Chapter 12

Probiotic Confectionery Products – Preparation and Properties

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Additional information is available at the end of the chapter

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1. Introduction

Proper orientation of the gastrointestinal tract biocenosis and consumption of probiotic products is becoming more and more important in the industrialized world as problems such as civilization diseases and population aging are spreading.

The word “probiotic” is derived from the Greek "pro bios" and means "for life". As defined by FAO /WHO, probiotics are specific strains of microorganisms, which when served to human in proper amount, have a beneficial effect on our body (improve health or reduce risk of getting sick) [1, 2]. Probiotic bacteria most commonly belong to *Lactobacillus* and *Bifidobacterium* species.

However, not all bacteria have equally strong effect on human health improvement. Activity of probiotic bacteria is a specific feature of the strain.

The effect of improving human health depends not only on strain (its probiotic activity) but also on media (a matrix on which bacteria are carried). The media should provide probiotic bacteria with a high viability and activity during transit through intestinal tract and at their final destination.

Probiotic bacteria support both, specific and nonspecific human and animal defense mechanisms.

Probiotics improve digestion of lactose in subjects suffering from disorders in its absorption and relieve symptoms of the gastrointestinal tract disorders. Additionally they may contribute to lowering of cholesterol as well as reduce adherence, and thereby prevent translocation of pathogenic microorganisms into the intestinal lumen. There are many different evidence that prove ability of probiotic bacteria to prevent or slow down the

processes leading to colorectal cancer. Lactic acid bacteria are also able to use (or bond) carcinogenic compounds derived from diet or produced by pathogenic bacteria in the intestines, such as nitrosamines, azo dyes, mycotoxins or amino acids pyrolisates. However, the strongest clinical evidence demonstrating the beneficial effect of probiotics on human health is immunity increase (immunomodulation) [4-9].

Probiotics may be consumed in the form of pharmaceutical preparations, food supplements or food additives.

LAB probiotic bacteria may play a role of a supplement in: vegetable, fruit and fruit and vegetable juices, breakfast cereals, different kinds of chips, mousses and creams, ice creams and fruit jellies. They may also serve as supplement when properly selected probiotic strain is added to fermented meats, vegetable silages and not soured dairy products, cottage and ripened cheeses as well as many other products. Probiotic bacteria are also used as an additive in nutrition products for children. Most commonly, however, they are used in process of manufacturing fermented dairy products such as yogurts or probiotic kefirs.

Fixation of lactic acid bacteria with the use of innovative processes, thanks to the elimination of characteristic sour taste allows to extend its possible application to a whole new group of products. LAB viability in this type of products is often caused by low water content and water activity, as well as leaving LAB in the state of anabiosis without performing fermentation. This criteria is met by a certain number of semi-finished and final products in confectionery industry. Under polish research projects no. 3 P06T 054 24 and no. R12 018 01 attempts were made to include LAB into the composition of such products as: chocolate and chocolate products, raisins coated with chocolate (dragees), confectionery cores from fatty masses, biscuits coated with chocolate couverture, interleaved wafers and bread spreads. In these products the number of bacteria as CFU $\cdot$ g^{-1} and LAB survival rate during several months of storage (depending on the type of testing material) was determined. This chapter describes the technology of manufacturing products such as interleaved wafers and chocolate covered raisins, biscuits and cores from peanut fatty masses, supplemented with lyophilized live bacterial cultures of lactic acid bacteria from *Lactobacillus* group [10-16]. The lyophilized preparation of LAB contained 3 strains:

- *Lactobacillus casei* strain no ŁOCK 0900 B/00019,
- *Lactobacillus casei* strain no ŁOCK 0908 B/00020,
- *Lactobacillus paracasei* strain no ŁOCK 0919 B/00021.

All these strains were derived from the Collection of Pure Industrial Microbial Cultures at the Lodz University of Technology ŁOCK 105. These strains were deposited in the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wroclaw.

The strains were selected on the basis of results of *in vitro* studies. They were resistant to the acidity of gastric juice, resistant to the bile, adhered to epithelial cells and displayed an antimicrobial activity. The studies were carried out according to FAO/WHO recommendations [1, 2]. On the basis of the sequence of the gene encoding 16S rRNA, the

examined bacterial strains were classified as *Lactobacillus casei* and *Lactobacillus paracasei* (97 ÷ 99% similarity). Both these species rank among the typical microflora of human intestines and can be safely used for production of fermented milk products and preparations of probiotics. The examined strains were tolerant to pH 3.5. Almost all cells survived 3 h incubation at pH 3.5 and at neutral pH (6.5) while 80 ÷ 100% cells survived at pH 2.5 (it depended on a strain) while in the presence of 4% bile salts only 60% cells survived. All the examined LAB strains exerted an inhibitory effect on pathogenic bacteria, both gram-negative and gram-positive. The *in vivo* studies employing 2-month old, immunocompetent mice Balb/c revealed no translocation of these bacteria to the blood and other internal organs. Minor amounts of these bacteria in mesenteric lymph nodes could be an evidence of activation of immune system. The safety of application of these strains was also proved through *in vivo* studies employing children suffering from the atopic skin inflammation [17].

2. Methods

Obtained probiotic confectionery products, namely: interleaved wafers, raisins coated in chocolate, as well as confectionery cores such as biscuits and peanut fatty masses were analyzed with the use of following methods:

- Casson viscosity and Casson yield value of couverture according to Casson method, with the use of digital rheoviscosimeter HADV – III+ from Brookfield Engineering Laboratories Inc. (USA), with co-axially arranged rotor SC4–27 (11.75 mm diameter), stator and an attachment for small volume samples (cylinder with diameter – 25.13 mm) [15, 18-20],
- percentage of couverture content established by a difference in weight of confectionery cores (biscuits, cores from peanut fatty masses, wafers, raisins) with coating and before coating,
- dry mass content by drying a sample with sand at a temperature 102 – 105°C,
- water activity with the use of a measuring instrument HYGROPALM AW 1 from Rotronic (Switzerland) with a digital probe AW-DIO at a temperature T=23 ± 1°C,
- total acidity by potentiometric titration to a pH value of 8.2 with the use of pH-meter from SCHOTT CG 843 with combined electrode – BlueLine 11 from SCHOTT GERÄTE GmbH (Germany),
- texture analysis at a temperature of 20°C, with the use of digital texture analyzer TA.XT Plus from Stable Micro Systems (UK) with driver and software, probes used: A/CKB – chocolate coated raisins, HDP/90 (heavy duty platform) – biscuits and peanut fatty masses coated with couverture, HDP/SR – wafer filling (consistency masses – spreadability), HDP/VB – wafer cores (hardness – crunchiness),
- changes in fat by DSC method with the use of DSC 111 apparatus from Setaram (France), according to the following procedure:
  - cooling a sample (with an initial room temperature) to a temperature of 10°C with cooling speed of $1°C \cdot min^{-1}$ to obtain a complete crystallization of fat,
  - leveling initial conditions by keeping a sample at a temperature of 10°C for 2 min,

- heating a sample to a temperature of 55°C with a heating speed of 3°C·min^{-1}, during which melting of fat in a sample occurred. Changes occurring during this stage were presented as melting curves. Maximum of peak created on developed curves describes as the melting point (Tm), meanwhile from a peak area melting enthalpy (ΔH) was calculated. Heating temperatures of samples were chosen from a range of melting temperatures of fat present in a product [21],

- organoleptic analysis covered evaluation of color, exterior surface, interior of product, consistency, as well as taste and smell with a hedonic 5-point scale [22],
- viability of lactic acid bacteria during storage of products at temperatures of 4, 18 and 30°C. The amount of *Lactobacillus* bacteria was determined by Koch's plate-cultivating method with the use of MRS growth medium. Products were suspended in a solution of physiological saline and peptone. With this manner first dilution was obtained. Prepared with this method samples were incubated in a water bath at a temperature of 37°C for 30 min, afterward samples were homogenized for 1 min. Next step included preparing serial decimal dilutions from which an inoculation of 1 ml of samples onto a Petri dish was performed (each dilution in triplicate). Plates were incubated for 48 h at a temperature of 37°C in a CO_2 WT3 Binder incubator (anaerobic conditions with an addition of 5% volume of CO_2).

Bacteria viability in studied confectionery products was calculated according to following formula:

$$\text{Viability}\left[\%\right] = \frac{N}{N_O} \times 100\%$$

N – log CFU · g^{-1} after a certain period of storage
N_0 – log CFU · g^{-1} directly after product preparation,

- statistical analysis, including a calculation of arithmetical average and standard deviation, was performed with a Microsoft Excel software. Results were obtained from at least three replicates.

3. Products coated with chocolate couverture supplemented with live cultures of lactic acid bacteria

3.1. Chocolate couverture as a media for lactic acid bacteria

Chocolate couvertures contain usually 30-40% of fat. Primary components of chocolate couverture are: cocoa fat, sugar, powder milk (in milk and white couvertures), cocoa liquor and lecithin. It has a fluid consistency during tempering and a solid form in a final product. Couverture can include bigger, possible to sense particles of additives, such as fragmented nuts, which can be found in couverture in a final product, although they were put on a product during processing before or after coating with couverture. Shelf life of couverture is usually 3 to 12 months, depending on its type, but ultimately shelf life of a couverture coated product depends on the kind of used filling. The content of chocolate couverture in

products consist at least 15% of products mass. The process of obtaining chocolate couverture, as an exterior layer on products, include: conching of couverture components, tempering of couverture, coating with a tempered couverture.

Conching takes place at a temperature of at least 40°C (in most of the times 60-70°C) and lasts for up to 48 h. In these conditions it is impossible to maintain high LAB viability, when they are introduced to a chocolate mass in form of a preparation. Tempering of milk chocolate couverture is performed at a temperature of 28°C, and dark chocolate couverture at 30°C [23]. Thus, temperatures used during tempering allow the possibility to introduce to the product probiotic additive in form of fixated LAB preparation. Additionally, low water activity of couverture – on a level of 0.3 – 0.5, allows quite high viability of LAB in products [15, 16]. In this studies LAB preparation fixated by freeze-drying on a powder milk as a carrier media. Obtained this way cultures of lactic acid bacteria, which in lyophilized preparation as well as in a final products, namely chocolate couvertures and chocolate products, were in a state of anabiosis, ready to return to normal life functions when found in proper environment, such as human digestive system.

The aim of this part of the study regarding supplementing of chocolate couverture, used for coating confectionery products, with a lactic acid bacteria preparation was to establish the possibility of obtaining such confectionery products with functional properties in the whole time of shelf life. Furthermore, to establish a minimal level of supplementation to maintain functional properties, for products with significant differences in used confectionery core. Finally, to study the most important properties of used couverture itself, as well as the whole coated with couverture product, which could lower the quality of final product, despite it maintaining full functional properties throughout the whole shelf life.

3.2. Obtaining chocolate couverture supplemented with live cultures of lactic acid bacteria used for coating of various confectionery cores

Obtaining chocolate couvertures enriched with live cultures of lactic acid bacteria was performed by adding lyophilized LAB to a industrially obtained chocolate couverture. Dark chocolate couverture produced by Union Chocolate Sp. z o. o. (Żychlin, Poland) and a preparation of live cultures of lactic acid bacteria (LAB) with a concentration of live bacterial cells from *Lactobacillus* species on a level of 9×10^{10} CFU · g^{-1} from Institute of Fermentation Technology and Microbiology, Lodz University of Technology (Poland) were used. Couverture to LAB preparation ratio was 96:4 (w/w).

In couverture supplemented with LAB, and in a control couverture, rheological properties were established (Table 1), which are extremely important from a technological standpoint, because an eventual increase in a couverture viscosity caused by LAB addition could significantly hinder latter stage of coating [15, 18-20].

Rheological properties analysis have shown an increase of viscosity caused by addition of LAB by about 5% (Table 1). From technological point of view this change is not big enough to cause any repercussions in a form of incomplete product coating. In this regard LAB

addition didn't cause any significant changes in a couverture. Thus, couverture without any further modifications (e.g. content of fat or emulsifier) can be used to selected confectionery products.

Type of couverture	Casson viscosity (Pa · s)	Casson yield value (Pa)
Dark	**1.33** ± 0.02	**8.86** ± 0.28
Dark +LAB	**1.40** ± 0.01	**8.47** ± 0.09

Table 1. Casson viscosity and yield value of couvertures used for confectionery cores coating.

3.3. Obtaining confectionery products coated with couverture supplemented with live cultures of lactic acid bacteria

For couverture coating, as cores, industrially produced biscuits and cores from peanut fatty masses, obtained in a laboratory, were used. Both these products significantly differed, both, in chemical composition, as well as an area to volume ratio (thus the development of couverture surface). Both factors could significantly influence the viability of bacteria present in LAB preparations during products manufacture and storage.

Obtaining confectionery cores used for couverture coating

As cores for couverture coating (with various thickness of its layer) Petit Beurre biscuits were used (Z.P.C. Piast Sp. z o. o., Głogówek, Poland), they contain of: wheat flour, sugar, eggs, confectionery fat and a raising agent in the mass ratio of 100:30:20:10:1. The second type of confectionery cores were candies from peanut fatty mass obtained in a laboratory. Raw materials used for obtaining this product originated from: sugar from Promyk Cukrohurt Sp. z o. o. (Siedlce, Poland) – 17 g · 100 g^{-1}, confectionery fat Efekt 40 MT "middle-tans" from Z.P.T. Kruszwica S.A. (Kruszwica, Poland) – 27 g · 100 g^{-1}, powdered skim milk from S.M. Spomlek (Radzyń Podlaski, Poland) – 17 g · 100 g^{-1}, peanut mash from Plus (Łódź, Poland) – 20 g · 100 g^{-1}, wafer production discards from Dybalski-Cukiernie (Łódź, Poland) – 19 g · 100 g^{-1}. Due to nutritional policy fat used in a recipe had a decreased amount of trans fatty acids [24]. Fat completely devoid of trans fatty acids didn't maintain proper rheological properties in the whole time of storage.

Confectionery fat was grind to a paste in a mixer with single work-load of 3 kg with a hook stirrer. Friable components i.e. powdered sugar, peanut mash, powdered milk and ground wafer discards were all mixed with each other in amounts featured in a recipe. To a ground to a paste fat, prepared mixture of components was gradually added. The pulp was mixed to obtain homogeneous consistency. Prepared pulp was carried to a rectangular mold. The surface of the pulp was leveled. Molds with pulp were cooled to a temperature 8-10°C, and then cut to single pieces with a size of 25×20×45 mm.

Obtaining confectionery cores for coating and the process of coating with a chocolate couverture

Peanut fatty mass cut to a shape of candies was lead to obtain a temperature of 15-18°C (to obtain a solid consistency). Biscuits were coated without cooling them beforehand. Prepared

cores were placed on a grid of coating machine and coated with a previously tempered couverture. Planned percentage of couverture layer on cores, i.e. 30%, 35% and 40% for biscuits and 16%, 25% and 30% for peanut fatty mass, was obtained by regulating the speed of movement of coating machines grid (Promet, Łódź, Polska) on which a layer of couverture poured on cores was blown away to a proper thickness by a stream of air. Chocolate couverture was heated to a temperature of 45-50°C. After the bulk liquidated, it was tempered to a temperature of 28-30°C, and next it was slowly heated to 31-32°C and finally then measured amount of LAB was added. The amounts of couverture on cores ware picked experimentally, to obtain a proper level of CFU of LAB per 1 gram of a whole coated product during storage time, with a possibly thinnest layer of couvurture. For couverture coated biscuits the amount of lyophilized LAB amounted a least 0.5% in relation to a weight of a product. This amount corresponded to 10^7 CFU of LAB per 1 gram of fresh product. To couverture used for peanut fatty mass coating the amount of lyophilized LAB was increased to 0.55% per mass of product to provide probiotic properties during the whole storage time. It corresponded to 10^8 CFU of LAB per 1 gram of fresh product.

