POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 32(2): 323–334, Y. 2017

EFFECT OF STEVIA ADDITION ON SELECTED PROPERTIES OF YOGHURT DURING REFRIGERATED STORAGE

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Key words: stevia, steviol glycoside, yoghurt, texture.

Abstract

The aim of this study was to investigate the effect of replacing sucrose 25%, 50%, 75% and 100% of the addition of stevia on yoghurt properties, during refrigerated storage. Reference sample was yoghurt sweetened with 8% added sucrose, the addition of stevia was 0,04%. The yoghurt was analysed for pH, titratable acidity, dry matter, and texture. It was also sensory assessed. With the increase in the concentration of stevia sweetener, the mixture pH decreased, and the increased acidity of the yoghurt. Despite the lower dry matter content of yoghurt with stevia exhibited higher curd hardness values, but the cohesiveness was slightly lower. Springiness and chewiness in yoghurt sweetened with stevia decreased remarkably after 7 days of storage. Yoghurt with stevia were less intense sweet flavor and considered to be more pronounced sweetness and persistent. The best was the yoghurt sweetened with sucrose, and the worst yoghurt with stevia. Mixtures of these sweeteners showed a higher degree of acceptance.

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WPŁYW ZASTOSOWANIA STEWII NA WYBRANE CECHY JOGURTU PRZECHOWYWANEGO CHŁODNICZO

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Słowa kluczowe: stewia, glikozydy stewiolowe, jogurt, tekstura.

Abstrakt

Celem pracy było zbadanie wpływu zastąpienia sacharozy 25%, 50%, 75% i 100% dodatkiem stewii na właściwości jogurtów w czasie przechowywania chłodniczego. Próbę odniesienia stanowił jogurt słodzony 8% dodatkiem sacharozy, dodatek stewii wynosił 0,04%. W jogurtach oceniono pH, kwasowość, suchą masę, parametry tekstury oraz poddano je ocenie sensorycznej. Wraz ze wzrostem stężenia stewii w mieszance słodzącej malało pH, a wzrastała kwasowość jogurtów. Pomimo niższej zawartości suchej masy jogurty ze stewią wykazywały wyższe wartości twardości skrzepu, jednak kohezyjność była nieznacznie niższa. Sprężystość i żujność w jogurtach słodzonych stewią spadła znacznie po 7. dniu przechowywania. Jogurty ze stewią wykazywały mniej intensywną słodycz, jednak bardziej wyraźną i uporczywą. Najlepszy okazał się jogurt słodzony sacharozą, a najgorszy jogurt ze stewią. Mieszanki tych środków słodzących wykazywały lepszy stopień akceptacji.

Introduction

For centuries, people have used various means of suitable sweet taste. A honey has been used for this purpose for a long time. Which over time has been replaced with sucrose produced industrially, and in recent years the glucose-fructose syrup (CICHOSZ et al. 2011, SZAJER 2011). However, it began to appear that eating sugar causes atherosclerosis and influences the increasing rates of type 2 diabetes. In addition, excessive intake of sugar and lack of physical activity triggers medical conditions such as obesity and tooth decay (CICHOSZ et al. 2011). A clear trend of replacing sugar with low-calorie sweeteners is being observed. These are synthetic ingredients or received by technological modification of saccharides characterised by high sweetened activity and low energy. The so-called sweeteners are increasingly at the no-or low-calorie alternative to natural sugars. Products based on them are a response to market demand for sweet, but sugar-free products (SIKORA 2012). Although sweeteners before being released for use in foods were subjected to a thorough evaluation, it still arouses a lot of controversy among scientists and consumers, in relation to information about their possible carcinogenic effects (WASZKIEWICZ-ROBAK et al. 2007).

