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DOI: 10.2478/auoc-2025-0005

Ovidius University Annals of Chemistry Volume 35, Number 1, pp. 42 - 50, 2025

Effects of natural pigments from flowers and leaves in non-heat-treated foods

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Abstract. The objective of this study is to investigate the addition of natural pigments extracted from flowers and leaves in food products. The following three non-thermally treated food products were used for the experimental study: nougat, cream cheese and butter. Flowers of *Viola odorata*, *Viola tricolor*, *Syringa vulgaris*, *Cucurbita maxima*, *Ranunculus bulbosus*, leaves of *Taraxacum officinale*, *Beta vulgaris* and petals of *Rosa* sp. were used to obtain red, green, blue and yellow pigments. Powdered pigments were added to three products marked N (nougat), C (cream cheese), and B (butter), at different concentrations: 0.5, 1.0 and 1.5% per 100 g of product. Chlorophyll (Chl) content in green leaves and total monomeric anthocyanin (TMA) content in flowers were determined. The pH, color and textural quality of nougat, cream cheese and butter were obtained. The highest amount of chlorophyll (0.587 mg/g) was found in dandelion leaves, while the highest total monomeric anthocyanin (TMA) content (5.978 mg cyanidin-3-glucoside /g fresh weight) was determined in *Rosa* petals. The pH of all nougat samples increased with the addition of pigments, while the pH of butter samples increased when blue and red pigments (1 and 1.5%) were added and decreased when yellow and green pigments were incorporated in butter samples. The lightness decreased with increasing pigment concentration for all samples. The addition of color pigments to the butter samples decreased the hardness of the samples. The nougat samples hardness decreased with increasing concentration of yellow, red and blue pigments, while addition of yellow and green pigments decreased the hardness of cheese cream samples.

***Keywords*:** anthocyanins; chlorophylls; food colors; pigments; texture.

1. Introduction

Food color and texture are sensory characteristics that play a significant role in food choice and acceptance, enhancing food quality. This has led to the need to understand what consumers want in terms of accepting or rejecting certain types of food. Therefore, many studies have shown that food quality is a central issue in today's food economy, and that food color enhances visual appeal, relative to presenting the same stimuli without a color cue [1-3]. The addition of food coloring may therefore influence sensory thresholds, such as intensity and acceptability of flavor, for certain staple foods, according to Spence [4]. Plant pigments such as chlorophylls, carotenoids and anthocyanins from natural sources, which contribute full hues to food color, have huge potential to replace many dyes. Natural food colorants represent a promising alternative to synthetic food dyes, with nutritional values associated with cytotoxic, antioxidant, antimicrobial, and anticancer activities [5, 6]. Consequently, ingredients (such as vegetable pigments) are increasingly introduced into food products, but the process is difficult because they are chemically unstable and have low bioavailability. The method of incorporating plant powders is the most common and practiced method of coloring food [7]. However, in recent years, research has focused on the possibilities of encapsulation and nanoencapsulation of plant pigments. Almeida et al. [8] evaluated curcumin (a polyphenol) powder and nano encapsulated curcumin powder formulations as colorants for yogurt. The results of their study showed that nano encapsulated curcumin has the strongest coloring ability and can be a solution with undeniable benefits for consumers. Also, Fierri et al. [9] nano encapsulated anthocyanins from red cabbage, Agarry et al. [10] encapsulated chlorophyll, while Gutiérrez et al. [11] discussed some methods for nanoencapsulation of β-carotene in the food sector.

Pigments that can be used to color food are found in fruits, stems, flowers, roots and leaves. Anthocyanins, a class of water-soluble compounds (pigments), offer a practical alternative to the most commonly used synthetic food dyes. They are a group of vacuolar pigments, and depending on the pH value of the environment, they have distinct colors from blue/purple to red /orange [12]. Consumption of these compounds is associated with health benefits, they have anti-cancer, anti-inflammatory, anti-diabetic, anti-obesity effects and may reduce the risk of myocardial infarction and oxidative stress [13]. The use of anthocyanins as food colorants has been limited because they have a low stability, which is easily influenced by light, pH and temperature, and in addition they interact with other compounds in food [14]. Chlorophyll, a highly valued bioactive compound is found in almost all green parts of plants and the process of photosynthesis uses these green pigments [15]. Viera et al. [16] observed that chlorophyll is bioavailable in commercial foods such as

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guacamole, virgin olive oil, tortellini, basil hummus, creamed spinach, creamed spinach, vegetable pasta, green tea with chocolate, avocado and kiwi juices and pesto sauce. They correlated the degradation of chlorophyll during in vitro digestion with the salt content of the digested food. Carotenoids, yellow or orange fat-soluble pigments, are divided into two groups: carotenes and xanthophylls [17]. Carotenes are hydrocarbons with no oxygen atoms in their chemical structure, while xanthophylls have oxygen atoms, which makes them more polar. From this subcategory, the most common xanthophylls are lutein, zeaxanthin and flavoxanthin. Carotenoids with hydroxyl groups (xanthophylls) are hydrolyzed and efficiently absorbed in humans, resulting in a bioavailability comparable to that of non-esterified compounds [18].