Storage of coated biscuits and candy from peanut fatty mass

Finished products were left at a temperature of 6-8°C to cool down and solidify. Next, products were wrapped in aluminum foil and stored at 4, 18 and 30°C for a period of time predicted as a suitable shelf life for given product, that is for 4 months in case of biscuits, and for 3 months for candy from peanut fatty masses.

3.4. Results

Water activity in couverture coated cores from biscuits and peanut fatty mass

Changes in water activity were presented only for fresh products and after the full period of storage, because of very small variation of this parameter (Table 2 and 3).

Storage temp.	**Content of couverture on biscuits (%)**					
	30	35	40	30	35	40
	Biscuits coated with couverture **supplemented** with LAB			Biscuits coated with couverture non-supplemented with LAB		
Water activity						
Fresh product	**0.229** ± 0.010	**0.336** ± 0.026	**0.275** ± 0.007	**0.233** ± 0.012	**0.338** ± 0.060	**0.235** ± 0.070
4°C	**0.282** ± 0.007	**0.266** ± 0.002	**0.307** ± 0.004	**0.314** ± 0.005	**0.292** ± 0.005	**0.307** ± 0.012
18°C	**0.314** ± 0.012	**0.319** ± 0.005	**0.303** ± 0.004	**0.302** ± 0.002	**0.316** ± 0.004	**0.300** ± 0.018
30°C	**0.303** ± 0.008	**0.308** ± 0.001	**0.300** ± 0.006	**0.294** ± 0.006	**0.308** ± 0.002	**0.312** ± 0.012

Table 2. Water activity in biscuits coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 4 months at temperatures 4, 18 or 30°C.

Storage temp.	Content of couverture on candy (%)					
	16	25	30	16	25	30
	Candy coated with couverture **supplemented** with LAB			Candy coated with couverture non-supplemented with LAB		
Water activity						
Fresh product	**0.221** ± 0.008	**0.365** ± 0.026	**0.210** ± 0.090	**0.221** ± 0.003	**0.323** ± 0.005	**0.281** ± 0.002
4°C	**0.346** ± 0.002	**0.328** ± 0.002	**0.339** ± 0.013	**0.329** ± 0001	**0.322** ± 0.002	**0.315** ± 0.006
18°C	**0.342** ± 0.001	**0.340** ± 0.004	**0.309** ± 0.005	**0.315** ± 0.004	**0.342** ± 0.003	**0.340** ± 0.003
30°C	**0.306** ± 0.004	**0.309** ± 0.003	**0.304** ± 0.001	**0.287** ± 0.002	**0.329** ± 0.001	**0.338** ± 0.002

Table 3. Water activity in peanut fatty mass candy coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 3 months at temperatures 4, 18 or 30°C.

An increase in water activity was observed, with an exception of 35% of couverture on biscuits and 25% of couverture on candy (both couvertures supplemented with LAB). It can be explained by re-crystallization of saccharose during storage, which is linked to releasing of water and increasing its activity, although in both candies and biscuits, water activity of products coated with couverture of middle thickness was relatively high [25]. Water activity in coated biscuits and candy in the whole time of storage was in a range of 0.210 – 0.340 and 0.229 – 0.338, respectively. The level of water activity allowed LAB to stay in a state of anabiosis, which provided stability and high viability of probiotic microorganisms [26].

Total acidity of couverture coated cores from biscuits and peanut fatty mass

Total acidity of couverture coated cores from biscuits and peanut fatty mass is presented in Table 4 and 5.

Storage temp.	**Content of couverture on biscuits (%)**					
	30	35	40	30	35	40
	Biscuits coated with couverture **supplemented** with LAB			Biscuits coated with couverture non-supplemented with LAB		
Total acidity (ml 1 M NaOH · 100 g^{-1})						
Fresh product	**2.67** ± 0.04	**2.74** ± 0.07	**2.82** ± 0.04	**2.64** ± 0.09	**2.70** ± 0.02	**3.06** ± 0.08
4°C	**3.02** ± 0.07	**3.14** ± 0.12	**3.18** ± 0.11	**3.00** ± 0.08	**3.10** ± 0.09	**3.12** ± 0.20
18°C	**3.12** ± 0.07	**3.20** ± 0.04	**3.22** ± 0.09	**3.08** ± 0.02	**3.26** ± 0.14	**3.28** ± 0.12
30°C	**3.19** ± 0.14	**3.39** ± 0.06	**3.43** ± 0.06	**3.17** ± 0.09	**3.32** ± 0.08	**3.38** ± 0.11

Table 4. Total acidity of biscuits coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 4 months at temperatures 4, 18 or 30°C.

Storage temperature	Content of couverture on candy (%)					
	16	25	30	16	25	30
	Candy coated with couverture **supplemented** with LAB			Candy coated with couverture non-supplemented with LAB		
Total acidity (ml 1 M NaOH · 100 g^{-1})						
Fresh product	**2.20** ± 0.07	**2.32** ± 0.09	**2.38** ± 0.04	**2.16** ± 0.02	**2.30** ± 0.11	**2.38** ± 0.08
4°C	**2.34** ± 0.03	**2.35** ± 0.02	**2.39** ± 0.07	**2.29** ± 0.12	**2.42** ± 0.03	**2.52** ± 0.08
18°C	**2.46** ± 0.09	**2.48** ± 0.05	**2.54** ± 0.07	**2.52** ± 0.04	**2.56** ± 0.06	**2.58** ± 0.03
30°C	**2.50** ± 0.03	**2.56** ± 0.07	**2.64** ± 0.06	**2.43** ± 0.12	**2.50** ± 0.12	**2.64** ± 0.03

Table 5. Total acidity of peanut fatty mass candy coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 3 months at temperatures 4, 18 or 30°C.

Both, coated biscuits and candy from peanut fatty mass directly after preparation showed an increase in total acidity along an increase in a amount of couverture on products. It indicates that a presence of couverture caused an increase in an amount of components with acidic properties. Couverture contains cocoa liquor, which is rich in volatile and non-volatile organic acids, thus acidity of couverture alone can amount to 8 ml 1 M NaOH·100 g^{-1}. Meanwhile coated biscuits and candy had total acidity in range of 2.7 – 3.4 ml 1 M NaOH · 100 g^{-1} and 2.2 – 2.6 ml 1 M NaOH · 100 g^{-1}, respectively. Higher values of total acidity in coated biscuits result from bigger amounts of couverture, comparing to candy. No noticeable influence of LAB addition on total acidity of products was observed. 3 months of storage caused total acidity to increase, more the higher temperature of storage was used. Furthermore, bigger increase of this parameter was observed in biscuits, which could be caused by two factors. Firstly, by longer storage time, which was dictated by normative requirements, and secondly by bigger area of surface of biscuits in relation to their weight. Because of that they had greater contact with external agents causing degradation changes, such as releasing of free fatty acids. In studied storage period LAB addition didn't cause any changes in total acidity, both in biscuits and candy. It can be considered to be a marker of keeping of probiotic microorganisms in a state of anabiosis, because their activity would cause a lactic acid production and it would influence the acidity of product, and consequently lead to its deterioration.

Hardness of couverture coated cores from biscuits and peanut fatty mass

In fresh biscuits an increase of hardness caused by the content of couverture was observed (Table 6). Biscuit itself was fresh, tender and rather brittle, but properly tempered couverture, with properly crystallized fat in its V polymorphic form, formed a hard surface, which decided about product hardness. Higher hardness values of biscuits coated with couverture supplemented with LAB, testify that it had good textural properties, which means that the addition of LAB didn't hinder cocoa fat crystallization in couverture. During biscuits storage decreasing of hardness was noticed. However no definite correlation

between hardness changes and LAB supplementation, storage temperature of couverture content was observed. It could be probably caused by the fact that changes in hardness are quite complex and a few factors affect it, including softening of cocoa fat in a couverture at temperatures above 15°C (especially at 30°C), an increase of water content in a couverture resulting from water diffusion from product, drying of biscuit core, re-crystallization of saccharose and retrogradation of starch in biscuits. More precise image of hardness changes in coated biscuits can be observed in a chart showing a cutting force (Figure 1).

Storage temp.	**Content of couverture on biscuits (%)**					
	30	35	40	30	35	40
	Biscuits coated with couverture **supplemented** with LAB			Biscuits coated with couverture non-supplemented with LAB		
Hardness (kg)						
Fresh product	**5.30** ± 0.09	**7.18** ± 0.02	**8.41** ± 0.08	**5.37** ± 0.04	**6.68** ± 0.07	**6.96** ± 0.04
4°C	**5.66** ± 0.08	**6.00** ± 0.09	**6.57** ± 0.20	**5.85** ± 0.07	**5.66** ± 0.12	**5.66** ± 0.11
18°C	**4.57** ± 0.02	**4.45** ± 0.14	**4.25** ± 0.12	**4.59** ± 0.07	**6.01** ± 0.04	**6.55** ± 0.09
30°C	**5.21** ± 0.09	**4.51** ± 0.08	**6.98** ± 0.11	**4.35** ± 0.14	**4.65** ± 0.06	**4.99** ± 0.06

Table 6. Hardness of biscuits coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 4 months at temperatures 4, 18 or 30°C.

During cutting of fresh biscuit the biggest action was observed after around ¾ of a second, that corresponds to a depth of about 1.5 mm. Thickness of couverture layer measured for this amount of couverture in a product amounted to 1 mm from each side. The biscuit was brittle, instantly breaking under the pressure of a cutting probe, and the couverture was less hard than the biscuit, and was gently cut by the blade. Cutting profile of a biscuit stored for 4 months at a temperature of 4°C was quite similar to the fresh biscuit, only with lower hardness peak, which was caused by a smoother cut caused by leveling of moisture in a whole product and by declining parts of tensions created during baking. Biscuits stored for 4 months at a temperature of 30°C showed a highest hardness after 1.5 s of the test, thus in deeper parts of the product. However, earlier in a cutting profile a local maximum with a lower values of hardness can be observed. This indicates that biscuit core dried to some degree, it crumbled not in a whole cut but in several layers. Overall hardness value was lower, however it was probably caused by a lower hardness of couverture. It can be observed that the beginning of diagram progresses with a slope at a lower angle comparing to fresh product, and the one stored at refrigeration conditions. After 4 months of storage at a temperature of 18°C similar tendency can be noticed, meaning a couverture is softer than on a fresh biscuit, a biscuit is dried and it crumbles unevenly. Above considerations lead to a conclusion that high biscuit hardness stored at a relatively high temperature is caused by its drying. From all samples stored for 4 month of biscuits coated with couverture supplemented with LAB in an amount of 40% showed statistically significantly higher hardness (about 30%) comparing to analogous samples in non-supplemented couverture, stored at temperatures of 18 and 30°C. Hardness of other samples coated in supplemented couverture, comparing to non-supplemented ones didn't differ by more than 20%.

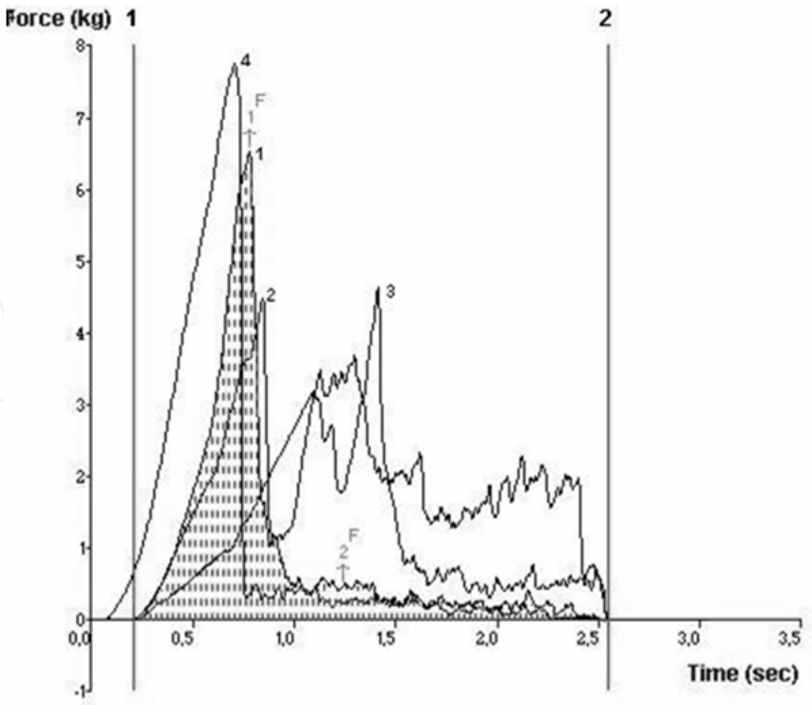

Figure 1. Exemplary profile of texture of fresh and stored for 4 months biscuits coated with couverture (35%) supplemented with LAB, maximal used force is the hardness of product; 1 – fresh product, 2 - stored at 4°C, 3 – stored at 18°C, 4 – stored at 30°C.

Fresh peanut fatty mass candy showed statistically similar hardness regardless of couverture content on cores or supplementation with LAB (Table 7). Noticeable decrease in hardness of candy stored in a period of 3 months at temperatures of 18 and 30°C was observed. Especially at the highest temperature, which resulted from plasticizing both, of cocoa butter in couverture and confectionery fat in candy core. Statistically higher hardness after storage was observed in candy coated with supplemented couverture. It can indicate that LAB preparation gives couverture additional rigidity, as well as makes couverture less susceptible to melting.

Storage temp.	**Content of couverture on biscuits (%)**					
	16	25	30	16	25	30
	Candy coated with couverture **supplemented** with LAB			Candy coated with couverture non-supplemented with LAB		
Hardness (kg)						
Fresh product	**0.66** ± 0.02	**0.62** ± 0.11	**0.63** ± 0.08	**0.66** ± 0.07	**0.66** ± 0.09	**0.66** ± 0.04
4°C	**0.53** ± 0.12	**0.65** ± 0.03	**0.69** ± 0.08	**0.52** ± 0.03	**0.56** ± 0.02	**0.55** ± 0.07
18°C	**0.35** ± 0.04	**0.35** ± 0.06	**0.36** ± 0.03	**0.28** ± 0.09	**0.28** ± 0.05	**0.35** ± 0.07
30°C	**0.12** ± 0.12	**0.17** ± 0.12	**0.14** ± 0.03	**0.13** ± 0.03	**0.14** ± 0.07	**2.64** ± 0.03

Table 7. Hardness of peanut fatty mass candy coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 3 months at temperatures 4, 18 or 30°C.

Cutting profile of coated candy shows that in fresh product the biggest hardness was present in a layer of couverture at a depth of almost 1 mm (Figure 2). Storage at a temperature of 4°C caused a lowering of hardness of couverture and an increase in deeper layers – at half-height, where its partial fracture took place [25]. In products stored at 18°C a lowering of hardness of both, couverture and core was observed. They showed local maximum of hardness on a similar level. Storage at a temperature of 30°C caused a significant softening of both, couverture and core. Cutting curve did not show any local hardness maximum. The blade evenly and gently delved into candy.

