

## Effect of the Addition of Polysaccharide Hydrocolloids on Sensory Quality, Color Parameters, and Anthocyanin Stabilization in Cloudy Strawberry Beverages

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This manuscript presents results of the qualitative characteristics of strawberry cloudy juice and beverages with the addition of 0.2% and 0.3% of carboxymethylcellulose (CMC), guar gum (GG), locust bean gum (LBG), and xanthan gum (XG). Fresh products were evaluated with reference to their sensory quality (5-point scale). Changes in  $L^*a^*b^*$  parameters and in the stability of anthocyanins (ultra-performance liquid chromatography–mass spectrometry) were monitored in the storage experiment (6 months, 4°C).

Most of the hydrocolloids have contributed to the improvement of the taste and the consistency of strawberry products. In overall taste evaluation, the highest scores were given to the samples with CMC, whereas in the consistency evaluation, to the samples with CMC, GG, and LBG addition at a dose of 0.2%. The study of color parameters of the products has indicated significant changes in their chromatic space during storage. After 6 months, beverages with CMC, GG, and LBG were darker in comparison to the control sample. The contribution of red color in beverages was higher, and of yellow color was lower than in the strawberry juice.

Strawberry juice was characterized by a high degree of anthocyanins degradation ( $Dd=84\%$ ), especially of pelargonidin-3-glucoside and cyanidin-3-malonylglucoside. The use of hydrocolloids has contributed to the partial reduction of this phenomenon.

In conclusion, the most beneficial protective effect on anthocyanins ( $Dd=65\%$ ) and the impact on the sensory characteristics in strawberry beverages was provided by LBG application.

### INTRODUCTION

Polysaccharide hydrocolloids are a large group of food additives with universal applications in the food industry. They are high-molecular-weight biopolymers and are obtained by extraction from terrestrial or sea plants, from plant exudates, or *via* the microbiological pathway. A number of derivatives of natural polysaccharide hydrocolloids, obtained by chemical or enzymatic treatment of raw materials, were identified as well [Dickinson, 2003]. Due to numerous functional properties, including stabilization, emulsification, thickening or gelling, they are used, among others, for the production of dairy products (including yogurts, desserts, beverages, cheeses) [Varela & Fiszman, 2013], ice creams, pastry and confectionery products, meat and convenience foods, soups, sauces, or salad dressings [Saha & Bhattacharya, 2010]. Fruit processing offers interesting application perspectives for the hydrocolloids. Texture and gelling properties of pectin are used in the production of bars and fruit

jams [Raju & Bawa, 2006]. Alginates are used, among others, for the production of structured fruits [de Almeida Lins *et al.*, 2014], whereas carboxymethylcellulose (CMC) is comprehensively used in concentrated fruit juices, as a filling in cakes, in juice dehydration, as an additive reducing syneresis and providing brighter appearance to the processed fruit products, and so on. Guar gum (GG) added to nectars acts as a thickener by increasing its viscosity [Somogyi, 2005]. Solutions of locust bean gum (LBG) are stable over a wide pH range, which makes them excellent stabilizers and thickeners in the production of beverages [Barak & Mudgil, 2014].

Functional properties of hydrocolloids, their mechanism of action, and their influence on the rheological properties of food are the well-known and well-described issues in previous studies. For example, some research have addressed the influence of these biopolymers on the stability and viscosity of cloudy apple juices [Genovese & Lozano, 2001] and peach nectars [Pastor *et al.*, 1996]. In turn, Chaikhram & Apichartsrangkoon [2012] described the dynamic, viscoelastic, and physicochemical properties of longan juices, while Mirhosseini *et al.* [2008] investigated the physical stability, turbidity loss rate, and cloudiness of orange bev-

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erage emulsion. However, in the case of juices, nectars or beverages production technology, the role of hydrocolloids requires consideration in a wider context. The following issues can be considered particularly important for the quality of these products: 1) the impact of hydrocolloids on the development of sensory characteristics (especially concerning the perception of sour and tart tastes [Pangborn *et al.*, 1978]), and 2) the effect they might exert on the stability of bioactive compounds, including anthocyanins [Heins *et al.*, 2001]. This problem is particularly important in strawberry processing. Anthocyanins that can be found in fruits are characteristic of undergoing strong degradation. The process of their decomposition in juices, nectars, drinks, or purees occurs very rapidly. After few weeks or even days of storage, strawberry products can change color from red to brown [Gössinger *et al.*, 2009]. Meanwhile, the scientifically proven beneficial effect of anthocyanins on human health [Tsuda, 2012; Gupta *et al.*, 2009; Ellingsen *et al.*, 2008] speaks for their preserving properties as best as possible in the fruit products. The protective effect of hydrocolloids on the stability of anthocyanins is possibly associated with intermolecular interactions or introduction of anthocyanins to the structure of hydrocolloids. They form a three-dimensional network in an aqueous phase, which leads to the compartmentation effect of colored compounds. Furthermore, there are known interactions between cationic molecules and certain hydrocolloids (*e.g.* pectin and sodium alginate). A similar mechanism could also apply to the flavylum cation, which is the parent structure of anthocyanidins [Heins *et al.*, 2001]. From the point of view of sensory attributes of fruit products, the important property of polysaccharides is their ability to reduce taste sensations, like astringency [Troszyńska *et al.*, 2010]. Because of their viscosity, hydrocolloids reduce the feeling of friction caused as a result of the reduction of oral cavity moistening during the consumption of astringent products. Another possible mechanism results from the interactions between polysaccharides and polyphenols [Taira *et al.*, 1997]. The formation of polysaccharide complexes with, *e.g.*, tannins prevents binding of the latter ones to salivary proteins.

Given the above premises, the objective of this study was to investigate the effect of selected polysaccharide hydrocolloids: carboxymethylcellulose (CMC), guar gum (GG), locust bean gum (LBG), and xanthan gum (XG) on sensory properties, color parameters, and stability of anthocyanin compounds in cloudy beverages made of strawberry of "Roxana" cultivar.

## MATERIALS AND METHODS

### Reagents and chemicals

Methanol and acetic acid (HPLC purity) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (UPLC; gradient grade) and *L*-ascorbic acid were from Merck (Darmstadt, Germany). Analytical standards of anthocyanins: pelargonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, pelargonidin-3-*O* rutinoside, and pelargonidin-3-*O*-galactoside were purchased from Extrasynthese (Lyon, France). Carboxymethylcellulose sodium, guar gum, locust bean gum, and xanthan gum were from Brenntag (Kędzierzyn Koźle, Polska).

### Strawberry cultivar for juice and beverages production

Fruits of strawberry ("Roxana" cultivar (cv); *Fragaria x ananassa* Duchesne) were collected at a commercial strawberry plantation (Smolna, Poland, 51°09'24"N, 17°25'56"E) in the 2016 season. Fully mature, expanded, free from stems, and undamaged fruits were hand washed and in this form used for juice and beverages production.

