

**CHLOROPHYLL, CAROTENOID AND FLAVONOID CONTENTS
IN VEGETATIVE ORGANS OF *VICIA FABA* L.**

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Keywords: *Vicia faba*, chlorophylls, carotenoids, flavonoids

ABSTRACT

*Leaf and stem tissues of broad bean (*Vicia faba* L.) were analyzed for chlorophyll, carotenoid and flavonoid concentrations.*

Total chlorophyll concentration was 6,258 mg/kg in leaves and 570 mg/kg in stems, with a dominance of chlorophyll a 2.2-2.4:1 ratio. Average carotenoid concentration reached 4,317 mg/kg in leaves and 404 mg/kg in stems. Flavonoids had elevated concentrations, with an average of 108,179 mg/kg in leaf tissue and five times lower, 20,423 mg/kg in broad bean stems. These values make broad bean a valuable forage plant.

INTRODUCTION

Broad bean (*Vicia faba* L.), also known as faba bean, horsebean etc. is one of the most important agricultural plants in the Fabaceae family (the third most cultivated, after soybean and peas. A native of the Near East and North Africa, it has quickly become a popular crop throughout the World (in China, for example, it has been cultivated for 5,000 years).

It is an annual herbaceous plant, with erect, 0.3-2 m tall stems, alternate compound leaves (with few or no tendrils), large, usually white flowers, with purple spots, grouped in axillary racemes and short, broad pods (Singh et al. 2013).

Broad bean is mostly cultivated for its seeds, a source of protein, carbohydrates, as well polyphenols and other antioxidants, its popularity in human alimentation increasing in recent times (Sathya Prabhu & Devi Rajeswari 2018). Other uses include soil regeneration through nitrogen fixation and forage.

Apart from seeds, vegetative organs (leaves and stems) are used as a forage. Good forage cultivars have stems with low branching and low lignin content and over 20 photosynthetically-active leaves at the time of cutting, with a high overall protein content (180-200 g/kg; Mihailović et al. 2011). This is why knowing the biochemical composition of broad bean vegetative organs is important in designing forage schemes.

Chlorophylls a and b are the main pigments found in green vegetative organs. They are an important part of human and animal nutrition, having antioxidant properties, limiting oxidative stress, digestive uptake of carcinogenic compounds and limiting kidney stone formation (Inanç 2011).

Carotenoids such as various carotenes, lutein, lycopene, zeaxanthin etc. are accessory photosynthetic and protective pigments in plant organs. For human and animal organisms, they function as precursors of retinol and melanin (essential compounds for eye and skin health), while also having strong antioxidant properties (Eldahshan & Singab 2013).

Phenolic and polyphenolic compounds form a wide group of phytochemicals that protect plants against various infections and insect attacks. Among these, flavonoids (low-mass polyphenols) are extremely valuable nutrients, with high antioxidant, antiviral, antibacterial, anti-inflammatory and antiproliferative capacities (Kivrak & Kivrak 2014).

MATERIAL AND METHODS

Plant material was collected from local farms in the Constanța County, Romania. Leaves and stems were sorted and analyzed separately.

For determining chlorophyll and carotenoid concentration, 0.1 g of fresh tissue were extracted in 10 mL acetone (80%), filtered and the spectrophotometric absorbance was determined at 470, 647 and 663 nm, using a S106 WPA spectrophotometer (Popoviciu et al. 2019). Concentrations were derived from absorbance values using specific equations (Lichtenthaler & Buschmann 2001).

For flavonoids, 1 g fresh tissue were extracted in 5 mL methanol, filtered and 0.5 mL aliquotes of the extract were diluted in 4:8 water:methanol solution. Spectrophotometric absorbance was read at 340 nm and concentrations were determined according to Szabo et al. 2012.

Dry biomass percentage was determined by drying fresh tissue at 105°C. All concentrations were expressed as mg/kg of plant dry weight.