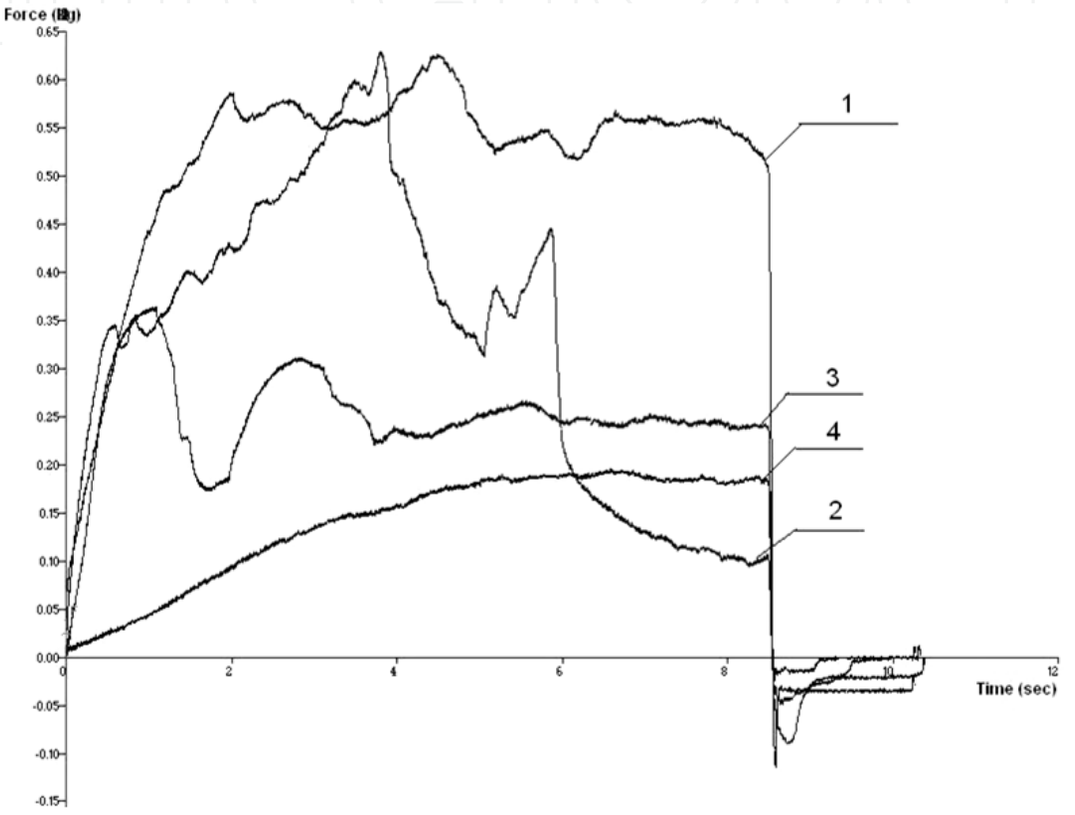

Figure 2. Exemplary profile of texture of fresh and stored for 3 months peanut fatty mass candy coated with couverture (25%) supplemented with LAB, maximal used force is the hardness of product; 1 – fresh product, 2 - stored at 4°C, 3 – stored at 18°C, 4 – stored at 30°C.

Thermal profile of fat from chocolate couverture from biscuits and peanut fatty mass candy

Melting enthalpy of cocoa butter from couverture, which coated biscuits increased with an increase of couverture content in a product (Table 8 and 9).

Furthermore, an increase of melting temperature with an increase of couverture thickness was observed, regardless if it was supplemented of non-supplemented with LAB. Supplemented couverture showed bigger values of melting enthalpy comparing to non-supplemented couverture. During 4 months of storage of coated biscuits melting enthalpy of couverture decreased both, in supplemented and non-supplemented product. This decrease was bigger when storage temperature increased, furthermore, bigger decrease was observed in couverture supplemented with LAB. In supplemented biscuits melting

temperature of cocoa butter remained at the same level during whole storage. In non-supplemented couverture a decrease of melting temperature during storage was noticed. Summarizing these changes it can be observed, that in both, supplemented and non-supplemented couverture fat remained in its stable V polymorphic form only when biscuits were stored at refrigeration temperature. At other temperatures it was partly in amorphous form with a lower melting temperature. Supplementing of couverture with LAB influenced positively maintaining of fats crystalline form. Exemplary thermogram can be seen in Figure 3.

Storage temp.	**Content of couverture on biscuits (%)**					
	30		35		40	
	Enthalpy ΔH (J · g^{-1})	Temperature Tm (°C)	Enthalpy ΔH (J · g^{-1})	Temperature Tm (°C)	Enthalpy ΔH (J · g^{-1})	Temperature Tm (°C)
Fresh product	**36.05** ± 0.18	**30.68** ± 0.09	**37.71** ± 0.28	**32.02** ± 0.12	**42.16** ± 0.37	**33.08** ± 1.06
4°C	**32.83** ± 0.78	**32.35** ± 0.55	**34.03** ± 0.46	**32.89** ± 0.25	**37.39** ± 0.71	**34.04** ± 0.69
18°C	**24.23** ± 0.65	**32.02** ± 0.37	**24.92** ± 0.41	**32.43** ± 0.55	**25.63** ± 0.18	**33.82** ± 0.91
18°C	**22.21** ± 0.72	**31.78** ± 0.75	**23.36** ± 0.61	**31.58** ± 0.38	**23.64** ± 0.58	**32.50** ± 0.29

Table 8. Enthalpy and maximal melting temperature of cocoa fat in dark couverture supplemented with LAB used for coating of biscuits during 4 months of storage at temperatures of 4, 18 and 30°C.

Storage temp.	**Content of couverture on biscuits (%)**					
	30		35		40	
	Enthalpy ΔH (J ·g^{-1})	Temperature T_m (°C)	Enthalpy ΔH (J ·g^{-1})	Temperature T_m (°C)	Enthalpy ΔH (J ·g^{-1})	Temperature T=(°C)
Fresh product	**35.07** ± 0.34	**32.92** ± 0.27	**36.55** ± 0.15	**33.35** ± 0.24	**37.29** ± 0.07	**33.35** ± 0.40
4°C	**31.09** ± 0.61	**31.34** ± 0.37	**31.66** ± 0.62	**32.50** ± 0.78	**31.71** ± 0.54	**33.76** ± 0.95
18°C	**28.22** ± 1.12	**26.53** ± 0.87	**28.26** ± 0.67	**33.27** ± 0.58	**29.30** ± 0.82	**33.85** ± 1.05
18°C	**23.77** ± 0.76	**25.28** ± 0.60	**26.64** ± 1.10	**27.99** ± 0.85	**28.35** ± 0.63	**31.99** ± 0.61

Table 9. Enthalpy and maximal melting temperature of cocoa butter in dark couverture non-supplemented with LAB used for coating of biscuits during 4 months of storage at temperatures of 4, 18 and 30°C.

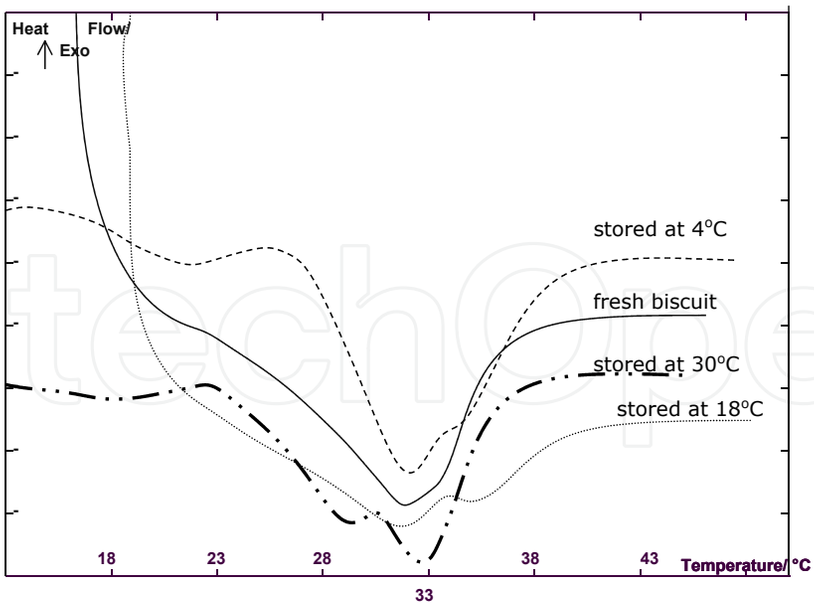

Figure 3. Exemplary DSC thermogram of cocoa butter in the couverture (in the amount of 35%) supplemented with LAB used for biscuit coating in fresh biscuit and biscuits stored for 4 months at temperatures of 4, 18 and 30°C.

In case of peanut fatty mass candy similar tendencies were observed.

Organoleptic analysis of couverture coated cores from biscuits and peanut fatty mass

The best rating of 5.00 obtained biscuits containing the least, namely 30% of couverture (Table 10). With increasing amounts of couverture on cores products obtained lower ratings. Similarly, the best rating 4.80 obtained cores from peanut fatty mass with the lowest amount of couverture, namely 16% (Table 11). Storage of products caused a decrease in organoleptic evaluation, especially those stored at 30°C, which were practically disqualified because of to soft consistency of couverture, and in case of candy also to soft consistency of cores. Organoleptic evaluation of products in supplemented and non-supplemented couverture was statistically on the same level.

Storage temperature	**Content of couverture on biscuits (%)**					
	30	35	40	30	35	40
	Biscuits coated with couverture **supplemented** with LAB			Biscuits coated with couverture non-supplemented with LAB		
Organoleptic rating (points 1- 5)						
Fresh product	**5.00** ± 0.11	**4.85** ± 0.16	**4.75** ± 0.14	**5.00** ± 0.18	**4.85** ± 0.17	**4.75** ± 0.30
4°C	**4.90** ± 0.27	**4.75** ± 0.10	**4.75** ± 0.11	**4.90** ± 0.14	**4.85** ± 0.09	**4.75** ± 0.24
18°C	**4.90** ± 0.24	**4.75** ± 0.31	**4.75** ± 0.24	**4.85** ± 0.11	**4.90** ± 0.14	**4.90** ± 0.31
30°C	4.00 ± 0.17	**4.00** ± 0.17	**3.50** ± 0.23	**4.00** ± 0.30	**3.90**± 0.19	**3.60** ± 0.08

Table 10. Organoleptic analysis of biscuits coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 4 months at temperatures 4, 18 or 30°C.

Storage temperature	Content of couverture on candy (%)					
	16	25	30	16	25	30
	Candy coated with couverture **supplemented** with LAB			Candy coated with couverture non-supplemented with LAB		
Organoleptic rating (points 1- 5)						
Fresh product	**4.80** ± 0.12	**4.80** ± 0.17	**4.95** ± 0.09	**4.70** ± 0.19	**4.75** ± 0.22	**4.75** ± 0.11
4°C	**4.75** ± 0.12	**4.75** ± 0.08	**4.70** ± 0.28	**4.75** ± 0.30	4.60 ± 0.17	**4.75** ± 0.24
18°C	**4.55** ± 0.13	**4.75** ± 0.14	**4.75** ± 0.27	**4.80** ± 0.19	**4.70** ± 0.18	**4.75** ± 0.14
30°C	**3.90** ± 0.19	**3.70** ± 0.17	**3.60** ± 0.22	**3.60** ± 0.18	**3.50** ± 0.18	**3.50** ± 0.17

Table 11. Organoleptic analysis of peanut fatty mass candy coated with various amount of couverture supplemented and non-supplemented with LAB in fresh product and after storage for 3 months at temperatures 4, 18 or 30°C.

Viability of Lactobacillus bacteria in couverture coated biscuits

Viability of bacteria from *Lactobacillus* species was established in biscuits coated with various amounts of couverture – 30%, 35% and 40%. Biscuits were stored at temperatures of 4, 18 and 30°C for a period of 3 months. The content of *Lactobacillus* bacteria in all products directly after their production amounted from 6.80×10^7 CFU · g^{-1} (30% of couverture) to 1.74×10^8 CFU · g^{-1} (35% of couverture). The amount of probiotic bacteria in biscuits stored for a period of 3 moths varied, depending on a storage temperature. After 4 months of storage at a temperature of 4°C of couverture coated biscuits, amount of probiotic bacteria in all studied products was 10^7 CFU · g^{-1}. Viability of *Lactobacillus* bacteria in coated biscuits after 4 month storage period at 4°C was at a level of 92.6% (30% of couverture) to 96.9% (35% of couverture) (Table 12). Storage at a temperature of 18°C caused a decrease of the amount of live probiotic bacteria in biscuits coated with couverture in amounts of 30% and 35% by two orders of magnitude, comparing to initial amounts. Only in biscuits coated with 40% of couverture bacteria amount maintained on the same level, and after 4 months amounted 2.3×10^7 CFU · g^{-1}. Viability of probiotic bacteria in a product stored at a temperature of 18°C after 4 months was lower than when stored at 4°C, and ranged from 75.7% (35% of couverture) to 92.6% (40% of couverture). The use of temperature of 30°C during storage caused a significant decrease in an amount of bacteria in a product, comparing to initial level of bacteria as well as to products stored at other temperatures. The content of probiotic bacteria, after 4 months of storage, lowered by 3 - 4 orders of magnitude – from10^8 CFU · g^{-1} to 10^3-10^4 CFU · g^{-1}. The highest viability of bacteria showed biscuits coated with 30% of couverture (64.9%), and the lowest biscuits coated with 40% of couverture (40.2%). On the basis of performed analyses it can be noticed that probiotic bacteria *L. casei* and *L. paracasei* show the best viability, in couverture coated biscuits stored for during 4 months, when kept at a temperature of 4°C. Confectionery products stored at this temperature also don't change their consistency and organoleptic properties. In case of products stored at temperatures of 18 and 30°C, obtained low amounts of live bacterial cells from *Lactobacillus* species, is not high enough to establish a product to be functional, with an exception of biscuits coated with couverture in an amount of 40%, stored at 18°C.

Couverture content	Storage temperature		
	4°C	18°C	30°C
	Viability of bacteria (%)		
30%	94.4 ± 3.7	78.5 ± 3.4	64.9 ± 4.1
35%	96.9 ± 4.1	75.7 ± 4.1	40.2 ± 4.0
40%	92.6 ± 2.8	92.6 ± 6.2	62.0 ± 3.9

Table 12. Viability of *Lactobacillus* bacteria in biscuits coated with various amounts of couverture after 4 months of storage.

Viability of Lactobacillus bacteria in candy from peanut fatty mass

Directly after product manufacture the amount of live cells of *Lactobacillus* bacteria in couverture amounted 1.6×10^8 CFU · g^{-1} and 1.4×10^8 CFU · g^{-1}, respectively. After 3 month storage period at a temperature of 4°C a slight decrease in an amount of live cells was observed, on average by 2.5%. Lactic bacilli in a couverture, coating candy from peanut fatty mass, in an amount of 16% and 30% maintained the highest viability, after 3 months of storage, at refrigeration temperature (4°C) and was 95.2% and 96.4%, respectively. At a temperature of 18°C after 3 month storage period amount of bacteria decreased by two orders of magnitude (from 10^8 CFU · g^{-1} to 10^6 CFU · g^{-1}), whereas storing at 30°C caused a decrease of three orders of magnitude – from 10^8 CFU · g^{-1} to 10^5 CFU · g^{-1} (Table 13). Increasing the amount of couverture of products slightly improved viability of bacteria, however these changes are not statistically significant. From performed experiments it can be concluded, that probiotic bacteria maintain the highest viability, after 3 month storage period, both at 4 and 18°C. However, the best temperature for storage of candy from peanut fatty mass coated with couverture with an addition of probiotic bacteria, was at the refrigeration temperature (4°C). At these conditions, after 3 months of storage, bacteria viability was the highest and amounted from 95.2% to 96.4%. High viability of bacteria, above 76%, was achieved during storage of candy at a temperature of 18°C. On the other hand, the lowest viability, from 68.1% to 67.8% was observed in products stored at 30°C.

Couverture content in candy from peanut fatty mass	Storage temperature		
	4°C	18°C	30°C
	Viability of bacteria (%)		
16%	95.2 ± 3.3	82.1 ± 4.3	68.1 ± 3.0
30%	96.4 ± 2.4	83.6 ± 3.3	67.8 ± 4.3

Table 13. Viability of *Lactobacillus* bacteria in candy from peanut fatty mass after 3 months of storage.

