THE ANALYSIS OF CHLOROPHYLL, CAROTENES AND ANTHOCYANINS EXTRACTS AS FOOD COLORINGS

ANALIZA UNOR EXTRACTE DE CLOROFILĂ, CAROTENI ȘI ANTOCIANI FOLOSITE DREPT COLORANȚI ALIMENTARI

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Abstract. *The dyes used in the food industry are mostly synthetic, due to their low cost and accessibility. However, natural dyes increase the quality of the products and represent an important selection factor among informed consumers. From the large number of natural dyes, we selected chlorophylls extracted from mint leaves, carotenes extracted from carrots and anthocyanins extracted from blackberries and blueberries. The extracts were made in 70% ethanol and the pigment content was determined spectrophotometrically. The change in values and colour in samples in acidic (lemon juice) and basic (sodium bicarbonate) mediums was monitored, considering the use of these dyes in acidified soft drinks or in pastry products. It was found that the extracts obtained from fresh material have higher levels of pigments than those obtained from dry material, except for those from carrots. The influence of the medium manifested differently, with chlorophyll extracts being more intense in alkaline solution, carotene extracts having comparable values in both mediums and anthocyanin extracts having values twice as big in acidic medium, in both blackberries and blueberries extracts.*
Key words: chlorophylls, carotenes, anthocyanins, food colorings

Rezumat. *Coloranții utilizați în industria alimentară sunt în mare parte sintetici, datorită costurilor reduse și accesibilității. Folosirea coloranţilor naturali creşte însă calitatea produselor și reprezintă un factor de selecţie important în rândul consumatorilor informați. Din numărul mare de coloranți naturali, am selectat clorofile extrase din frunze de mentă, caroteni extrași din morcovi și antociani extrași din mure și afine. Extractele au fost realizate în etanol 70% iar conținutul de pigmenți a fost determinat spectrofotometric. S-a urmărit modificarea valorilor și a culorii în probe în mediu acid (suc de lămâie)și bazic (soluție dicarbonat de sodiu), considerând utilizarea acestor coloranți în băuturi răcoritoare acidulate sau în produse de patiserie. S-a constatat că extractele obținute din material proaspăt au nivele mai ridicate de pigmenți decât cele obținute din material uscat, cu excepția celor din morcovi. Influența mediului s-a manifestat diferit, extractele de clorofile fiind mai intense la reacție alcalină, cele de caroteni având valori comparabile iar extractele de antociani având valori duble în mediul acid, atât la mure cât și la afine.*
Cuvinte cheie: clorofile, caroteni, antociani, coloranți alimentari

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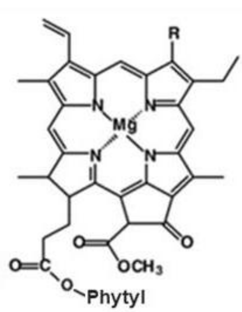
INTRODUCTION

Colour influences the acceptance of products by consumers. The dyes used in the manufacture of food products, medicines, textiles, cosmetics are classified into natural, identically natural and synthetic.

Chlorophylls (Figure 1) are green pigments that have been used as a natural food ingredient. High chlorophyll content is found in green leafy vegetables, algae and green fruits. Ethanol is the most suitable extraction solvent in order to use the solutions for food applications. Studies showed that chlorophylls could be preserved in ethanolic solutions at room or lower temperatures when protected from light, obtaining a suitable natural food colouring. (Ferreira A. *et al.*, 2023) Although chlorophyll is a natural food colouring agent, there are disadvantages associated with its use. Its content is not precisely known and it tends to be unstable under the different pH conditions of the foods to which it is added. [Irede L. E. et al., 2020]. Chlorophyll is reported to be stable at alkaline conditions and thermally stable at pH 7.5, An increase in extraction time and temperature results in higher chlorophyll content, which will decrease, though, with the prolonged postharvest storage. (Ngcobo, S. *et al.*, 2024)