The pigments used in the present study were extracted from flowers (*Viola odorata*, *Viola tricolor*, *Syringa vulgaris*, *Cucurbita maxima*, *Ranunculus bulbosus*, *Rosa* sp.), and leaves (*Taraxacum officinale*, *Beta vulgaris*), which have nutritional properties for a healthy diet.

The chemical composition of *V. odorata* is complex, compounds such as polysaccharides, anthocyanins, flavonoids have been isolated. In *Viola odorata* the main anthocyanins are violanin (delphinidin-3-(4″-p-coumaroyl)-rutinoside-5-glucoside) and cyanidin-3-*O*-glucoside [19], while *Viola tricolor* contains beside violanin, cyanidin-3-(coumaroyl)-methylpentosyl-hexosyl-5-hexoside, cyanidin-3-*O*-rutinoside and delphinidin-3-*O*-rutinoside [20]. The color of *Viola odorata* extract is red at pH 1–6, purple at pH 7–8, green at pH 9–10, and brown at pH 11–12 due to pH-dependent changes in the anthocyanin molecular structure [19]. The leaves and flowers of *V. odorata* are also used in perfumery, for their content in volatile oils (yield 0.02%) with a dark green color, smell of earth and leaves, but they are generally not economical products [21]. Pansy flowers (*Viola tricolor* L.) are used in food preparations, such as salads, teas, and various desserts, they contain bioactive and nutraceutical compounds, being also recognized for their beneficial effects on human health [22]. *Syringa vulgaris* L. (common lilac) is one of the edible flowers, and a source of bioactive compounds with significant antioxidant, antitumor, anti-inflammatory, and cytotoxic potential [23]. These flowers contain between 0.03 and 3.71 mg/100 g dry weight anthocyanin pigments according to Deeva et al. [24].

The edible flowers of *Cucurbita maxima* (common pumpkin) are an attractive food source of macronutrients (carbohydrates, proteins and fats) and micronutrients (vitamins and minerals) with antimicrobial, anti-inflammatory and osteosarcoma potential, respectively [25]. The total carotenoid content in fresh *Cucurbita maxima* flowers is 2.81 μg/mL, while the chlorophyll content is 3.82 μg/mL according to Gargi et al. (2023). The carotenoid (1.82 - 2.78 μg/mL) and chlorophyll (0.32 - 1.66 μg/mL) content in dried samples depends on the drying method [26]. *Ranunculus bulbosus* L. species are widely distributed in wetlands and grasslands and have nutraceutical and pharmaceutical potential, being used in traditional medicine for arthritic pain and neuralgia [27]. Valdivielso et al. [28] determined a total content of carotenoid compounds of 106 ± 2.48 mg of β-carotene per 100 g dry matter DM.

The edible petals of *Rosa canina* L. are very popular and their use is increasing because they are sources of bioactive compounds, and also contain large amounts of carbohydrates and proteins [29]. The young leaves of *Taraxacum officinale*, commonly called dandelion, are also used as food in salads and vegetable dishes, but also as a traditional medicinal plant, due to its nutritional value as they contain high concentrations of fiber, minerals, vitamins, and essential fatty acids [30].

Beetroot (*Beta vulgaris*) and its functional products are grown for food use (pickles, salad, juice) but are also used as a food coloring or additive. Beetroot leaves vary in size, shape and color and contain carbohydrates (5 g/100 g), starch (4.5 g/100 g) and protein (14.8 mg/100 g), vitamin A (3.93 mg/100 g), vitamin K (280 mg/100 g) and calcium (2220 mg/100 g), iron (16.90 mg/100 g), magnesium (350 mg/100 g), potassium (1400 mg/100 g) and phosphorus (330 mg/100 g) [31].

In the present study, the pigment powders were obtained from different flowers and leaves and were characterized in terms of anthocyanin or chlorophyll content. The aim of this work was to evaluate the coloring effect of natural pigments from vegetable sources in non-thermally treated foods with different pH values.

2. Experimental

2.1. Materials and sample preparation

The pigments were obtained from flowers of *Viola odorata*, *Viola tricolor*, *Syringa vulgaris*, *Cucurbita maxima*, *Ranunculus bulbosus*, leaves of *Taraxacum officinale*, *Beta vulgaris* and petals of *Rosa* sp. (Figure 1). The plants were harvested in the summer of 2022 from extremely low polluted environments, gradually dried by dehydration using the Hendi Profi Line dehydrator, 1000 W, and then ground.