In Table 14 the amounts of live bacterial cells, after storage for 3 months at different temperatures are presented. Results are calculated per final product, namely per a single candy from peanut fatty mass coated with couverture with a weight of 15 g. Storing this product at temperatures of 4 and 18°C, provides a high level of live *Lactobacillus* bacterial cells, above 10^7 CFU · 15 g^{-1}. Consumed with a confectionery product amount of lactic bacilli is high enough, to provide a beneficial effect of health and well-being of a consumer. During

final product storage at a temperature of 30°C level of live bacterial cells (10^6 CFU · 15 g^{-1}) is not high enough, for a product to become functional.

Couverture content	Storage temperature		
	4°C	18°C	30°C
	The amount of live bacterial cells in a single piece of candy (CFU · 15 g^{-1})		
16%	9.6×10^8	8.0×10^7	5.8×10^6
30%	1.1×10^9	9.8×10^7	5.1×10^6

Table 14. The amount of live bacterial cells of *Lactobacillus* species in candy from peanut fatty mass, with a weight of 15g, after 3 months of storage.

Full summary of results of analysis regarding all stages of storage can be found in a report from research project supported by Polish Ministry of Science and High Education within development project [11].

Possibility of application of live bacterial cultures of lactic acid preparation for supplementation of chocolate couverture used for confectionery cores coating

Biscuits and cores from fatty masses coated with chocolate couverture supplemented with cultures of lactic acid bacteria, with various percentage content on cores were characterized by correct physicochemical and organoleptic properties for this kind of products. Couverture supplementation with LAB didn't cause any deterioration of physicochemical and organoleptic properties of coated candy and biscuits. For both products, temperatures of 4 and 18°C were proper to achieve high viability of LAB and to classify them as functional food during the whole storage time.

4. Wafers supplemented with lactic acid bacteria

4.1. Wafers

Wafer cream, as an environment for LAB, is a confectionery semi-product with a moisture content below 3%, obtained by aerating of fillings such as: praline, sugar-fat, received from oil seeds, and others (e.g. sugar-protein). Consistency of cream is a sticky and smooth paste, it gives wafer products their characteristic taste. Main components of creams are fat and powdered sugar. The amount of fat in cream depends on relative costs of sugar and fat and on the nutritional purpose of a product. Most of the times 30% of fat are used, but this amount can vary between 23 and 45% of fat in a cream. A certain content of sugar is not exceeded, because it weakens cohesion of a cream. Cream consistency depends on the amount of fat used in a recipe. Other components of creams are: powdered milk, organic acids, flavoring and coloring agents. The addition of wafer production discards gives creams brown color and lowers the concentration of water in the mass. As a partial saccharose substitute glucose can also be used. As a thickening agent and a stabilizer of consistency starch can be added, however it can hinder mass aeration. Water present in a

mass causes an increase of viscosity, which can be lowered by an addition of lecithin in an amount of 0.2% per products mass. When producing a cream, to the sticky fat all friable components predicted with a recipe are added, which lowers the temperature of fat. Later, while mixing and aeration the temperature of the mass increases again. After finished mixing process, cream with a definite temperature, density and consistency is received. Density of cream varies between 0.75 and 1.15 $g \cdot ml^{-1}$. Latter squeezing of cream, under increased pressure, onto a product provides further aeration. In case of wafer creams, it is necessary for them to have high nutritional value, proper taste, flavor and color, smooth and spongy consistency, low water content, or to have bounded structure so they won't soften the wafer. Creams should provide good adhesion to wafers, plasticity and an ability to harden after cooling down of final products. It is important for a cream to be stable at room temperature and to have certain melting characteristics, namely to be solid at a temperature of 20°C, and to melt quickly in the mouth [27-30].

To a cream, used for interleaving wafers, lactic acid bacteria were introduced, making it a product with probiotic properties [31]. Certain physicochemical, organoleptic and textural properties of this cream, compared to non-supplemented cream are described. Research-development works in this subject area were conducted under project no. R12 018 01 [11].

4.2. Obtaining interleaved wafers supplemented with lactic acid bacteria

Production of wafer product includes following steps: preparation and measurement of raw materials, mixing and graining of components, grinding, pouring semi-fluid mass onto individual wafers, sticking of wafer with one another, cutting wafer to required size, optional coating with tempered couverture and finally storage. Main, and the longest process from all mentioned above is the process of mass grinding. It is performed until solid phase particles do not exceed 30 μm. In case of probiotic creams, lyophilized lactic acid bacteria preparation is added to the mass (with a temperature of 40°C) in the final stage of its grinding. Interleaved wafers were received by sticking together three individual wafers (Wafer factory "MIRAN WAFEL" Sp. z .o. o., Poland) with a filling consisting 70% of core mass. Final products comprised of wafer cores coated and non-coated with a dark couverture (Union Chocolate Sp. z o. o., Żychlin, Poland). In coated wafers couverture was added in an amount of 30% per final product mass.

Wafer fillings differed by the type of used fat and its concentration. For human health it is preferable that fats used in confectionery industry have as few trans-configured fatty acids as possible. To realize the idea of nutritional policy for producing wafer fillings transless fats: Akomic 2000 (AarhusKarlshamn, Sweeden) and Akotres S30 (AarhusKarlshamn, Sweeden) and medium-trans fat Efekt 40 (Z.T. Kruszwica S.A., Kruszwica, Polska) were used, in amounts of 34.44, 37.44 and 40.44%, respectively. In supplemented fillings amount of added powdered milk was lowered proportionally to the amount of added lactic acid bacteria lyophilisate, with a concentration of live bacterial cells of *Lactobacillus* species on a level of 9×10^{10} $CFU \cdot g^{-1}$. Initially 3.5% of lyophilisate was used, however bacteria content was so high, that for economic reasons, this amount was lowered to 0.5% per products

weight. This amount, with ease provided a probiotic character of wafer products during whole storage time. Other materials used in wafer filling production are: powdered sugar (Promyk Cukrohurt Sp. z o.o., Siedlce, Poland), wafer discards (Dybalski-Cukiernie, Łódź, Poland), powdered skim milk (S.M. Spomlek, Radzyń Podlaski, Poland), lecithin (Cargill S.A., Bielany Wrocławskie, Poland), ethyl vanillin (Plus, Łódź, Poland). In Table 15 whole recipe for obtaining probiotic wafer fillings in presented. Finished wafer cores were stored at temperatures of 4, 18 and 30°C for a period of 3 months, during which the changes in physicochemical properties and LAB viability were established.

Raw material	Concentration of raw material (%)		
Fat	40.44	37.44	34.44
Sugar	25.71	28.71	31.71
Powdered milk	27.60	27.60	27.60
Production wafer discards	6.18	6.18	6.18
Lecithin	0.05	0.05	0.05
Ethyl vanillin	0.02	0.02	0.02

Table 15. Recipe for obtaining probiotic creams, used as a filling for interleaving wafers, with the use of Efekt 40, Akomic 2000 and Akotres S30 fats.

Considering required physicochemical and organoleptic properties of wafer products for it to be a probiotic product, i.e. proper texture (mainly crunchiness of final product and spreadability of filling), right amount of LAB and unchanged sensory properties, comparing to product without LAB, products were analyzed to establish following parameters: water activity, spreadability of cream, hardness (crunchiness) of product and finally organoleptic evaluation.

4.3. Physicochemical analysis of interleaved wafers supplemented with lactic acid bacteria

Considering the great amount of obtained results of physicochemical analyses of wafers only selected were chosen and presented in a following chapter, namely only those for products stored at 18°C. This temperature was chosen because of the fact that confectionery products are stored at it most of the times on a store shelf. Full results are presented in a report from a research-development project no. R12 018 01 [11].

Water activity

The content of easily accessible water in food as well as the amount and type of solute influences microorganism development in a product. Most of microorganisms prefer a_w from 0.9 to almost 1. Xerophilic mold on a solid surface are able to develop still when a_w values 0.65, and osmophilic yeast are able to expand with a_w of 0.61. Most microorganisms can endure conditions, when water activity of environment is lower than needed for their development. This is the case with lactic bacteria, which during the state of anabiosis, while in lyophilized form were added to wafer fillings. In Figures 4 and 5 variability of water

activity of wafer products stored at 18°C for 3 months, which fillings were supplemented with lactic acid bacteria.

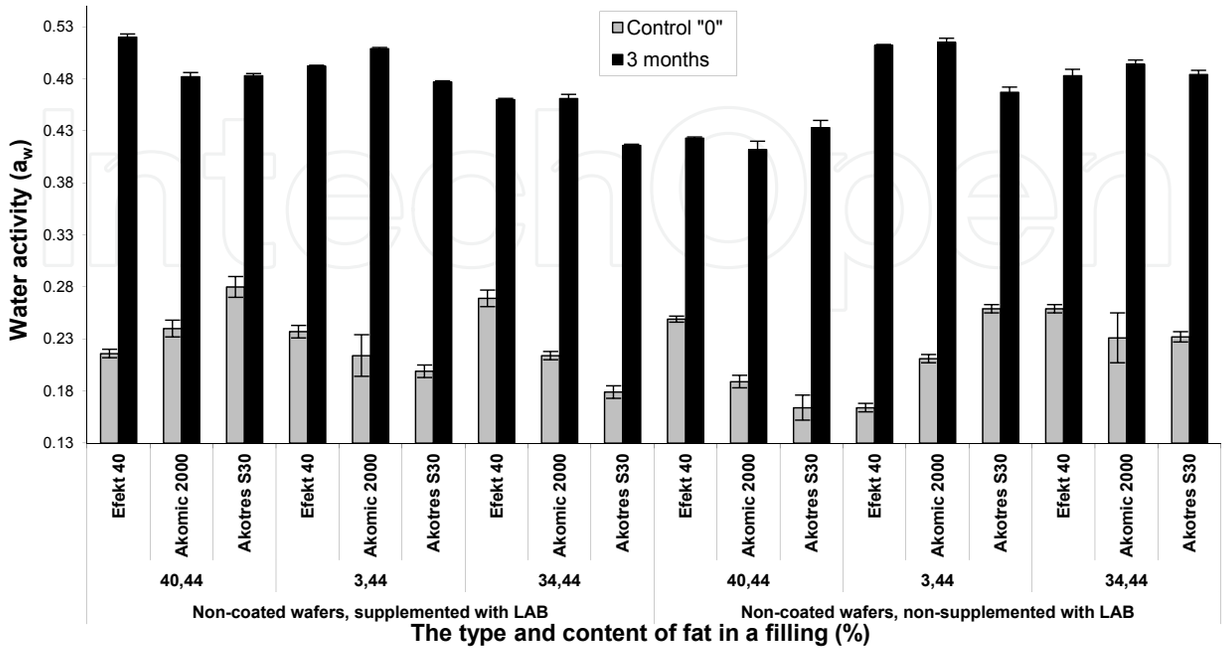

Figure 4. Water activity in **non-coated wafers interleaved with cream supplemented and non-supplemented with LAB**, stored at a temperature of **18°C** for a period of **3 months,** depending on the type and the amount of used fat.

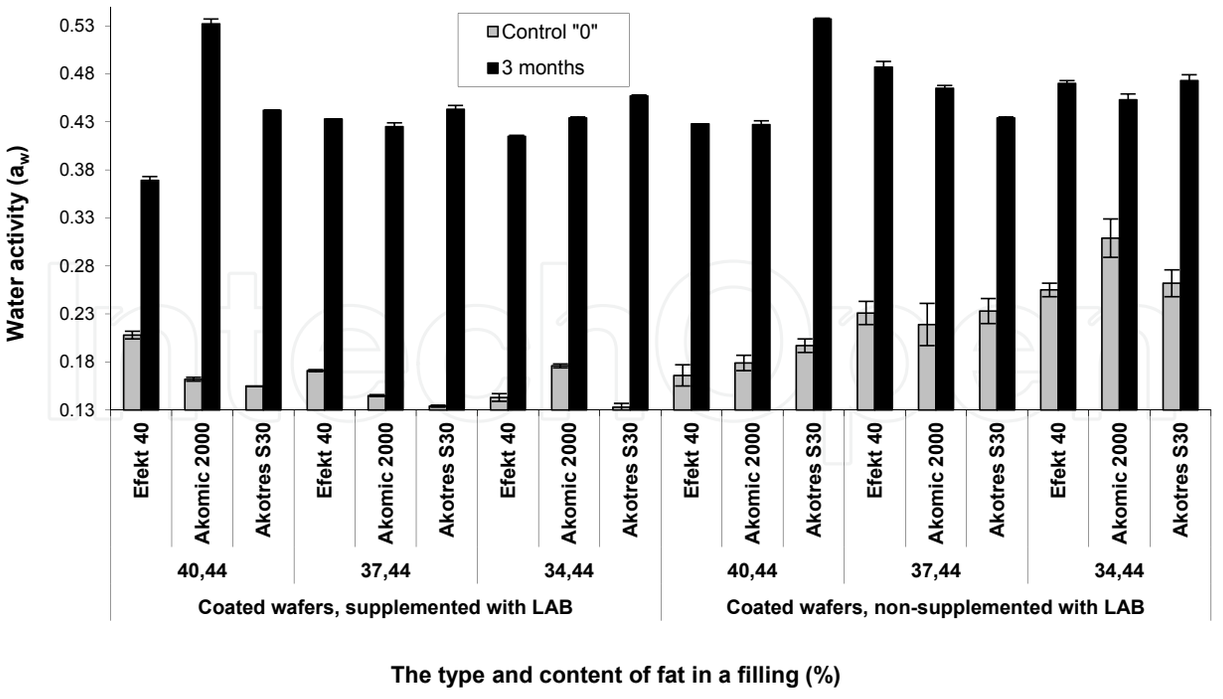

Figure 5. Water activity in **coated wafers interleaved with cream supplemented and non-supplemented with LAB**, stored at a temperature of **18°C** for a period of **3 months**, depending on the type and the amount of used fat.

Initial samples of studied wafers had low water activity, and it ranged between 0.133 and 0.280. The lowest a_w value had wafers coated with couverture supplemented with LAB. During storage wafers showed an overall tendency to absorb moisture from the environment, especially when stored at 18°C. Final values of a_w of products stored in these conditions were in a range of 0.405 – 0.520. It is a level at which LAB are still in a state of anabiosis. Those conditions guaranteed high viability of probiotic bacteria. Judging by obtained results of water activity in wafers: coated and non-coated, supplemented and non-supplemented with LAB, it can be concluded that 3 month storage period at a temperature of 18°C won't have any negative influence on microbiological stability of studied product. Protective role of couverture on wafer core clearly showed in couverture coated wafers, both supplemented and non-supplemented with LAB, when stored at 18°C.

No noticeable correlation between a_w and the type and amount of used fat was observed.

Consistency (spreadability) of confectionery fillings derived from fatty masses

For wafers to be properly, evenly glued together the filling used for their interleaving must have proper consistency (spreadability). This parameter was expressed as a force necessary to immerse in a mass (spreadability) and emerge (adhesiveness) from a mass a conical probe, moving with a constant speed. In Tables 16 and 17 spreadability of masses used as wafer fillings, depending on the type and amount of used fat, is presented. Obtained results for spreadability of fatty masses used as fillings for interleaving wafers show a distinct dependency from the type and amount of used fat.

The biggest hardness, which is equal to the worst spreadablity showed masses received with 34.44% of fat (in this case force values often were non-determinable, above 40.000 g). With an increasing content of fat in a filling its spreadability was improving (force values necessary for immersing the probe averaged between 5.000 and 37.500 g). On the other hand, considering the type of fat used in masses, and the spreadability of those fillings, it can be observed that the least hard, which is equal to the best spreading fillings were obtained with the use of Akotres S30 fat, regardless of the amount of fat. Even in a concentration of 34.44% masses were quite spreadable (force value ranged between 5.000 and 28.000 g), while for fats Efekt 40 and Akomic 2000 in this amount spreadability could not be determined.

