

Acta Sci. Pol. Technol. Aliment. 18(4) 2019, 361–371

eISSN 1898-9594 http://dx.doi.org/10.17306/J.AFS.2019.0724

ORIGINAL PAPER

Received: 19.09.2019 Accepted: 25.11.2019

# THE EFFECTS OF THE ADDITION OF STARTER CULTURES AND STEVIOSIDE ON TECHNOLOGICAL LOW-FAT FERMENTED SHERBET ICE-CREAM WITHOUT SUGAR

pISSN 1644-0730

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#### ABSTRACT

**Background.** The maintenance and strengthening of public health and the prevention of diseases associated with the malnutrition of children and adults is an urgent and acute problem facing the population of the whole world. Diabetes type 2 has become a serious problem for modern medicine. This disease is widespread throughout the world among children and adolescents. There are substantial grounds to believe that this global incidence is related to obesity and physical inactivity. There is a diverse assortment of ice-creams and frozen desserts available all around the world. Even with the development of the ice-cream industry, frozen desserts for people suffering from diabetes type I and II have not been sufficiently developed. Therefore, this study aims to select low-calorie components for use in the manufacture of sherbet ice-cream without sucrose in their composition, with a low glycemic index and with a high content of protein and vitamins.

**Materials and methods.** To develop the technology and formulation of the product, a combination of appropriate starter cultures and their ratios were determined. The most suitable fruit mix with a low glycemic index was chosen to maintain the product with properties desirable to consumers.

**Results.** Combined starter cultures, consisting of CHN-22 and St-Body 1 at a ratio of 7:3 were selected experimentally. The best thixotropic properties were shown by the test samples with a titratable acidity of  $60-65^{\circ}T$  at a fermentation temperature of  $33 \pm 1^{\circ}C$ . The fruit mixture for the sherbet ice-cream was made from fruits and berries recommended for people with diabetes, including cherries, blueberries and lingonberries at a ratio of 3:4:3, respectively. The part of the mixture that was inserted into the sherbet ice-cream was evaluated as 25% of the weight of the final mixture. Stevioside and syrup of Jerusalem artichoke were selected at amounts of 0.05% and 7.5% by weight of the mixture respectively. The resulting sherbet was not inferior in sweetness to the control sample with 21% sucrose. The shelf life of the low-fat fermented sherbet ice-cream without sugar was obtained according to the results of research on organoleptic, physicochemical and microbiological properties and was substantiated as 3 months at 18°C.

**Conclusions.** The presented production procedure enables the manufacture of a low-fat, sugar-free product with preventive and therapeutic properties for people who suffer from diabetes and obesity. Studies were conducted on the influence of the introduced starter cultures and sweeteners on the organoleptic, physico-chemical and rheological parameters of the developed low-fat frozen sherbet. Starter cultures and doses of stevioside which had a favorable effect on the indicators of the finished product were selected.

Keywords: sherbet ice-cream, stevioside, overrun, fermented ice-cream

Funding: This work was financially supported by the Government of the Russian Federation.

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# INTRODUCTION

Functional nutrition problems are related to the health and life expectancy of the entire world population. The relevance of these problems are reflected in many foreign and Russian sources, including in the documents and materials of the International Dairy Congress. The analysis of the actual nutrition of the Russian population indicates a number of structural violations associated with unfavorable environmental conditions, changes in lifestyle and socio-economic conditions. These violations are expressed in a deficit of high-grade proteins, vitamins, minerals and dietary fiber with an excess intake of carbohydrates and animal fats, which lead to various forms of disruption of the normal state of the human body (Kaminsky and Kovalenko, 2010).

Diabetes mellitus is a so-called "non-infectious epidemic", which has affected more than 160 million people worldwide (Kaminsky and Kovalenko, 2010; Uotkins, 2006). Therefore, the development of functional products has become a problem with a practical relevance. One of the rational ways of solving this problem is an expansion of the assortment of icecreams and, as an option, the development of low-fat fermented sherbet ice-creams without sugar.

