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Application of Acid Whey in Orange Drink Production

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Summary

The aim of this study is to compare qualitative changes in orange and orange beverages containing whey during 12 months of storage. The beverages contained 12 % extract, half of which was orange concentrate, the rest was sugar or sugar and whey extract. Acid whey was used in the production of beverages, added at a rate of 50 % of the used water. Orange beverages with whey contained more protein, ash, glucose, lactose and vitamin B_2 than the orange beverages, but less sucrose, fructose and vitamin C, and also showed lower antioxidant activity against the DPPH radical. No significant differences between the two types of beverages were found in the polyphenolic content or activity against the ABTS cation radical. The type of beverage had a significant effect on the colour parameter values under the CIELAB system, although no significant differences were found between the beverages in the sensory evaluation of colour desirability. The overall sensory evaluation of orange beverages with whey was 2–10 % lower than of other orange beverages. The intensity of orange, sweet and refreshing taste was greater in orange beverages, while that of sour and whey taste was greater in orange beverages containing whey. There were significant decreases in sucrose, lactose, all indicators of antioxidant activity and sensory quality during storage. Levels of glucose and fructose rose with the storage period, while the intensity of sour, orange and refreshing taste decreased.

Key words: orange, whey, beverages, antioxidants, vitamins, sensory analysis

Introduction

The most popular fruit beverages among consumers are those which contain orange juice. Average global orange production is about 67.6 million tonnes (*1*), a substantial proportion of which is used in the production of orange juice concentrate. In countries with large seasonal production of fresh fruit, juices and fruit drinks provide valuable nutritional constituents throughout the year. Because of their high level of consumption, oranges, whether fresh or processed, constitute a significant source of antioxidants (chiefly vitamin C and polyphenolic compounds) in the diet (*2*). Vitamin C content in orange juice is about 30 mg per 100 mL (*3*), which means that one glass (250 mL) provides 60–70 % of the recommended daily allowance. Apart from vitamin C, the other main contribution to the antioxidant properties of orange juice comes from polyphenolic compounds, among which hydroxycinnamic acid and flavanones (a class of flavonoids) are the most abundant. These compounds have shown such therapeutic properties as anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities (*2*).

Acid whey contains components which, apart from their high nutritional value, exhibit biological activity: mainly proteins, mineral salts, vitamins and the prod-

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ucts of lactic acid fermentation. In particular, whey proteins are a rich source of exogenous amino acids. Additional effects of whey proteins or the peptides deriving from them are: antimicrobial, antioxidant, hypotensive, hypocholesterolemic, immunomodulative, insulinotropic and anticancer (*4*). During acid coagulation of milk, calcium originally bound in the form of calcium phosphate in casein micelles dissociates into the solution. As a result, acid whey is rich in the nutritionally valuable elements calcium and phosphorous.

Around half the global production of whey is not recycled but treated as a waste product. Acid whey is difficult to recycle because of its low pH. In effect, acid whey is mainly used as animal fodder or treated as a polluting waste product. In Poland 90 % of acid whey is used as fodder and only 3 % in food production (*5*). So far there have been a few attempts to use acid whey in combination with fruit drink; Shekilango *et al.* (*6*) produced whey and banana beverages from overripe bananas.

Consumers pay increasing attention to the nutritional value and potential health benefits of food (*7*). Orange and whey beverage could be an interesting and nutritious product in the developing market for functional foods. The aim of this work is to compare qualitative changes of physicochemical, antioxidant and sensory properties in orange and orange beverages containing whey during 12 months of storage.

Materials and Methods

Production of beverages

Two types of beverages were produced on a laboratory scale: orange, and orange containing whey. The ingredients were: low-mineral natural spring water; orange concentrate (Citrusco, Matão, Brazil) comprising 64.5 % extract with 4.5 g of citric acid per 100 g; acid whey from a local dairy, a by-product of twarog (quark) cheese production (comprising in %: water 93.7, protein 0.6, fat 0.5, lactose 4.3 and ash 0.6); sugar and citric acid. Directly after twarog cheese production, whey was filtered, centrifuged to remove any remaining fat, and heated to 65 °C for several seconds to ensure microbiological stability. For optimal balance of extract and acidity, it was decided that the beverages would contain (12±0.2) % extract, 50 % of which would be orange concentrate, the rest would be the sugar (in orange beverages) or sugar and whey extract (in orange beverages with whey), and that the acidity of the beverages would be 0.6 g per 100 g of citric acid. In orange beverages with whey, the whey was added at the rate of 50 % of the amount of added water. The citric acid was added to reach the acidity of 0.6 g per 100 g in both types of beverages, taking into consideration the acidity of whey added to the orange beverages. The juices were pasteurised for several seconds at 80 °C, then filtered while hot and bottled (in 0.33-litre glass bottles). After sealing, the bottled beverages were pasteurised for 15 min at 80 °C, then stored at 4 °C pending analysis. The beverages were produced in three independent experimental series and analysed after 2 weeks, and again after 6 and 12 months of storage at 4 °C.

Physicochemical analysis

The content of dry matter, extract, ash and protein was determined according to AOAC standards (*8*). Total acidity was determined using the titration method described in AOAC official methods (*8*) and expressed as g of citric acid per 100 mL of product. Citric acid content was determined using the colorimetric method described by Marrier and Boulet (*9*). Glucose content was determined by an enzymatic colorimetric oxidase assay; the absorbance was read at a wavelength of 500 nm according to Yuen and McNeill (*10*). Fructose content was assessed according to Hofer and Jenewein (*11*) by an enzymatic method with spectrometric determination at a wavelength of 340 nm. Sucrose content was determined following Holmes' enzymatic assay (*12*) entailing the coupling of invertase-catalysed sucrose hydrolysis with a fructose dehydrogenase-catalysed oxidation of the liberated fructose, followed by spectrometric determination of fructose with tetrazolium salt at a wavelength of 570 nm. Lactose content was assessed according to Shapiro *et al.* (*13*) using an enzymatic method and thio-nicotinamide-adenine dinucleotide (thio-NAD) as an oxidizing agent, with spectrometric determination at a wavelength of 405 nm.