In the face of the growing demand for low-energy products, food manufacturers are looking for natural substances that could reflect the sweet taste of sugar without causing adverse effects on health. A plant – *Stevia rebaudiana* Bertoni, commonly called stevia, can be a new solution. This plant belongs to the family *Asteraceae* used for centuries by South Americans as a sweetener, as well as the drug substance. Stevia owes its sweet taste to steviol glycosides contained in its leaves. Over eight glycosides had been identified, two of which (stevioside and rebaudioside A) are the most important. Stevioside is present in the leaves in the largest amounts (4–13% DM). It is 150–300 times sweeter than sucrose. However, there is more bitter and licorice flavor than rebaudioside A. It is the less well soluble in water as well. Rebaudioside A is present in smaller amounts than stevioside (2–4% DM). It is the sweetest of steviol glycosides (200–400 times sweeter than sucrose) and has the best taste. It dissolves in water better than stevioside and it is resistant to various pH environments (CARAKOSTAS et al. 2008, LEMUS-MONDACA et al. 2012).

In December 2008, the U.S. Food and Drug Administration (FDA) stated that steviol glycosides can be considered GRAS (Generally Recognized As Safe). The introduction of stevia to the European market took much longer because, according to Regulation (EC) No 258/97 it required an opinion on the use of this plant as a new food additive. The European Food Safety Authority (EFSA) in the process of stevia toxicological assessment stated, that the glycosides found in the stevia are not genotoxic or carcinogenic, and that the plant is safe and can be used as an additive in food and beverage (KUŹMA at al. 2012). The acceptable daily intake (ADI) was set at 4 mg kg^{-1} body weight/day (JUSZCZAK 2012). In Europe, steviol glycosides have been approved for use by Regulation No 1131/2011 of 11 November 2011, the new food additive has been marked with number E 960. Stevia is currently used in many countries, including Japan, Brazil, Australia, Switzerland, the United States, Mexico and the European Union. It can be used in the production of reduced-calorie meals, in addition to drinks, sweet dishes and desserts. The high sweetening power steviol glycosides in comparison to sucrose makes it to achieve the desired sweetness with using small amount of this additive.

Among the arguments in favor of allowing the use of steviol glycosides most important are their natural origin, non-toxic, high solubility, stable in aqueous solution over a wide range of pH values and temperatures, non-fermentative, safety for diabetics and people with phenylketonuria (JUSZCZAK 2012). Having the potential of the sweetener it can be expected that the market will demand more and more products with stevia as ingredient. Also in the dairy industry it had been allowed to be used in flavored fermented milk, which can improve the image of health-promoting these products.

The aim of this study was to investigate the effect of substitution of sucrose with different ratios of steviol glycosides, on the physicochemical, textural and sensory properties of set style yoghurt, during 21 days of refrigerated storage.

Material and Methods

The study was based on white sugar (sucrose from sugar beets) and Truvia®, stevia produced by Cargill (min. 95% Rebaudioside A). Yoghurt produced by thermostatic method from pasteurized milk containing 2% fat, concentrated skim milk powder, up to about 11.5% non-fat dry matter. The prepared milk was divided into five parts and added to each the sweeteners, according to Table 1. Reference sample was the yoghurt sweetened with sucrose. In subsequent trials 25%, 50%, 75% and 100% sucrose was replaced stevia, wherein the total addition of sucrose was 8%, and stevia due to the high sweetness of 0.04%. After addition of sweeteners, milk was repasteurised in 85°C for 10 minutes and then cooled down to 45°C. Yoghurt cultures FD-DVS YC-X16-YoFlex® Chr. Hansen (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) were incubated in 2% fat UHT milk at 43°C, 4,5h. Each sample was inoculated with 5% of starter culture, dispensed into containers with a capacity of 100 ml and incubated at 43°C to about pH 4.7, and then cooled to 5°C. Assessment of the effect of the addition of sweeteners to the parameters of the yoghurt conducted immediately after the fermentation, designated as the "0 days storage", and at 3, 7, 14 and 21 days of refrigerated storage. The yoghurts were determined titratable acidity in °SH, pH and conductivity measured by microcomputer pH/conductivity meter Elmetron CPC-411, equipped with a combination electrode type OSH 12-00 and 60 EC conductivity sensor. The dry matter was determined by drying yoghurt at 130°C. Analysis was performed in duplicate, the mixed samples, at a temperature of 20°C +/- 1°C. The texture was determined with the analyser Brookfield CT3 equipped with Brookfield Texture Pro CT software. For determination there was selected the TPA test with the following settings: sample – cylinder 66.00 mm x 33.86 mm, force 0.1 N, head speed 1 mm/s, table TA-BT-KIT, probe TA3/100. The test was performed on intact curd yoghurt, immediately after taking it from the cold, in triplicate. Texture qualities like hardness [N], chewiness [mJ], springiness [mm] and cohesiveness were assessed. The results are presented as arithmetic mean and standard deviation.