### Production of cloudy strawberry juice (laboratory scale)

Strawberry fruits were homogenized and heated (10 s, 75°C,) in a Thermomix device (Vorwerk, Wuppertal, Germany). The pulp was pressed in a hydraulic basket press (SSRE, Warsaw, Poland) for 5 min at a piston thrust of 5000 KG/cm<sup>2</sup>. Fresh juice was heated to 90°C for 2 min (Thermomix), poured into 80-mL colorless jars, left for 10 min for pasteurization, and cooled in a water bath to 20°C. Three replicates of cloudy strawberry juice preparation were carried out. Juices were analyzed twice: after processing and after 6 months of cold storage (4°C, no light exposure).

### Selection of hydrocolloids

The choice of hydrocolloids for the present experiment was based on the analysis of literature data on the functional properties of these biopolymers and consultations with an adviser from Brenntag Polska Sp. z o.o. company (use of selected substances for fruit and vegetable processing). First, the hydrocolloids were added to the model product in the concentration range from 0.1 to 0.5%. Taking into account the changes in the consistency of the analyzed samples (sensory evaluation, viscosity measurement), two concentrations of hydrocolloids were selected for further studies (0.2 and 0.3%).

### Production of strawberry beverages with hydrocolloids

Fresh, unpasteurized cloudy strawberry juice was heated in the Thermomix to 40°C. When this temperature had been reached, hydrocolloids (CMC, GG, LBG, XG) were added at the dose of 0.2% and 0.3%, mixed, and heated with juice in the Thermomix to 90°C. Beverages were poured into 80 mL colorless jars, left for pasteurization (10 min), and cooled in a water bath to 20°C. Three replicates of beverages preparation were carried out.

### Sensory evaluation of juice and beverages

The sensory evaluation of fresh strawberry cloudy juice and beverages was carried out by a group of 12 trained panelists (10 women, 2 men) using a universal 5-point scale [ISO 13299:2003]. The evaluators had methodical (theoretical and practical) preparation in the field of sensory analysis (senses, sensory language, properties and techniques).

The evaluation included the following sensory attributes: color, overall taste, aroma, consistency (I part of evaluation), as well the intensity of sweet/ sour/ tart/ foreign taste (II part of evaluation). Accordingly, an average score of 1 was equivalent to non-detectable taste in the sample, whereas score 5 denoted the maximum intensity. The intensity scales for the descriptors were provided in Table 1 and developed by the panelists.

Coded samples (four-digit codes) were provided to the panelists for the evaluation at a temperature of *ca.* 20°C

TABLE 1. Sensory parameters of strawberry juice and beverages evaluated in descriptive analysis.

Quality parameter	Score				
	1	2	3	4	5
Color	Strongly changed or atypical	Changed, not very intense or with a brown shade	Clearly darker or lighter than the color of strawberry flesh	Intense, slightly darker or lighter than the color of strawberry flesh	Very intense, slightly darker or lighter than the color of strawberry flesh
Aroma	Strongly foreign	Unperceptible or weak, foreign	Poorly perceptible, no foreign odour	Aromatic, harmonized	Very aromatic, fresh, harmonized
Overall taste	Foreign	Changed, strawberry taste- unperceptible, bitter, empty	Low-intensity, non-harmonized (eg. too sweet, too sour)	Intense, strawberry, harmonized	Very intense, strawberry, harmonized
Consistency	Strongly changed (undrinkable, too thick) or atypical	Changed (too thick) or atypical (heterogeneous, clearly stratified)	Drinkable, changed (thick)	Semi-liquid, homogeneous	Smooth, delicate, homogeneous, semi-liquid

in uniform, transparent, 50-mL plastic containers in complete randomized order. The members of sensory panel were seated in individual booths in a light and temperature controlled room. Distilled water was used to clean the palate between samples. All of strawberry products were evaluated in one session (9 samples).

#### Analysis of anthocyanins content in juice and beverages by UPLC-PDA

The content of anthocyanins in strawberry products was analyzed directly after processing and after 6 months of storage (4°C, no light exposure). Juice and beverages (2 mL) were centrifuged for 10 min (4°C, 15,000 rpm). The MillexSamplicity® Filters System (Merck Millipore, Darmstadt, Germany) was used for samples filtration. The filtered samples were kept at 4°C, whereas the analytical column was thermostated at 30°C (column oven). The mobile phase consisted of 4.5% formic acid (solvent A) and acetonitrile (solvent B). The program parameters were as follows: 0–1 min – isocratic elution with 99% of 4.5% formic acid; 12 min – linear gradient, lowering of solvent A to 0%; 12.5–13.5 min – return to 99% of solvent A (the initial composition). The injection volume was 5 µL and the flow rate was 0.45 mL/min. The runs were monitored at 520 nm. Calibration curves of anthocyanins (concentrations ranging from 0.05 to 5 mg/mL;  $r^2 \leq 0.9998$ ) were made from cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, pelargonidin-3-*O*-galactoside, and pelargonidin-3-*O*-rutinoside as standards. Pelargonidin-3-malonylglucoside and cyanidin-3-malonylglucoside were expressed as pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, respectively. The samples were analyzed in triplicate. Results were expressed in mg/L of juice/ beverages.

#### Identification of anthocyanins by the ultra-performance liquid chromatography–mass spectrometry (LC–ESI–MS) method

According to the procedure previously described by Wojdyło et al. [2014], identification of anthocyanins in strawberry juice and beverages was carried out using an ACQUITY Ultra Performance LCTM system (UPLC™) with a binary solvent manager and a Waters Micromass Q-ToF Micro mass spectrometer

(Manchester, U.K.) equipped with an ESI (electrospray ionization) source operating in the positive ion mode.

#### Color measurements

Color properties of juice and beverages ( $L^*$ ,  $a^*$ ,  $b^*$ ) were determined with a Color Quest XE colorimeter (Hunter Lab, Reston, Virginia, USA). The samples were filled into a 1-cm cell, and  $L^*a^*b^*$  parameters were determined using 10° observer angle and Illuminant D65. The color measurement was done in triplicate. Products were analyzed directly after processing and after 6 months of cold storage (4°C, no light exposure).

#### Statistical analysis

Statistica version 12.5 (StatSoft, Poland) was used for statistical analyses of the results of color measurements and anthocyanin content determinations. One-way analysis of variance (ANOVA) by Duncan's test was used to compare the means. Differences were found significant at  $p < 0.05$ . Results were presented as mean  $\pm$  standard deviation of three determinations.

## RESULTS AND DISCUSSION

#### Sensory evaluation of strawberry juice and beverages with added hydrocolloids

Members of the sensory panel evaluated a total of nine strawberry products (one juice and eight beverages) with 0%, 0.2%, and 0.3% of hydrocolloids of plant origin, that is CMC, GG, LBG, and the microbiological XG. The first part of the study included the evaluation of color, aroma, taste, and consistency, whereas in the second part, the panelists were focused on the evaluation of the intensity of taste sensations (Figure 1a-1d).

