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Influence of sweetener stevia on the quality of strawberry flavoured fresh yoghurt

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Summary

Stevia is a natural, non energetic sweetener, 200-300 times sweeter than sucrose, and is obtained by leaves extraction of the *Stevia rebaudiana*. According to the low energy value of stevia to sweeten food products, it is a great possibility of its use in the dairy industry. This study examined the differences in the sweetness of the strawberry flavoured yoghurt with the addition of sucrose, stevia and equal portions of sucrose and stevia; each combination was used at three different concentrations (3, 4.5 and 6 % / 100 g yoghurt). Stevia was diluted in a concentration which, according to the literature, matches the sweetness profile of sucrose. Viscosity of the yoghurts was determined by the rheometer, and sensory profiling of the products was evaluated by a panel using the ranking test and weighted factors methods. The level of sweetness of all yoghurt samples (using sucrose, stevia and mixture of sucrose and stevia) was judged by a test panel, and products were rated in the terms of degree of sweetness as sucrose > sucrose + stevia > stevia. The recommended level by panelists of any type or combination of sweeteners for strawberry yoghurt was 4.5 g sweetner/100 g. The apparent viscosity was lower in sucrose yoghurts compared to products made with stevia or stevia + sucrose which was also reflected in the sensory scores.

Key words: strawberry flavoured yoghurt, sensory analysis, stevia, sucrose, viscosity

Introduction

Stevia rebaudiana Bertoni is a shrub-like plant of the Asteraceae family, originating from Paraguay and Brazil, where it is used as a sweetener for therapeutic preparations, such as teas and other beverages, for hundreds of years. It is often called "sweet Paraguay honey" (Genus, 2003). The sweetener compound is obtained from the leaves of the plant by extraction (Prakash et al., 2008). The sweet taste of stevia is derived from glycosides that are located in the leaves. The most represented glycosides are steviosides, rebaudiosides A and F, steviolbiosides and dulcosides as shown in Figure 1 (Gardan et al., 2003). The most represented are steviosides and rebaudiosides and, depending on variety and growing conditions, their amount varies between 4-20 %. The sweetness of steviosides and rebaudiosides is 200-300 times higher than sucrose. Stevia does not cause undesirable tolerance to the taste.

Stevia is stable at the high temperatures and in aqueous solutions. It has no energy value and is suitable for use in the diabetic's diet, people with phenylketonuria and obesity (Figlewicz et al., 2009). Stevia is also used to sweeten soft drinks, soy sauce, yoghurt and many other foods. In Korea and Japan, it estimated that the use of stevia is around 115 tonnes per year. Although the use of stevia in Europe (except France) is not permitted, it is considered that stevia has a beneficial effect on human health (Gardan et al., 2003). Some studies have shown that stevia decreases the blood pressure, does not cause allergic reactions and acts as an antioxidant. In addition, stevia

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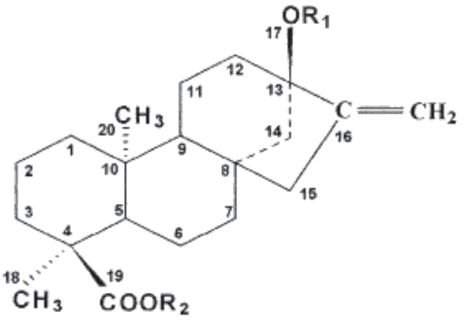

Glycosides	R1	R2
Stevioside	-glc^2-glc	-glc
Steviolbioside	-glc^2-glc	-H
Rebaudioside A	-glc^3-glc^2-glc	-glc
Rebaudioside B	-glc^3-glc^2-glc	-H
Rebaudioside C	-glc^3-glc^2-rha	-glc
Dulcoside A	-glc^2-rha	-glc

Figure 1. Chemical structures of the main *S. rebaudiana* sweeteners and their aglycones (Gardana et al., 2003)

improves the kidney function by increasing water excretion (Atteh et al., 2007), and as an immune stimulant by stimulating the cellular immunity (Sehar et al., 2008). Other studies have shown that: (a) stevia reduces blood sugar levels and does not affect the metabolism of insulin (Kinghorn, 2002) and, therefore, is recommended to diabetics, and (b) in human food, stevia does not damage the genetic material, or the deoxy ribonucleic acid (DNA) of the cells (Brusick et al., 2008).