Sensory analysis was performed by a team of 21 people trained in the evaluation profile yoghurt, at 3, 7, 14 and 21 days of storage. Evaluators

Table 1

Addition of the sweeteners during yoghurt manufacture

Sweetener content [%]	Addition of sweetener [%]
Sucrose 100%	Sucrose 8%
Sucrose 75% + stevia 25%	Sucrose 6% + stevia 0.01%
Sucrose 50% + stevia 50%	Sucrose 4% + stevia 0.02%
Sucrose 25% + stevia 75%	Sucrose 2% + stevia 0.03%
Stevia 100%	Stevia 0.04%

ranked coded samples of yoghurt from the best to the worst (JĘDRYKA 2001). The sensory profile of yoghurt during storage was compared. The study consisted of nine descriptors selected in accordance to *Sensory analysis*... PN-ISO11035:1999, consistency, color, aroma (sweet, sour, stranger), taste (sweet, sour, cream, off-flavor). Evaluators made their assessment on nine-point scale, with markings edge: 1 point "very characteristic" (for color and consistency) or "undetectable" (in the case of evaluation of taste and aroma) and 9 points: accordingly "very characteristic" or "very intense".

Results and Discussion

The basis for the production of yoghurt fermentation process is taking place with the participation of lactic acid bacteria, where the metabolic activity largely contributes to the development of organoleptic and rheological characteristics. Cooling the yoghurt after production limits such activity, but does not inhibit it entirely, because during storage of yoghurt refrigerant still occur changes in its composition and structure. Yoghurts with added stevia were characterised by slightly lower pH values (Figure 1) and higher titratable acidity (Figure 2) compared to yoghurt sweetened with sucrose. With increasing concentration of stevia the pH values of yoghurts decreased and titratable acidity increased more quickly during storage. Yoghurt sweetened with stevia also showed higher electrical conductivity. Therefore, the last day of storage, yoghurt with stevia had the lowest pH (4.2) and the highest titratable acidity (54.2°SH). GUGGISBERG et al. (2011) were examining yoghurt with sucrose and stevia in the 7th day of storage, and tests showed a slightly lower pH and higher content of organic acids in yoghurt sweetened with sucrose, however, the content of aromatic compounds formed during fermentation was at a similar level and in the case of diacetyl even higher in stevia sweetened yogurt. PRAKASH et al. (2008) reported that rebaudioside A is stable during the manufacturing process and during storage of yoghurt containing live micro-

flora. So it is not fermented by lactic acid bacteria. Similarly, in the human digestive tract, bacteria from the lower gastrointestinal tract convert it to steviol, which is then excreted (CARAKOSTAS et al. 2008). Studies have reported on the impact of anti-bacterial extracts of stevia in relation to pathogens of the digestive system, as well as its potential antioxidant properties (LEMUS-MONDACA et al. 2012). In light of these reports, it can be hypothesized that steviol glycosides stimulate the yoghurt bacteria, but it has to be examined further.

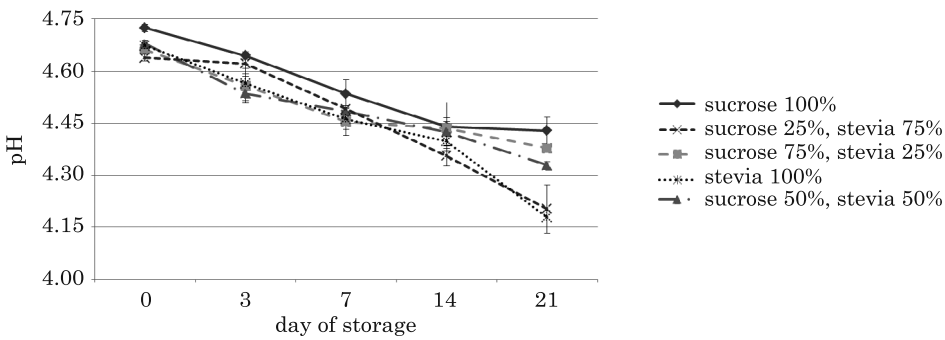

Fig. 1. pH of yoghurts with sweeteners during storage ($n = 2$, $\bar{x} \pm$ SD)