Chlorophyll *a*, R=CH_3
Chlorophyll *b*, R=CHO

Fig. 1 Chlorophylls

Carotenes are obtained by solvent extraction, usually in acetone or ethanol combined with n-hexane. β–carotene is a precursor of vitamin A and is used in various research due to its strong antioxidant effect (Figure 2). All carotenoids, especially β–carotene, are used in the food and cosmetic industries, also in medicine and pharmaceutical industry [Rifqi, M. et al., 2023]. The highest content of β–carotene is found in carrots, apricots, tomatoes, asparagus, broccoli, pumpkins.

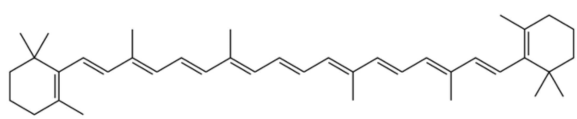


Fig. 2 Structure of β –carotene

Tiwari and Sarkar (2018) established that the β–carotene amounts in carrots extracted with organic solvents varies between 371.90 mg/L and 1631.90 mg/L. β –carotene (the major component of the carotenes extracts) can be used in a large variety of food and beverages including cider, brews, flavoured drinks, margarines and oils, milk-based products like cheese or yoghurt, sweet creams and nuts, various fast-food products. [Smajlagić, A. et al., 2024]

In nature, anthocyanins are the pigments that color many fruits (especially berries) and flowers. Their amount reaches 1.15 mg/g in blueberries and up to 20 mg/g in some red and black vegetables. They are used as dyes from E163 series and their color is mainly red, blue and purple, depending on the source, temperature, air exposure, light and pH of the extract. [Li, X. et al., 2021; Taghavi, T. et. al., 2023]

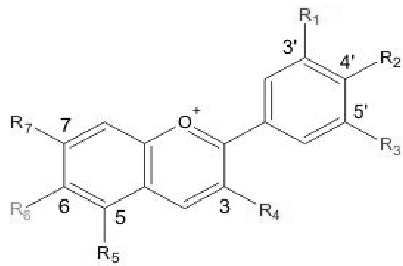


Fig. 3 Structure of flavylium ion

Over 500 different anthocyanins have been isolated, but the basic structure of an anthocyanin is always the flavylium ion (Figure 3) (Kalt, W. *et al.*, 2020). Anthocyanins have multiple biological activities (antioxidant, antitumoral, liver support, anti-inflammatory, antiaging). Therefore, they have been used in food, health products, cosmetics, medicine and pharmaceutical industry (Tan, J. *et al.*, 2022).

The aim of this paper was to extract these three pigments from a natural source and compare the quantity and stability of the extracts' color obtained from fresh or dry materials in acidic or alkaline medium.

MATERIAL AND METHOD

The products used in this study were acquired from a local supermarket and the amounts of specific pigments were analyzed. The following samples were taken into consideration: F_m – fresh mint leaves (*Mentha piperita*): D_m – dry mint leaves: F_c – fresh carrots (*Daucus carota*): D_c – dry carrots: F_{bkb} – fresh blackberries (*Rubus fruticosus*): D_{bkb} – dry blackberries: F_{beb} – fresh blueberries (*Vaccinium myrtillus*): D_{beb} – dry blueberries.

Specific methods were used to determine the content of dry matter and moisture by oven drying at 105^0C using a Biobase hot air oven, the pH values using a pH-meter from Hanna Instruments and the content of the three considered pigments by colorimetric method using a Spekol 1100 spectrophotometer.

Taking into consideration the use of the extracts in baking products or beverages, they were obtained using 25 mL ethanol 70% per sample, for 72 hours at room temperature in a dark place, as follows: 2 g fresh mint leaves and the equivalent dry amount, 5 g of carrots, blackberries and blueberries and their equivalent dry samples. The extracts were treated with lemon juice 10% (pH = 2.47) and baking powder 10% (pH = 7.95). The extracts were maintained, after preparation, at 4^0C.