Cream cheese and butter (80% fat) were purchased from a commercial producer, while nougat was obtained in the faculty laboratory. The cream cheese has 24.0% (g/100 g) fat, 7% (g/100 g) protein and 1.5% (g/100 g) NaCl. 10% stable plus premix (Pakmaya) stabilizers were added and the cream was homogenized before packaging. The following ingredients were used to prepare the nougat: 500 g of white sugar, 250 g of acacia honey, 5 egg whites, 25 mL lemon juice, 2 mL vanilla essence, 80 mL of water. The egg whites were beaten until foamy, then the essence, water, acacia honey, and sugar were added, and boiled for 10 minutes. In a tray with pastry sheets, the composition was placed and compartmentalized (Figure 2) so that the pigment was introduced in different amounts.

2.2. Experimental design

To highlight the pH changes, 3 types of food (nougat, cream cheese and butter) were chosen. The obtained samples were marked according to the color of the pigment used: Y (yellow), B (blue), R (red) and G (green).

From each batch, three samples corresponding to a quantity of pigment: 0.5, 1 and 1.5% were taken and introduced in three products labeled N (nougat), C (cream cheese), and B (butter) (Table 1).

Figure 1. Sources of vegetable raw materials used

Figure 2. Food products are enriched by increasing levels (0.5, 1.0, 1.5%) of colored pigments (butter with red and green pigments; cream cheese with yellow and blue pigment) and nougat without color pigment

Table 1. Samples with color pigments

Type of pigment added (50:50, except red)	Nougat	Cheese cream	Butter
Yellow (flower powder of *Ranunculus bulbosus* and *Cucurbita maxima*)	Y_N_0.5	Y_C_0.5	Y_B_0.5
	Y_N_1.0	Y_C_1.0	Y_B_1.0
	Y_N_1.5	Y_C_1.5	Y_B_1.5
Blue (flower powder of *Violo odorata* and *Syringa vulgaris*)	B_N_0.5	B_C_0.5	B_B_0.5
	B_N_1.0	B_C_1.0	B_B_1.0
	B_N_1.5	B_C_1.5	B_B_1.5
Red (*Rosa sp.* petals)	R_N_0.5	R_C_0.5	R_B_0.5
	R_N_1.0	R_C_1.0	R_B_1.0
	R_N_1.5	R_C_1.5	R_B_1.5
Green (leaves powder of *Taraxacum officinale and Beta vulgaris*)	G_N_0.5	G_C_0.5	G_B_0.5
	G_N_1.0	G_C_1.0	G_B_1.0
	G_N_1.5	G_C_1.5	G_B_1.5

2.3. Extraction and determination of chlorophyll

Chlorophylls (Chl) content was extracted from 1 g of fresh leaves homogenized with 20 mL acetone 85%, while small amount of pure Silica Quartz and 0.5 g $CaCO_3$ was used to equalize the cellular sap acidity and to stop the formation of pheophytin with oxygen from the air. The chlorophyll content of the extract with 90% acetone samples was determined by using spectrophotometric methods (spectrophotometer UV-VIS-NIR HR 4000 Ocean Optics), according to Helaly et al. [32].

Chlorophylls pigments were calculated using the following Eqs. (1-3):

$$\text{mg Chlorophyll } a/\text{g tissue} = \frac{12.7\ (A663) - 2.69\ (A645) * V}{1000 * W} \tag{1}$$

$$\text{mg Chlorophyll } b/\text{g tissue} = \frac{22.9\ (A645) - 4.68\ (A663) * V}{1000 * W} \tag{2}$$

$$\text{mg total Chlorophylls}/\text{g tissue} = \frac{20.2\ (A645) + 8.02\ (A663) * V}{1000 * W} \tag{3}$$

where, A - absorbance at specific wavelength, V - final volume of chlorophyll extract in 80% acetone, W - fresh weight of tissue extracted.

2.4. Extraction and determination of total monomeric anthocyanins

Flowers of *Viola odorata*, *Viola tricolor*, *Syringa vulgaris*, *Cucurbita maxima*, *Ranunculus bulbosus* and *Rosa* sp. petals, were used for determination of anthocyanins. Anthocyanins were extracted using 2 g of flower powder and ethanolic solvent. The samples were allowed to stand for one hour and then centrifuged. After centrifugation was completed, 5 mL of sample was measured and the total monomeric anthocyanins (TMA) content was determined spectrophotometrically at 530 nm and 657 nm. TMA were determined using a spectrophotometric pH-differential method assay according to Perez et al. [33].