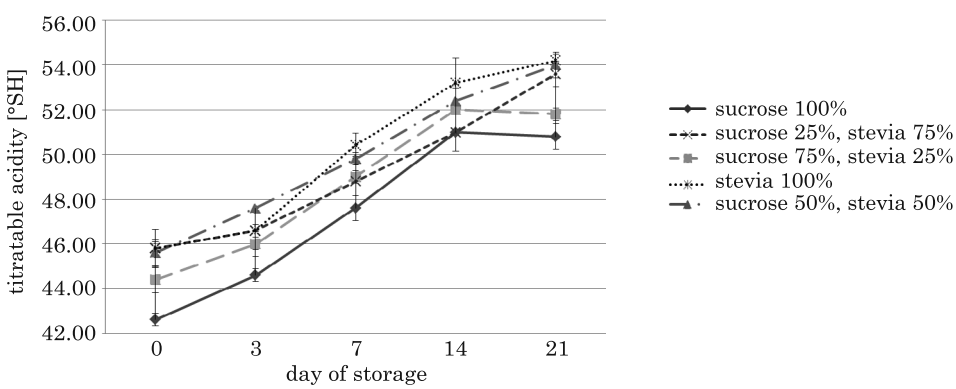

Fig. 2. The titratable acidity (°SH) of yoghurts with sweeteners during storage ($n = 2$, $\bar{x} \pm$ SD)

For a consumer products, texture is an important parameter of the quality, it, makes an impact on the pleasure of eating. After fermentation of yoghurt texture was not formed yet, because the products were not fully mature and chilled. They reached their final form after 72 hours. The research of ŻBIKOWSKI (2012) showed that changes in texture of yoghurt occur most

intensively in the first week of storage. This causes an increase in viscosity or thickening of the curd. These changes may result from post acidification of yoghurt occurring during storage and microbial activity leading to the strengthening of the protein matrix by increasing of lactic acid and bacterial exopolysaccharides production. In this study, yoghurt sweetened with stevia, despite a significantly lower dry matter content (Figure 3) showed higher curd hardness values (3.23 N – 14 days) than yoghurt sweetened with sucrose (2.97 N – 14 days) during the entire storage period (Figure 4). However chewiness (Figure 5) and springiness (Figure 6) in yoghurt sweetened with stevia alone decreased significantly after 7 days of storage. Another texture parameter of product containing stevia, that showed lower values than the reference sample, was cohesiveness (Figure 7). GUGGISBERG et al. (2011) believe that the addition of stevia is too small to affect the texture. In the studies showed that yoghurts with sucrose at 14th day of storage had higher hardness (2.79 N) than yoghurts with stevia (2.41 N) but only in the case of higher fat content (3.5%), while non-fat yoghurt hardness was at a similar level.

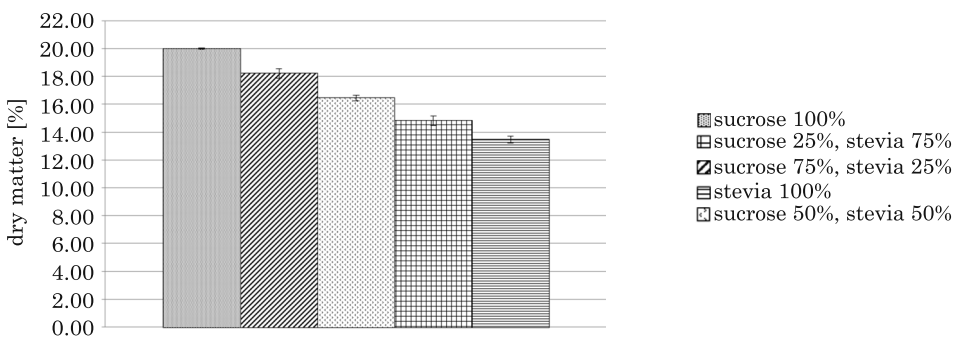

Fig. 3. Avarage dry matter [%] of yoghurts with sweeteners (n = 10, $\bar{x}$ ± SD)