Stevioside is a glycoside from an extract of plants of the genus *Stevia*. It exceeds sucrose in sweetness by 250–300 times. Stevioside is considered to be a harmless natural sugar substitute which has a low-energy value, is non-toxic, non-mutagenic and carcinogenic, and resistant to heat, long-term storage and the effects of acids and alkalis. Stevioside is used in small doses and is harmless over long-term periods of use (Chatsudthipong and Muanprasat, 2009; Pavlova et al., 2005; Petrov and Podgornova, 2015; Ponomarev et al., 2005).

Jerusalem artichoke syrup is made from the tubers of the plant of the same name, *Heliánthus tuberósus*. It is a natural and beneficial sweetener which has sugar-lowering capabilities, and contains prebiotics – specific substances that are needed for nutrition and the healthy activity of intestinal cultures. Biologically active substances of Jerusalem artichoke gently stimulate the heart and reduce blood pressure and the level of LDL cholesterol in the blood (Arseneva, 2011; Fleming et al., 1979; Kleessen et al., 2003).

Including cherries (Prúnus subg. Cérasus), blueberries (Vaccínium myrtíllus) and lingonberries (Vaccinium vitis-idaéa) as part of a diet is associated with lower blood pressure and CHD risk and plays an important role in the management of type 2 diabetes (Jenkins et al., 2011; Vaccinium myrtillus..., 2001). Consumption of cherries decreases markers for oxidative stress, inflammation, exercise-induced muscle soreness and loss of strength, blood pressure, arthritis, and bad sleep (Kelley et al., 2018). The berries' ability to improve night vision, halt the progression of cataracts, and protect against glaucoma has also been shown (Vaccinium myrtillus..., 2001). The consumption of blueberries helps in maintaining healthy blood flow and protects against cardiovascular disorders (Postolova et al., 2004). Lingonberries have shown potential antimicrobial effects and have also shown beneficial effects against urinary tract infections (Kalidindi, 2014). These berries are well known to be rich in antioxidants and have high amounts of phenolic compounds (Cho et al., 2004; Huang et al., 2012).

The objective of this research was to develop the composition and optimise the technology of icecreams and frozen desserts by selecting a starter culture for fermented sherbet ice-cream with a low fat content without sugar. The selection of prescription components and the impact of their doses on the control parameters of the product were analysed.

### MATERIALS AND METHODS

### Materials

Based on an analysis of literature data, components for ice-cream sherbet were selected according to the following criteria: component functional properties, the absence of sucrose, economic viability, consumer preferences (based on market research data) and compatibility of components.

Skimmed milk and dry buttermilk were purchased at a local market and meet the demands of GOST 31658-2012 and GOST R 53513-2009, respectively. The quality of whey protein concentrate was assessed according to GOST R 53456-2009. The following DVS starter cultures were used: FD-DVS CHN-22 (*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*) – Chr.

Hansan, Denmark; FD-DVS YC-X16 - Yo-Flex (Lactobacillus subsp. bulgaricus and Streptococcus thermophilus) - Chr. Hansan, Denmark; FD-DVS St--Body-1 - Yo-Flex (L. thermophilus) - Chr. Hansan, Denmark. Stabilizer Cremodan SE 334 VEG - "Danisco A/S" (Edwin Rahrs Vej 38 DK-8220 Brabrand, Denmark). Stevioside powder (food additive-natural sweetener "Stevia 31834003920000"; manufactured by 'Rudolf Wild GmbH & Co/KG') was scaled and added in dry form. Stevia made in accordance with the Unified sanitary-epidemiological and hygienic requirements for goods subject to sanitary-epidemiological supervision (control) was used, along with Jerusalem artichoke syrup TU 9164-001-17912573-2001. Quick-frozen fruits and berries - cherries, lingonberries, blueberries - were purchased from a local supermarket according to GOST 33823-2016.

#### Methods

Standard physicochemical, microbiological and rheological research methods generally accepted in research practice were performed.