Total monomeric anthocyanins (TMA) method was based on a cyanidin-3-*O*-glucoside molar extinction coefficient of 26.900 and a molecular weight (Mw) of 449.2 g/mol, and was calculated based on Eq. (4):

$$\text{mg TMA/g} = \frac{A*Mw*DF*V}{\varepsilon*OP*Wt} \tag{4}$$

where, *A* corresponds to the difference of absorbance measures at 530 and 657 nm (Eq. 5):

$$A = A_{530\ nm} - 0.25\times A_{657\ nm} \tag{5}$$

where, *DF* is referred to the dilution factor; ε is the molar absorptivity of cyanidin-3-*O*-glucoside (26.900 L cm^{-1} mol^{-1}); *V* is total sample volume (5 mL) and *OP* represents the optical path (1 cm); W_t is sample weight.

Results were expressed in mg of anthocyanins per g of dry sample.

2.5. pH, color and texture determination

The pH was evaluated in the initial products using a Mettler Toledo pH meter (Mettler Toledo GmbH, Greifensee, Switzerland) at 20 °C, then determined after the addition of each pigment type (0.5, 1.0 and 1.5% per 100 g of product) in powder form. Color measurements (L*, a*, b*) were performed using the CIELab system (Konica-Minolta 200, Tokyo, Japan). 2 g of each sample was placed in transparent Petri dishes which were then moved to a white surface. Calibration was done using black and white calibration plates [34]. A TVT 6700 texturometer (Perten Instruments, Stockholm, Sweden) was used to determine the stickiness and hardness of the samples by the penetration test.

2.6. Statistical analysis

All experiments were performed in triplicate and the results were further investigated using statistical software (Minitab version 17), considering an analysis of variance (ANOVA) with a 95% confidence interval ($p<0.05$) and Tukey test. Principal Component Analysis was also performed.

3. Results and discussion

3.1. Determination of chlorophyll and total monomeric anthocyanin contents

Chlorophyll (Chl) content of leafy vegetables was presented in Table 2. The highest amount of chlorophyll (0.587 mg/g) was found in the dandelion leaves.

Table 2. Chlorophylls in selected leafy vegetables (means ± S.D) (mg/g)

Type of chlorophylls	Samples	
	Dandelion leaves	**Beetroot leaves**
Chlorophyll *a*	0.352±0.05	0.0364±0.07
Chlorophyll *b*	0.0526±0.08	0.0593±0.01
Total chlorophylls	0.587±0.05	0.400±0.03

The difference between chlorophyll *a* and chlorophyll *b* is found at carbon 7, where chlorophyll *a* present a methyl group while chlorophyll *b* includes a formyl group. The use of chlorophylls in food products is often limited because they are unstable at acidic pH, and the chemical conditions during the food processing accelerate the degradative process [35]. Chlorophyll and its derivatives have multiple potential health benefits, such as anti-inflammatory and antimicrobial effects, but also can regulate the immune system [36]. The initial color of the sample was pale green due to the presence of chlorophyll. The color change is more noticeable at a pH of 3, as the dark greens become practically white. When chlorophyll is subjected to acidic conditions, it breaks down into a compound called pheophytin, which gives an olive-brown color. During the acid reaction, hydrogen ions can convert chlorophyll into pheophytins and pheophorbides which results in a change from bright green to dull olive green or yellow [37].

The content of total monomeric anthocyanins determined in the selected flowers was presented in Table 3.

Table 3. Total monomeric anthocyanin (TMA) content (mg cyanidin-3-*O*-glucoside/g fresh weight) in selected flowers (means ± S.D)

Samples	TMA
Syringa vulgaris flowers	4.164±0.141
Violo odorata flowers	4.188±0.169
Rose petals	5.978±0.119

Anthocyanin is naturally formed as a red pigment contained in the flowers of *Rosa canina* L. [38], and the content of red anthocyanins were 5.978 ±0.119 mg cyanidin-3-*O*-glucoside/g fresh weight. Co-pigmentation is specific to anthocyanins and cannot be observed in other classes of polyphenols or other non-phenolic compounds. Thus, pigments form molecular or complex associations with other colorless organic compounds or metal ions, resulting in a change or increase in color intensity. Anthocyanins are sensitive to pH and change color depending on acidity. Under acidic conditions (pH 1.0), they display intense colors in the form of flavylium cations. At neutral pH (4.5), colorless structures appear. Alkaline conditions (pH > 7) lead to the formation of quinoidal structures, resulting in intense coloration [39, 40]. Based on observations of some anthocyanins in vitro, the following scheme is accepted, related to changes in pH: at a pH of about 3 or lower, the orange, red or purple flavylium cation predominates; at pH between 6 and 7 with the formation of quinoid anions leading to a bluish color. At pH values typical for fresh and processed fruits and vegetables, each anthocyanin will be represented by a mixture of components that will determine the basic color of the products [39, 40].