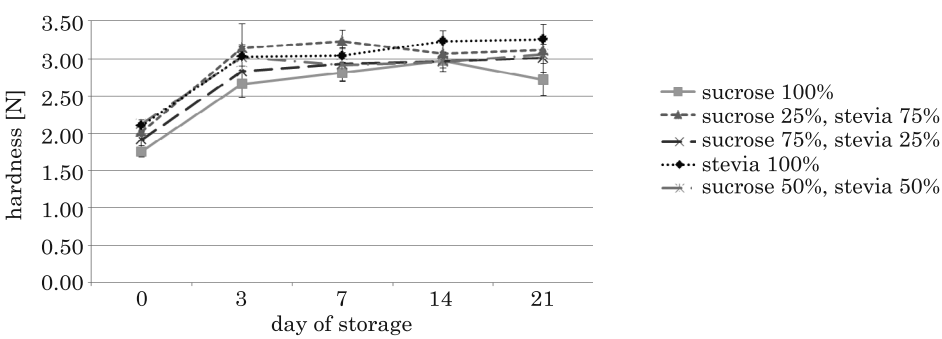

Fig. 4. Hardness [N] – the force necessary to compress the sample in the first compression cycle, in yoghurts with sweetening agents

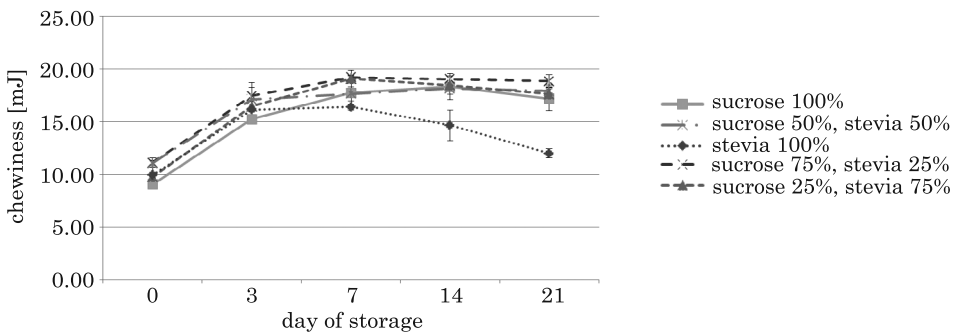

Fig. 5. Chewiness [mJ] energy required to chew yoghurts with sweetening agents during storage ($n = 3$, $\bar{x} \pm$ SD)

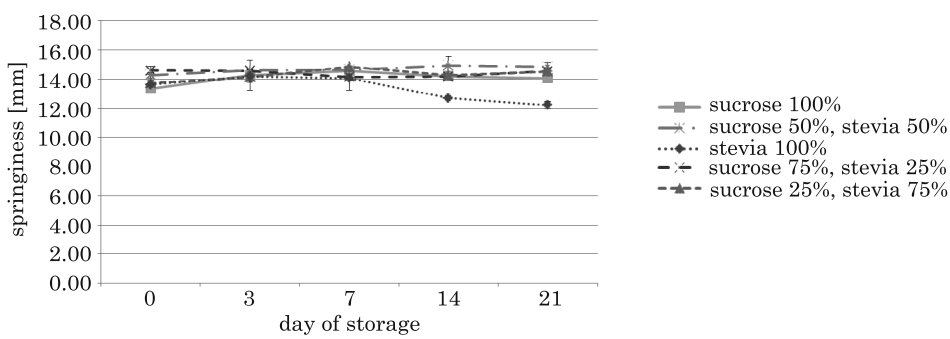

Fig. 6. Springiness [mm] expressed as a difference between the height of sample before and after compression cycle in yoghurts with sweetening agents during storage, ($n = 3$, $\bar{x} \pm$ SD)