The strawberry products were characterized by attractive red color, however, its intensity was significantly changing as a result of the addition of hydrocolloids. Hence, for the evaluators the color attributed to 100% strawberry juice was considered best (average score 4.88), whereas the color of other beverages was perceived as less desirable. This effect was observed in all the products containing hydrocolloids. At the same time,

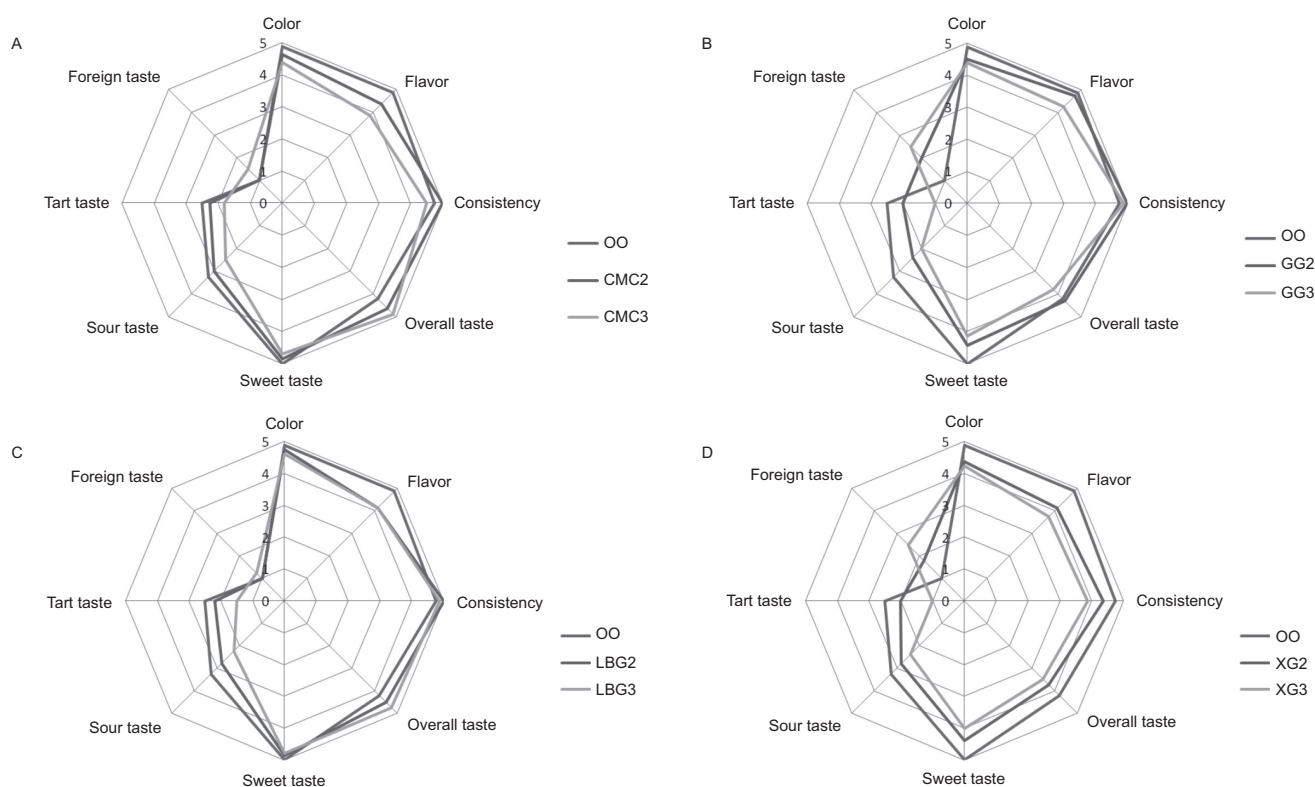


FIGURE 1. Sensory evaluation of strawberry juice and beverages with the addition of polysaccharide hydrocolloids.

OO – control sample (juice), CMC – beverages with carboxymethylcellulose, GG – with guar gum, LBG – with locust bean gum, XG – with xanthan gum; 2 and 3 – 0.2% and 0.3% addition of hydrocolloids.

with an increase in their concentration, the scores in color evaluation were particularly lower. The least favorable effect on the color of beverages was reported in the case of addition of XG (average scores of 4.38 and 4.25 for products with 0.2% and 0.3% of XG, respectively).

In the sensory evaluation, higher scores were given to beverages containing plant hydrocolloids, especially LBG (average scores 4.75 and 4.63 for products with 0.2% and 0.3% of LBG, respectively). Products with CMC (average scores 4.63 and 4.38) and GG (average scores 4.50 and 4.38) were characterized by a less intense color.

In the concentration ranges studied, these substances also affected the assessment of aroma of the products. With the increase in the proportion of hydrocolloids in beverages, their aroma became less perceptible. However, it did not result from the emergence of foreign, undesirable odor in the tested samples, but only from masking the natural strawberry aroma. Sensory evaluation results indicated that such properties were attributed only to XG (at both concentrations) and among the substances of plant origin — to LBG and CMC at a dose of 0.2% and 0.3%, respectively. In the case of XG and LBG, their 0.2% addition to beverages caused a significant weakening of their aroma in relation to the control sample (in both cases, the score was 4.13). However, increasing the dose of both the hydrocolloids up to 0.3% appeared to be disadvantageous only in the case of XG (average score 3.75). The use of 0.2% additive of guar gum (GG2; average score 4.75) and 0.2% carboxymethylcellulose

(CMC2; average score 4.38) affected the quality of strawberry beverages to the least extent. At their higher concentration, the effect of aroma masking was more prominent; however, the sample containing carboxymethylcellulose was given lower scores (CMC3; average score 3.88).

Interesting information was provided by the results of overall taste evaluation of strawberry beverages. Evaluators recognized samples containing plant hydrocolloids (except for guar gum at a concentration of 0.3%) as more tasty, in comparison to the control (100% cloudy juice made of strawberries; average score: 4.20). Simultaneously, the greater was the addition of hydrocolloids, the higher scores were given to the products by the evaluators. The highest scores were given to beverages made of CMC (average scores: 4.63 and 4.88 for CMC2 and CMC3, respectively). A beneficial effect on the overall taste was also reported upon the addition of LBG (average scores: 4.50 and 4.75 for LBG2 and LBG3, respectively). In the case of GG, a slight improvement in the flavor of the beverages was observed only at a dose of 0.2% (average score: 4.30). Unfavorable taste modification was caused by the addition of XG to the strawberry juice. In sensory evaluation, beverages with this additive were given the lowest scores (3.75 for XG2 and 3.50 for XG3). This proves that the impact of hydrocolloids on the food taste is not just a matter of the dose used, but above all of the specific, individual properties of these substances. The analysis of the intensity of taste sensations associated with the consumption of juice and strawberry beverages appeared to be helpful in the understanding of this phenomenon.

The “Roxana” cultivar of strawberries, used to produce juice in this experiment, is a dessert type cultivar. Hence, in all the products, sweet taste was predominant. Although the use of hydrocolloids significantly reduced the sweetness of strawberry drinks (proportionally to the dose used), it did not have a decisive influence on the results of previously described overall taste evaluation (Figure 1a-1d). It can be clearly observed in the case of the control sample containing 100% of cloudy juice. This product was the sweetest of all the products tested (average score: 5.00); however, in terms of overall taste perception, it cannot be equal to the beverages containing even CMC or LBG. It was more sour (average score: 3.25) and more tart (average score: 2.50; Figure 1a-1d), compared to the products with hydrocolloids (more balanced and milder taste).