3.2. pH determination

Figures 3-5 illustrate the pH changes after addition of color pigments (Y (yellow), B (blue), R (red) and G (green)) in nougat, cheese cream and butter. The pH of the nougat product is initially 5.34±0.01, after the introduction of a quantity of 1.5% yellow pigment/100 g product the pH increased by 8.98%. Similarly, the same trend was noted for the red, green and blue pigment. In all cases, the higher the amount of pigment, the higher the pH of the nougat product (Figure 3). The pH of cheese cream was 4.58±0.05, the addition of pigments led to a slight increase in pH value, but there were no statistically significant differences between cheese cream samples with different pigments (Figure 4). Similar to the other two products, the pH of butter (initially 6.46±0.02) increases with increasing amounts of blue and red vegetable pigment, and decreased when yellow and green pigments were added (Figure 5).

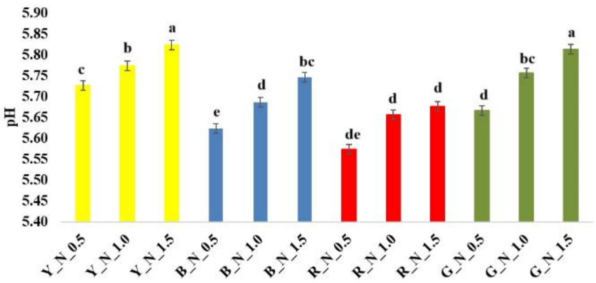

Figure 3. pH of nougat samples with color pigments

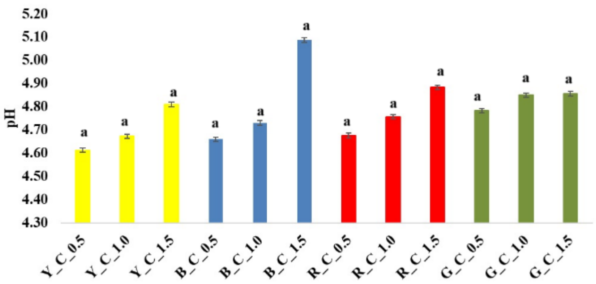

Figure 4. pH of cheese cream samples with color pigments

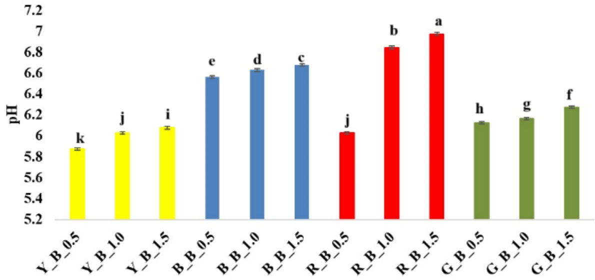

Figure 5. pH of butter samples with color pigments

The yellow pigment determines the highest pH values in the case of the butter and nougat products and the low ones in the cream cheese. The addition of green pigment to cream cheese also changes the pH of this product, leading to a higher increase in pH values. The blue pigment shows the highest values in butter. Red pigment in nougat samples does not show significant increases.

3.3. Color parameters

Color parameters of nougat, cheese cream and butter samples with added pigment were determined and presented in Table 4. The color parameter L* indicating lightness decreased with increasing pigment concentration for all samples (nougat, cheese cream and butter). For 1.5%of yellow pigment added to the 100 g samples, the highest brightness value is recorded for cream cheese, L* 81.10±0.02, and the lowest value for butter, 75.06±0.02. Similarly for the red pigment added 1.5% the maximum value for L* is for nougat, 80.76±0.02, and the lowest for cheese cream, 66.73±0.01. Among all samples, the darkest one was the nougat G_1.5, showing the lowest L* value, probably because it was the most concentrated pigment green added sample. These results are confirmed by Kaur et al. [41], who used lycopene extracted from the skin of tomato residues in butter, ice cream and lycopene extracted from mayonnaise. Kaur et al. [41] indicated that there were insignificant changes in the L* value of both control and lycopene-treated butter during 4 months of storage. Positive values of a* color parameter were determined for nougat samples with yellow pigment, for cheese cream samples with red and green pigment, indicating redness. For these samples, it was observed that a* values increased with increasing pigment concentration. Also, a* had positive values for cheese cream samples with 1 and 1.5% blue pigment, and for butter samples with 1.5% yellow pigment and 0.5% red pigment. For the other samples, the values recorded for a* were negative, indicating greening. In case of parameter b* positive values were obtained for all samples, indicating a tendency toward yellow. The highest values for b* (between 26.69±0.03 and 34.70±0.01) were recorded for cheese cream samples with added yellow pigment (values increasing with increasing concentration of pigment added), while the highest value of b* for butter samples was obtained when 1.5% red pigment was added. In the case of nougat samples, the highest (15.44±0.01) and the lowest (5.98±0.01) b* values were determined when 1% green pigment and 0.5% red pigment were added.