Organoleptic characteristics were determined by the scaling method using a 5-point scale (Clark, Costello, Drake, Bodyfelt, 2009). A sensory evaluation of the samples was carried out by a tasting group of 25 employees and students of the Applied Biotechnology Faculty. Tasters had good thresholds of sensitivity and did not consume food, drinks or cigarettes within an hour of tasting. During the profile analysis, score scales were used to evaluate the intensity of certain properties. The samples were randomly coded with three-digit numbers and fed accordingly from the sample with the least saturated taste and aroma to the sample with the most saturated taste and aroma. Participants were asked to evaluate a number of specific features. All samples were at the same temperature of 15°C in portions of 15–20 g. Each member of the Commission put their points on a five-point scale. Then all the points were added together and divided by the number of experts. All studies were carried out in three-fold repetitions. The final data on the mean value were presented graphically in the form of a profile. A description of the organoleptic attributes of the sherbet ice-cream with fruit mixes is shown in Table 1.

The titratable acidity was measured according to GOST 32256-2013. The water-holding capacity of the final mixture was determined using the amount of released whey by centrifuging 10 g of the sample for 30 min at a speed of 1500 rpm. The total solids content was determined gravimetrically according to GOST 32256-2013. The sherbet overrun was determined by a method based on measuring the mass of a fixed volume of the sherbet mixture entering the freezer and the same volume of air-saturated sherbet mixture leaving the freezer (GOST 32256-2013).

Table 1.	The cha	racteristics o	of organole	ptic indicato	rs of sherbet	ice-cream
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taste	odour	consistency	colour	Score
too sweet, sugary	unpleasant	too liquid	too dark	1
too sweet	flat fruit-berry aroma	too dense	uneven, does not match the chosen fillers	2
not sweet enough	insufficient fruit-berry aroma	heterogeneous	slightly uneven, match- es the chosen fillers	3
sweet enough	moderate fruit-berry aroma	not dense enough	even enough, matches the chosen fillers	4
sweet, pleasant	pronounced fruit-berry aroma	homogeneous, dense, with small inclusions of berry mix	even, matches the cho- sen fillers	5

The overrun of the mixture (S) was calculated using the formula:

$$S = \frac{M_C - M_M}{M_M - M_W} \tag{1}$$

where:

- $M_{c}$  the mass of the glass with the initial mixture, g
- $M_{_M}$  the mass of the glass with a mixture after freezing, g
- $M_W$  empty glass mass, g.

The resistance of ice-cream to melting was characterized by the time taken for the accumulation of 10 ml of the mixture by melting a sample of soft or hardened ice-cream in a thermostat at 25°C (Bogatova et al., 2013).

The size of lactose crystals in the soft and in the hardened ice-cream were measured at  $-6 \pm 1^{\circ}$ C according to the microscopic method using the eyepiece graticule (Arseneva, 2011). The division value had previously been determined using an object micrometer with a microscope magnification of 630 times. Crystals were counted for each microscope slide, in groups which were characterized by the size of their constituent crystals, in seven fields of view.

Using the number of groups *n*, the average size (diameter;  $\mu$ m) of crystals for each  $d_m$  was calculated using the formula:

 $d_m = \sqrt[3]{\sum_{1}^{n} d_i^3} \frac{N_i}{N}$ 

where:

 $d_i$  – average diameter of crystals in each group,  $\mu$ m  $N_i$  – the number of crystals in each group,

 $N_i$  total number of crystals in all groups.

Determination of the size of air bubbles was performed using a Goryaev chamber. A sample of icecream was applied to a calibrated grid with a known division value. The air bubbles were observed using a microscope in transmitted light immediately after preparation of the microscope slide. The sizes of the bubbles were calculated using the following formula (Bogatova et al., 2013).

Using the number of groups *n*, the average size (diameter;  $\mu$ m) of air bubbles for each  $d_m$  was calculated using the formula:

$$d_m = \sqrt[3]{\sum_{1}^{n} d_i^3} \frac{N_i}{N}$$
(3)

where:

- $d_i$  average diameter of air bubbles in each group, µm,
- $N_i$  the number of air bubbles in each group,
- N total number of air bubbles in all groups.

The consistency of the mixtures before freezing was measured using a laboratory viscometer VZ-246 according to GOST 9070-75.