Modern lifestyle, unhealthy diet and stress are the biggest factors that often lead to some digestive problems and even diseases, especially cancer of the digestive systems. Consumption of the products containing probiotic bacteria can be renewed or re-establish a complete intestinal microflora, which is often damaged or disrupted due to stress or due to infectious diseases, chemotherapy, etc (Božanić et al., 2002).

The aim of this work was to investigate the differences in sweetness profile and viscosity of the strawberry flavoured yoghurt (fresh) with the addition of sucrose, stevia and equal portions of sucrose and stevia.

Materials and methods

Milk

Pasteurized and homogenized milk with 3.2 % of fat ("Dukat", Dairy Industry Inc. Croatia) was used, and experiment was replicated three times.

Microorganisms

The DVS (Direct Vat Set) culture ABT 5 was used for direct inoculation of milk (Christian Hansen A/S, Denmark). The culture consists of bacterial strains: *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium animalis* subsp. *lactis*.

Sweeteners

Sucrose ("Viro", Croatia) and stevia powder (Naturex, France) were used during the manufacture of different yoghurts.

Stevia powder consists of 90 % of steviosides and prior to its addition to the milk base was diluted with water to 1 % concentration.

Aroma

For flavouring of produced yoghurt, aroma identical to natural strawberry aroma was used, which contained carmine red A (E124) and tartrazine (E102) (Döhler, Germany).

Sample preparation

The inoculum was prepared by dissolving 1 g of microbial culture in 100 mL of milk (40 °C) and activated for 30 minutes at 40 °C. Milk samples were inoculated at rate 2.5 % (v / v) followed by fermentation until the pH reached 4.6. After fermentation, the samples were cooled, sweetened, and stored in a refrigerator (+4 °C) for 24 hours until analysis. The strawberry aroma (0.2 mL/100 g) and different amounts of sweeteners (see Table 1) were added to cold fermented samples. Average values were calculated.

Table 1. Sweetener amounts added in yoghurt samples

Sweetener amount (%)	A Samples	B Samples	C Samples
3	A1: 3 g of sucrose	B1: 1 mL 1 % dilution of stevia	C1: 1.5 g of sucrose + 0.5 mL of stevia
4.5	A2: 4.5 g of sucrose	B2: 1.5 mL 1 % dilution of stevia	C2: 2.25 g of sucrose + 0.75 mL of stevia
6	A3: 6 g of sucrose	B3: 2 mL 1 % dilution of stevia	C3: 3 g of sucrose + 1 mL of stevia

Table 2. Sweetness scale (intensity scale)

Not sweet	Light sweet	Medium sweet	Very sweet	Extreme sweet
0	1	2	3	4

Sensory evaluation

The sensory properties of yoghurts were evaluated by a trained panelists consisting of 5 members using two different methods. Firstly the ranking method compared the sweetness between samples containing the same proportion of sweeteners, regardless of type (3, 4.5 and 6%). The samples were presented to the sensory panelists in sequences (from lower sweetener amount to the highest), and they ranked each sample according to an appropriate scale (Table 2). Secondly the 20-point scaling system with weighted factors evaluated the taste, odour, consistency and colour produced in the strawberry flavoured yoghurts. The weighted factors (Fv) were for: taste 1.4, odor 1; consistency and color 0.6. The maximum number of weighted factors was 20, and grades for each property were in the range of 1 to 5.

Determination of viscosity

All measurements of rheological properties were carried out using a rotating rheometer, (Rheometric Scientific RM 180 Inc. Piscataway, USA). The rheometer consisted of cylindrical spindle and the outer sheath in which was placed 32 mL of sample. The apparent viscosity, flow index and the regression coefficient for each sample, by linear regression were measured.

Results and discussion

The fermentation time of milk base using starter culture ABT 5 to reach pH ~ 4.6 was 5.5 hours, and the pH values of strawberry flavoured yoghurts were measured after the first and second day of storage at 4 °C. The pH values of the products dropped by 0.12 units (pH ~ 4.4 after the first day and pH ~ 4.28 after the second day), which was due to the metabolic activity of the starter culture ABT 5 known as post-acidification. However, all the pH values of all the yoghurts were approximately similar, which could be assumed that the addition of sweeteners (sucrose, stevia, or combinations of sucrose and stevia) had no effect on the metabolic activity on the starter organisms (Figure 2).