Polyphenolic content and antioxidant activity

Polyphenolic content was determined using the Folin-Ciocalteu reagent (*14*) in 80 % methanol extract acidified with 0.5 % HCl. Absorption was measured 60 min after adding the Folin-Ciocalteu reagent at a wavelength of 675 nm. Polyphenolic content was measured by reading off the catechin standard curve. Antioxidant activity against the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid]) cation radical was determined by the methods described by Pekkarinen *et al.* (*15*) and Re *et al.* (*16*) in the extracts prepared for polyphenolic determination. Absorption was measured at 516 nm for DPPH and at 734 nm for ABTS cation radical, directly after adding the reagent and again 10 min later. Antioxidant activity was expressed in μmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent in 1 mL of beverage.

Vitamin content

The content of vitamins C, B_1 and B_2 was determined by the HPLC method according to EN 14130:2003 (*17*), EN 14122:2003 (*18*) and EN 14152:2003 (*19*) standards, respectively. Determination of vitamin content was performed using HPLC liquid chromatograph (Merck Hitachi, Manchester, UK) equipped with an online L-7612 degasser, L-7250 programmable autosampler, L-7100 pump, L-7480 fluorescent detector, L-7420 UV-VIS detector, Merck L-7360 column thermostat, Interface D-7000, and with D-7000 HPLC System Manager (HSM) software. For vitamin C and L-ascorbic acid content, beverages were diluted with 0.1 M metaphosphoric acid (5 mL of beverages with 45 mL of 0.1 M metaphosphoric acid) and centrifuged. Samples were injected into a Merck LiChrospher® RP-18 column (250×4.6 mm; Merck Millipore, Billerica, MA, USA). The elution was carried out using 0.1 M metaphosphoric acid at a flow rate of 1 mL/min. A UV-

-VIS detector (Merck Hitachi) was used. Absorbance was monitored at 254 nm. The sum of L-ascorbic and L-dehydroascorbic acids was determined after reduction with L-cysteine according to EN 14130:2003 standard (*17*). Identification and quantitative analysis of vitamin C was performed using an external standard of L-ascorbic acid in metaphosphoric acid. The standard curve was prepared for 5–50 μg/mL of L-ascorbic acid solution. The standard of ≥99 % purity was purchased from Sigma-Aldrich Chemie (Munich, Germany). The 87 % average recovery level was taken into account in calculations. Analysis of vitamins B_1 and B_2 was carried out on a Phenomenex Onyx Monolithic C18 Column (100×4.6 mm; Phenomenex, Torrance, CA, USA). A fluorescence detector (Merck Hitachi) was used for both vitamins. Analysis was conducted at wavelengths of excitation and emission of 366/435 for thiamine and 370/520 for riboflavin. Water and acetonitrile (in the ratio of 88:12 at t=0 and 100 % acetonitrile after 12 min) were used as mobile phase in gradient elution. For the determination of thiamine and riboflavin as well for the quantitative analysis of these vitamins, thiamine hydrochloride dissolved in hydrochloric acid and riboflavin dissolved in acetic acid were used as external standards, respectively. The standard curve was prepared for 0.2–5.0 μg/mL of thiamine hydrochloride and riboflavin solutions. The standards of ≥99 % (thiamine hydrochloride) and ≥98 % (riboflavin) purity were purchased from Sigma-Aldrich Chemie. In calculations, the so-called recovery level was taken into account, which amounted to 94 and 91 % on average for thiamine and riboflavin, respectively.

Colour parameters

Colour measurement was carried out by the transmittance method (measuring geometry $d/0°$, illuminant D65, range 400–700 nm at 20 nm intervals, optical path length 20 mm) in the CIELAB system using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Inc., Tokyo, Japan). Based on that measurement, the following parameters were established: L^* – colour brightness (L^*=0 black, L^*=100 white); a^* – green colour (a^*<0), red colour (a^*>0); b^* – blue colour (b^*<0), yellow colour (b^*>0); C^* – colour saturation; and h^* – hue angle.

Sensory analysis

Sensory analysis was conducted according to the Polish standard PN-ISO 6658 (*20*) using a 5-point scale (5=excellent, 4=very good, 3=good, 2=bad, 1=very bad) by a panel of 5 members, all of whom fulfilled the basic requirements for sensory sensitivity (*21*). The analysis took into account the basic quality descriptors: appearance (colour, sediments and suspension), odour acceptability, odour intensity, and taste. The descriptor of overall sensory quality for a product was established by using the importance factor for particular quality descriptors. The number of points obtained after dividing the total points for a given product by the sum of the importance factors was accepted as the overall result.

Descriptive flavour analysis was conducted by a team of 8 people, all acknowledged experts according to ISO 8586/2 standard (*22*), whose discussion was moderated by a person with considerable experience in using this method of food quality assessment and highly knowledgeable in the field of fruit beverages and juices. The analysis took into account the following descriptors of flavour: sour taste, sweet taste, orange taste, refreshing taste, tart taste, whey taste, bland taste, orange odour, whey odour and total flavour assessment. The intensity of individual descriptors was rated on a scale from 0 to 5 (0=not perceptible, 5=very high intensity).