The rheological properties of coagulums were investigated using a «Rheotest-2» type rotating viscosimeter (VEB-MEDINGEN, Germany). For this study, the coaxial cylinder device, N, was used. To determine the properties which characterize the structure's resistance to destruction under mechanical action and its ability for thixotropic recovery, the measurement was carried out at a constant shear rate for two minutes. The sample (10 ml) of coagulum was put into the gap between the coaxial cylinders and the viscosity was measured every 15 s. Then, the coagulum was left for 15 min for structure recovery and the viscosity was measured.

$$B\eta = (\eta_r / \eta_n) \cdot 100\% \tag{4}$$

where:

(2)

 $B\eta$  – degree of structure recovery, %

 $\eta_n$  – viscosity of the unbroken structure,

 $\eta_r$  – viscosity measured after recovery of the structure.

Selection of starter cultures. The fermentation starters were added to the milk base without filler. The amount of ferment that was introduced from the mass of the mixture was selected in accordance with the recommendations of the suppliers. The duration of fermentation was determined before the formation of a visual clot. At the end of the fermentation, the samples were cooled to a temperature of  $4-6^{\circ}$ C and subjected to maturation for 12 hours. After maturation, the test samples were kept at room temperature and subjected to measurements when the temperature of the samples reached  $10-12^{\circ}$ C.

**Manufacturing of sherbet ice-cream.** The dry components (dry buttermilk, whey protein concentrate, stabilizer) were stirred and mixed with skimmed milk.

The amount of each component was determined using the calculation method, based on the requirements for the total solids content in the product according to GOST 32256-2013. Reconstitution of the skimmed milk powder was carried out using distilled water at 45°C. The mixture was pasteurized at 85°C for 1 min, cooled and subjected to the fermentation process using the selected starter culture. The fermentation process was used to increase the biological value of the sherbet ice-cream. The fermentation was stopped by rapid cooling.

The fruit-berry mix was pureed. The sweeteners were introduced into the puree and the fruit mix was pasteurized at 80°C for 60 s. The fruit mix was added to the fermented mixture at  $6 \pm 2^{\circ}$ C. The resulting mixtures were stirred and frozen using the batch freezer without a forced air supply. For the analysis, soft ice-cream after freezing and hardened ice-cream were used. For ice-cream hardening, the samples were placed in the hardening chamber at a temperature of -35–(-50)°C until a temperature of  $-18 \pm 1^{\circ}$ C was reached.

**Setting the application time.** Setting of the application time of the frozen sherbet was carried out according to the methodical instructions of the sanitary-epidemiological assessment of the validity of shelf life and storage conditions of food products. In addition, the finished product was subjected to microbiological studies. The finished hardened product was stored for 5 months. With a periodicity of 1 month, studies were carried out according to the above methods.

#### **RESULTS AND DISCUSSION**

The results concerning the selection of starter cultures are presented in Table 2.

Analyzing the data presented, we can conclude that there is a need to combine the starters FD-DVS CHN--22 and FD-DVS St-Body-1-Yo-Flex and determine their ratio. Since the samples with the *CHN 22* had an excellent taste, they were combined with FD-DVS St-Body-1 in ratios of 5:5; 6:4; 7:3; 8:2; 8:1, respectively. Further investigations on the organoleptic properties of the samples showed that the one most preferred

Samples							
FD-DVS YC-X16 – Yo-Flex	FD-DVS St-Body-1 – Yo-Flex	FD-DVS CHN-22					
	Body and texture						
homogeneous, insufficient thickness	homogeneous, thick	homogeneous, insufficient thickness					
	Appearance						
	glossy						
	Flavour and smell						
not pronounced enough	clean, not sufficiently pronounced fer- mented milk	clean, fermented milk, with a pronounced taste and aroma of pasteurization					
	Colour						
white, with a cream shade, uniform throughout the mass							
Titratable acidity, °T							
71 ±2	76 ±1	73 ±1					
Spreading diameter, mm							
69 ±3 60 ±2 66 ±2							

Table 2. Characteristics of the quality of test samples



**Fig. 1.** Water-holding properties of the combined starters: FD-DVS CHN-22 and FD -DVS St-Body 1-Yo-Flex in ratios: no 1 - 5:5, no 2 - 6:4, no 3 - 7:3, no 4 - 8:2, no 5 - 9:1

by the panelists was the chosen starter cultures at the ratio of 7:3.