RESULTS AND DISCUSSIONS

The dry matter content of the analyzed samples was calculated and their values are presented by comparison with the data from literature in Figure 4.

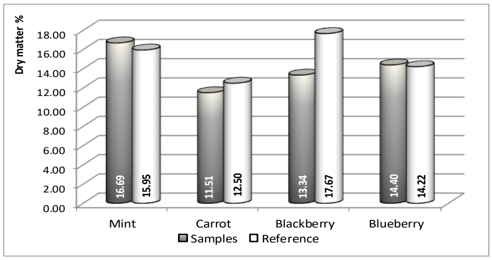


Fig. 4 Dry matter values compared to literature reference

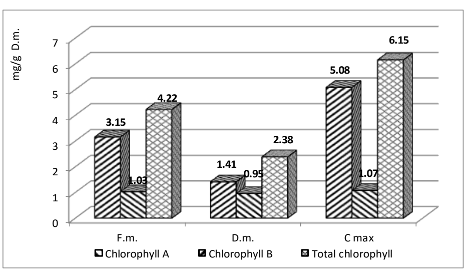
Apart from blackberries, which presented a slightly lower content of dry matter, the samples had similar values to the ones found in literature for the considered species.

Chlorophyll is used as a colouring agent due to its selective absorbance of light of certain wavelengths and its consequent green colour. It absorbs light mostly in the blue range of the electromagnetic spectrum, followed by the red one. For the obtained extracts, absorbance was measured at 645 nm and 663 nm wavelength and the concentrations in chlorophyll a, b and total were expressed in mg/g dry matter, calculated with the following formulas [Aminot, A., Rey F., 2001]:

$$Chlorophyll\ a = 12.7 \cdot A_{663} - 2.59 \cdot A_{645}$$

$$Chlorophyll\ B = 22.9 \cdot A_{645} - 4.7 \cdot A_{663}$$

$$Total\ chlorophyll = 8.2 \cdot A_{663} + 20.2 \cdot A_{645}$$



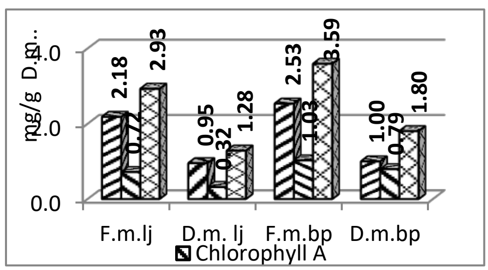


Fig. 5 Chlorophylls values in samples

Fig. 6 Influence of medium on chlorophylls

Fresh samples registered higher contents in all chlorophyll types, no matter the pH of the medium, and the alkaline extracts had increased values than the acidic ones (Figures 5 and 6).

The dosage of carotenes from the alcoholic extracts used a calibration curve (Figure 7) and the following equation, where b – cuvette length (cm), d – dilution factor and C = concentration.

$C (mg/g) = 1/453.59 \times (A_{436} \times 454) / (196 \times b \times d)$

The concentrations of the dried sample registered a higher value compared to the fresh one, and the pH of the extract had no significant influence, as presented in Figure 8:

