the characteristically fermentation curves were for selected strains and natural fermentation.

Figure 1 presents the fermentation curves for S.E.7 stalk at the mentioned sugar concentration and at an incubation temperature of 16ºC. From figure 1 representation can be observed that at 16ºC temperature S.E.7 stalk starts the fermentation process very quickly when the sugar concentration from the medium is not exceeding 240 g/L.

At a sugar concentration of 320 g/L the fermentation process starts after 2 days from cells insemination. In compare with the fermentation induced by S.E.7 stalks, natural fermentation at 16ºC, starts rapidly only if sugar concentration is reduced (180 g/L). A sugar concentration between 240 and 320 g/L determines a delay of natural fermentation with 5 days, comparing with the induced fermentation by S.E.7 stalk. It can be observed that at this temperature, the fermentation curves presents minimum points just in the natural fermentation case on a sugar concentration of 320 g/L.

The figure 2 presents the fermentation curves of S.E.7 stalk, at 25ºC and at three concentrations of sugar. At this temperature, the fermentation induced by the S.E.7 stalk starts in the same time at all three sugar concentrations. The maximum of metabolized sugars in 24 hours (53 g/L) is registered at 320 g/L sugar concentration, only in 24 hours from the insemination.

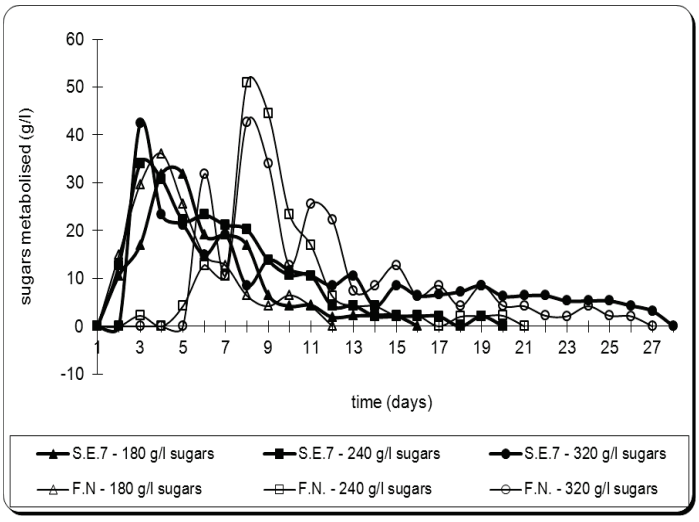


Figure 1. The fermentative curves of natural fermentation and with S.E.7 at three concentrations of sugars and to 16°C.

It can be observed that at this temperature, on small concentrations of sugars in medium the fermentation process works slowly in comparison with variants when sugar concentration is bigger, explained by the fact that although the 25 temperature permits a intensification of the process of cells multiplication, then when the growing medium does not contain sufficient C and N, this process is greater slowed or even stopped.

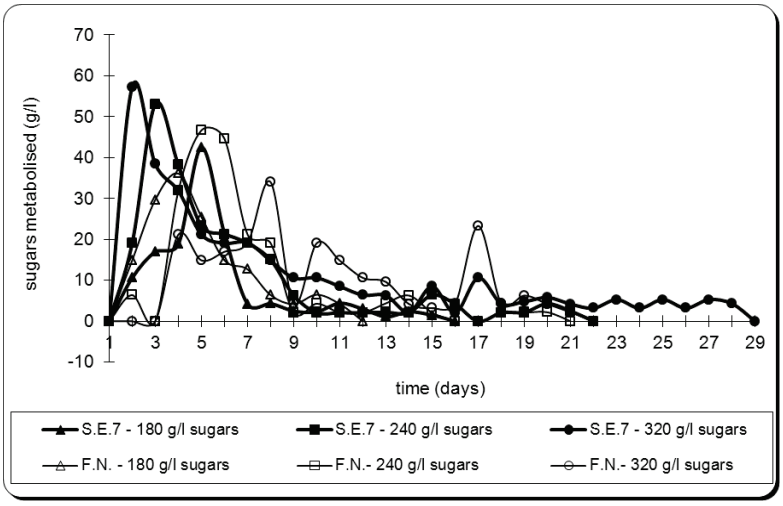


Figure 2. The fermentative curves of natural fermentation and with S.E.7 at three concentrations of sugars and to 25°C.