The strength of sour taste masking by hydrocolloids was most evident in the beverages containing GG. With the addition of 0.2% GG, the detection of sour taste was expressed on a 5-degree scale by an average score of 2.40. By comparison, the control product obtained the score of 3.25 for the same property. Increasing the amount of GG up to 0.3%, the degree of reduction of sour taste was already so important that evaluators have identified its intensity as weakly perceptible (average score: 2.00). Guar gum effectively masked the tartness of strawberry products. This taste was not strongly constituted in the products tested (in the case of 100% cloudy juice, the average score was 2.50), although evaluators have clearly indicated the differences in the intensity of tartness among them. On the addition of 0.3% GG, the tart taste in the strawberry beverages was not identified at all (average score: 1.00). The use of XG provided equally strong effect; on addition to strawberry juice, it caused a decrease in the flavor intensity in the final product to a level equal to 2.0 (dosage: 0.2%) and then to 1.0 on a 5-point intensity scale (dosage: 0.3%). Despite these advantageous properties of both GG and XG, beverages with their addition were not as attractive in terms of taste as those containing CMC or LBG. It may probably be due to the perception of undesirable flavor in the products with GG and XG. However, all hydrocolloids used in the experiment provided a foreign flavor to the products, only for GG and XG was this effect not neutral among evaluators and had a negative impact on the overall assessment of the desirability of the taste of the beverages.

During the consumption of products containing hydrocolloids, a major role in the perception of taste and aroma is attributed to their rheological properties. The higher the values of viscosity (synonymous with increasing concentration of colloids), the lesser the ability of man to respond to sensory stimuli. This relationship was described by Pangborn *et al.* [1978], who studied the effect of the addition of xanthan gum and CMC on sensory properties of tomato juice, orange drink, and instant coffee. They have observed that increasing the concentration of hydrocolloids reduces the taste and favors the intensity of all products, regardless of the test temperature. The use of gums has significantly reduced the sourness and saltiness of tomato juice, sourness of orange drink, and bitterness of coffee. Baines & Morris [1987] found a similar effect of GG on sweetness and flavor of strawberry. In order to decrease the sensibility of this flavor by three fold,

it was necessary to increase the viscosity by at least 2 orders of magnitude. Similar properties are also attributed to other hydrocolloids such as sodium alginate, pectins [Hayashi *et al.*, 2005; Sun-Waterhouse & Wadhwa, 2013], and carrageenan [Calton & Wood, 2002]. Reduced perception of sour and tart taste in the strawberry products was probably due to the inhibition of hydrocolloids interactions with substances imparting the sensory properties to food in the cell membrane and taste receptors of the oral cavity [Sun-Waterhouse & Wadhwa, 2013].

Given the influence of hydrocolloids on the sensory quality of juices and fruit beverages, tartness masking appears to be a particularly important aspect. Although consumers occasionally consume products with intensively tart taste, for example, bitter chocolate, tea, or dry wine, in general, this taste is considered undesirable and largely contributes to the lack of acceptance of new products. The issue of tartness is the more problematic, since consumers are increasingly looking for products with reduced content of, for example, sugar, which naturally masks the undesirable tastes. In general, it is assumed that tartness is a sensory sensation derived from complexes of polyphenols with salivary proteins [Kallithraka *et al.*, 2001]. Due to significant differences in the chemical structure of hydrocolloids, providing explanation for these interactions is difficult. For instance, due to the presence of functional groups in the molecule derived from acetic acid and pyruvic acid, XG is classified as an anionic compound [Sun *et al.*, 2007; García-Ochoa *et al.*, 2000]. In this case, it can be assumed that the suppression of astringency reflects interactions between the above mentioned groups and the ionic form of tannic acids. In addition, the spiral structure of XG allows “trapping” substance molecules, which are the carriers of tart taste. Another mechanism of action is attributed to nonionic hydrocolloids, for example, GG composed of polymannan chain randomly substituted with galactose molecules. The masking of astringency is based on the physical adsorption of substances with astringent properties to the polysaccharide surface [Troszyńska *et al.*, 2010]. Hydrocolloids improve the sensory characteristics of food with no need of using *e.g.* sweetening agents. Consequently, they facilitate the processing of raw materials rich in bioactive compounds, but characterized with a specific and intense flavor profile.

Hydrocolloids are commonly used to concentrate fruit juices. This property leads to changes in the specific physical parameters (*i.e.* viscosity and stability of cloudiness), which can be visually observed as the change of consistency. Under the influence of addition of hydrocolloids, the tested strawberry products resembled more of smoothies than the cloudy juice. Nevertheless, the members of the sensory panel positively evaluated their texture, especially when these substances were used at a lower dose. Beverages containing 0.2% of CMC, GG, and LBG received the highest scores in the assessment of consistency among all samples analyzed (average value 5.00). At higher concentration of colloids (0.3%), this effect was not that positive. However, the consistency of the products with GG and LBG (average score: 4.88) was still perceived as better in comparison with the control product (average score: 4.75). The lower scores (XG2=4.38;

TABLE 2. Changes of color parameters of strawberry juice and beverages during storage.

Sample code	$L^*$		$a^*$		$b^*$	
	0 months	6 months, 4°C	0 months	6 months, 4°C	0 months	6 months, 4°C
OO	34.64±0.01 <sup>e</sup>	39.75±0.00 <sup>c</sup>	18.93±0.05 <sup>h</sup>	14.59±0.10 <sup>i</sup>	9.03±0.05 <sup>e</sup>	11.94±0.00 <sup>b</sup>
CMC2	34.65±0.01 <sup>e</sup>	38.12±1.50 <sup>e</sup>	19.36±0.10 <sup>c</sup>	18.21±0.30 <sup>a</sup>	8.97±0.00 <sup>h</sup>	11.02±0.10 <sup>c</sup>
CMC3	34.82±0.30 <sup>c</sup>	37.53±0.00 <sup>i</sup>	18.96±0.00 <sup>e</sup>	14.92±0.00 <sup>f</sup>	8.92±0.10 <sup>g</sup>	10.59±0.00 <sup>e</sup>
GG2	34.50±0.20 <sup>h</sup>	38.63±0.80 <sup>f</sup>	19.23±0.40 <sup>d</sup>	16.70±0.01 <sup>c</sup>	9.39±0.10 <sup>c</sup>	10.83±0.05 <sup>f</sup>
GG3	34.84±1.40 <sup>d</sup>	39.19±0.01 <sup>d</sup>	19.07±0.00 <sup>f</sup>	16.10±0.05 <sup>d</sup>	9.15±0.00 <sup>c</sup>	10.87±0.00 <sup>c</sup>
LBG2	34.70±0.10 <sup>f</sup>	38.95±0.20 <sup>e</sup>	19.13±0.01 <sup>e</sup>	15.23±0.50 <sup>e</sup>	9.26±0.06 <sup>d</sup>	10.91±0.00 <sup>d</sup>
LBG3	35.00±0.02 <sup>c</sup>	38.08±0.00 <sup>h</sup>	19.12±0.00 <sup>e</sup>	17.96±0.20 <sup>b</sup>	9.07±0.20 <sup>f</sup>	10.42±0.00 <sup>h</sup>
XG2	35.67±0.03 <sup>b</sup>	40.21±0.10 <sup>b</sup>	20.86±0.20 <sup>b</sup>	14.66±0.00 <sup>h</sup>	10.53±0.30 <sup>b</sup>	11.93±0.08 <sup>b</sup>
XG3	38.46±0.10 <sup>a</sup>	40.86±1.30 <sup>a</sup>	24.42±0.00 <sup>a</sup>	14.80±0.00 <sup>e</sup>	12.82±0.00 <sup>a</sup>	12.92±0.20 <sup>a</sup>