When observing obtained results of consistency measurement of filling used for interleaving wafer, no significant influence of LAB supplementation was noticed. It can be concluded that supplementation with LAB of masses used for interleaving wafers won't hinder the ability to properly bind them together. Obtained results of hardness analysis of wafer cores, showed a lack of clear dependency of this parameter from the type and amount of fat used for obtaining creams, as well as from its supplementation with LAB.

Comparing force values from a "biting test" of control samples, it can be noticed that the least amount of force necessary to break, showed wafers interleaved with masses obtained with Akotres S30 fat.

In case of non-coated wafers it can also be noticed, that harder values were obtained by products received with Akomic 2000, comparing to wafers with Efekt 40 fat in its material composition. Similar dependency from the type of used fat was observed for consistency (spreadability) studies of fillings (Table 16).

Type of mass	Type and amount of fat (%)								
	Efekt 40			Akomic 2000			Akotres S30		
	40.44	37.44	34.44	40.44	37.44	34.44	40.44	37.44	34.44
1. Supplemented masses; non-coated	**7.064** ± 0.162	**19.456** ± 1.032	*	**35.116** ± 0.777	**23.491** ± 0.330	*	**11.892** ± 1.245	**17.505** ± 0.411	**25.110** ± 0.078
2. Non-supplemented masses; non-coated	**37.434** ± 0.177	**37.505** ± 0.093	*	**30.811** ± 0.202	**36.438** ± 0.459	**37.531** ± 0.074	**16.149** ± 0.351	**21.036** ± 0.073	**18.904** ± 0.181
3. Supplemented masses; coated	**9.123** ± 0.329	**14.957** ± 1.374	**37.549** ± 0.050	**32.507** ± 0.549	**37.559** ± 0,230	**37.559** ±0.557	**4.399** ± 0.623	**6.656** ± 0.473	**5.822** ± 0.814
4. Non-supplemented masses; non-coated	**34.594** ± 0.529	**22.505** ± 0.220	**37.559** ± 0.042	**26.870** ± 0.395	**34.489** ± 0.304	**37.561** ± 0,050	**13.171** ± 0.048	**4.065** ± 0.375	**28.120** ± 0.294

Table 16. Consistency (spreadability) of cream used for interleaving wafers, expressed as force (g) necessary to immerse a conical probe in a mass, depending on material composition.
* Masses, in which consistency could not be determined, the value of applied force above 38 000 g

Type of mass	Type and amount of fat (%)								
	Efekt 40			Akomic 2000			Akotres S30		
	40.44	37.44	34.44	40.44	37.44	34.44	40.44	37.44	34.44
1. Supplemented masses; non-coated	**-4.952** ± 0.156	**-6.206** ± 0.351	*	**-7.471** ± 0.252	**-8.776** ± 0.080	*	**-6.074** ± 0.682	**-5.674** ± 0.174	**-5.769** ± 0.029
2. Non-supplemented masses; non-coated	**-4.847** ± 0.444	**-3.938** ± 0.109	*	**-7.266** ± 0.548	**-6.451** ± 0.505	**-6.821** ± 0.626	**-6.487** ± 0.201	**-5.761** ± 0.095	**-6.489** ± 0.197
3. Supplemented masses; coated	**-6.205** ± 0.242	**-6.274** ± 0.519	**-0.002** ± 0.650	**-7.529** ± 0.267	**-0.003** ± 0.001	**-3.858** ± 0.342	**-4.849** ± 0.282	**-4.489** ± 0.030	**-4.276** ± 0.012
4. Non-supplemented masses; non-coated	**-5.347** ± 0.036	**-6.447** ± 0.257	**-3.619** ± 0.022	**-8.737** ± 0.172	**-6.850** ± 0.631	**-0.002** ± 0.000	**-7.882** ± 0.151	**-4.195** ± 0.424	**-5.075** ± 0.162

Table 17. Consistency (adhesiveness) of cream used for interleaving wafers, expressed as force (g) necessary to emerge a conical probe from a mass, depending on material composition.
* Masses, in which consistency could not be determined

An increase of hardness of wafer products during storage, might be caused by an increase of individual wafers hardness caused by moisture absorption as well as changes occurring in consistency of filling resulting from shifting proportion between the content of solid to liquid phase. Polymorphic form, in which initially fat components crystallized (change of melting temperature – DSC measuring) could change, and an altering of crystalline network structure of masses used for interleaving wafers could occur. As a result of new crystals emergence from already present crystal germ, the filling could harden, or as a result of crystal aggregation – soften (an increase of a decrease of solid phase surface). It seems probable, that those changes in quite significant degree, could be the reason of hardness changes in final products.

Hardness (crunchiness) of wafer cores, interleaved with LAB supplemented cream

In case of wafer type products, one the most important organoleptic property, which consumer pays close attention to when choosing his favorite product, is wafer hardness (crunchiness). This parameter in established in a "biting test". It is expressed as a force value necessary to fully cut the wafer core. In Figures 6 and 7 hardness values of wafers: supplemented and non-supplemented with LAB, coated and non-coated with couverture, after 3 months of storage at a temperature of 18°C are presented.

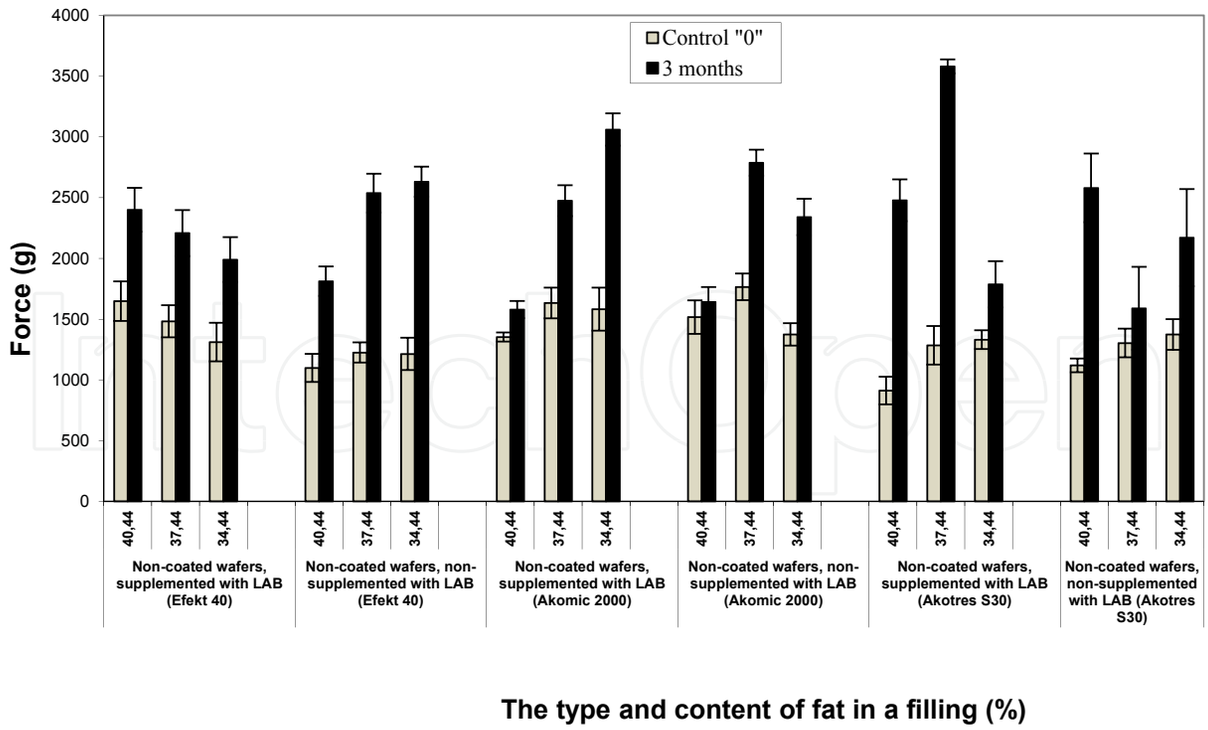

Figure 6. Comparison of hardness values of **non-coated wafers interleaved with cream supplemented and non-supplemented with LAB**, stored for **3 months** at a temperature of **18°C**, depending on the type and amount of used fat.

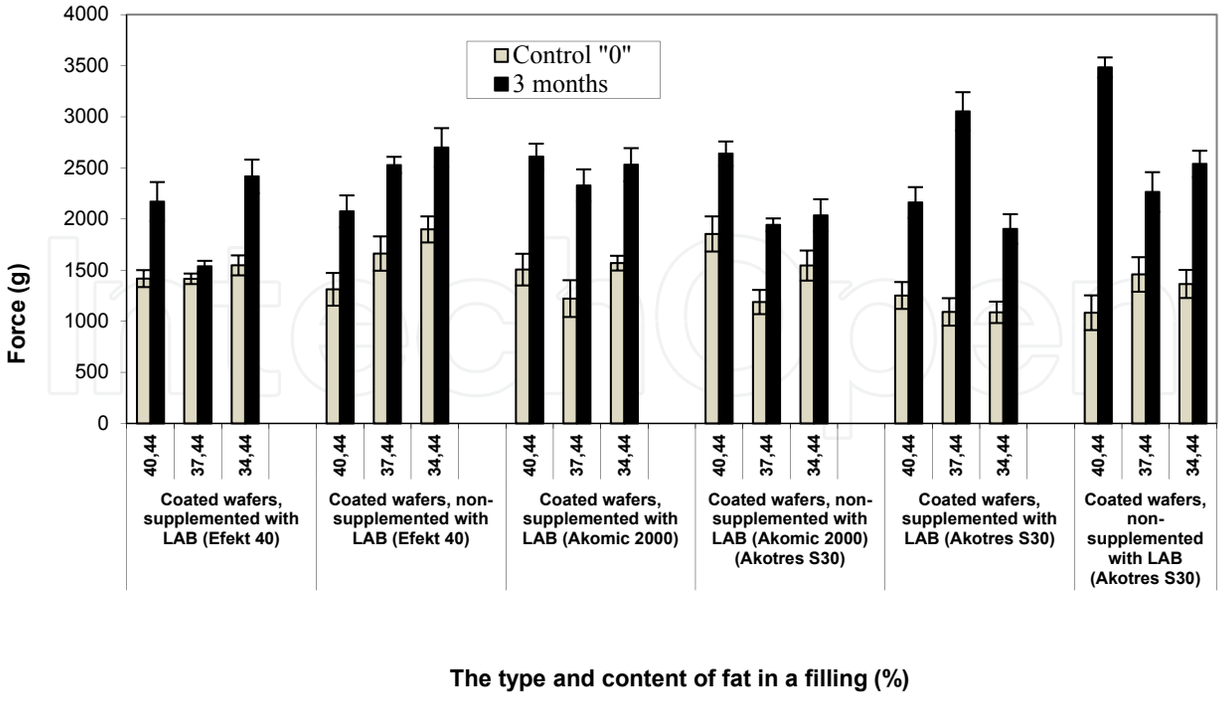

Figure 7. Comparison of hardness values of **coated wafers interleaved with cream supplemented and non-supplemented with LAB**, stored for **3 months** at a temperature of **18°C**, depending on the type and amount of used fat.

Polymorphic changes of fats

Thermal analysis of fatty mass fillings used for interleaving wafers indicated differences in polymorphism of fats used for obtaining products, mainly depending on temperature and storage period. No significant influence of LAB supplementation on the amounts, or temperatures of disintegration of polymorphic forms of used fats was observed. On average, 3 polymorphic forms of used fats occured, regardless of their type. In filling received with Akotres S30 fat (transless) 3 peaks on an endothermic curve were observed, there were temperatures of 12.50, 24.8 and 34.6°C, for Akomic 2000 fat (transless) those temperatures were: 13.98, 26.7 and 33.34°C. The highest melting temperatures of polymorphic forms were obtained for fillings with Efekt 40 fat (trans-containing), namely 16.78, 27.95 and 34.9°C. With an increasing storage temperature and storage time changes occurring in polymorphism of fats used for producing fillings were observed. An increase of melting temperatures was observed, also a new polymorphic form of fat in products stored at 30°C was noticed. The biggest changes occurred in products made with medium-trans Efekt 40 fat, and the least significant ones in products with transless Akotres S30 fat.

The lower melting temperatures of polymorphic forms of fats are, the more it is possible for it to contain a significant amount of unsaturated fatty acids. Whereas, the higher melting temperature of a polymorphic form of fat, the more saturated fatty acids can be found in its composition. Taking this criteria into account, the most beneficial it is to use Akotres S30 fat for obtaining fatty mass fillings. Endotherms obtained for melting of this fat in control samples didn't indicate any significant changes in shape of values of melting temperatures

and enthalpies, compared to samples obtained from products stored for 6 and 12 weeks. It indicates that studied wafers are suitable for consumption during a whole 3 month period of storage. This tendency was noticed for wafers both, supplemented and non-supplemented with LAB. DSC analysis of fillings used for interleaving wafers also revealed that additional supplementation with LAB didn't influence significantly physicochemical properties of final product.

Organoleptic evaluation

The best flavor and appearance properties had product both, supplemented and non-supplemented directly after production. During storage in all products crunchiness parameter decreased, also in case of coated wafers, an appearance of couverture was changing (grey coating on a surface of couverture appeared). In Tables 18 and 19 organoleptic rating of coated wafers supplemented and non-supplemented with LAB is presented. Rating of non-coated wafer cores and a wide description of all types of products can be read in a report from a project [11].

Storage time / **Fat concentration**	Efekt 40			Akomic 2000			Akotres S30		
	40.44%	37.44%	34.44%	40.44%	37.44%	34.44%	40.44%	37.44%	34.44%
Coated wafers supplemented with LAB lyophilisate at concentation of 0.5%									
Control „0"	**3.9**±0.3	**3.9**±0.1	**3.8**±0.1	**3.8**±0.2	**3.9**±0.1	**3.9**±0.3	**3.9**±0.1	**3.9**±0.1	**3.8**±0.1
Stored at 4°C									
6 weeks	**3.8**±0.2	**3.8**±0.1	**3.8**±0.1	**3.8**±0.1	**3.9**±0.2	**3.9**±0.2	**3.8**±0.1	**3.8**±0.1	**3.8**±0.1
12 weeks	**3.7**±0.2	**3.7**±0.1	**3.7**±0.2	**3.7**±0.2	**3.7**±0.2	**3.7**±0.2	**3.7**±0.2	**3.7**±0.2	**3.7**±0.3
Stored at 18°C									
6 weeks	**3.5**±0.1	**3.4**±0.2	**3.4**±0.1	**3.4**±0.1	3.5±0.3	**3.5**±0.1	**3.3**±0.2	**3.5**±0.1	**3.5**±0.1
12 weeks	**3.0**±0.2	**2.7**±0.3	**2.7**±0.2	**2.7**±0.2	**3.0**±0.3	**3.0**±0.2	**3.7**±0.1	**3.0**±0.2	**3.0**±0.1
Stored at 30°C									
6 weeks	**3.0**±0.0	**3.0**±0.1	**2.7**±0.3	**3.0**±0.1	**3.0**±0.3	**2.7**±0.1	**3.0**±0.2	**3.0**±0.2	**2.7**±0.2
12 weeks	**2.7**±0.1	**2.5**±0.2	**2.6**±0.1	**2.5**±0.2	**2.4**±0.3	**2.7**±0.1	**2.6**±0.1	**2.6**±0.2	**2.7**±0.1

Table 18. Organoleptic rating of **coated wafers supplemented with LAB**, differing by a material composition, depending on the storage time and temperature.