3.4. Texture analysis

Three textural parameters (hardness, stickiness and adhesiveness) were determined. Table 5 shows the textural properties of nougat samples with 0.5, 1 and 1.5% color pigments. It was observed that the hardness of the samples decreased with increasing concentration of yellow, red and blue pigments, while the addition of green pigment in higher concentrations increased the hardness of the samples. The highest value of hardness (1628.71±0.26) was obtained for the nougat sample with 1.5% green pigment, while the lowest value (400.26±1.09) was determined for the nougat sample with 1.5% yellow pigment.

Table 4. Color parameters of samples according to the amount of pigment added

Pigment %	Nougat samples			Cheese cream samples			Butter samples		
	Color parameters								
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Y-0.5	82.18 ± 0.02^{bc}	0.31 ± 0.03^{b}	13.95 ± 0.01^{d}	84.22 ± 0.01^{a}	-2.75 ± 0.02^{j}	26.69 ± 0.03^{b}	79.60 ± 0.47^{a}	-0.22 ± 0.16^{b}	21.40 ± 0.73^{c}
Y-1.0	81.22 ± 0.01^{cd}	0.39 ± 0.01^{ab}	14.03 ± 0.02^{d}	83.63 ± 0.02^{c}	-2.66 ± 0.05^{i}	26.59 ± 0.01^{c}	78.99 ± 0.02^{b}	-0.35 ± 0.02^{b}	20.07 ± 0.02^{d}
Y-1.5	80.57 ± 0.02^{d}	0.44 ± 0.03^{a}	14.81 ± 0.01^{b}	81.10 ± 0.02^{d}	-3.13 ± 0.03^{k}	34.70 ± 0.01^{a}	75.06 ± 0.02^{e}	0.79 ± 0.01^{a}	26.20 ± 0.03^{b}
R-0.5	82.35 ± 0.02^{b}	-0.67 ± 0.01^{de}	5.98 ± 0.01^{i}	76.60 ± 0.02^{g}	6.81 ± 0.01^{c}	4.12 ± 0.01^{j}	76.68 ± 0.01^{c}	0.79 ± 0.02^{a}	25.88 ± 0.01^{b}
R-1.0	81.46 ± 0.01^{bcd}	-0.72 ± 0.01^{e}	6.02 ± 0.01^{i}	67.39 ± 0.02^{j}	11.87 ± 0.03^{b}	3.60 ± 0.02^{k}	76.43 ± 0.02^{c}	-3.45 ± 0.01^{f}	26.10 ± 0.01^{b}
R-1.5	80.76 ± 0.02^{d}	-0.69 ± 0.01^{de}	6.46 ± 0.01^{h}	66.73 ± 0.01^{k}	13.06 ± 0.03^{a}	3.95 ± 0.01^{l}	75.89 ± 0.01^{d}	-3.66 ± 0.01^{g}	27.59 ± 0.06^{a}
B-0.5	83.56 ± 0.01^{a}	-0.70 ± 0.02^{de}	6.89 ± 0.01^{g}	83.88 ± 0.01^{b}	-0.37 ± 0.03^{h}	10.29 ± 0.01^{e}	76.37 ± 0.02^{c}	-2.37 ± 0.01^{d}	15.39 ± 0.01^{h}
B-1.0	82.04 ± 1.13^{bc}	-0.59 ± 0.10^{d}	7.24 ± 0.30^{f}	80.91 ± 0.01^{e}	0.21 ± 0.02^{g}	10.94 ± 0.01^{d}	74.90 ± 0.02^{e}	-2.60 ± 0.03^{e}	16.02 ± 0.01^{g}
B-1.5	78.71 ± 0.01^{e}	-0.26 ± 0.02^{c}	8.72 ± 0.01^{e}	80.14 ± 0.01^{f}	0.54 ± 0.02^{e}	10.05 ± 0.01^{f}	65.74 ± 0.02^{g}	-1.24 ± 0.04^{c}	17.80 ± 0.01^{e}
G-0.5	60.41 ± 0.10^{f}	-3.23 ± 0.02^{g}	14.34 ± 0.00^{c}	72.42 ± 0.13^{h}	0.55 ± 0.03^{d}	8.78 ± 0.01^{h}	70.31 ± 0.02^{f}	-4.68 ± 0.02^{h}	16.57 ± 0.01^{fg}
G-1.0	56.31 ± 0.07^{g}	-2.91 ± 0.03^{f}	15.44 ± 0.01^{a}	70.94 ± 0.05^{i}	0.42 ± 0.03^{f}	8.73 ± 0.02^{i}	63.80 ± 0.02^{h}	-5.03 ± 0.02^{i}	20.29 ± 0.02^{d}
G-1.5	51.17 ± 0.05^{h}	-2.93 ± 0.04^{f}	14.29 ± 0.02^{c}	66.22 ± 0.01^{l}	1.04 ± 0.03^{d}	9.02 ± 0.01^{g}	56.42 ± 0.01^{i}	-3.44 ± 0.02^{f}	16.91 ± 0.01^{f}