Statistical analysis

The results of the investigation were analyzed statistically using two-way analysis of variance based on the Snedecor's F-test and Student's t-test, and the least significant difference (LSD) was calculated at the probability level α=0.01. Linear correlation coefficiency between the value of antioxidant activity and the content of vitamin C and polyphenols was also established. The STATISTICA v. 8.0 (StatSoft Inc, Tulsa, OK, USA) program was used for statistical calculations.

Results and Discussion

Chemical composition of beverages

The results of the basic analysis of chemical composition for the beverages are given in Table 1. The level of extract in both beverages was, as planned, approx. 12 %, which is comparable to the levels found in commercial orange juice. According to Michalak-Majewska *et al.* (*23*), the level of extract in the most popular orange juices on the Polish market is (11.3±0.7) %. The level of dry matter in the investigated beverages was approx. 0.3 % higher than that in the extract; this was probably connected with the presence of protein compounds, part of which are in colloidal dispersion and do not affect the extract. The reason why levels of these two parameters did not vary between orange and orange beverages with whey was because the whey extract was taken into account when balancing the formulae of the beverages.

There was a significant difference in protein levels between the beverages, with orange drinks with whey containing 67 % more protein than orange drinks. Mean protein content in acid whey is between 0.6 and 0.8 g per 100 mL (*24*). It should be noted that the addition of whey, a high-quality protein, to beverages contributes to biological activity. Among the protein fractions in milk, whey protein has the highest content of essential amino acids (*25*). Orange beverages containing whey had nearly three times as much ash as non-whey drinks. Acid whey is particularly rich in minerals, mainly calcium and phosphorous. Mineral content in acid whey varies from 0.43 to 0.72 g per 100 mL (*24*).

The acidity of juices and beverages is one of the main factors affecting their sensory traits. Acidity in orange juices ranges from 0.6 to 1.6 g of citric acid per 100 mL (*26*). Acidity in the produced beverages was at the lower end of this range, which, however, met with a favourable response from the sensory evaluation panel. According to Michalak-Majewska *et al.* (*23*), the most popular orange juices among Polish consumers have an average acidity of (0.69±0.06) g of citric acid per 100 mL. No significant differences were found in the acidity of the tested beverages, which was due to an equivalent amount of

Table 1. Physicochemical parameters of orange, and orange and whey beverages

m/V	*t*(storage)	Beverage			LSD
g/100 mL	month	Orange	Orange and whey	Mean	α=0.01
Dry matter	0	12.1±0.1	12.1±0.1	12.1±0.1	ns
	6	12.2±0.1	12.1±0.1	12.1±0.1	
	12	12.1±0.1	12.1±0.1	12.1±0.1	
	mean	12.1±0.1	12.1±0.1		I×II
LSD α=0.01			ns		ns
Extract	0	11.9±0.1	11.9±0.1	11.9±0.1	ns
	6	11.9±0.1	11.9±0.2	11.9±0.1	
	12	11.9±0.1	11.9±0.2	11.9±0.1	
	mean	11.9±0.1	11.9±0.1		I×II
LSD α=0.01			ns		ns
Protein	0	0.38±0.01	0.56±0.01	0.47±0.10	ns
	6	0.37±0.01	0.57±0.01	0.47±0.11	
	12	0.38±0.01	0.57±0.01	0.47±0.10	
	mean	0.38±0.01	0.57±0.01		I×II
LSD α=0.01			0.01		ns
Ash	0	0.19±0.02	0.56±0.04	0.37±0.20	ns
	6	0.18±0.01	0.56±0.02	0.37±0.20	
	12	0.19±0.01	0.57±0.03	0.38±0.20	
	mean	0.19±0.01	0.56±0.03		I×II
LSD α=0.01			0.03		ns
Acidity (g of citric acid)	0	0.58±0.01	0.60±0.01	0.59±0.01	ns
	6	0.58±0.02	0.60±0.01	0.59±0.02	
	12	0.59±0.01	0.59±0.01	0.59±0.01	
	mean	0.58±0.01	0.60±0.01		I×II
LSD α=0.01			0.01		ns
Citric acid	0	0.56±0.04	0.48±0.03	0.52±0.05	ns
	6	0.57±0.04	0.49±0.05	0.53±0.06	
	12	0.57±0.03	0.48±0.04	0.52±0.06	
	mean	0.57±0.03	0.48±0.04		I×II
LSD α=0.01			0.05		ns

results are expressed as mean values±standard deviation; ns=not significant difference; I×II=interaction

citric acid added to non-whey drinks to compensate for the additional acidity in beverages containing acid whey. Orange drinks contained a significantly higher level of citric acid than orange beverages containing whey. A proportion of the acidity in the latter product came from lactic acid, one of the main products arising from the fermentation process involved in the manufacture of twarog cheese. The content of citric acid in orange beverages varies considerably, as does that of total acidity. Kelebek *et al.* (*27*) found 1.26 g of citric acid per 100 mL of juice obtained from Turkish oranges, while Saavedra *et al.* (*28*) determined 0.84–1.26 g of citric acid per 100 mL of juice made from Spanish oranges. Apart from citric acid, malic acid also occurs in significant amounts in orange juice, while the remaining organic acids, such as oxalic, tartaric, galacturonic and quinic, occur in smaller concentrations (*29*).

The above descriptors of chemical composition remained constant in the beverages throughout the entire duration of the experiment, which confirmed the lack of signifficant differences ($p \leq 0.05$).