In the same medium conditions the natural fermentation work normal at sugar concentrations between 180 and 240 g/L while at a sugar concentration of 320 g/L the fermentation curve characteristic for natural fermentation presents an numerous minimum points determinate by different resistance of yeast's strains.

At plasmoliza phenomenon because of a higher sugar concentration in the medium. At 35°C S.E.7 stalk, although starts the fermentation process, first at 320 g/L sugar concentration, presents an maximum of quantity of metabolized sugars in 24 hours, at an 180 g/L sugar concentration in medium.

This can be explained by the fact that, when the temperature is higher and favorable to the process of respiration, the development of the cells is deranged by the higher sugar concentrations (Figure 3).

At the maximal concentration of sugars and to the temperature 16ºC, the prefermentative phase is very long (36 hours) but the residual sugars is small (42 g/L) comparative with the same concentration and the 35ºC were the prefermentative phase is small (6 hours) but the sugars residual is 156 g/L.

At the concentration of sugars of 240 g/l, the alcoholic degree is maxim at the termed level of 25ºC. The medium is clear at the final of fermentation and the warehouse is adhered at glass.

When the extrinsic factor inhibits the cells metabolism, the development of the growing medium is much more intense at lower values of the intrinsic (sugar concentration). At this temperature natural fermentation started with 3-5 days delay comparative with induced fermentation by the S.E.7 strains.

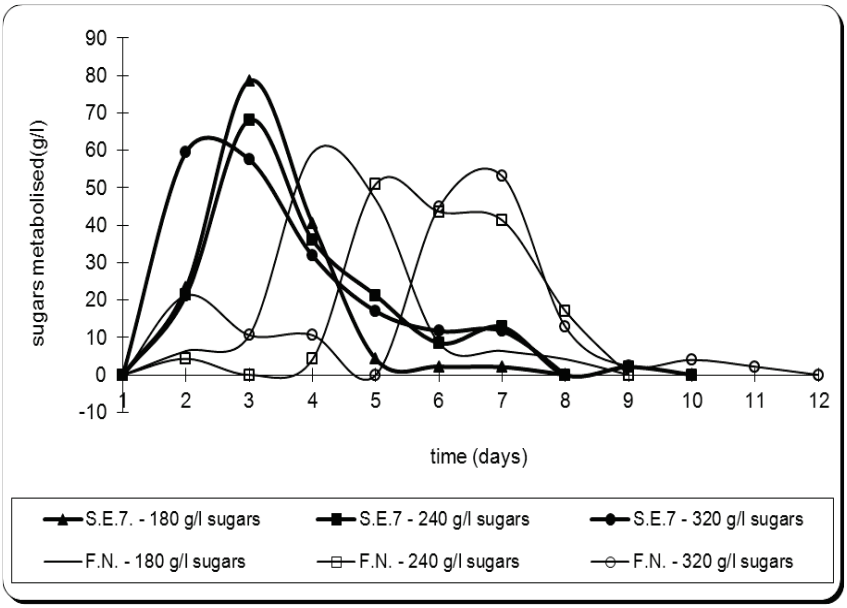


Figure 3. The fermentative curves of natural fermentation and with S.E.7 at three concentrations of sugars and to 35°C

## CONCLUSIONS

At lower sugar concentrations the fermentation induced by S.E. 7 strains doesn't differentiate a lot in comparison with natural fermentation, when the fermentation temperature was between 16 and 25°C. Then, in grape juice with low sugar concentration is not necessarily the use of S.E.7 stalk for making the fermentation process.