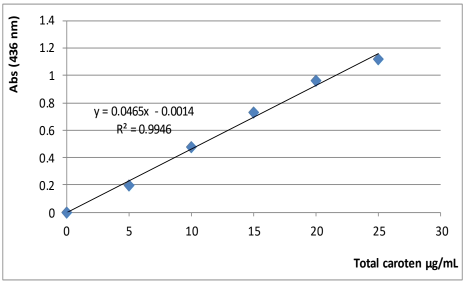


Fig. 7 Calibration curve

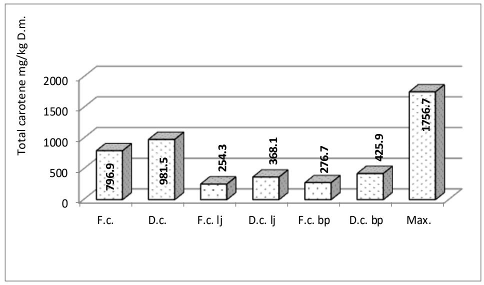


Fig. 8 Influence of medium on carotenes

In what regards anthocyanins, they are easily soluble in polar solvents and have strong absorption in the UV (280 nm) and visible (500–550 nm) region. They are the most effective antioxidants and free radicals scavengers, 50 times more powerful than vitamin E and 20 times than vitamin C. (Li, X. *et al.*, 2021)

The measurements were conducted at 520 respectively 700 nm wavelengths in the two buffer solutions of pH 1 and 4.5, and the contents were calculated with the following formula:

$$A\ (mg/g)=(A_{520:pH1}-A_{700:pH1})-(A_{520:pH4,5}-A_{700:pH4,5})\cdot 449.2\cdot V_B\cdot V_E\cdot 1000/26900\cdot m\cdot d$$

where V_B = the volume of the buffered extract (mL), V_E = the volume of the initial extract (mL), m = sample mass (g), d – cuvette length (cm).

It was observed that fresh samples had higher anthocyanins values than the dried ones and the acidified extracts had values twice as big as the alkaline ones, proving the stability of these pigments at lower pH (Figures 9 and 10).

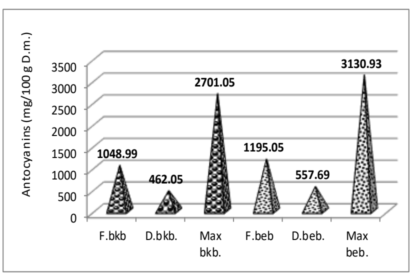


Fig. 9 Anthocyanins values in samples

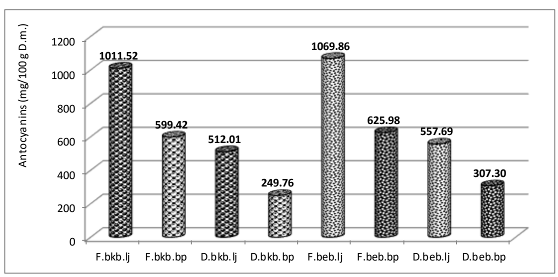


Fig. 10 Influence of medium on anthocyanins

The extracts are easy to prepare and maintained the colour intensity for more than one month when refrigerated at 4^0C, therefore they can be used as food colourings in households and industry, in food products like cold beverages (especially lemonade, for mint extract) or various sweet products like custard, pudding, cookies or cake filling.

CONCLUSIONS

1. Alcoholic extracts of mint leaves, carrots, blackberries and blueberries were prepared and tested for the influence of acidic (lemon juice) and alkaline (baking powder solution) mediums on the pigments' content.

2. The fresh samples for all products registered higher levels of pigments, except for carrots, where the dry sample had 981.48 mg compared to 796.89 mg for the fresh sample, both expressed in mg/Kg dry matter.

3. In what regards the influence of pH on chlorophylls content, the alkaline sample registered a higher value of 3.59 mg total chlorophylls/g dry matter compared to the acid extract, of only 2.93 mg total chlorophylls/g dry matter.

4. Both extracts of carotenes from fresh and dry samples had lower values in acidic and alkaline mediums, slightly bigger for the dry sample extract.

5. The anthocyanins extracts from blackberries and blueberries registered values much lower than maximum presented in literature, most likely due to the early harvesting, before consumption maturity. For both fruits, the acidic extracts had values almost twice as big as the alkaline extracts (blackberries - 1011.52 compared to 599.42 mg/100 g dry matter¸ blueberries - 1069.86 compared to 625.98 mg/100 g dry matter).

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