Table 5. Textural properties of nougat samples with 0.5, 1 and 1.5% color pigments

Samples	Hardness, g	Stickiness	Adhesiveness, g·s
Y_N_0.5	718.69 ± 0.28^{g}	-190.62 ± 0.54^{i}	-190.79 ± 0.26^{i}
Y_N_1.0	648.26 ± 1.09^{i}	-110.48 ± 0.23^{h}	-110.58 ± 0.38^{h}
Y_N_1.5	400.26 ± 1.09^{k}	0	0
R_N_0.5	719.72 ± 0.25^{g}	0	-78.80 ± 0.26^{e}
R_N_1.0	678.26 ± 0.25^{h}	-88.71 ± 1.66^{e}	-85.24 ± 0.25^{f}
R_N_1.5	645.36 ± 0.32^{k}	-98.45 ± 0.40^{g}	0
B_N_0.5	1301.28 ± 0.33^{b}	-94.63 ± 0.11^{f}	-94.71 ± 0.03^{g}
B_N_1.0	1044.18 ± 1.05^{c}	-28.86 ± 0.14^{a}	-28.71 ± 0.10^{a}
B_N_1.5	904.54 ± 0.50^{d}	-73.82 ± 0.10^{d}	-73.68 ± 0.16^{d}
G_N_0.5	780.99 ± 0.99^{f}	0	0
G_N_1.0	788.65 ± 0.29^{e}	-31.2 ± 0.26^{b}	-31.21 ± 0.25^{b}
G_N_1.5	1628.71 ± 0.26^{a}	-56.81 ± 0.28^{c}	-56.96 ± 0.04^{c}

The addition of yellow pigment in lower concentration in the nougat samples, but also the addition of green pigment in high concentration, increases hardness and decreases stickiness and adhesiveness. In the case of cream cheese samples, the addition of yellow and green pigments led to a decrease in hardness with increasing pigment concentration (from 39±0.5 (Y_C_0.5) to 26±0.2 (Y_C_1.5) in the case of yellow pigment samples and from 315±0.23 (G_C_0.5) to 173±0.59 (G_C_1.5) in the case of green pigment samples), while the hardness of the samples increased with increasing concentrations of red (from 137±0.58 (R_C_0.5) to 294±0.99 (R_C_1.5)) and blue (from 68±0.45 to 93±0.23) pigments. Stickiness and adhesiveness decreased with increasing hardness. All butter samples showed a decrease in hardness and an increase in adhesiveness with increasing concentration of added pigment. The highest hardness was determined for the butter sample with 0.5% green pigment (881±0.36), while the lowest was observed for the butter sample with 1.5% red pigment (50±0.64). The increase in product hardness with the addition of color pigments from natural sources has also been described in other studies [42, 43]. These studies demonstrated by texture analysis that the addition of anthocyanins in a percentage higher than 1% led to increased bread hardness and lower specific volume due to the increase of phenolic compounds in the product mass. The phenolic compounds interact with the disulfide groups, weakening the gluten network and resulting in a product with high hardness [42]. The addition of pumpkin powder to the sponge cake formula influenced the textural properties of the sponge cake. For example, the density, viscosity, consistency, and firmness of the dough increased when pumpkin powder was used in the preparation of sponge cake. Tounsi et al. [43] incorporated carob powder into a Halva-type confectionery product at a percentage of 5% and significantly increased the hardness of the product from 139.35 ± 49.86 to 340.17 ± 78.08, which was not appreciated by the manufacturer. The effect of carob powder on the texture of Halva is mainly explained by its oil-retaining power, related to its high fiber content [43].

3.5. Principal component analysis

The relationships between pH, textural and color parameters of nougat, cheese cream and butter samples with color pigments are illustrated in Figures 6, 7 and 8.

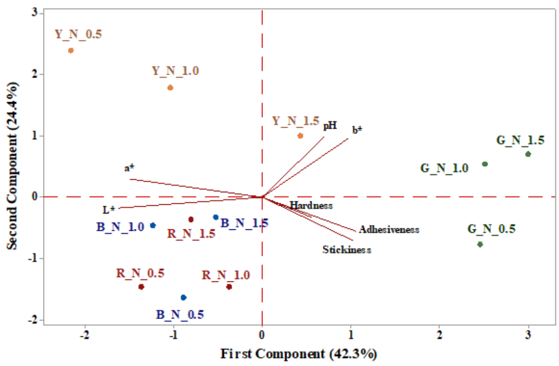

Figure 6. Principal component analysis - biplot depicting the relationship between parameters of nougat samples

From Figure 6 it can be observed that principal component 1 (PC1) accounted for 42.3% of the total variation, while PC2 accounted for 24.4% of the total variation. Color parameters L* (-0.546) and a* (-0.505) had negative loadings on PC1, while the others parameters had positive loadings. pH (0.580), a* (0.175) and b* (0.564) had positive loadings on PC2, while others parameters had negative loadings. Nougat samples with red and blue pigments had negative values for both PC1 and PC2, nougat samples with green pigment had positive values on PC1, while nougat samples with yellow pigment (0.5 and 1%) had positive values PC2 and negative values for PC1 (Figure 6).