In case of non-coated wafers, regardless of storage period and temperature, a few parameters remained at the same level, namely: wafer color, filling color and filling consistency at room temperature. Tastiness of products didn't change, but overall taste impressions were worse than in control samples, resulting from changes which occurred in products. Wafers stored at a temperature of 4°C lost their crispiness. Products stored at 18°C dried, or lost their crispiness, and in some cases became harder than control samples. Wafers stored at 30°C showed good crunchiness and crispiness, but at the same time were very fragile. Coated wafers, even then freshly made were not evenly coated with couverture, "overcoatings" were observed, and because of that organoleptic rating of those products

suffered, receiving grades below 4 (desirable quality). Coated wafers stored at 18 and 30°C received in a rating values of "tolerable" or below. Factor that disqualified wafers stored at a temperature of 30°C were changes of color and consistency of couverture and taste of a whole product.

Storage time / Fat concentration	Efekt 40			Akomic 2000			Akotres S30		
	40.44%	37.44%	34.44%	40.44%	37.44%	34.44%	40.44%	37.44%	34.44%
Model wafers – coated, non-supplemented with LAB									
Control „0"	**3.9**±0.2	**3.9**±0.1	**3.8**±0.2	**3.9**±0.2	**3.9**±0.1	**3.8**±0.3	**3.8**±0.2	**3.9**±0.1	**3.9**±0.1
Stored at 4°C									
6 weeks	**3.9**±0.1	**3.9**±0.1	**3.8**±0.1	**3.8**±0.2	**3.8**±0.1	**3.8**±0.2	**3.8**±0.2	**3.9**±0.2	**3.8**±0.1
12 weeks	**3.7**±0.1	**3.9**±0.1	**3.7**±0.2	**3.7**±0.1	**3.7**±0.1	**3.7**±0.3	**3.7**±0.1	**3.9**±0.1	**3.7**±0.2
Stored at 18°C									
6 weeks	**3.7**±0.1	**3.5**±0.3	**3.5**±0.1	**3.4**±0.1	**3.7**±0.2	**3.5**±0.1	**3.5**±0.2	**3.7**±0.1	**3.4**±0.3
12 weeks	**3.0**±0.1	**3.0**±0.2	**3.0**±0.1	**2.7**±0.2	**2.7**±0.1	**3.0**±0.1	**3.0**±0.2	**3.0**±0.1	**3.0**±0.1
Stored at 30°C									
6 weeks	**3.0**±0.2	**3.0**±0.1	**3.0**±0.1	**3.0**±0.2	**2.7**±0.2	**3.0**±0.1	**3.0**±0.1	**3.0**±0.3	**3.0**±0.2
12 weeks	**2.5**±0.2	**2.7**±0.2	**2.5**±0.3	**2.7**±0.1	**2.4**±0.1	**2.6**±0.2	**2.7**±0.1	**2.5**±0.1	**2.7**±0.1

Table 19. Organoleptic rating of **coated wafers non-supplemented with LAB**, differing by a material composition, depending on the storage time and temperature.

No noticeable influence of the type and amount of fat used in fillings on changes occurring during storage of products was observed. In no way, in products supplemented with LAB, the presence of lactic acid bacteria was noticed during organoleptic evaluation.

Viability of Lactobacillus bacteria in wafers

Similar to previously described products, wafers were also stored at temperatures of 4, 18 and 30°C. 27 types of wafers with various composition were examined, including 18 wafers non-coated with couverture (W1 - W18) and 9 types of wafers with a couverture coating (W19 - W27).

Lactic acid bacteria in a form of lyophilisate were introduced into the filling of wafers. Initially 3.5% of lyophilisate was used (wafers W1 - W5), however bacteria level proved to be so high, that for economic reasons the amount of added lyophilisate was reduced to a range of 0.71% (W10 - W18) to 0.5% (wafers W6 - W9 and W19 - W27).

Initial level of LAB in non-coated wafers W1 – W5 ranged between 4.0×10^8 CFU · g^{-1} and 7.0×10^8 CFU · g^{-1}, in wafers W6 - W18 it ranged from 2.8×10^7 CFU · g^{-1} to 1.7×10^8 CFU · g^{-1}. Storage of non-coated wafers at a refrigeration temperature (4°C) allowed to maintain a high viability of probiotic bacteria from *Lactobacillus* species. In all types of wafers, regardless of its composition, after three months of storage viability was quite high and ranged from 92.2% (W6) to 98.3% , which is equal to an amount of live cells from 3.8×10^7 CFU · g^{-1} to

8.4×10^7 CFU · g^{-1}. Very high viability of bacteria was maintained in wafers non-coated with chocolate, with 40.44% and 37.44% of Akomic 2000 fat in its composition (W13 – 98.1%, W14 – 98.0%), and in non-coated wafers with 34.44% of Akotres S30 fat (W18 – 99.4%) (Table 20).

During storage at a temperature of 18°C lactic acid bacteria viability was at a similar level in all studied types of wafers. The biggest amount of live cells of probiotic bacilli was observed in non-coated wafers, to which 3.5% of lyophilisate was added (W1 – W5), and in non-coated wafers with Akotres S30 fat in a concentrations of 34.44% and 37.44% (W8 – W9), ranging from 80.3% (W8) to 87.9% (W9) (Table 20). In other wafers viability of probiotic bacteria kept on a level of 72-7 – 77.8% (Table 23). Low level of live cells of *Lactobacillus* bacteria was observed after storage of wafers at a temperature of 30°C. Beside wafers W1 – W5, in which viability of bacteria amounted from 70.4% (W1) to 72.2% (W5), in other non-coated wafers viability of lactic bacilli ranged from 62.5% (W18) to 69.4% (W9) (Table 20). The rest of the products, i.e. W19 – W27 contained of wafers with chocolate coating, in which initial levels of probiotic bacteria ranged from 2.5×10^7 CFU · g^{-1} (W25) to 8.1×10^7 CFU · g^{-1} (W23). The addition of couverture was performed to hinder oxygen access to the filling, containing live cells of probiotic bacteria, and therefore to improve the viability of bacteria in the product. The biggest viability, from 95.69% (W23) to 98.64% (W21), was achieved by storing couverture coated wafers at a temperature of 4°C. In couverture coated wafers containing Efekt 40 fat, at a concentration of 37.44% and 34.44% (W20, W21) and in coated wafers containing Acomic 2000 fat at a concentration of 40.44% (W22), bacteria viability after 3 months of storage was the biggest and amounted 98.2%, 98.6% and 98.0%, respectively (Table 20). Also on a high level was viability of live cells of probiotic bacteria in wafers stored at 18°C. At this temperature, after 3 months of storage viability of bacteria ranged between 72.2% (W23) and 83.6% (W19). A temperature of 30°C proved to be the least desirable for couverture coated wafers storage. After 3 months of storage at this temperature, the amount of live cells of *Lactobacillus* bacteria ranged from 7.1×10^4 CFU · g^{-1} to 1.6×10^5 CFU · g^{-1}, which equaled to a viability range from 61.6% (W26) to 67.2% (W20) (Table 20).

Examined wafers differed not only in the amounts of added lyophilisate, but also in the content of fat and sugar. Fat can be a substance protecting cells, and sugar participates in lactic fermentation. However, no influence of those constituents on viability of *Lactobacillus* bacteria in wafers was observed. Similarly, coating wafers with couverture did not influence bacteria viability in examined wafers in a significant manner.

Obtained results allowed to conclude, that preferable temperatures for storage of wafers, both coated and non-coated with courertuve, which provides high probiotic bacteria viability are two temperatures, i.e. refrigeration temperature (4°C) as well as a temperature suggested by normative legislations for storage of this type of products, namely 18°C.

The amount of live cells of *Lactobacillus* bacteria consumed with one piece of wafer, stored for 3 months at a temperature of 4°C reached 10^9 CFU per wafer (Table 21). It was the highest in wafers non-coated with chocolate, in which lyophilisate content was 3.5% (W1 – W5) and amounted from 5.3×10^9 CFU · 28 g^{-1} to 9.8×10^9 CFU · 28 g^{-1} (Table 21). High level of live cells of probiotic bacteria, i.e. from 10^7 CFU to 10^8 CFU per individual product, is also

maintained when products are stored for 3 months at a temperature of 18°C (Table 21). In case of wafers stored at a temperature of 30°C, the amount of live and active cells consumed by a potential buyer, would be lower than 10^7 CFU per wafer, for most of examined products. With an exception of wafers W1 – W5, in which this level was from 4.3×10^7 CFU · 28 g^{-1} to 5.8×10^7 CFU · 28 g^{-1}, but this requires an addition of 3.5% of lyophilisate to the product.

Couverture	Type of fat	Fat content (%)	Sugar content (%)	Symbol of product	Lyophilisate content in final product (%)	Storage temperature		
						4°C	18°C	30°C
						Viability of bacteria (%)*		
Non-coated	Efekt 40	40.44	25.71	W1	3.50	97.2	81.6	70.4
	Efekt 40	37.44	28.71	W2	3.50	96.9	80.7	70.7
	Efekt 40	34.44	31.71	W3	3.50	96.6	80.5	72.1
	Akomic 2000	40.44	25.71	W4	3.50	94.1	84.5	70.8
	Akomic 2000	37.44	28.71	W5	3.50	96.1	85.7	72.2
	Akomic 2000	34.44	31.71	W6	0.50	92.2	77.5	63.6
	Akotres S30	40.44	25.71	W7	0.50	93.,5	76.2	63.9
	Akotres S30	37.44	28.71	W8	0.50	95.5	80.3	65.9
	Akotres S30	34.44	31.71	W9	0.50	97.2	87.9	67.4
	Efekt 40	40.44	25.71	W10	0.71	95.7	75.7	64.7
	Efekt 40	37.44	28.71	W11	0.71	94.5	77.4	65.6
	Efekt 40	34.44	31.71	W12	0.71	97.7	75.0	65.1
	Akomic 2000	40.44	25.71	W13	0.71	98.1	77.8	65.1
	Akomic 2000	37.44	28.71	W14	0.71	98.0	76.5	64.6
	Akomic 2000	34.44	31.71	W15	0.71	96.6	74.2	64.1
	Akotres S30	40.44	25.71	W16	0.71	97.3	75.0	64.4
	Akotres S30	37.44	28.71	W17	0.71	98.0	72.7	63.0
	Akotres S30	34.44	31.71	W18	0.71	98.4	76.7	62.5
Coated	Efekt 40	40.44	25.71	W19	0.50	97.6	82.6	65.4
	Efekt 40	37.44	28.71	W20	0.50	98.2	81.4	67.2
	Efekt 40	34.44	31.71	W21	0.50	98.6	77.6	66.9
	Akomic 2000	40.44	25.71	W22	0.50	98.0	72.6	62.7
	Akomic 2000	37.44	28.71	W23	0.50	95.7	72.2	63.0
	Akomic 2000	34.44	31.71	W24	0.50	97.2	73.4	63.0
	Akotres S30	40.44	25.71	W25	0.50	96.3	74.1	64.0
	Akotres S30	37.44	28.71	W26	0.50	96.1	74.7	61.6
	Akotres S30	34.44	31.71	W27	0.50	98.0	75.2	63.2

Table 20. Viability of *Lactobacillus* bacteria in wafers after 3 months of storage.
*Weight of an average wafer without couverture coating is 28 g, and with couverture coating - 39 g.

Couverture	Type of fat	Fat content (%)	Sugar content (%)	Symbol of product	Lyophilisate content in final product (%)	Storage temperature		
						4°C	18°C	30°C
						The amount of live bacterial cells		
Non-coated	Efekt 40	40.44	25.71	W1	3.50	9.8×10^{9}	4.2×10^{8}	4.3×10^{7}
	Efekt 40	37.44	28.71	W2	3.50	8.6×10^{9}	3.3×10^{8}	4.4×10^{7}
	Efekt 40	34.44	31.71	W3	3.50	8.3×10^{9}	3.2×10^{8}	5.8×10^{7}
	Akomic 2000	40.44	25.71	W4	3.50	5.9×10^{9}	8.4×10^{8}	5.2×10^{7}
	Akomic 2000	37.44	28.71	W5	3.50	5.3×10^{9}	6.7×10^{8}	4.6×10^{7}
	Akomic 2000	34.44	31.71	W6	0.50	1.1×10^{9}	6.6×10^{7}	4.8×10^{6}
	Akotres S30	40.44	25.71	W7	0.50	1.2×10^{9}	4.6×10^{7}	4.5×10^{6}
	Akotres S30	37.44	28.71	W8	0.50	1.1×10^{9}	6.6×10^{7}	4.8×10^{6}
	Akotres S30	34.44	31.71	W9	0.50	1.4×10^{9}	5.2×10^{7}	5.4×10^{6}
	Efekt 40	40.44	25.71	W10	0.71	1.4×10^{9}	3.5×10^{7}	4.6×10^{6}
	Efekt 40	37.44	28.71	W11	0.71	1.7×10^{9}	3.5×10^{7}	4.1×10^{6}
	Efekt 40	34.44	31.71	W12	0.71	1.7×10^{9}	2.6×10^{7}	4.2×10^{6}
	Akomic 2000	40.44	25.71	W13	0.71	1.9×10^{9}	4.5×10^{7}	4.4×10^{6}
	Akomic 2000	37.44	28.71	W14	0.71	1.9×10^{9}	3.6×10^{7}	4.0×10^{6}
	Akomic 2000	34.44	31.71	W15	0.71	1.6×10^{9}	2.5×10^{7}	4.1×10^{6}
	Akotres S30	40.44	25.71	W16	0.71	1.3×10^{9}	2.2×10^{7}	3.1×10^{6}
	Akotres S30	37.44	28.71	W17	0.71	1.8×10^{9}	1.8×10^{7}	3.0×10^{6}
	Akotres S30	34.44	31.71	W18	0.71	1.8×10^{9}	3.4×10^{7}	2.6×10^{6}
Coated	Efekt 40	40.44	25.71	W19	0.50	1.9×10^{9}	1.0×10^{8}	4.8×10^{6}
	Efekt 40	37.44	28.71	W20	0.50	1.9×10^{9}	8.0×10^{7}	6.3×10^{6}
	Efekt 40	34.44	31.71	W21	0.50	2.0×10^{9}	4.1×10^{7}	6.0×10^{6}
	Akomic 2000	40.44	25.71	W22	0.50	1.5×10^{9}	1.7×10^{7}	2.8×10^{6}
	Akomic 2000	37.44	28.71	W23	0.50	1.4×10^{9}	2.0×10^{7}	3.8×10^{6}

	Akomic 2000	34.44	31.71	W24	0.50	1.5×10^{9}	2.1×10^{7}	3.2×10^{6}
	Akotres S30	40.44	25.71	W25	0.50	1.3×10^{9}	1.9×10^{7}	3.2×10^{6}
	Akotres S30	37.44	28.71	W26	0.50	1.4×10^{9}	3.0×10^{7}	2.8×10^{6}
	Akotres S30	34.44	31.71	W27	0.50	1.5×10^{9}	2.6×10^{7}	3.1×10^{6}

Table 21. The amount of live bacterial cells of Lactobacillus species in non-coated wafer, with a weight of 28 g, and in couverture coated wafer, with a weight of 39 g, after 3 months of storage.

Applications

Received probiotic product in a form of wafers interleaved with a mass supplemented with LAB had similar organoleptic properties, i.e. color, structure, exterior appearance, consistency, balanced taste and smell to wafers produced with a mass without lactic bacteria lyophilisate. Additional presence of bacterial preparation didn't influence in any significant manner water activity in products. Supplemented wafer products maintained proper conditions, which provided lactic acid bacteria with an environment, and allowed it to stay on a level, so that it can be considered to be a product with functional properties. In case creams used for interleaving wafers, besides proper organoleptic rating, they have to have certain textural properties, such as: adhesiveness, hardness and spreadability. In this regard supplemented masses were very similar to non-supplemented ones. According to above observations it can be concluded that it is safe to use masses supplemented with lactic acid bacteria for interleaving wafers, increasing this way health benefits of final wafer products.