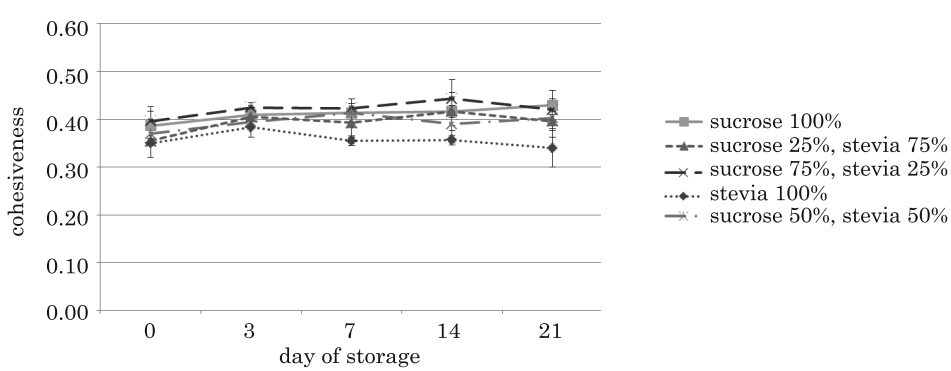

Fig. 7. Cohesiveness – The strength of internal bonds making up the body of the yoghurts with sweetening agents during storage ($n = 3$, $\bar{x} \pm$ SD)

Addition of stevia did not cause changes in the color and noticeable changes in the consistency of yogurt, which has been assessed in all trials as a very characteristic. Stevia did not affect the smell of the products, which were characterized by natural, sweet and sour flavor. Truvia® stevia Cargill is a white or off-white powder, very finely ground, odorless, with an intensely sweet taste. During storage of yoghurt, both sweet and acidic aroma were enhanced (Figures 8–10). The evaluators concluded that stevia sweetened products were characterized by a sweet aroma weaker when compared with the reference (Figure 11). Decreasing pH and progressive acidity of yoghurt (called post acidification), observed during storage can lead to modified physical and chemical interactions, which can modify fragrances as a result and also the reduction of release of the aroma and flavor ingredients in yoghurt. Changes in odor intensity of refrigerated yoghurts are also explained by hypothesis of transformation of some of aroma components by bacterial enzyme system (ŻBIKOWSKI 2012). The sweet taste of yogurt was the strongest during the entire period of storage and a sour taste in all samples was average and not very noticeable (Figure 11). The evaluators found that yoghurt sweetened with stevia do not differ in the intensity of sweetness from the other, but the sweet taste of stevia was felt to be more persistent, and considered as less natural. According to ŻBIKOWSKI (2012) sensory profile of stored yoghurt does not depend directly on changes in pH and noticeable acidity, but the balance

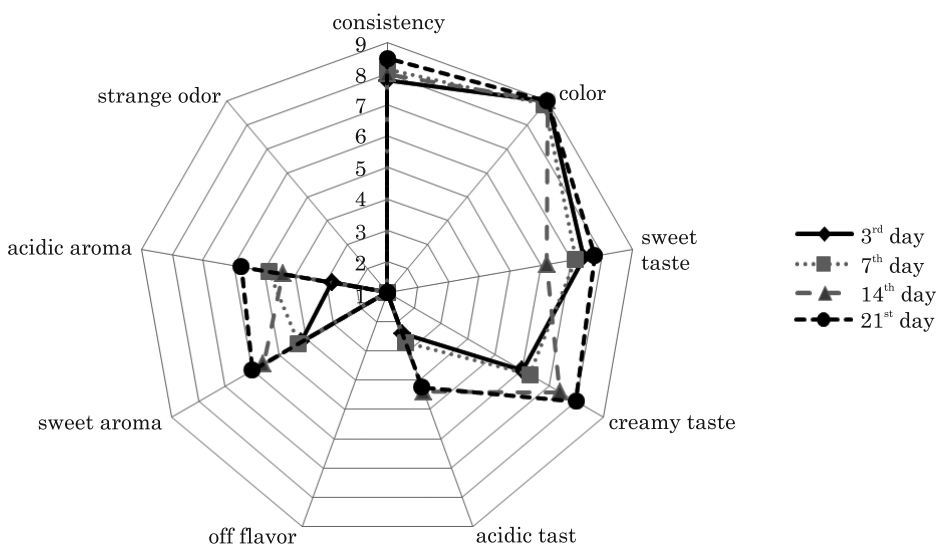

Fig. 8. Sensory evaluation of yoghurt with sucrose during storage in nine-point scale, with markings edge: 1 point "very characteristic" (for color and consistency) or "undetectable" (for taste and aroma) and 9 points: respectively "very characteristic" or "very intense"