After 24 hours of storage and gel stabilization, the yoghurt samples were sensory profiled. The ranking test was presented to a panel in a series of samples and they were graded according to sweetness scale (Table 2). The first samples presented contained the least amount of sweeteners (3 % / 100 g), followed by the medium (4.5 % / 100 g) and highest amount of sweeteners (6 % / 100 g). As expected, the yoghurts with the same amount of sweeteners, regardless of type, were equally graded. However, the sweetness ranking of the samples was as follows: yoghurt made with sucrose > sucrose + stevia > stevia (least) (Table 3). Increase of the amount of added sweeteners to yoghurt tended to increase the sweetness intensity of all samples regardless of the

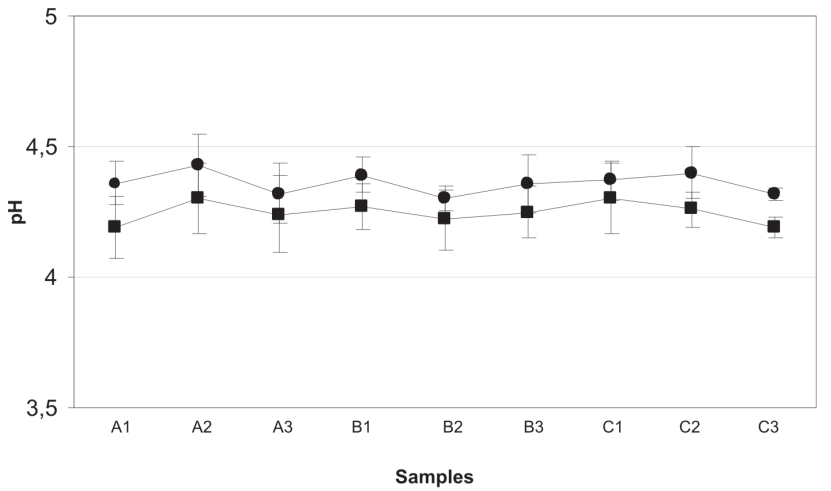

Figure 2. Average pH values of strawberry flavoured yoghurts with its standard deviations after storage for one (●) and two (■) days

Table 3. Ranking effect of sweetness of strawberry flavoured yoghurts by the panelists according to sweetness scale (Table 2)

Sweetener amount (%)	A Samples - sucrose ($\bar{x} \pm \delta$)	B Samples - stevia ($\bar{x} \pm \delta$)	C Samples - mixture ($\bar{x} \pm \delta$)
3	A1: 1.27±0.28	B1: 0.47 ± 0.51	C1: 1.13±0.38
4.5	A2: 2.33±0.34	B2: 0.87±0.38	C2: 1.97±0.28
6	A3: 2.87±0.51	B3: 1.20±0.65	C3: 2.47±0.38

sweetener type used (sucrose, stevia or a combination of sucrose and stevia) (Table 3).

Afterwards, the sensory profiling was performed (using the 20-point scoring system with weighted factors) (Table 4). Scores for the odour and colour attributes of all the yoghurt samples were similar because the amount of added flavour was the same in all the products, and statistically there were no significant differences ($p>0.05$). The viscosity of yoghurts made with only sucrose were the least viscous (~ 3.75 score) compared to the other samples, regardless of the amount added. Scores for taste attribute ranged between 2.7 to 5.7 with a maximum 7 points (Table 4), and the yoghurts most liked contained 4.5 % / 100 g of sucrose, while the least liked samples contained 3 % / 100 g of stevia. The low scores awarded to stevia yoghurts was attributed to the after-mouth taste of the sweetening compound, which is reminiscent of artificial sweeteners; however, samples sweetened with a mixture of stevia and sucrose had no after-mouth taste effect. The highest score (16.08, optimal) was awarded to yoghurt containing equal ratio of stevia and sucrose in the product (4.5 % / 100 g), and the least score was for stevia yoghurt (3 % / 100 g) (Table 4). Yogurts containing 6 % of sweetening compounds (stevia + sucrose) had a better taste score (5.50) compared to the products sweetened with sucrose only. In addition, the consistency scores of sucrose yoghurts were lower compared to the other products because they were less viscous. Also, the consistency scores of yoghurts (stevia + sucrose) were slightly lower than stevia yoghurt.