The composition of the carbohydrate fraction in the tested beverages depended on the amounts of these compounds present in orange juice concentrate and whey, as well as on the amount of added sucrose (see Table 2). The dominant sugar in both types of beverages was sucrose, although its level was significantly lower in orange drinks with whey due to the presence of lactose. The main monosaccharides in the beverages were glucose and fructose. As was the case in the tested beverages, sucrose

Table 2. The level of carbohydrates in orange, and orange and whey beverages

m/V	*t*(storage)	Beverage			LSD
g/100 mL	month	Orange	Orange and whey	Mean	α=0.01
Glucose	0	1.4±0.1	1.8±0.1	1.6±0.22	0.15
	6	1.7±0.1	1.9±0.1	1.8±0.1	
	12	1.9±0.1	1.9±0.1	1.9±0.1	
	mean	1.7±0.2	1.9±0.1		I×II
LSD α=0.01			0.12		0.21
Fructose	0	1.5±0.1	1.5±0.1	1.5±0.1	0.18
	6	1.9±0.1	1.6±0.1	1.8±0.2	
	12	2.3±0.1	1.9±0.1	2.1±0.3	
	mean	1.9±0.4	1.7±0.2		I×II
LSD α=0.01			0.14		ns
Sucrose	0	7.1±0.2	4.9±0.2	6.0±1.4	0.22
	6	6.7±0.1	4.6±0.2	5.7±1.1	
	12	6.5±0.1	4.5±0.1	5.5±1.1	
	mean	6.8±0.4	4.7±0.2		I×II
LSD α=0.01			0.18		0.31
Lactose	0	0.0±0.0	1.9±0.1	0.9±1.0	0.05
	6	0.0±0.0	1.8±0.0	0.9±1.0	
	12	0.0±0.0	1.7±0.1	0.9±0.9	
	Mean	0.0±0.0	1.8±0.1		I×II
LSD α=0.01			0.04		0.08

results are expressed as mean values±standard deviation; ns=not significant difference; I×II=interaction

was the dominant sugar in the orange extract. Orange juice contains 4.71–5.93 g of sucrose per 100 mL (*27*,*30*, *31*). The ratio of sucrose/glucose/fructose in orange juices is approx. 2:1:1 (*32*). When sucrose was added, the ratio in the orange beverage was 4.1:1:1.1, while that in the orange beverages containing whey was 2.8:1.1:1. Levels of sucrose and lactose fell significantly with storage time, accompanied by an increase in glucose and, probably, galactose. This phenomenon was connected with the hydrolytic decomposition of sucrose and lactose into monosaccharides. At the beginning of the storage period, the glucose content of orange and whey beverages was greater in comparison with orange beverages; after 12 months of storage, however, similar levels of glucose were found in both types of beverages. This was probably due to the fact that the source of glucose in whey beverages was, apart from orange concentrate, the whey itself, in which the presence of glucose resulted from the hydrolysis of lactose by β-galactosidase synthesised by lactic acid bacteria. A slightly different process was involved in fructose content changes, which, at the beginning of the storage period, was similar in both types of beverages. However, the rate of increase in fructose content during storage was considerably greater in orange beverages than in orange beverages with whey. After 12 months, the difference in fructose content between the two types of beverages amounted to 0.41 g per 100 mL. The principal source of fructose in fresh beverages was orange concentrate. As the amount of concentrate was the same, similar levels of fructose in both types of beverages directly after production were measured. During storage, the proportion of fructose increased as a result of the hydrolysis of sucrose. Since orange beverages contained substantially more sucrose than orange beverages with whey, sucrose hydrolysis was faster in the former, and the products of this process, such as fructose, were correspondingly greater in quantity. Glucose and fructose content in orange juices can vary widely depending on their source, ranging from 1.75 to 3.23 and from 1.90 to 2.86 g per 100 mL, respectively (*27*,*30*,*31*). The lactose in orange beverages with whey derived solely from whey, so this sugar was not found in orange beverages. Jelen (*24*) states that acid whey contains 4.4–4.6 g of lactose per 100 mL.

Antioxidant activity of orange juices

The most important antioxidant compounds in orange juices are polyphenols and vitamin C. Polyphenols comprise a fairly wide group of compounds. Kelebek *et al.* (*27*) identified 13 polyphenolic compounds in orange juice, of which 2 were hydroxybenzoic acids, 5 hydroxycinnamic acids and 6 flavanones, totalling 31.74 mg per 100 mL. Klimczak *et al.* (2) found 63.5–68.4 mg of total polyphenols per 100 mL of orange juices. The analysed beverages contained 14.8–59.8 mg of total polyphenols per 100 mL depending on the type and storage time (see Table 3). The addition of whey did not have a

significant effect on total polyphenols; however, their level fell significantly with increased storage time. After 6 and 12 months of storage, total polyphenolic content fell by 62 and 73 %, respectively, indicating that the greatest decrease occurred in the first 6 months of storage. Klimczak *et al*. (2) found far smaller losses of polyphenolic compounds in commercial orange juices stored for 6 months, recording decreases of 2–18 % depending on the storage temperature. Contradictory data on the changes of the content of polyphenols during storage can be found in literature. Significant decrease in the level of polyphenols in the orange juices during relatively short storage period was demonstrated also by Del Caro *et al*. (*33*). These authors observed 41–65 % decrease in the total content of polyphenols in unpasteurized juices after 15 days of cold storage at 4 °C. On the other hand, Caliskantürk *et al*. (*34*) reported slight changes in the content of polyphenols in the whole, freshly squeezed and pasteurized orange juices stored for 4 weeks at 6, 15 and 30 °C.