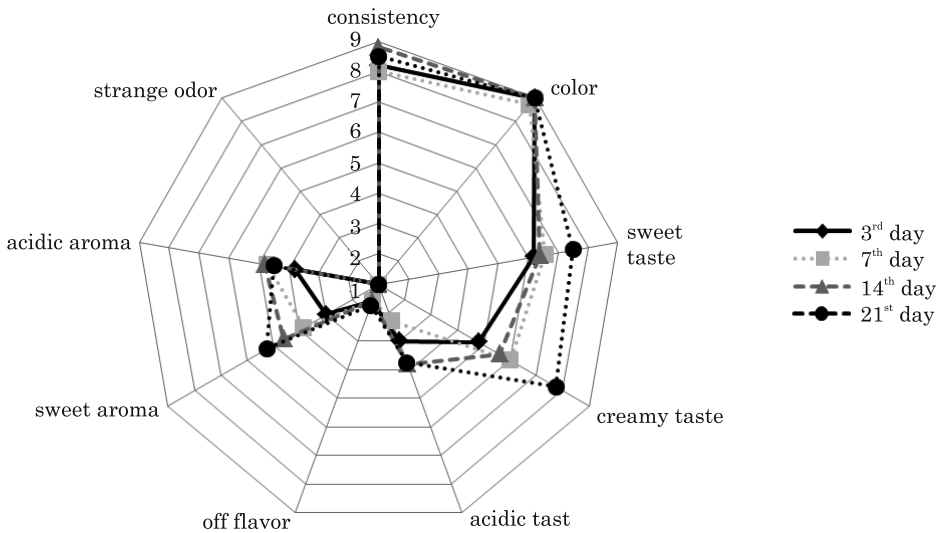

Fig. 9. Sensory evaluation of yoghurt with stevia during storage in nine-point scale, with markings edge: 1 point "very characteristic" (for color and consistency) or "undetectable" (for taste and aroma) and 9 points: respectively "very characteristic" or "very intense"

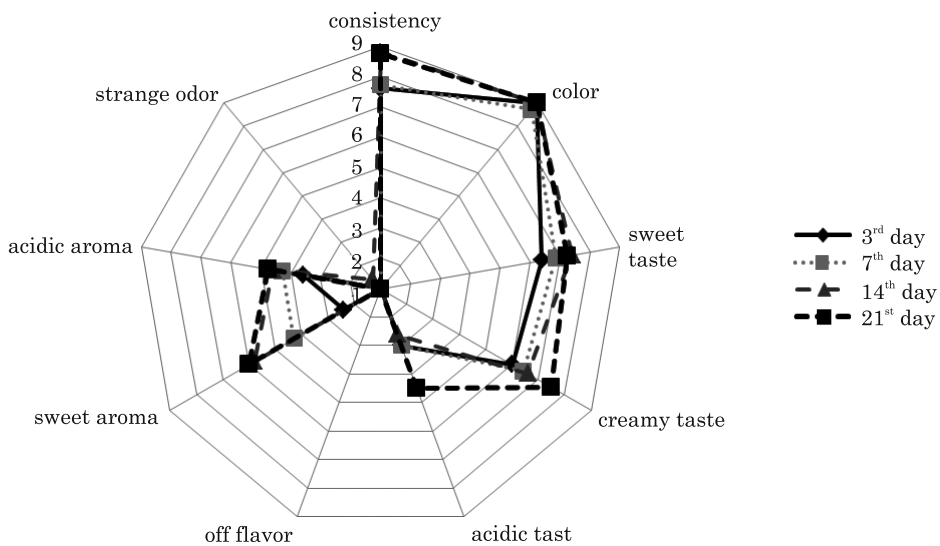

Fig. 10. Sensory evaluation of yoghurt with 50% stevia addition, during storagein nine-point scale, with markings edge: 1 point "very characteristic" (for color and consistency) or "undetectable" (for taste and aroma) and 9 points: respectively "very characteristic" or "very intense"