OO – control sample (juice), CMC – beverages with carboxymethylcellulose, GG – with guar gum, LBG – with locust bean gum, XG – with xanthan gum; 2 and 3 – 0.2% and 0.3% addition of hydrocolloids.

Values were expressed as mean ± standard deviation (n=3); a.b.c... – statistically homogenous groups according to values in column (Duncan test,  $p \leq 0.05$ ).

XG3=3.88) were given by the panelists to the beverages with XG. Consistency of these products was strongly changed (to thick, undrinkable). Moreover, other sensory quality indicators, *i.e.*, color, aroma, and taste, were not favorably perceived by evaluators, which potentially also affected the results of consistency assessment.

#### Color measurement in the CIE $L^*a^*b^*$ system

Measurements of color parameters of juice and strawberry beverages were conducted using fresh products and these stored at 4°C for 6 months. Colorimetric analysis results are shown in Table 2 and Figure 2. In the control sample, the value of parameter  $L^*$  (lightness) was 34.64, whereas that of  $a^*$  parameter (proportion of red/green color) was 18.93 and that of  $b^*$  parameter (proportion of yellow/blue color) was 9.03. As a result of the addition of hydrocolloids, individual color components of beverages were shifted to a chromatic space, and the direction of these changes was dependent on both the dose and the type of the substance used. In the samples before storage, a positive and proportional relationship was observed between the dose of the hydrocolloid and the values of parameter  $L^*$  (Table 2).

Among products containing hydrocolloids of plant origin, an increase in the value of  $\Delta L^*$ , that is brightness in relation to the standard (100% strawberry juice), was observed under the influence of CMC and LBG (Figure 2). However, in both the cases these values were close to zero, which indicates that in the range of concentrations tested, these hydrocolloids do not cause significant changes in the brightness of the products. A significant increase in the brightness was reported in the beverages containing XG, in which the values of  $\Delta L^*$  were 1.03 and 3.82 (for doses of 0.2% and 0.3%, respectively). Considering the changes in the other parameters of the chromatic space ( $a^*$ ,  $b^*$ ), always the same relationship could be observed in these products always, that is, increasing the contribution of a particular color component in relation

to the control sample, proportional to the hydrocolloid dose used (Figure 2).

Thus, both red and yellow color were more strongly constituted with XG in comparison to the control, and the values of  $\Delta a^*$  and  $\Delta b^*$  coefficients were 1.93 and 1.50 (0.2% XG) as well as 5.49 and 3.75 (0.3% XG), respectively. The addition of plant hydrocolloids (CMC, GG, LBG) to strawberry juice did not cause such dynamic color changes. Furthermore, the values of  $\Delta a^*$  and  $\Delta b^*$  estimated in the colorimetric test suggested that the mechanism of action of these substances differs from that observed in the case of XG. In the samples with added CMC, the contribution of red color was indeed higher than in the control sample; however, with an increase of CMC additive, this difference decreased (Figure 2). A similar regularity was found in the samples containing GG and LBG. The direction of changes in the values of parameter  $b^*$  was different. In beverages with added CMC, slightly smaller proportion of yellow color in relation to 100% strawberry juice was observed, wherein the greater the amount of hydrocolloid in the product, the greater was the color difference  $\Delta b^*$ . In the beverages with added GG and LBG, the contribution of  $b^*$  was higher than in the control sample, although with a decreasing trend in the value of color difference and increasing concentrations of hydrocolloids.

As a result of storage of strawberry products (6 months at 4°C), dynamic changes were observed in their chromatic space. All samples analyzed were lighter, less red, and more yellow in relation to the samples prior to storage (Table 2). In the control product, changes within the parameters  $L^*$  and  $b^*$  were more advanced in comparison to beverages containing hydrocolloids. This trend was partially confirmed by the results of red color intensity measurement. In beverages with added plant hydrocolloids (CMC, GG, LBG), differences between the values of parameter  $a^*$  before and after storage were lower than these observed in the control prod-