Apart from polyphenols, vitamin C is one of the principal antioxidants in orange juices. Vitamin C levels in orange and orange and whey beverages at the beginning of storage were 32.4 and 30.6 mg per 100 mL respectively, of which L-ascorbic acid comprised 93 and 83 %, respectively. In view of the fact that orange concentrate comprised only 50 % of the beverage extract, these levels may be considered satisfactory, indicating that good quality concentrate was used. Kelebek *et al.* (*27*) found 50.6–53.2 mg of ascorbic acid per 100 mL of fresh orange juice, whereas Meléndez-Martínez *et al.* (*35*) found considerable variation in the levels of L-ascorbic acid in commercial juices made from concentrate, rang-

Table 3. Polyphenols, antioxidants and vitamins in orange, and orange and whey beverages

Parameter	*t*(storage)	Beverage			LSD
	month	Orange	Orange and whey	Mean	α=0.01
L-ascorbic acid (mg per 100 mL)	0	30.1±0.2	25.4±0.2	27.7±2.6	
	6	17.0±0.2	11.9±0.7	14.5±2.8	0.62
	12	14.0±0.7	3.8±0.2	8.9±5.5	
	mean	20.4±7.3	13.7±9.3		I×II
LSD α=0.01			0.51		0.88
Vitamin C (mg per 100 mL)	0	32.4±0.9	30.6±0.6	31.5±1.2	
	6	18.9±0.2	13.1±0.5	16.0±3.1	0.98
	12	16.2±1.2	8.9±0.3	12.6±3.9	
	mean	22.5±7.5	17.6±9.8		I×II
LSD α=0.01			0.80		1.39
Polyphenols (mg of catechin per 100 mL)	0	58.5±1.3	59.8±0,5	59.1±1.1	
	6	22.8±1.5	22.5±0.6	22.6±1.1	1.40
	12	16.8±0.5	14.8±1.0	15.8±1.3	
	mean	32.7±19.3	32.3±20.5		I×II
LSD α=0.01			ns		1.98
Antioxidant activity DPPH (μmol of TE per 1 mL)	0	11.3±1.1	10.9±0.5	11.1±0.8	
	6	6.1±0.1	5.6±0.1	5.9±0.3	0.77
	12	5.3±0.3	2.1±0.2	3.7±1.7	
	mean	7.6±2.8	6.2±3.8		I×II
LSD α=0.01			0.63		1.09
Antioxidant activity ABTS (μmol of TE per 1 mL)	0	17.7±1,7	18.5±0.5	18.1±1.2	
	6	17.3±0.4	18.0±0.4	17.7±0.5	1.30
	12	8.3±1.0	7.9±0.5	8.1±0.8	
	mean	14.4±4.7	14.8±5.0		I×II
LSD α=0.01			ns		ns
Vitamin B_1 (mg per 100 mL)	12	0.030±0.001	0.030±0.001		
LSD α=0.01			ns		
Vitamin B_2 (mg per 100 mL)	12	0.012±0.001	0.041±0.002		
LSD α=0.01			0.003		

results are expressed as mean values±standard deviation; ns=not significant difference; I×II=interaction; TE=Trolox equivalent

ing from 19.6 to 63.4 mg per 100 mL, depending on the producer. Vitamin C content in orange, and orange beverages containing whey decreased significantly during storage, falling by 42 and 57 %, respectively, after 6 months, and 50 and 71 % after 12 months. During storage, vitamin C levels in orange beverages were almost two times higher than in orange and whey beverages. Vitamin C loss in fruit juices during storage is a widely known phenomenon, the extent of which depends on the composition of the product, the type of packaging and conditions of storage. Esteve *et al.* (*29*) found that vitamin C degradation in pasteurised orange juices (produced using FMC extraction method) stored at 10 °C was twice as fast as that in juices stored at 4 °C. These authors calculated the daily loss at 2.26–4.59 and 1.42–2.47 mg per 100 mL respectively. According to Klimczak *et al.* (*2*), vitamin C content in commercial juices stored for 6 months at 18 °C was 28.0–33.3 mg per 100 mL, which is 18–22 % lower than the initial levels.

No significant differences were found between the beverages in respect of antioxidant activity against the ABTS radical cation, which remained constant for the first 6 months of storage. The following 6 months a significant reduction of antioxidant activity by 53 and 56 % in orange and orange drinks with whey was noted, respectively. Galaverna *et al.* (*36*) and Arena *et al.* (*37*) investigated antioxidant activity against the ABTS radical cation in fresh squeezed orange juice, obtaining values of 8.65 and 5.08–5.18 μmol of TEAC per mL, respectively. The values for antioxidant activity found in orange, and orange and whey drinks in the present study were higher than those cited by Galaverna *et al.* (*36*) and Arena *et al.* (*37*). Antioxidant activity against the DPPH radical was 23 % on average higher in orange beverages than in orange and whey beverages, which was a significant difference. The level of this activity fell significantly during storage, decreasing by 53 and 81 %, respectively, in orange, and orange beverages with whey after 12 months. Del Caro *et al.* (*33*) found that antioxidant activity against the DPPH radical in the fresh squeezed juice from two varieties of oranges, Shamouti and Salustiana, was 4.69 and 4.12 μmol of TEAC per mL, respectively. After 15 days of storage at 4 °C, the level remained unchanged in Shamouti but fell by 14.6 % in Salustiana. An increase in the DPPH antioxidant activity was found in orange juices after 40 days of storage at 4 °C by Plaza *et al.* (*38*). Klimczak *et al.* (*2*) found a non-significant increase of this parameter in two brands of orange juice during the first two months of storage; however, during the following four months of storage at 18 °C, the level fell by 17 and 24 %. The increase in the antioxidant activity during the first stage of storage can be attributed to the presence of the Maillard reaction products or the indirect products of polyphenol oxidation, which exhibit a greater capacity for antioxidant activity than the original form (*39*). The decrease in the antioxidant activity during storage of orange juices can usually be explained by the degradation of vitamin C, polyphenols or carotenoids, which was corroborated by the data obtained in the present study. A significant decrease in vitamin C and polyphenolic content that occurred during storage was accompanied by a decrease in the antioxidant activity in the tested beverages. The calculated correlation coefficients confirmed this dependency. A particularly strong correlation ($p<0.01$) was found between DPPH antioxidant activity and the levels of vitamin C ($R=0.97$) and polyphenols ($R=0.95$). There was also a significant correlation ($p<0.01$) with the activity against the ABTS radical cation, although the values were considerably lower at $R=0.61$ and 0.62 respectively. The differences in the results for antioxidant activity between the two methods are probably due to the fact that the DPPH radical is less sensitive to hydrophilic compounds than the ABTS radical cation (*40*). Plaza *et al.* (*38*) also found a significant correlation ($R=0.848$, $p<0.001$) between DPPH antioxidant activity and vitamin C content in orange juices.