5. Raisins coated with chocolate supplemented with live cultures of lactic acid bacteria

To receive raisins coated with chocolate sultana raisins from Iran and chocolate couvertures (dark, milk and white) from Union Chocolate (Żychlin, Poland) supplemented with lyophilized live cultures of lactic acid bacteria from *Lactobacillus* species on a level of 9×10^{10} CFU · g^{-1} were used. For polishing raisins in chocolate polishing agent was used, prepared according to a recipe: distilled water (56.4% w/w), citric acid (0.35% w/w), glucose-fructose syrup (5.275% w/w), saccharose (16.082% w/w), acacia gum (47.47% w/w), edible oil (0.3% w/w) and soy lecithin (0.1% w/w) [32].

In a table below results of analysis of Casson viscosity and yield value of couverture are presented.

Using chocolate couverture supplemented with LAB in an amount of 0.5% based on the weight of the product, at a level of about 50% in relation to raisin core, caused an increase in couverture viscosity. The increase was the highest when white couverture was LAB supplemented. Addition of lactic acid bacteria preparation also influenced the yield value of

couvertures. A significant drop of yield value was observed in white couverture supplemented with LAB. Smaller changes were noticed in dark couverture. Whereas in milk couverture yield value was practically the same.

Type of couverture	η_{CA} (Pa·s)	τ_{CA} (Pa)
Dark	**1.333** ± 0.023	**8.86** ± 0.28
Dark + LAB	**1.398** ± 0.011	**8.47** ± 0.09
White	**1.913** ± 0.008	**4.31** ± 0.19
White + LAB	**2.616** ± 0.032	**2.45** ± 0.02
Milk	**2.189** ± 0.009	**1.02** ± 0.02
Milk + LAB	**2.586** ± 0.047	**1.08** ± 0.01

Table 22. Casson viscosity (η_{CA}) and yield value (τ_{CA}) of couvertures.

5.1. Obtaining chocolate coated raisins

To receive chocolate coated raisins following procedure was used. Raisins were washed, dried at a temperature of 30°C, sorted according to size and placed in a coating drum heated previously to 25°C. Temperature was kept constant during the whole process of coating. Onto rotating in a drum raisins subsequent portions of tempered couverture with a temperature of 33°C were poured, total in an amount of 50% in relation to the weight of the product. In raisins in chocolate supplemented with lactic acid bacteria, before coating, to a couverture LAB lyophilisate in an amount of 0.8% based on the weight of the product, was added and stirred for 5 min to provide a full distribution. First portion of couverture was laid on raisins without the use of cool air stream. Latter layers of couverture were placed on raisins with cool air blowing on it while coating. The time of coating of one layer of couverture was 30 s. Total time of coating amounted to 65-150 min, depending on a temperature and air humidity. After coating raisins in chocolate were placed on sieves (in a single layer) and left for 24 hours to obtain a full solidification and consolidation of chocolate couverture structure. After 24 hours, ready product was polished in a spinning coating machine by gradually pouring portions of polishing agent onto it. After each polishing layer product was left in a spinning coating machine for 2 min with cool air blower turned off. After this period cool air was turned on again. Next layer of polishing agent was used when polished product was dry. After finished polishing process, dry chocolate coated raisins were placed on sieves for at least 2 hour period. Afterward, product was packed into plastic bags and kept for storage at temperatures of 4, 18 and 30°C for a period of 3 months. Analyses were performed at monthly intervals [11, 32].

In obtained raisins coated with chocolate supplemented with LAB and with normal (control) chocolate couverture content was established to verify the degree of stratification (which should be about 50%). Obtained results are presented in Table 23.

From obtained results of average percentage content of couverture in received raisins coated with chocolate it can be noticed, that this parameter was at a level of about 50% (w/w), as

planned. Raisins coated with supplemented white chocolate were coated in the smallest degree. It was probably caused by bigger losses in a coating machine.

Average content of couverture (%) in chocolate coated raisins					
Dark + LAB	**Dark**	**White + LAB**	**White**	**Milk + LAB**	**Milk**
50.47 ± 2.21	**49.68** ± 0.75	**46.36** ± 2.80	**49.99** ± 1.59	**50.55** ± 0.04	**50.64** ± 1.32

Table 23. Average percentage content of couverture in raisins with diffetent types of couverture.

5.2. Physicochemical analysis of chocolate coated raisins

Dry mass content in chocolate coated raisins

Results of dry mass analysis in chocolate coated raisins are placed in Table 24.

Storage time	Dry mass content (%) in chocolate coated raisins:					
	Dark + LAB	**Milk + LAB**	**White + LAB**	**Dark**	**Milk**	**White**
Control „0"	**92.85** ± 0.04	**92.72** ± 0.07	**92.45** ± 0.03	**92.15** ± 0.02	**91.80** ± 0.04	**92.76** ± 0.03
Stored at 4°C						
1 month	**91.56** ± 0.03	**91.80** ± 0.09	**91.43** ± 0.07	**92.23** ± 0.04	**91.85** ± 0.07	**91.76** ± 0.07
2 months	**92.38** ± 0.02	**91.24** ± 0.06	**92.33** ± 0.03	**92.69** ± 0.03	**92.33** ± 0.09	**92.03** ± 0.04
3 months	**92.36** ± 0.06	**92.84** ± 0.07	**92.93** ± 0.04	**92.69** ± 0.07	**93.20** ± 0.10	**92.23** ± 0.06
Stored at 18°C						
1 month	**91.70** ± 0.02	**91.90** ± 0.03	**90.64** ± 0.04	**93.04** ± 0.04	**91.56** ± 0.04	**92.19** ± 0.02
2 months	**92.70** ± 0.06	**92.53** ± 0.09	**92.56** ± 0.03	**92.52** ± 0.03	**91.95** ± 0.07	**92.60** ± 0.07
3 months	**93.12** ± 0.05	**92.46** ± 0.02	**92.97** ± 0.02	**92.91** ±0.06	**93.10** ± 0.03	**92.47** ± 0.05
Stored at 30°C						
1 month	**92.23** ± 0.02	**93.00** ± 0.07	**92.28** ± 0.02	**92.93** ± 0.09	**92.44**± 0.06	**92.08** ± 0.03
2 months	**93.89** ± 0.04	**93.66** ± 0.04	**94.47** ± 0.04	**92.99** ± 0.04	**93.66** ± 0.02	**93.76** ± 0.05
3 months	**94.41** ± 0.03	**94.25** ± 0.02	**94.73** ± 0.03	**94.47** ± 0.07	**94.95** ± 0.05	**94.18** ± 0.04

Table 24. Dry mass content (%) in chocolate coated raisins, received with the use of different types of couverture **supplemented**, and as a comparison **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C for 3 months.

Directly after obtaining the biggest dry mass content was noticed in raisins coated with dark chocolate supplemented with LAB.

Supplementation of dark and milk couvertures caused an increase of dry mass content in final products, comparing to analogous products with non-supplemented couvertures. In case of raisins coated with white couverture supplemented with LAB, dry mass content was slightly smaller than in non-supplemented one, namely by about 0.3% percentage point. During storage dry mass content in chocolate coated raisins changed. Usually after slight decrease after the first month, dry mass content increased during latter storage month. Higher temperature used during storage of supplemented raisins in chocolate caused an

increase of dry mass content, regardless of the type of couverture used for coating (dark, white, milk).

Water activity in chocolate coated raisins

Results of water activity (a_w) in chocolate coated raisins are presented in Table 25.

Storage time		Water activity in chocolate coated raisins:					
		Dark + LAB	**Milk + LAB**	**White + LAB**	**Dark**	**Milk**	**White**
Control „0"	whole	**0.427** ± 0.001	**0.408** ± 0.001	**0.414** ± 0.001	**0.414** ± 0.002	**0.420** ± 0.011	**0.386** ± 0.003
	crushed	**0.472** ± 0.007	**0.474** ± 0.003	**0.507** ± 0.004	**0.486** ± 0.003	**0.510** ± 0.020	**0.389** ± 0.007
Stored at 4°C							
1 month	whole	**0.491** ± 0.002	**0.457** ± 0.002	**0.480** ± 0.001	**0.476** ± 0.003	**0.429** ± 0.001	**0.390** ± 0.001
	crushed	**0.535** ± 0.009	**0.496** ± 0.004	**0.490** ± 0.007	**0.512** ± 0.012	**0.512** ± 0.006	**0.550** ± 0.004
2 months	whole	**0.540** ± 0.001	**0.433** ± 0.001	**0.499** ± 0.001	**0.523** ± 0.001	**0.533** ± 0.004	**0.490** ± 0.003
	crushed	**0.527** ± 0.004	**0.524** ± 0.009	**0.522** ± 0.004	**0.533** ± 0.007	**0.563** ± 0.008	**0.537** ± 0.013
3 months	whole	**0.545** ± 0.001	**0.541** ± 0.001	**0.514** ± 0.004	**0.550** ± 0.001	**0.490** ± 0.005	**0.510** ± 0.001
	crushed	**0.521** ± 0.003	**0.565** ± 0.004	**0.547** ± 0.009	**0.545** ± 0.009	**0.524** ± 0.011	**0.527** ± 0.019
Stored at 18°C							
1 month	whole	**0.494** ± 0.001	**0.460** ± 0.001	**0.483** ± 0.002	**0.416** ± 0.001	**0.426** ± 0.004	**0.358** ± 0.003
	crushed	**0.546** ± 0.001	**0.488** ± 0.003	**0.493** ± 0.007	**0.504** ± 0.014	**0.513** ± 0.009	**0.532** ± 0.004
2 months	whole	**0.514** ± 0.002	**0.416** ± 0.001	**0.497** ± 0.001	**0.505** ± 0.001	**0.532** ± 0.001	**0.498** ± 0.001
	crushed	**0.520** ± 0.003	**0.511** ± 0.002	**0.522** ± 0.011	**0.521** ± 0.006	**0.541** ± 0.017	**0.529** ± 0.007
3 months	whole	**0.535** ± 0.001	**0.555** ± 0.002	**0.551** ± 0.001	**0.566** ± 0.001	**0.539** ± 0.001	**0.555** ± 0.004
	crushed	**0.516** ± 0.007	**0.548** ± 0.009	**0.552** ± 0.007	**0.541** ± 0.008	**0.528** ± 0.011	**0.521** ± 0.003
Stored at 30°C							
1 month	whole	**0.397** ±	**0.396** ±	**0.400** ±	**0.415** ± 0.06	**0.358** ±	**0.345** ±

		0.007	0.009	0.001		0.001	0.004
	crushed	**0.499** ± 0.011	**0.463** ± 0.003	**0.477** ± 0.006	**0.495** ± 0.014	**0.472** ± 0.003	**0.513** ± 0.002
2 months	whole	**0.406** ± 0.003	**0.295** ± 0.004	**0.404** ± 0.004	**0.476** ± 0.050	**0.481** ± 0.005	**0.493** ± 0.001
	crushed	**0.495** ± 0.007	**0.451** ± 0.004	**0.461** ± 0.002	**0.483** ± 0.020	**0.490** ± 0.005	**0.474** ± 0.011
3 months	whole	**0.433** ± 0.002	**0.478** ± 0.002	**0.469** ± 0.002	**0.472** ± 0.070	**0.457** ± 0.002	**0.462** ± 0.001
	crushed	**0.456** ± 0.004	**0.487** ± 0.007	**0.488** ± 0.005	**0.480** ± 0.009	**0.469** ± 0.007	**0.469** ± 0.009

Table 25. Water activity in chocolate coated raisins, received with the use of different types of couverture **supplemented**, and as a comparison **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C for 3 months.

Raisins coated with dark and milk couverture without LAB addition showed similar water activity values (for whole chocolate coated raisins). Slightly lower value of a_w had raisins coated with white couverture. Supplementation of couvertures with lactic acid bacteria only very slightly increased the values of a_w in final products, obtained with the use of dark and milk couvertures. More noticeable increase of a_w – from 0.389 to 0.414 was observed for raisins coated with white couverture.

During storage of raisins coated with all types of couverture supplemented with LAB at refrigeration and room temperatures water activity increased (whole raisins in chocolate). Only at higher storage temperature of 30°C water activity was decreasing for 2 months of storage to finally increase during third month. Similar changes of a_w during storage were observed for raisins coated with non-supplemented couverture. A difference was noticed for a_w changes of chocolate coated raisins stored at 30°C, in which during first month of storage a_w decreased, and during following months of storage rose to values higher than in initial samples (directly after production).

Water activity in crushed products was generally higher comparing to the values of this parameter analyzed in a whole product. Comparing water activity values in whole and crushed raisins coated with chocolate, obtained with the use of supplemented with LAB and non-supplemented couvertures – dark, milk and white, directly after production and during 3 months of storage at temperatures of 4, 18 and 30°C it can be concluded that they kept under the value of 0.6. Due to that fact, it is probable that during the whole time of storage no bacterial activity in both, supplemented and non-supplemented with LAB, will be maintained.

Total acidity in chocolate coated raisins

Total acidity changes of chocolate coated raisins during 3 months of storage in various temperatures is presented in Table 26.

Storage time	Total acidity (ml 1 M NaOH · 100 g^{-1}) in chocolate coated raisins:					
	Dark + LAB	Milk + LAB	White + LAB	Dark	Milk	White
Control „0"	**17.0** ± 0.1	**15.0** ± 0.2	**14.2** ± 0.2	**17.7** ± 0.8	**14.9** ± 0.5	**13.6** ± 0.1
Stored at 4°C						
1 month	**15.6** ± 0.4	**14.8** ± 0.2	**14.2** ± 0.1	**16.2** ± 0.4	**15.7** ± 0.1	**14.0** ± 0.2
2 months	**16.1** ± 0.2	**13.9** ± 0.2	**14.1** ± 0.1	**15.5** ± 0.2	**15.1** ± 0.2	**14.0** ± 0.1
3 months	**15.3** ± 0.1	**15.1** ± 0.2	**14.2** ± 0.2	**14.9** ± 0.1	**16.8** ± 0.2	**13.5** ± 0.2
Stored at 18°C						
1 month	**16.6** ± 0.1	**14.8** ± 0.3	**13.2** ± 0.3	**16.7** ± 0.1	**14.7** ± 0.1	**13.8** ± 0.3
2 months	**15.7** ± 0.3	**13.6** ± 0.3	**14.1** ± 0.2	**14.8** ± 0.2	**14.5** ± 0.1	**13.4** ± 0.1
3 months	**16.0** ± 0.1	**13.1** ± 0.3	**13.1** ± 0.1	**15.5** ± 0.2	**15.2** ± 0.1	**14.4** ± 0.2
Stored at 30°C						
1 month	**15.2** ± 0.2	**14.6** ± 0.1	**14.6** ± 0.2	**15.8** ± 0.2	**15.1** ± 0.1	**13.8** ± 0.3
2 months	**14.3** ± 0.2	**14.1** ± 0.2	**15.1** ± 0.2	**14.0** ± 0.2	**15.7** ± 0.1	**13.8** ± 0.2
3 months	**14.8** ± 0.2	**12.7** ± 0.1	**13.9** ± 0.1	**15.0** ± 0.1	**15.9** ± 0.1	**13.6** ± 0.1

Table 26. Total acidity in chocolate coated raisins, received with the use of different types of couverture **supplemented**, and as a comparison **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C for 3 months.

Directly after obtaining the highest total acidity, amounting 17.7 ml 1 M NaOH · 100 g^{-1}, had raisins coated with dark couverture non-supplemented with LAB, and the lowest, amounting 13.6 ml 1 M NaOH · 100 g^{-1}, was observed in raisins coated with white couverture.

Supplementation of dark, milk and white couvertures with bacteria from *Lactobacillus* species didn't influence significantly the total acidity of products after production. The biggest changes of this parameter after supplementation of couverture with LAB, namely by 0.7 ml 1 M NaOH · 100 g^{-1}, were noticed in raisins coated with dark couverture.