RESULTS AND DISCUSSIONS

Average dry biomass percentage was 21% in stems and 35% in leaves.

Chlorophyll concentrations are shown in Fig. 1. Fig. 2 shows total carotenoid concentrations, while Fig. 3 shows flavonoid content in plant organs.

Total chlorophyll concentration was 6,258 mg/kg in leaves and 570 mg/kg in stems, with a dominance of chlorophyll a 2.2-2.4:1 ratio. Chlorophyll concentrations found in broad bean leaves are thus similar to those found in vegetative organs of some common pasture grasses ranging from 3,220 to 15,300 mg/kg, also with a dominance of chlorophyll a (Bokari 1983). It was higher than in lettuce and nettle leaves (226, respectively 456 mg/kg FW; Morna 2015).

Average carotenoid concentration reached 4,317 mg/kg in leaves and 404 mg/kg in stems. This is higher, for instance, than values found in forage rye (*Secale cereale*) – up to 271 mg/kg (Zhao et al. 2020) – or lettuce and nettle (45-74 mg/kg FW; Morna 2015).

Flavonoids had elevated concentrations, with an average of 108,179 mg/kg in leaf tissue and five times lower, 20,423 mg/kg in broad bean stems. For comparison, red clover, *Trifolium pratense*, contains up to 37,800 mg/kg flavonoids (Tava et al. 2015), alfalfa, *Medicago sativa* – 12,000 mg/kg (Karimi et al. 2013). Mulberry leaves (*Morus* sp), used as forage in many Asian countries contain up to 58,400 mg/kg total flavonoids (Hassan et al. 2020). Such high flavonoid contents are even comparable to those in tea leaves (Blumberg et al. 2015).

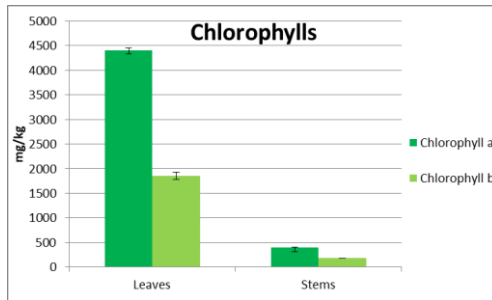


Figure 1. Chlorophyll a and b concentration in *Vicia faba* tissues (mg/kg).

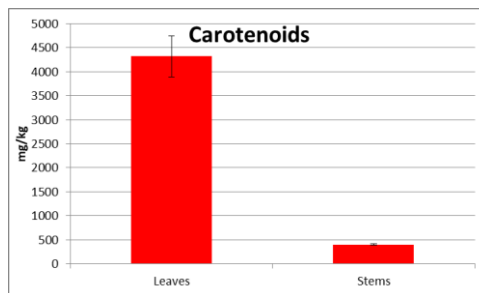


Figure 2. Total carotenoid concentration in *Vicia faba* tissues (mg/kg).

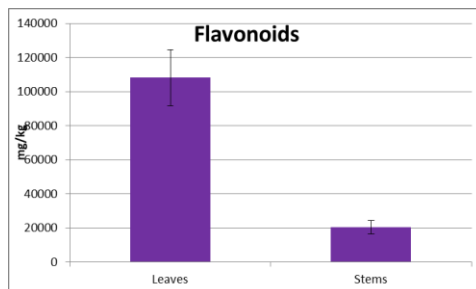


Figure 3. Total flavonoid concentration in *Vicia faba* tissues (mg/kg).

CONCLUSIONS

With high chlorophyll and carotenoid content (up to 6,258 mg/kg, respectively, 4,317 mg/kg), broad bean tissues, especially foliar ones, are comparable to common forage plants.

Flavonoid contents are even higher, with up to 108,179 mg/kg in leaf tissues. These values are higher than most forage plants and offer a valuable source of antioxidants.

These results show a high potential for broad bean vegetative organs as a forage, but also for other possible applications.

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