The apparent viscosity of all sucrose yoghurts was lower than in the other samples (Table 5). Dissolution of sucrose had diluted the samples and, as a consequence, the viscosity was decreased, which was also reflected in the sensory scores (Table 4). For no apparent reason, the viscosity measurement of stevia + sucrose yoghurt were not low, a phenomenon which was difficult to explain. The average flow index measurements for all samples were

Table 4. Weighed scores for certain properties of strawberry flavoured yoghurts

Sweetener amount (%)	Sample	Taste (max 7)	Odour (max 5)	Consistency (max 5)	Colour (max 3)	Total (max 20)
3	A1	4.20	3.93	3.80	2.72	14.65
	B1	2.70	3.47	4.20	2.60	12.97
	C1	3.92	3.87	4.20	2.60	14.59
4.5	A2	5.70	3.74	3.73	2.70	15.87
	B2	3.82	3.53	4.33	2.72	14.40
	C2	5.22	3.93	4.20	2.73	16.08
6	A3	5.14	3.93	3.73	2.68	15.48
	B3	3.18	3.73	4.13	2.56	13.60
	C3	5.50	3.80	3.93	2.67	15.87

Table 5. Average values with its standard deviations of flavoured yoghurts rheological properties

Sweetener amount (%)	Sample	Apparent viscosity, m_p (mPas) ($x \pm \delta$)	Flow index, n ($\bar{x} \pm \delta$)	Regression coefficient, R^2 ($\bar{x} \pm \delta$)	Consistency coefficient, k (Pas^n) ($\bar{x} \pm \delta$)
3	A1	20,00±1,73	0,279±0,07	0,979±0,02	6,32±3,52
	B1	20,67±1,53	0,242±0,06	0,972±0,03	7,46±2,04
	C1	21,00±3,61	0,264±0,08	0,963±0,03	8,36±1,33
4.5	A2	20,33±2,08	0,292±0,06	0,977±0,02	7,36±2,36
	B2	20,67±2,52	0,258±0,06	0,961±0,02	6,74±3,02
	C2	21,33±2,08	0,261±0,05	0,966±0,01	6,78±2,45
6	A3	21,33±3,05	0,272±0,09	0,969±0,02	7,09±2,97
	B3	22,00±2,65	0,270±0,08	0,953±0,02	8,29±1,07
	C3	22,33±2,08	0,274±0,06	0,969±0,01	6,42±2,69

around 0.27, which indicated that all of the samples were non-Newtonian pseudo plastic fluids. Regression coefficients for all yoghurts were very high (over 0.96), which implied that accuracy of the calculated rheological properties of yoghurt by means of linear regression was good.

Conclusions

Additions of equal concentration of sweeteners (sucrose, stevia, and sucrose + stevia) to yoghurt were evaluated by a sensory panelist to assess the level of sweetness in the products. The sweetest yoghurt was made with sucrose, followed by stevia + sucrose yoghurt and the least sweet was ste-

via yoghurt, and the recommended level was 4.5 g sweetener/100 g regardless of the type. The regression profile for highest sensory score was awarded to yoghurt made with the mixture of stevia and sucrose, followed by yoghurt with sucrose; while stevia sweetened yogurts had the lowest sensory scores. The apparent viscosity of sucrose yoghurts was lower compared to other products, which also reflected in the sensory score. Addition of stevia in yoghurt did not cause a decrease in viscosity measurement opposite to sucrose yoghurt.

Utjecaj sladila stevie na kvalitetu aromatiziranog jogurta od jagode

Sažetak

Stevia je prirodno sladilo, bez kalorijske vrijednosti, 200-300 puta slađa od saharoze, a dobiva se ekstrakcijom lišća biljke *Stevia rebaudiana*. S obzirom na manju kalorijsku vrijednost proizvoda zaslađenih steviom velika je mogućnost njenog korištenja u mliječnoj industriji. U ovom je radu ispitivana razlika u slatkoći aromatiziranog jogurta od jagode sa dodatkom saharoze, stevie te jednakih udjela saharoze i stevie. Jogurti su zaslađeni u tri različite koncentracije (3; 4,5 i 6 %/100 g jogurta). Stevia je prethodno razrijeđena u omjeru koji prema literaturi odgovara profilu slatkoće saharoze. Viskoznost proizvedenih uzoraka jogurta određivana je reometrom, a senzorska svojstva jogurta ocijenio je panel ranking testom i metodom ponderiranih bodova. Dodatak jednake koncentracije saharoze, stevie te kombinacije saharoze i stevie ocjenjeno je različitim stupnjem slatkoće (saharoza>saharoza + stevia > stevia). Senzorski su najbolje ocjenjeni jogurti zaslađeni sa 4,5 % sladila/100 g. Jogurtima kojima je dodana samo saharoza prividna viskoznost je bila manja u odnosu na uzorke sa dodatkom stevie ili kombinacije stevie i saharoze, što se odrazilo i na senzorsku ocjenu.

Ključne riječi: aromatizirani jogurt od jagode, senzorska procjena, stevia, saharoza, viskoznost

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