Vitamin B_1 and B_2 content in the beverages was evaluated after 12 months of storage. Vitamin B_1 content in both types of beverages was the same, 0.030 mg per 100 mL, which suggests that the principal source of this vitamin was orange concentrate. Biesalski and Back (*41*) reported that vitamin B_1 content in cow's milk was 37 μg per 100 g, but that losses arising from standard thermal treatment can be as much as 45 %. During the processing of cheese slurry, most of the water-soluble vitamins, such as B-complex vitamins, pass into the whey. However, the addition of whey did not have an effect on the level of vitamin B_1 in the analysed beverages, which may have been due to the considerable losses resulting from repeated thermal processing of milk and whey before the latter was added to beverages. Orange beverages with whey contained almost 3.5 times as much vitamin B_2 as orange beverages. Whey, like milk, is rich in riboflavin, which may be seen in its yellow-green colour, imparted by the vitamin. According to Biesalski and Back (*42*), whey contains 0.15 mg of vitamin B_2 per 100 g of whey. High resistance of riboflavin to thermal processing (unlike vitamin B_1) and its stability during storage without light explains its relatively high content in the analysed whey beverages, even after 12 months of storage.

Colour analysis of orange juices

Food colour is the sensory quality parameter that consumers assess first and is the most important in determining consumer acceptance (*43*). This particularly applies to foods with distinctive and attractive colours, such as orange juices and beverages, whose colour is determined by the carotenoid pigments they contain. The addition of whey to orange beverages led to a significant reduction (of 3.73 units or 7.1 % on average) in the value for the brightness parameter L^* (Table 4). The first 6 months of storage brought about a significant decrease in the L^* parameter values of the beverages, although further storage caused only a slight reduction. The decrease in L^* parameter values over the entire storage period was decidedly greater in orange and whey beverages than in orange beverages, being 2.81 (5.6 %) and 0.84 (1.6 %) units, respectively. The parameter values of a^* and b^* were positive for the examined beverages, reflecting the presence of red and yellow hues. Parameter values of a^* were significantly higher in orange drinks with whey than in orange beverages, being 4.55–7.02 and 4.56–5.64 respectively, depending on the storage time. Storage had the effect of increasing the level of the a^* parameter. Significant differences were found between a^*

parameter values directly after production and those after 6 and 12 months of storage, with no differences between the beverages after 6 and 12 months of storage. Changes were more pronounced in orange and whey beverages. Parameter values of b^* fluctuated between 55.8 and 57.7, confirming intensive yellow hues in the beverages. The differences in b^* parameter values between the beverages were statistically significant; these values were not significantly affected by storage time. A similar tendency was found for colour saturation parameter C^*. The addition of whey brought about a significant average increase of 1.84 units (3.3 %) in the value of this parameter. A 12-month storage had no significant effect on the level of this parameter. Orange beverages exhibited values for the h^* parameter 0.8 % on average higher than orange beverages containing whey; however, the level decreased by an average of 2 % during storage. Despite being relatively small, the changes in the h^* parameter in relation to the type of beverage and storage time were, however, statistically significant due to the high repeatability of the values obtained for this parameter.

Esteve *et al.* (*29*) found significant differences in the values for a^*, b^*, L^* and h^* between orange juices depending on the producer and storage time. The values recorded by the above authors for these parameters in fresh juices were 5.17–11.74, 62.33–65.42, 57.00–60.73 and 79.33–85.43, respectively. During 5-week storage at 10 °C, they found a significant increase in the values for L^* and a^* parameters, a decrease in the b^* parameter and no significant change in the h^* parameter. Cortés *et al.* (*44*) found variations in the values for L^* and a^* parameters in pasteurised orange juices during 7-week storage, with the final values for L^* and a^* parameters being respectively slightly lower and significantly higher than their initial values. The same authors noted a systematic decrease in b^* and C^* parameter values over the entire storage period in the juices they examined. The differences in colour parameter values between orange and orange beverages with whey were connected with the addition of whey, a liquid characterised by cloudiness due to the presence of colloidal protein, and yellow-green hue arising mainly from the riboflavin dissolved in it. Changes in colour parameters in orange juices occurring during storage are probably caused by the precipitation of sedi-