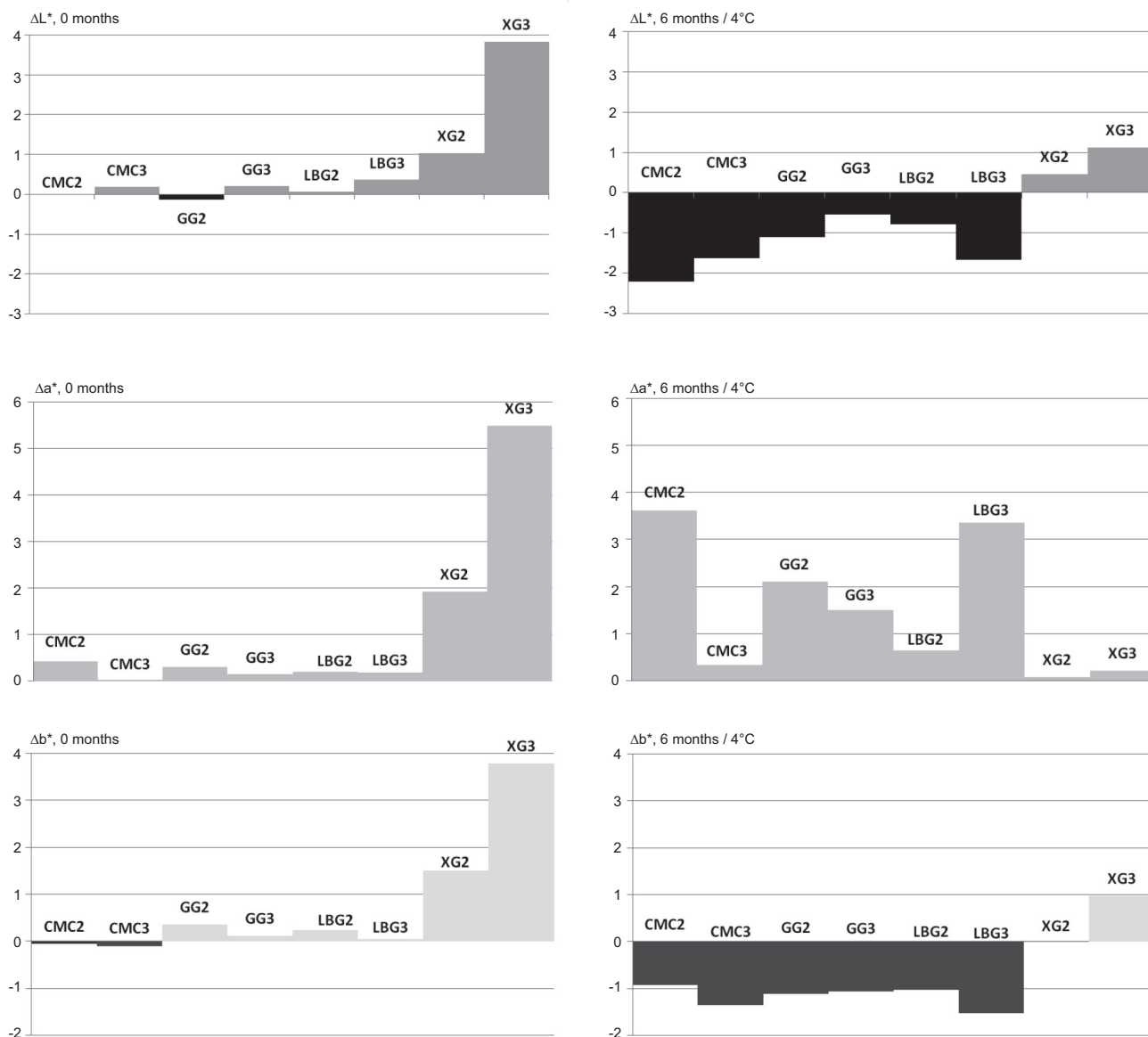


FIGURE 2. Effect of polysaccharide hydrocolloids addition on color parameters of strawberry beverages compared to control sample (100% juice) after and before storage (6 months, 4°C).

$\Delta L^*$  – changes of brightness;  $\Delta a^*$  – changes of redness/greenness;  $\Delta b^*$  – changes of yellowness/blueness.

uct. The proportion of red color was significantly reduced in the samples containing XG (Table 2).

Color conversion in the products containing hydrocolloids was also considered in relation to the parameters of the control sample, determining the values of  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  (Figure 2). After 6 months of storage, beverages containing CMC, GG, and LBG were darker than the control sample and (in case of CMC and GG addition) these differences were smaller at a higher dose of these substances. In all the other beverages, the contribution of red color was greater than that of 100% strawberry juice. Depending on the type and dose of hydrocolloids, the values of  $\Delta a^*$  ranged from 0.07 (XG2) to 3.62 (CMC2). In turn, the contribution of yellow color was observed to be lower in all the other beverages, except for those containing XG, in comparison with the control product. However, the influence of hydrocolloid dose on the direction

of these changes appeared to be ambiguous. In drinks containing CMC and LBG, the values of  $\Delta b^*$  decreased with an increase in the proportion of hydrocolloids. In turn, in beverages with 0.2% and 0.3% addition of GG, the contribution of yellow color was similar, which resulted in slightly different values of  $\Delta b^*$ .

The ambiguous impact of hydrocolloids on the color of strawberry drinks could be related to the analytical technique used involving the measurement of radiation reflected from the surface. The control product contained mainly water in the free form, and the light was reflected from its surface differently than in the case of strawberry beverages characterized with a structure altered by the addition of hydrocolloids. Water can adsorb the radiation resulting in its lesser reflection from the surface [Paślawska *et al.*, 2010]. Another factor affecting the measured values of color components could

also be an increase in samples turbidity caused by the addition of hydrocolloids and intensification of the phenomenon of oxygen incorporation into the products [Laaman, 2011].

Chaikham & Apichartsrangkoon [2012] have observed that pasteurized juice made of longan fruits (*Euphoria longana*) containing 0.15% of XG was characterized by lower brightness and a higher proportion of yellow and red color compared to the control sample. In the paper by Azoubel *et al.* [2011], concerning the restructuring of passion fruit (*Passiflora cincinnata*) pulp using hydrocolloids (gelatin, pectin, and alginate), changes were observed in the  $L^*a^*b^*$  color components. The addition of structure-forming substances caused an increase in the proportion of red color and a decrease in the proportion of yellow one, whereas the values of  $L^*$  parameter were changing ambiguously. Color changes observed in our study during the storage of strawberry products were probably associated with advanced polymerization of anthocyanins into brown compounds for which, among others, the degradation products of sugars and ascorbic acid are responsible for. The conversion of the monomeric forms of the anthocyanins into oligo- or polymeric pigments induces significant color changes toward the reddish brown color [Monagas *et al.*, 2006], which may result in an increase in  $+a^*$  parameter value. This phenomenon is characteristic for long stored products [Piątkowska *et al.*, 2011; Krifi & Metche, 2000]. Polyphenol compounds are largely responsible for color conversion of juices, concentrates, beverages, and other fruit products. The final products of their decomposition can have different colors, which can be quantified using instrumental methods. As a result of the reaction between hydroxycinnamic acid and flavan-3-ols, colorless or slightly yellow caffeine, dehydrodicatichin A (yellow) and B (colorless), procyanidin A (colorless), or hetero dimers (some of them are red) are formed [Alonso-Salces *et al.*, 2005]. Changes in the pH value (from acidic to neutral) adversely affect the stability of the anthocyanins, causing even a com-

plete loss of color [Giusti & Wrolstad, 2001]. The formation of *o*-quinones, as a result of the reaction of anthocyanins and polyphenoloxidase, leads to color change of food from red through blue to brown [Fang *et al.*, 2007]. All these processes affect color intensity and colloidal stability of the fruit products [Saucier *et al.*, 1997].

### Content and storage stability of anthocyanin compounds

In the strawberry products, five anthocyanin compounds were identified including three pelargonidin glycosides (3-*O*-glucoside, 3-*O*-rutinoside, and 3-malonyl-glucoside) and two cyanidin glycosides (3-*O*-glucoside and 3-malonyl-glucoside) (Figure 3, Table 3). Their structure was confirmed *via* UPLC-MS/MS analysis, in which the retention times and mass spectra of anthocyanins present in the beverages were compared to the retention times and mass spectra of the standards (Table 3). In the case of acylated anthocyanins, their identification was based on a comparison of results obtained in our study to the data from previous studies. The total content of anthocyanins (TA) in a fresh 100% cloudy strawberry juice was 114 mg/L (Table 4). More than 90% of this value was mainly due to the two pelargonidin glycosides:

TABLE 3. Identification of anthocyanins in strawberry juice and beverages by UPLC-MS/MS method.