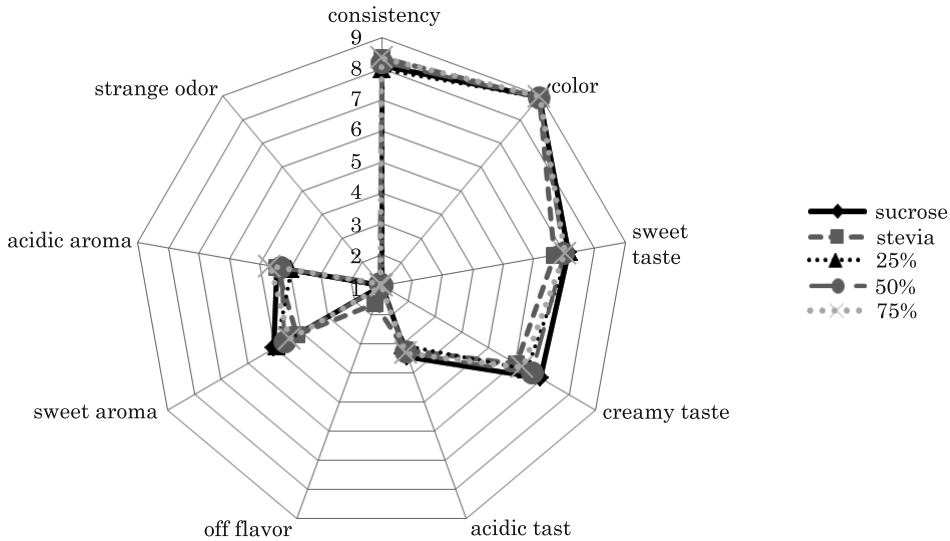

Fig. 11. Average assessment in sensory evaluation of yoghurts with sweeteners in nine-point scale, with markings edge: 1 point "very characteristic" (for color and consistency) or "undetectable" (for taste and aroma) and 9 points: respectively "very characteristic" or "very intense"

between sweet and sour flavors. There were no off-flavor or it was not defined in any of yoghurts during the entire period of storage (Figure 11). The other observation was made by GUGGISBERG et al. (2011) using the same amount of additives sweeteners, observed that yoghurt sweetened with sucrose have been recognized as sweeter than sweetened with stevia. Author also stated that bitterness was significantly higher in the sample containing stevia alone which was also the sample showing a significantly higher off-flavour described by the panel as "artificial", "cardboard" and "metallic". In this test, however, stevia contained purified stevioside, not a rebaudioside A, and the sweetening power of the first glycoside is lower and it taste more bitter and licorice (LEMUS--MONDACA et al. 2012).

The taste of control sample with sucrose was the best and 53% of the evaluators gave it the first place. The worst was the yoghurt contains only stevia, 38% put it on the last place. But even a small addition of sugar to the sweetening mixture caused the increased acceptability of yoghurt. Most second preferences were yoghurt with 75% addition of stevia (33%).

Conclusion

1. The acidity of the products with the addition of stevia were higher than the reference yoghurt.

2. Yoghurts with stevia had lower dry matter content compared to yoghurt with sucrose, which has not affected the consistency.

3. Hardness of yoghurts with stevia added was higher than yoghurt base in the entire period of storage. Other texture parameters, such as cohesiveness, springiness and chewiness of curd decreased after 7 days of storage in yoghurt containing stevia.

4. Yoghurt sweetened with stevia did not differ in the intensity of sweetness from the other, but the sweet taste of stevia was felt more by what it considered to be less natural than the taste of sucrose.

5. Yoghurt with stevia were considered worse than control yoghurt, but yoghurt contains 75% addition of stevia in the mixture, was usually selected as a second choice.

Translated by Jacek Wołk

Accepted for print 22.12.2014

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