Table 4. Colour parameters of orange, and orange and whey beverages

Parameter	*t*(storage)	Beverage			LSD
	month	Orange	Orange and whey	Mean	α=0.01
L^*	0	53.0±0.1	50.6±0.1	51.8±1.2	
	6	52.3±0.5	47.8±0.5	50.0±2.4	0.36
	12	52.1±0.1	47.8±0.1	49.9±2.3	
	mean	52.4±0.5	48.7±1.4		I×II
LSD α=0.01			0.29		0.50
a^*	0	4.5±0.2	4.6±0.0	4.6±0.2	
	6	5.6±0.0	7.0±0.0	6.3±0.7	0.11
	12	5.6±0.0	7.0±0.0	6.3±0.7	
	mean	5.3±0.5	6.2±1.2		I×II
LSD α=0.01			0.09		0.16
b^*	0	56.4±0.0	57.2±0.1	56.8±0.4	
	6	55.8±0.1	58.0±0.5	56.9±1.2	ns
	12	55.8±0.1	57.9±0.1	56.8±1.1	
	mean	56.0±0.3	57.7±0.4		I×II
LSD α=0.01			0.19		0.32
C^*	0	56.6±0.1	57.4±0.1	57.0±0.4	
	6	56.1±0.1	58.4±0.4	57.2±1.2	ns
	12	56.1±0.1	58.5±0.4	57.3±1.3	
	mean	56.3±0.3	58.1±0.6		I×II
LSD α=0.01			0.21		0.36
h^*	0	85.3±0.0	85.5±0.0	85.4±0.1	
	6	84.3±0.0	83.2±0.0	83.7±0.6	0.02
	12	84.2±0.0	83.1±0.0	83.7±0.6	
	mean	84.6±0.5	83.9±1.1		I×II
LSD α=0.01			0.02		0.03

results are expressed as mean values±standard deviation; ns=not significant difference; I×II=interaction

ment and the interaction of constituents (*44,45*). This explains the greater degree of change occurring in orange and whey than in orange beverages during storage. Whey beverages contained considerably more protein and mineral constituents, compounds prone to interactive processes, coagulation and sedimentation.

Sensory attributes of orange juices

The results of the 5-point evaluation are given in Table 5. With the exception of colour desirability, whey beverages scored significantly lower for all the evaluated attributes. In addition, storage time led to significantly lower scores for all the sensory quality descriptors evaluated. Both types of beverages achieved a very good score for colour at the beginning of the storage period. Although the scores decreased with the length of storage, the evaluation after 12 months was still above good. Sediment and suspension were found in both types of beverage, which to a large extent was due to the unclarified orange concentrate used, and the filtering method applied during production. At the beginning of storage, this parameter was evaluated as above good. During storage, the amount of sediment and suspension increased at a considerably higher rate in beverages containing whey. This meant that after 12 months, the evaluation of this quality descriptor was reduced by 0.5 and 0.7 points, respectively, for orange and orange beverages with whey. In their analysis of fruit beverages containing whey, Djurić *et al.* (*46*) concluded that sedimentation is a characteristic feature of this type of beverage and should be taken into consideration when setting evaluation criteria.

Table 5. Sensory analysis on a 5-point scale of orange, and orange and whey beverages (scale 0–5 points)

Parameter	Importance factor	*t*(storage) month	Beverages			LSD α=0.01
			Orange	Orange and whey	Mean	
Colour	5	0	5.0±0.0	5.0±0.0	5.0±0.0	
		6	4.7±0.2	4.7±0.2	4.7±0.2	0.16
		12	4.4±0.3	4.3±0.3	4.3±0.3	
		mean	4.7±0.3	4.6±0.4		I×II
LSD α=0.01				ns		ns
Sediments and suspension appearance	2	0	4.6±0.1	4.3±0.2	4.4±0.0	
		6	4.3±0.3	4.2±0.2	4.2±0.2	0.24
		12	4.1±0.2	3.6±0.4	3.9±0.4	
		mean	4.3±0.3	4.0±0.4		I×II
LSD α=0.01				0.19		ns
Odour acceptability	2	0	5.0±0.1	4.8±0.2	4.9±0.2	
		6	5.0±0.0	4.5±0.3	4.7±0.3	0.21
		12	4.8±0.2	4.1±0.4	4.5±0.5	
		mean	4.9±0.2	4.5±0.4		I×II
LSD α=0.01				0.17		0.30
Odour intensity	3	0	5.0±0.0	5.0±0.0	5.0±0.0	
		6	5.0±0.0	4.5±0.3	4.7±0.3	0.15
		12	4.7±0.2	4.2±0.2	4.4±0.3	
		mean	4.9±0.2	4.6±0.4		I×II
LSD α=0.01				0.12		0.21
Flavour	7	0	4.9±0.2	4.7±0.4	4.8±0.3	
		6	4.9±0.2	4.5±0.2	4.7±0.3	0.23
		12	4.7±0.3	4.1±0.2	4.4±0.4	
		mean	4.8±0.2	4.5±0.4		I×II
LSD α=0.01				0.19		ns
Overall score	20	0	4.9±0,1	4.8±0.2	4.9±0.2	
		6	4.8±0.1	4.5±0.3	4.7±0.3	0.20
		12	4.6±0.3	4.1±0.2	4.4±0.4	
		mean	4.8±0.2	4.5±0.4		I×II
LSD α=0.01				0.16		ns

results are expressed as mean values±standard deviation; ns=not significant difference; I×II=interaction

The differences in the evaluation of surface appearance by sensory analysis were partly confirmed by instrumental colour analysis. During storage, the reduction in points awarded for surface appearance was greater in orange beverages with whey; similarly, these beverages showed the greatest changes in values for the L^* and a^* parameters in the instrumental colour analysis.

The examined beverages were evaluated with high scores for odour, ranging from 4.7 to 5.0 points. During storage, odour was significantly less stable in orange and whey than in orange beverages. At the begining of the storage period, scores for desirability and intensity of odour were nearly very good, but after 6 and 12 months of storage decreased to above good and good, respectively. There was a similar tendency in the evaluation of taste. Directly after production and after 6 months of storage, the taste of orange beverages was assessed as very good and that of orange beverages with whey as above good. After 12 months of storage, the scores for taste were respectively 0.2 and 0.6 points lower than the initial evaluation.

In the overall sensory evaluation, orange beverages obtained very good scores after 0 and 6 months of storage and above good after 12 months, while orange beverages with whey were rated very good, above good and good at the same three stages of storage. These results suggest that the beverages containing whey maintain the high sensory quality in the storage period not shorter than 6 months.