Peak number	$\lambda_{\max}$ (nm)	MS (m/z)	MS <sup>2</sup> (m/z)	Compound
1	516	449.109	287.056	Cyanidin-3- <i>O</i> -glucoside
2	518	433.113	271.061	Pelargonidin-3- <i>O</i> -glucoside
3	506	579.171	271.061	Pelargonidin-3- <i>O</i> -rutinoside
4	518	535.104	287.057	Cyanidin-3-malonylglucoside
5	520	519.113	271.069	Pelargonidin-3-malonylglucoside

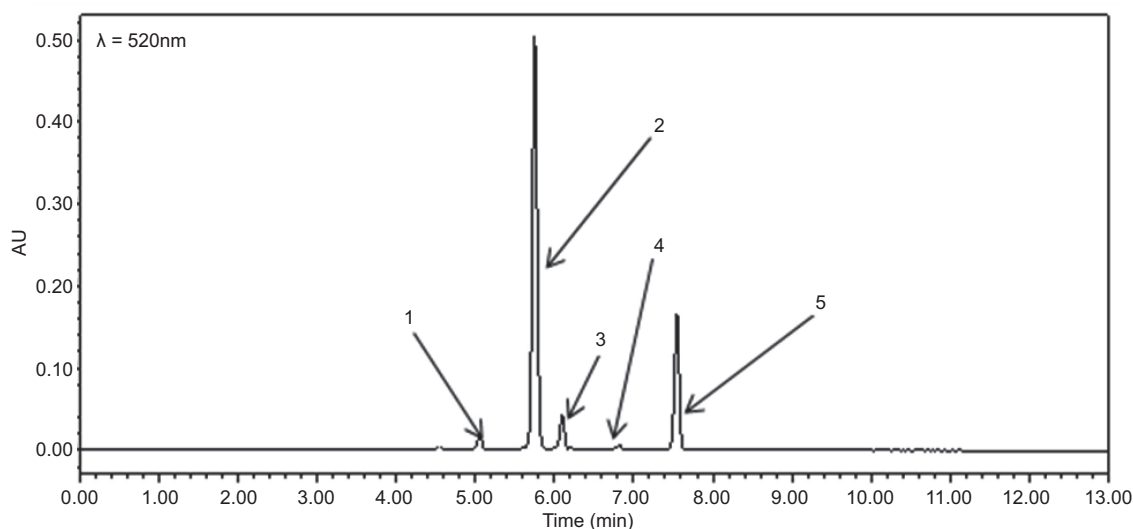


FIGURE 3. LC-PDA chromatogram of strawberry juice/beverages anthocyanins.

1 – cyanidin-3-*O*-glucoside, 2 – pelargonidin-3-*O*-glucoside, 3 – pelargonidin-3-*O*-rutinoside, 4 – cyanidin-3-malonylglucoside, 5 – pelargonidin-3-malonylglucoside.



TABLE 4. Changes in anthocyanins content (mg/L) in strawberry juice and beverages during storage

Sample code	Storage conditions	C-3-Glu	Dd (%)	P-3-Glu	Dd (%)	P-3-Rut	Dd (%)	C-3-mGlu	Dd (%)	P-3-mGlu	Dd (%)	TA	Dd (%)
OO	0 months	3.70 <sup>a</sup>		86.0 <sup>a</sup>		3.23 <sup>a</sup>		1.32 <sup>b</sup>		19.7 <sup>a</sup>		114 <sup>a</sup>	
	6 months, 4°C	0.652 <sup>G</sup>	82.4	14.1 <sup>G</sup>	83.5	0.00	100	0.00	100	3.41 <sup>G</sup>	82.7	18.2 <sup>G</sup>	84.0
CMC2	0 months	3.44 <sup>d</sup>		82.4 <sup>d</sup>		2.93 <sup>f</sup>		1.38 <sup>a</sup>		18.5 <sup>d</sup>		109 <sup>c</sup>	
	6 months, 4°C	1.04 <sup>B</sup>	69.7	23.4 <sup>B</sup>	71.6	0.00	100	0.00	100	4.70 <sup>B</sup>	74.4	29.2 <sup>B</sup>	73.2
CMC3	0 months	3.16 <sup>h</sup>		73.8 <sup>i</sup>		3.20 <sup>b</sup>		0.797 <sup>g</sup>		17.2 <sup>i</sup>		98.2 <sup>i</sup>	
	6 months, 4°C	1.25 <sup>A</sup>	60.5	16.6 <sup>E</sup>	77.5	0.00	100	0.00	100	4.69 <sup>C</sup>	72.7	22.5 <sup>E</sup>	77.0
GG2	0 months	3.57 <sup>c</sup>		82.5 <sup>c</sup>		2.83 <sup>g</sup>		0.917 <sup>f</sup>		18.8 <sup>c</sup>		108 <sup>d</sup>	
	6 months, 4°C	0.997 <sup>C</sup>	72.1	17.7 <sup>D</sup>	78.5	1.15 <sup>C</sup>	59.3	0.00	100	3.81 <sup>E</sup>	79.7	23.7 <sup>D</sup>	78.2
GG3	0 months	3.18 <sup>e</sup>		75.2 <sup>h</sup>		2.50 <sup>j</sup>		1.09 <sup>e</sup>		17.6 <sup>h</sup>		99.6 <sup>h</sup>	
	6 months, 4°C	0.719 <sup>E</sup>	77.4	18.5 <sup>C</sup>	75.4	1.20 <sup>A</sup>	52.1	0.00	100	3.95 <sup>D</sup>	77.5	24.4 <sup>C</sup>	75.5
LBG2	0 months	3.60 <sup>b</sup>		82.8 <sup>b</sup>		3.19 <sup>c</sup>		1.33 <sup>b</sup>		19.3 <sup>b</sup>		110 <sup>b</sup>	
	6 months, 4°C	0.725 <sup>E</sup>	79.9	15.9 <sup>F</sup>	80.8	0.250 <sup>D</sup>	92.2	0.00	100	3.45 <sup>F</sup>	82.2	20.3 <sup>F</sup>	81.5
LBG3	0 months	3.41 <sup>e</sup>		77.9 <sup>f</sup>		2.96 <sup>e</sup>		1.05		18.1 <sup>e</sup>		103 <sup>f</sup>	
	6 months, 4°C	0.862 <sup>D</sup>	74.7	26.3 <sup>A</sup>	66.3	1.18 <sup>B</sup>	60.2	0.00	100	7.80 <sup>A</sup>	56.8	36.1 <sup>A</sup>	65.1
XG2	0 months	3.00 <sup>i</sup>		80.8 <sup>c</sup>		3.07 <sup>d</sup>		1.18 <sup>d</sup>		17.9 <sup>g</sup>		106 <sup>e</sup>	
	6 months, 4°C	0.676 <sup>F</sup>	77.5	13.9 <sup>H</sup>	82.8	0.00	100	0.00	100	3.40 <sup>G</sup>	81.1	17.9 <sup>H</sup>	83.1
XG3	0 months	3.20 <sup>f</sup>		77.4 <sup>e</sup>		2.83 <sup>h</sup>		1.21 <sup>c</sup>		18.0 <sup>f</sup>		102 <sup>g</sup>	
	6 months, 4°C	0.592 <sup>H</sup>	81.5	13.5 <sup>I</sup>	82.6	0.00	100	0.00	100	3.13 <sup>h</sup>	82.6	17.2 <sup>I</sup>	83.2