The effects of the composition of the starters on the water-holding properties of the fermented mixtures is presented in Figure 1.

The highest water-holding properties were shown by test sample no 3. This ratio allowed the formation of the densest coagulum and a more viscous consistency than the other experimental samples.

As the optimal fermentation process temperature of mesophilic and thermophilic cultures is different, the

next step of this research was to determine the appropriate fermentation temperature for the selected combination of FD-DVS CHN-22 and FD-DVS St-Body-1 at a ratio of 7:3. The treatment changes in the titratable acidity of the treatment samples are shown in Figure 2. As can be seen in Figure 2, an increase in the fermentation temperature resulted in an increase in the rate of lactic acid accumulation and led to an increase in titratable acidity values after 12 hours of fermentation.

It was also determined that the most pronounced taste and aroma were marked in the treatment sample



**Fig. 2.** Influence of temperature on titratable acidity during fermentation: no 1:  $25 \pm 1^{\circ}$ C, no 2:  $29 \pm 1^{\circ}$ C, no 3:  $33 \pm 1^{\circ}$ C, no 4:  $37 \pm 1^{\circ}$ C, no 5:  $41 \pm 1^{\circ}$ C



**Fig. 3.** Recoverability of the sample structure with the combined fermentation starter FD-DVS CHN-22 and FD-DVS St-Body-1 at a ratio of 7:3 at various values of titrated acidity

fermented at  $33 \pm 1^{\circ}$ C. Experimental data on the determination of the flow time showed that the test sample fermented at this temperature demonstrated the shortest flow time  $19 \pm 1$  s.

To identify the end of the fermentation process of coagulum, the recoverability of the structure was determined at various values of titratable acidity. This property is very important due to the need for structural recovery after incorporation of the fruit-berry mixture into the dairy base. Experimental data are presented in Figure 3. In Figure 3, it can be seen that an increase in titratable acidity of the treatment samples led to a decrease in recoverability.

Analyzing the experimental data presented in Figures 2 and 3, the most pronounced taste and aroma, a thick consistency, and good thixotropic properties were found in prototypes fermented to acidity in the range of  $60-65^{\circ}$ C at  $33 \pm 1^{\circ}$ C.

The results of the sensory assessment of the fermented milk mixtures with different ratios of cherries, blueberries and lingonberries are given in Figure 4. According to the data in Figure 4, the highest score was obtained for the analysed sample with a mix of cherries, blueberries and lingonberries at a ratio of 3:4:3, respectively.

During the experiment, the influence of the concentration of the chosen fruit-berry mix was investigated.



**Fig. 4.** Organoleptic evaluation of test samples with different ratios of cherries/blueberries/lingonberries: 1 - 4:3:3, 2 - 3:4:3, 3 - 3:3:4, 4 - 2:2:6

The concentration of the fruit-berry mix varied from 20 to 45% of the fermented milk base with increments of 5%. The data are presented in Figure 5. Figure 5 shows that the sample with 25% (w/w) of fruit-berry mix was the most acceptable. This sample had the highest scores for taste, odour, consistency and colour. A further increase in fruit-berry mix led to the



**Fig. 5.** The effects of the concentration of the fruit-berry mix on the organoleptic characteristics of the samples

appearance of such defects of consistency as a snowy texture.

At the next stage of experimental studies, the concentration of sweeteners such as stevioside and Jerusalem artichoke syrup were determined. The concentration of stevioside varied from 0.01 to 0.07% of sherbet ice-cream weight with increments of 0.01%. Organoleptic characteristics, such as colour and consistency, did not change depending on the concentration of stevioside. Table 3 shows the influence of stevioside concentration on the taste and smell of the mixture of frozen dessert. The control sample was sherbet ice-cream made according to GOST 32256-013 with a sucrose content of 21%.