In their investigation of the sensory quality of fruit beverages containing whey, Djurić *et al.* (*46*) rated the following flavours in descending order: peach, pear, orange and apple. Among the orange and whey beverages, the best was that with 6 % of orange extract, 4 % sucrose and pH=3.8. On a 20-point scale, these authors rated the beverage 16.3 or 81.5 % of the maximum. The orange drinks containing whey examined in the present work achieved slightly higher scores: 96 % directly after production and 83 % after storage.

The results of descriptive flavour analysis are shown in Figs. 1 and 2. In the sensory profile of flavour, orange odour was dominant in orange beverages over the entire storage period, being rated as above good directly after production and after 6 months, and good after 12 months.

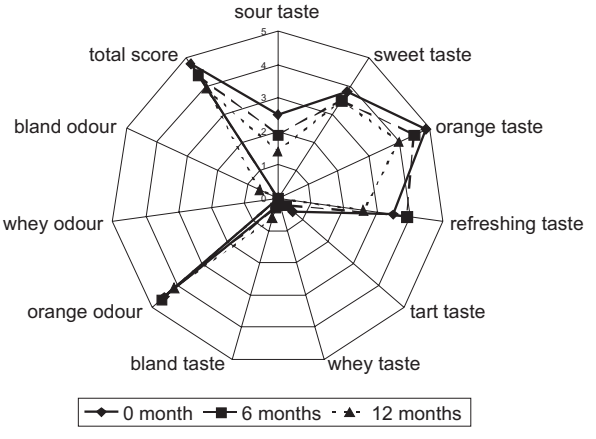

Fig. 1. Descriptive flavour analysis of orange beverages

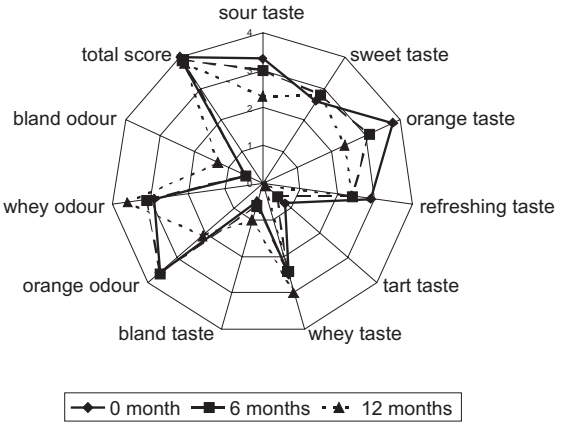

Fig. 2. Descriptive flavour analysis of orange and whey beverages

Whey odour was not detected in orange beverages, although bland odour was just perceptible after 12 months. Orange beverages containing whey scored significantly lower for orange odour and had pronounced whey and bland odours. The intensity of orange odour in these beverages after 6 months of storage remained unchanged at above fair; however, the evaluation was 2.1 points lower after 12 months. The reduction in the intensity of orange odour was accompanied by a considerable increase in whey and bland odours, with the intensity of whey odour being evaluated as fair after 0 and 6 months of storage, and above fair after 12 months, by which time it had become the dominant odour. The role of bland odour in the formation of bouquet in orange beverages containing whey was of the third order, its intensity being evaluated at 0.5–1.3 points.

The dominant tastes forming the flavour of the analysed beverages were orange, sweet, refreshing and sour. In orange beverages with whey, an additional and key role was played by whey taste. Orange beverages exhibited significantly greater intensity of orange, sweet and refreshing tastes than orange beverages with whey, but lesser intensity of sour and whey tastes. The intensity of orange taste in orange and orange beverages with whey decreased with storage by 0.9 and 1.4 points, respectively. The intensity of refreshing taste fell significantly (by 1.3 points) in non-whey beverages between the 6th and 12th month of storage. In the beverages containing whey, the level of this descriptor of quality was substantially lower, scoring 0.5 of a point. Changes in the intensity of sweet taste in both types of beverage during storage were not significant. The impression of sour taste decreased significantly in orange and orange beverages with whey over 12 months of storage by 1.1 and 1.0 points respectively. The intensity of whey taste in whey beverages rose by a non-significant 0.5 of a point after 12 months of storage. The significance of tart and bland tastes in forming the overall flavour of the examined beverages can be regarded as marginal. The values for these descriptors did not exceed 1 point and did not depend on the type of beverage or the storage time.

The overall evaluation summarising the flavour profile analysis showed that orange beverages obtained significantly higher scores than the whey-containing bever-

ages. There was a significant deterioration in the flavour of orange beverages during storage from very good to good, whereas the deterioration in orange beverages containing whey was not significant; the evaluation of flavour remained at good throughout the storage period.

Conclusion

Orange beverages with whey contained higher levels of protein, ash, vitamin B_2 and lower level of sucrose than orange beverages. Adding whey to beverages had no significant effect on polyphenolic content or antioxidant activity against ABTS radical cation; however, orange beverages contained significantly higher levels of vitamin C and greater antioxidant activity against the DPPH radical than the orange beverages containing whey. During storage polyphenolic and vitamin C contents as well as antioxidant activity significantly decreased. The addition of whey significantly influenced colour when measured instrumentally, although the sensory evaluation of desirable colour did not discern any differences between the beverages. The presence of whey had a negative effect on taste and odour, becoming more noticeable beyond 6 months of storage. It is worth pointing out that for overall sensory quality, orange beverages containing whey obtained scores of very good to good, which opens the possibility of these beverages being acceptable to consumers. In view of their functional properties, arising from the bioactive constituents in fruit and whey, orange drinks containing whey could be an interesting product in the constantly growing market for functional food.

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