C-3-Glu – cyanidin-3-glucoside, P-3-Glu – pelargonidin-3-glucoside, C-3-mGlu – cyanidin-3-malonylglucoside, P-3-mGlu – pelargonidin-3-malonylglucoside, TA – total anthocyanins, Dd – degradation degree

a,b,c... – statistically homogenous groups according to values in column for fresh samples (Duncan test.  $p \leq 0.05$ ); A, B, C... – statistically homogenous groups according to values in column for samples stored for 6 months at 4°C (Duncan test.  $p \leq 0.05$ ).

the dominant pelargonidin-3-*O*-glucoside (P-3-Glu; 74% TA) and pelargonidin-3-malonyl-glucoside (P-3-mGlu; 17% TA). Significantly lower concentrations were observed for cyanidin-3-*O*-glucoside (C-3-Glu; 3.25% TA). In strawberry beverages obtained by adding hydrocolloids to 100% cloudy juice, lesser amounts of anthocyanins were determined. As expected, in these products, the detectable concentration of the test compounds was inversely proportional to the dose of the hydrocolloids. Therefore, probably, these substances may trap the colored compounds, limiting the degree of their extraction to solutions that are subjected to chromatographic analysis. Of all the beverages tested, the most anthocyanins were determined in samples with LBG (LBG2=110 mg/L and LBG3=103 mg/L, respectively;  $p < 0.05$ ). In the samples with the addition of XG, their concentration was lower by 7% and 10% in relation to the control (for the XG2 and XG3, respectively). Beverages with CMC and GG were characterized by a similar content of TA (Table 4). In the concentration ranges of hydrocolloids under study, 5%–14% less anthocyanins were determined compared to strawberry juice. The key issue of the study was to determine how the addition of hydrocolloids can reduce the distribution of anthocyanins in beverages. As demonstrated by our previous study, the cloudy strawberry juice (“Roxana” cv.) obtained after

the addition of hydrocolloids was characterized by a high degree of degradation of anthocyanin pigments [Teleszko *et al.*, 2016]. At the same time, during the stage of fruit processing (laboratory scale), no addition of inactivators/PPO inhibitors was observed, which could exclude the potential impact of the additional factors or their interactions with hydrocolloids on the color stability of the products. The pasteurization process commonly used in fruit and vegetable industry was selected as a method of food preservation. The cloudy juice obtained and the beverages were stored at 4°C in the dark for 6 months. The storage experiment has shown that the addition of hydrocolloids to cloudy strawberry juice led to the partial degradation of anthocyanins. This effect, however, was directly dependent on the type of hydrocolloid.

After 6 months, only 16% of the initial content of the test compounds remained in 100% cloudy strawberry juice (Table 4). There was no presence of P-3-Rut and C-3-mGlu, and the degree of degradation (*Dd*) of other anthocyanin monomers was found in the range between 82.4% (C-3-Glu) and 83.5% (P-3-Glu). The addition of hydrocolloids did not prevent the degradation of C-3-mGlu, which is revealed due to the presence of a trace amount of this compound in the products tested (1% TA on average); however, this observation was not so relevant for the present experiment. Nev-

ertheless, the ability of hydrocolloids to stabilize compounds such as C-3-Glu or P-3-Rut differs significantly. It was also observed that increasing the concentration of hydrocolloids in the samples did not ensure an increase in the stability of active compounds during storage.

The lowest degree of degradation of anthocyanins was observed in the beverage containing 0.3% of LBG. After 6 months of storage, 36.1 mg TA/L was determined in this product, which corresponded to the amount of *Dd* at the level of 65.1%. Interestingly, when using a lower dose of LBG (0.2%), the protective effect of anthocyanins was negligible, and the degree of their decomposition was similar to that observed in the control sample (*Dd*=81.5%). Such strong diversity in the stability of TA depending on the concentration of hydrocolloid was only observed in the samples with LBG (Table 4). By comparison, in the beverages with GG, the values of *Dd* were 78.2% and 75.5% (for GG2 and GG3, respectively). In the products with CMC, the decomposition of anthocyanins was more advanced in the sample with 0.3% addition of this hydrocolloid, and *Dd* value was in the range of 73.1%–77.0% (for CMC2 and CMC3, respectively). There was, however, no significant effect of the use of XG on the inhibition of anthocyanins degradation in beverages.

In this case, the rate of TA degradation was not only the highest among samples containing the addition of hydrocolloids, but also the least diverse (XG2=83.1% and XG3=83.2%). The degree of degradation of anthocyanins was investigated not only in relation to their total content in the products, but also in terms of individual monomers. The average values of *Dd* coefficient of three major anthocyanins identified in beverages were comparable and were found between 74.1% (C-3-Glu) and 76.9% (P-3-Glu). Decomposition of C-3-Glu was reduced to the greatest extent by the addition of CMC, wherein the higher the concentration of this hydrocolloid in the product, the lower was the degree of degradation of C-3-Glu (CMC2=69.7%; CMC3=60.5%).

By comparison, in a control product stored under the same conditions, the value of *Dd* calculated for C-3-Glu was 82.4% (Table 4). P-3-Glu and P-3-mGlu were best preserved in the beverage containing 0.3% of LBG (*Dd* accounted for 66.3% and 56.8%, respectively). Moreover, the effect of hydrocolloids on the stability of P-3-Rut appeared to be interesting. It was observed that only the use of GG contributed to a satisfactory reduction in decomposition of this compound. By adding 0.2% GG, the degree of degradation of P-3-Rut in the product tested was reduced to 59.3%, while by adding 0.3% of GG, the value of *Dd* was reduced to 52.1% (Table 4). The protective effect against P-3-Rut was observed neither in beverages containing CMC, nor in those with XG. This effect was partially indicated in the beverage containing 0.3% LBG, for which the value of *Dd* was 60.2%.

Hubbermann *et al.* [2006] studied the effect of hydrocolloids (sodium alginate, citrus pectin, locust bean gum, carrageenan, and corn starch) on the stability of anthocyanins contained in elderberry concentrates and black currant. They demonstrated that the majority of hydrocolloids tested caused only slight color changes. Only sodium alginate significantly

affected color stability of elderberry concentrate. In the case of longer storage, a trend for *a\** value stabilization was also observed for pectin, corn starch, and sodium alginate in black currant concentrate. The molecular mechanism of anthocyanins binding with hydrocolloids is not well-understood. Fernandes *et al.* [2014] have described this process in relation to low-methylated pectin and pure anthocyanin preparations: cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside. Their studies have indicated that hydrogen bonds and hydrophobic interactions are involved in pectin–anthocyanins interactions.

## CONCLUSION

The use of hydrocolloids in fruit processing allows obtaining new, sensory-attractive products with increased stability of anthocyanin compounds. The key issue is the selection of these substances, both in terms of quantity and quality. In the case of strawberry beverages, the best results were obtained using the addition of LBG. These products (at both concentration ranges of LBG) obtained higher scores in the taste and consistency assessment in comparison to 100% cloudy strawberry juice, which was the control sample. Above all, however, the use of LBG at a dose of 0.3% has contributed to the reduction of anthocyanins degradation up to 65%. Given their advanced decomposition in juice (*Dd* 84%), this effect can be considered satisfactory and important from the perspective of the quality of the analyzed products.

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