According to the data obtained (Table 3), the concentration of stevioside of 0.05% by weight of sherbet

Table 3.	Effects o	of stevioside	concentration	on taste and	smell	of sherbet	ice-cream
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The concentration of stevioside, %	Taste and smell
Control sample	pure sour-sweet
0.01	pure, too sour, with the smell of a fruit-berry mix
0.02	sour, with a smell of fruit-berry mix
0.03	unsweetened, with a smell of fruit-berry mix
0.04	sweetish, with a smell of fruit-berry mix
0.05	pure, fermented, not sweet enough in comparison with the control, with a smell of fruit-berry mix
0.06	not sweet enough with the specific flavour of stevioside
0.07	not sweet enough with a stevioside bitter taste



Fig. 6. Effect of Jerusalem artichoke syrup concentration on organoleptic characteristics of ice-cream sherbet

ice-cream was preferred. At this concentration, the taste of the sample was pure fermented, but not sweet enough. With a further increase in the concentration of stevioside, a pronounced, specific flavour was tasted.

The results concerning the effects of Jerusalem artichoke syrup concentration on the organoleptic characteristics of the sherbet ice-cream are presented in Figure 6. It can be concluded that, when stevioside and Jerusalem artichoke syrup were introduced at amounts of 0.05% and 7.5% by weight of the mixture, respectively, the test sample was similar to the control sample in all aspects of organoleptic properties.

Table 4 presents the composition and quality indicators of the sherbet ice-cream. According to the

Table 4. Composition and quality indicators of the sherbet ice-cream

Composition and indicators	Prototype		
Mass fraction of dry matter, %	$30\pm0.8$		
Mass fraction of fat, %	$0.8 \pm 0.1$		
Mass fraction of lactose, %	$8.8\pm0.5$		
Mass fraction of sucrose, %	0		
Taste and smell	pure sour-sweet, with a pleasant fruit mix aftertaste		
Consistency	moderately dense, with small fruit mix inclusions		
Overrun, %	33 ±1		
Resistance to thawing, min	39 ±1		
Freezer outlet temperature, °C	6 ±1		
The average diameter of air bubbles, $\mu m$	63 ±1		

	Storage time, month						
Indicator -	0	1	2	3	4	5	
Taste and smell	and smell pure sour-sweet, with a pleasant aftertaste fruit mix						
Consistency	moderately dense, with small fruit mix inclusions						
The average diameter of air bubbles, $\mu m$	$63 \pm 1$	$63 \pm 1$	$62\pm1$	$61 \pm 1$	$59\pm 1$	$57\pm1$	
The average diameter of lactose crystals, $\mu m$	0	0	2	4	7	9	
Yeast, CFU g <sup>-1</sup>	no more than 10 <sup>2</sup>						
Mould, CFU g <sup>-1</sup>	no more than 10 <sup>2</sup>						
Coliform bacteria per 0.01 cm <sup>3</sup>	no coliform bacteria						

data presented in Table 4, it may be concluded that the sherbet meets the requirements of GOST 32256-2013. The average diameter of the bubbles and the resistance to thawing are important criteria during transportation and meet the basic consumer desired properties.

As can be seen from the data presented in Table 5, during the five months of product storage there were no significant changes in its quality indicators. However, according to the technique (Gapparov et al., 1999) the recommended period of storage for the product is three months at a temperature  $-18 \pm 1^{\circ}$ C.

## CONCLUSION

During the fermentation of the milk base, combinations of starting lyophilized DVS ice cultures were experimentally selected. They consisted of mesophilic and thermophilic cultures CHN-22 and St-Body 1 at a ratio of 7:3. The fermented mixture with an acidity of 60–65°T at 33  $\pm$ 1°C has the most pronounced taste and aroma, a thick consistency and a good thixotropic properties. Based on the recommendations of diabetologists for diabetics with a low glycemic index, a mix of cherries, blueberries and lingonberries at a ratio of 3:4:3, respectively, at an amount of 25% by mixture weight, received the highest score in organoleptic qualities. The recommended doses of stevioside and Jerusalem artichoke syrup are 0.05% and 7.5% by mixture weight, respectively. The experimental samples obtained were not inferior in organoleptic characteristics to the control with a mass fraction of sucrose

of 21%. The advantages of this ice-cream sherbet are its low fat content, no sugar, the use of natural fillers and the enrichment of the product with healthy microflora. According to the results of organoleptic, physicochemical and microbiological indicators, the shelf life of a low-lactose fermented sherbet ice-cream without sugar is 3 months at  $-18 \pm 1^{\circ}$ C.

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