When sugar concentration is more than 240 g/L, the start of the natural fermentation is delayed producing a number of perturbations of the fermentation process (minimum points). The induced fermentation by S.E.7 stalk did not presented minimum points at this sugar concentration which demonstrates that the selected stalk determinates a regularization of the process.

## REFERENCES


Anghel I. et al., 1991, Biologia si tehnologia drojdiilor. Vol.II, Ed. Tehnica, Bucuresti.

Banbalov G.K., Tzvetanov O.S, 1995, The effect of storage on some characteristics of yeasts used for sparkling wines. Journal of Wine Research 6(2): 123-132.

Barnett J.A. et al., 2000, Yeast's. Characteristic and identification. 3rd edition. Cambrige Univ. Press.

Belloch C., Orlić S., Barrio E., Querol A., 2008, Fermentative stress adaptation of hybrids within the Saccharomyces sensu stricto complex. International Journal of Food Microbiology 122: 188-195.

Charoenchai, C., Fleet, G., Henschke, P.A., 1998. Effects of temperature, pH and sugar concentration on the growth rates and cell biomass of wine yeasts. American Journal of Enology and Viticulture 49: 283-288.

D'Amato D., Corbo M.R., Del Nobile M.A., SinigagLia M., 2006, Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system. International Journal of Food Science & Technology 41: 1152-1157.

D'Amore T., Punchal C.J., Russell. J., Stewart G.G, 1998, Osmotic pressure effects and intracellular accumulation of ethanol in yeast during fermentation. J. Ind. Microbiol 2:365.

Hohmann S., 2002, Osmotic stress signaling and osmoadaptation in yeasts. Microbiology and Molecular Biology Reviews 66:300-372.

Laurent B., Inigo M., Maran N., Pierre A., Marechal G., and Patrick G., 2000, Influence of thermal and osmotic stresses on the viability of the yeast Saccharmyces cerevisiae. International Journal of Food Microbiology 55: 275279.

Morris, G.J., Winters, L., Coulson, G.E. and Clarke, K.J.,1986, Effect of osmotic stress on the ultrastructure and viability of the yeast Saccharomyces cerevisiae. J. Gen. Microbiol., 129: 2023–2034.

Nagodawithana T.W., Castellano C., Steinkraus H.K.,1974, Effect of dissolved oxygen, temperature, initial cell count, and sugar concentration on the viability of Saccharomyces cerevisiae in rapid fermentations, Applied Microbiology, 28(3): 383-391.

Owades, J.L., 1981, The role of osmotic pressure in high and low gravity fermentations. Tech. Q. Master Brew. Assoc. Am., 4:163–165.

Patricia L., Pratt, James H., Bryce, Graham G., Stewrt, 2003, The effects of osmotic pressure and ethanol on yeast viability and morfology. J.Inst. Brew. 109 (3): 218-228.

Punchal C.J., Stewart G.G., 1980, The effect of osmotic pressure on the production and excretion of ethanol and gLycerol by a brewing yeast strain. J. Inst. Brew.86:207.

Tzvetanov O.S., Bambalov G.K., Luchev S.S., 1994, Effect of cultural conditions on some of the most important technological properties of champagne yeasts used for making red sparkling wines. Journal of Wine Research, vol.4, Issue 3, 177-185.

Safri I., Learmonth R.P., Kennedy U.J., 2011, Fermentation performance of the yeast Saccharomyces cerevisiae in media with high sugar concentration Proceedings of the 2ndInternational Seminar on Chemistry, pp. 379-385

Stefan H., 2002, Osmotic Stress Signaling and Osmoadaptation in yeasts. Microbiology and Molecular Biology Reviews; 66: 300372.

Ribereau-Gayon P. et al., 1998,Traite d·Oenologie, vol.I. Microbiologie du vin.Vinification. Dunod, Paris.

Younis, O.S. and Stewart, G.G.,1998, Sugar uptake and subsequent ester and higher alcohol production by Saccharomyces cerevisiae. J. Inst. Brew., 104:283–287.