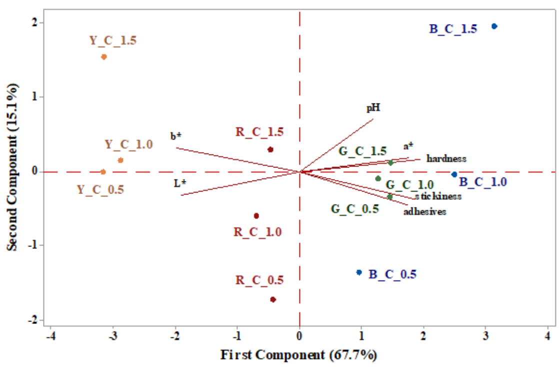

Figure 7. Principal component analysis - biplot depicting the relationship between parameters of cheese cream samples

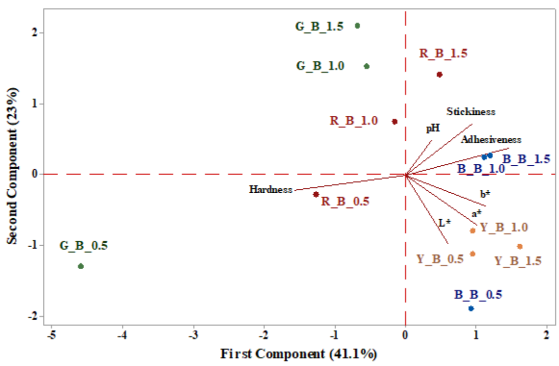

Figure 8. Principal component analysis - biplot depicting the relationship between parameters of butter samples

In the case of cheese cream samples with color pigments (Figure 7) the PC1 accounted for 67.7% of the total variation, while PC2 accounted for 15.1% of the total variation. All parameters had positive loadings on PC1 except L* (-0.404) and b* (-0.419), while adhesiveness (-0.422), stickiness (-0.349) and L* (-0.295) had negative loadings on PC2. Cheese cream samples with green and blue pigments had positive values for PC1, while cheese cream samples with yellow and red pigments had negative values for PC1 (Figure 7). All cheese samples with 0.5 and 1% color pigments (except sample with 1% yellow pigment) had negative value for PC2, while samples with 1.5% color pigments had positive values for PC2.

Figure 8 indicates that PC1 and PC2 accounted for 41.1% and 23% of the total variation. Hardness is the only parameter which had negative loadings (-0.545) on PC1, while pH, stickiness, and adhesiveness had positive loadings on PC2. The butter samples with yellow pigment had positive values for PC1 and negative values for PC2, while butter samples with green pigment had negative values for PC1. All butter samples with blue pigment had positive values for PC1, butter with 0.5 and 1% red pigment had negative values for PC1, while butter with 1.5% red pigment had positive values for PC1.

4. Conclusions

The addition of natural pigments (yellow, blue, red and green) extracted from flowers and leaves to foods with different pHs, such as nougat, cream cheese and butter, was investigated. The chlorophyll content determined was higher in dandelion leaves than in beetroot leaves, while the total monomeric anthocyanin content was higher in *Rosa* petals, followed by *Violo odorata* flowers and *Syringa vulgaris* flowers. In general, the addition of pigments to the samples led to an increase in their pH. The results showed that the highest increase in nougat samples was observed when yellow and green pigments were added at a concentration of 1.5%, and the lowest when blue pigments were added at a concentration of 0.5%. In the case of cheese samples with pigments the increase in pH values was lower compared to the initial sample and there were no statistically significant differences between cheese samples with different pigments. Also, for butter samples the pH values increased with the addition of blue and red pigments, the highest increase was observed for the butter sample with blue pigment at 1.5% concentration. The color of the samples was influenced by the type of pigment added and its concentration. Therefore, addition of yellow and green pigments decreased the pH values of butter samples. Addition of yellow, red and blue pigments decreased the hardness of nougat samples, addition of yellow and green pigments decreased the hardness of cheese cream samples, while addition of all color pigments in butter samples decreased the samples hardness.

Conflict of interest

Authors declare no conflict of interest.

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Received: 16.01.2025
Received in revised form: 20.03.2025
Accepted: 22.03.2025