In raisins coated with white and milk couvertures total acidity after supplementation with LAB increased by 0.1 and 0.6 ml 1 M NaOH · 100 g^{-1}, respectively.

During storage of chocolate coated raisins only slight decrease of total acidity was observed. With an exception in product containing milk couverture non-supplemented with LAB, in which total acidity increased by 1 ml 1 M NaOH · 100 g^{-1} after 3 months of storage at a temperature of 30°C. The magnitude of total acidity changes in chocolate coated raisins depended on storage temperature.

In raisins coated with dark, white and milk couverture supplemented with LAB total acidity decrease during storage. The biggest decrease of this parameter was noticed in chocolate coated raisins stored at 30°C and in products in dark, milk and white couverture supplemented with LAB was 2.2, 2.3 and 0.3 ml 1 M NaOH · 100 g^{-1}, respectively.

Analysis of fat quality in a coating of chocolate coated raisins by DSC method

In obtained chocolate coated raisins, analyses of changes occurring in fat from a couverture (which is a coating of the product), by differential scanning calorimetry method were performed. These changes are presented in tables 30 and 31. Exemplary thermograms of fats from dark couverture supplemented and non-supplemented with LAB, which are a coating of chocolate coated raisins, stored during 3 months period at a temperature of 18°C are presented in Figures 8 and 9, respectively.

Storage time	**Enthalpy and melting temperature of fat from coatings of chocolate coated raisins:**					
	Dark + LAB		**Milk + LAB**		**White + LAB**	
	ΔH (J/g)	Tm (°C)	ΔH (J/g)	Tm (°C)	ΔH (J/g)	Tm (°C)
Control „0"	23.014	34.30	22.906	34.30	24.020	34.20
Stored at 4°C						
1 month	35.480	34.78	16.530	34.51	22.357	34.47
2 months	32.550	35.00	20.794	32.03	27.496 4.487 + 23.009	Tm_1=30.58 Tm_2=34.54
3 months	25.261	34.51	25.218	34.03	33.653	34.15
Stored at 18°C						
1 month	35.742	34.54	24.230	33.94	17.124	34.73
2 months	34.312	34.77	22.906	33.46	25.591	34.00
3 months	29.820 16.564 + 13.255	Tm_1=32.47 Tm_2=34.10	34.744	32.48	33.698	34.74
Stored at 30°C						
1 month	34.000	36.45	16.353	34.96	29.053	35.08
2 months	32.475 2.440 + 30.034	Tm_1=29.57 Tm_2=35.82	18.786 7.561+11.229	Tm_1=31.48 Tm_2=35.12	23.137	35.60
3 months	36.578 22.555+14.022	Tm_1=33.01 Tm_2= 4.57	24.102 5.988+18.113	Tm_1=29.67 Tm_2=34.42	35.622 8.977+26.645	Tm_1=31.11 Tm_2=35.34

Table 27. Enthalpy (ΔH) and melting temperature (Tm) of fat from coatings of chocolate coated raisins obtained with the use of different types of couvertures **supplemented** with LAB, stored at temperatures of 4, 18 and 30°C during 3 months.

In samples of chocolate coated raisins directly after production the value of melting enthalpy of fat extracted from product coating, in all types of couvertures supplemented with lactic acid bacteria was lower than in analogous products coated with non-supplemented couverture. This phenomena can be explained by the fact, that LAB preparation influenced fat crystallization, namely in supplemented couverture more liquid phase of fat was present than in analogous products without LAB.

During storage of chocolate coated raisins melting enthalpy value of fat from coatings of dark and white chocolate coated raisins, increased regardless of storage conditions. It can be caused by crystallization of previously present sources of crystallization or by increasing the area of already existing fat crystals.

Storage time	Enthalpy and melting temperature of fat from coatings of chocolate coated raisins:					
	Dark		Milk		White	
	ΔH (J/g)	Tm (°C)	ΔH (J/g)	Tm (°C)	ΔH (J/g)	Tm (°C)
„0" control	30.157	35.51	27.559	34.34	34.93	34.25
Stored at 4°C						
1 month	31.568	35.90	25.400	33.94	22.026	34.61
2 months	33.064 0.911+32.153	Tm_1= 28.64 Tm_2= 35.56	33.148	34.57	31.872	35.03
3 months	37.718	34.25	23.874 3.744+20.129	Tm_1=30.28 Tm_2=33.69	37.027	34.93
Stored at 18°C						
1 month	36.765	34.54	26.622	33.57	32.684	34.86
2 months	31.847	36.20	28.173 1.580+26.59	Tm_1=26.84 Tm_2=34.17	33.455	34.61
3 months	38.068 25.119+12.917	Tm_1= 32.32 Tm_2= 34.54	34.592	32.48	34.568 13.273+21.294	Tm_1= 31.64 Tm_2= 34.75
Stored at 30°C						
1 month	36.373	35.35	24.059 5.225+18.834	Tm_1=31.29 Tm_2=34.86	21.614	35.31
2 months	29.962 0.741+29.22	Tm_1=29.14 Tm_2=35.94	18.764 5.244+13.519	Tm_1=32.39 Tm_2=35.06	31.425	36.07
3 months	33.30 4.558+28.743	Tm=31.04 Tm=35.95	24.259 6.087+18.171	Tm_1=29.69 Tm_2=34.43	26.953 3.650+23.302	Tm_1= 31.65 Tm_2= 35.71

Table 28. Enthalpy (ΔH) and melting temperature (Tm) of fat from coatings of chocolate coated raisins obtained with the use of different types of couvertures **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C during 3 months.

Temperature and storage time of chocolate coated raisins had an influence on changes of polymorphic forms of fat from couverture coating products. In coatings without LAB addition, bigger tendency to two polymorphic forms creation was observed, mainly in raisins stored at temperatures of 18 and 30°C. The range of maximal melting temperatures of first polymorphic form of fat from couverture from chocolate coated raisins non-supplemented with LAB was from 26.80 to 32.30°C. Whereas for second polymorphic form it ranged from 33.69 to 35.95°C. Range of melting temperatures of fat from couverture from chocolate coated raisins supplemented with LAB, for first polymorphic form was from 29.57 to 33.01°C, and for second form, from 34.10 to 38.82°C. Range of melting temperatures of

first and second polymorphic forms of fat was similar in raisins coated with both, supplemented and non-supplemented couvertures. At a temperature of 30°C in dark and milk couvertures both, supplemented and non-supplemented, appearance of second polymorphic form of fat was observed after 2 months of storage. In white couverture appearance of second polymorphic form was noticed after 3 month storage of product. In can concluded that lack of cocoa liquor and bigger content of milk in a white couverture delayed polymorphic changes of fats in this couverture.

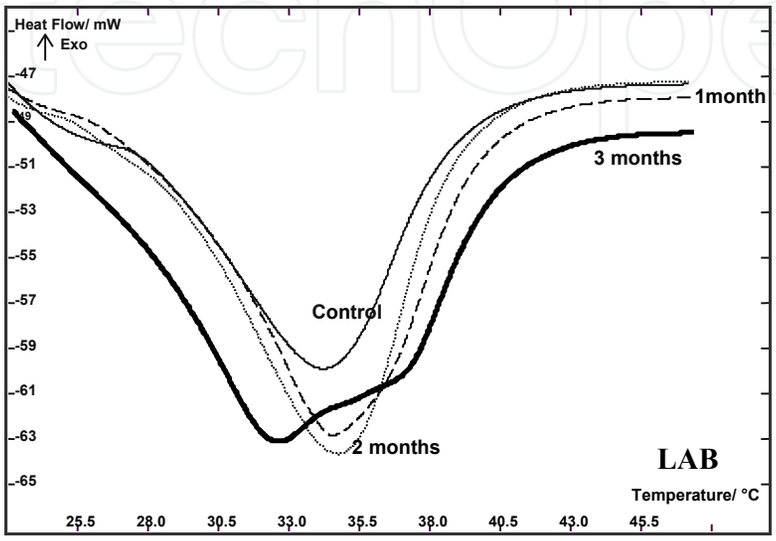

Figure 8. Thermogram of fat from coatings of chocolate coated raisins obtained from dark couverture **supplemented** with LAB stored at a temperature of 18°C during 3 months.

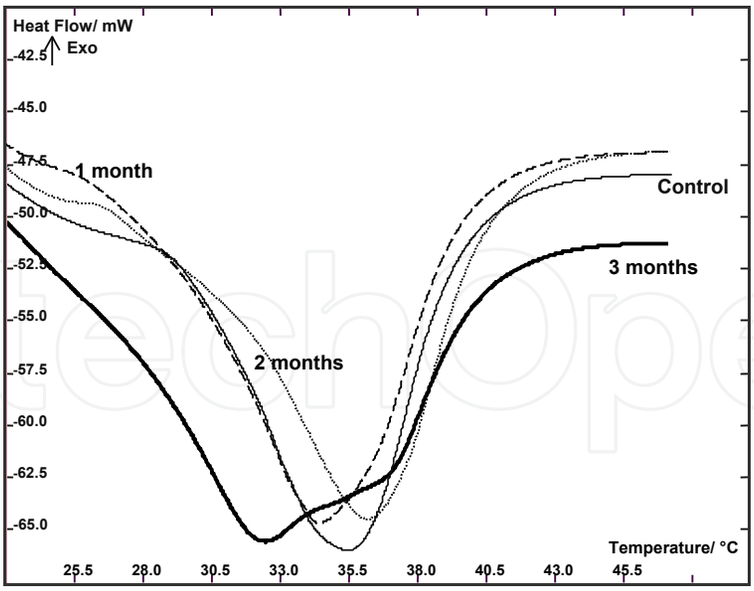

Figure 9. Thermogram of fat from coatings of chocolate coated raisins obtained from dark couverture **non-supplemented** with LAB stored at a temperature of 18°C during 3 months.

Texture of chocolate coated raisins

In Table 29 results of cutting test of chocolate coated raisins are presented.

Dark and milk chocolate coated raisins supplemented with lactic acid bacteria became harder. On the other hand raisins in white chocolate after supplementation with LAB softened.

During storage, from all supplemented products, raisins coated with white couverture became the hardest. During storage of all studied chocolate coated raisins (with and without LAB addition) at temperatures of 4 and 18°C hardness gradually decreased, which can be associated with water diffusion. During storage of supplemented and non-supplemented chocolate coated raisins at temperature of 30°C hardness initially rose (drying of surface), next it decreased (water diffusion from raisin to coating and from environment into product), and finally to increase after third month. Additionally hardness of supplemented products was higher than hardness analyzed directly after production. With an exception of raisins coated with white chocolate, in which hardness was significantly lower than in fresh products.

Storage time	**Force (kg) required to cut chocolate coated raisins:**					
	Dark + LAB	**Milk + LAB**	**White + LAB**	**Dark**	**Milk**	**White**
Control „0"	3.002	2.949	3.781	2.777	2.850	3.861
Stored at 4°C						
1 month	3.005	3.125	3.531	2.461	2.596	2.344
2 months	2.821	2.731	2.424	2.247	2.492	2.067
3 months	2.078	2.068	2.329	2.227	2.271	1.838
Stored at 18°C						
1 month	2.712	3.007	3.319	2.023	3.110	1.898
2 months	2.512	2.597	2.785	1.772	2.316	1.723
3 months	2.125	2.136	2.015	2.180	2.527	2.037
Stored at 30°C						
1 month	3.010	3.154	3.337	2.832	3.480	2.156
2 months	2.786	2.727	2.751	2.314	3.321	2.139
3 months	3.068	3.404	3.608	2.649	2.641	2.413

Table 29. Force required to cut chocolate coated raisins received with the use of different types of couverture **supplemented**, and as a comparison **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C for 3 months.

Organoleptic evaluation of chocolate coated raisins

In received raisins coated with couverture supplemented with LAB organoleptic analysis was performed, according to a 5-point scale, and it was compared to products obtained with non-supplemented courevture (Table 30).

The highest note in a 5-point scale received raisins coated with white chocolate and then with milk couverture. Lowest ratings (below 4 points) received raisins coated with dark chocolate both, fresh and during the whole storage period, regardless of LAB supplementation. To high grade of raisins coated with white couverture was caused by their

delicate, gentle taste, and soft, elastic consistency. Addition of lactic acid bacteria to couvertures coating raisins didn't influence significantly sensory properties of products, which is favorable from the point of view of a consumer, who highly appreciates sensory quality of chocolate. However, although in first month of storage of chocolate coated raisins stored at all temperatures organoleptic rating didn't change, in latter months this parameter degraded, especially when stored at 30°C. It was caused by the changes occurring in products during storage. Most noticeably these changes were observed in chocolate coated raisins stored at 30°C. They included changes of taste, caused by modifications of fat in a coating, an increase of dry mass content in cores (raisins), increase of hardness of raisins coated with dark and milk chocolates, connected to an increase of dry mass content in chocolate couvertures and surfaces of products, which became less shiny with time.

Organoleptic ratings of raisins coated with dark, milk and white chocolates stored at temperatures of 4 and 18°C were practically identical in a first month of storage (differences of 0.0 – 0.1 points) comparing to fresh product, they were slightly different after second month (by 0.0 – 0.2 points) and third month (0.0 – 0.1 points) of storage. In case of 3 month storage period of non-supplemented products, differences in organoleptic evaluation between fresh product and product stored for a 3 month period were more noticeable and reached 0.7 points. Considering similar organoleptic evaluation of raisins coated with chocolate stored at 4 and 18°C it can be concluded, that examined raisins coated with chocolate don't have to be kept at refrigeration conditions and can be stored at a store shelf as well, where they can easily be found by a consumer next to analogous traditional products.

Storage time	Grades (points) of chocolate coated raisins:					
	Dark + LAB	**Milk + LAB**	**White + LAB**	**Dark**	**Milk**	**White**
Control „0"	**4.0** ±0.1	**4.2** ± 0.1	**4.7** ± 0.2	**3.9** ± 0.0	**4.0** ± 0.1	**4.6** ± 0.1
Stored at4°C						
1 month	**3.7** ± 0.1	**4.1** ± 0.1	**4.6** ± 0.3	**3.9** ± 0.2	**3.9** ± 0.2	**4.5** ± 0.3
2 months	**3.6** ± 0.2	**4.1** ± 0.1	**4.6** ± 0.1	**3.8** ± 0.2	**3.9** ± 0.1	**4.6** ± 0.1
3 months	**3.1** ± 0.0	**3.9** ± 0.1	**4.5** ± 0.2	**3.6** ± 0.1	**3.8** ± 0.2	**4.5** ± 0.2
Stored at18°C						
1 month	**3.8** ± 0.2	**4.0** ± 0.2	**4.6** ± 0.2	**3.8** ± 0.2	**3.9** ± 0.1	**4.5** ± 0.3
2 months	**3.7** ± 0.2	**4.0** ± 0.1	**4.5** ± 0.2	**3.7** ± 0.2	**3.8** ± 0.2	**4.3** ± 0.2
3 months	**3.0** ± 0.1	**3.7** ± 0.2	**4.4** ± 0.3	**3.4** ± 0.1	**3.1** ± 0.3	**4.0** ± 0.2
Stored at30°C						
1 month	**3.9** ± 0.2	**4.1** ± 0.2	**4.6** ± 0.2	**3.9** ± 0.1	**3.8** ± 0.2	**4.5** ± 0.1
2 months	**3.7** ± 0.2	**3.9** ± 0.3	**4.4** ± 0.2	**3.4** ± 0.2	**3.7** ± 0.1	**4.2** ± 0.2
3 months	**2.8** ± 0.3	**3.6** ± 0.2	**3.5** ± 0.2	**2.9** ± 0.2	**3.0** ± 0.1	**3.4** ± 0.2

Table 30. Organoleptic evaluation of chocolate coated raisins received with the use of different types of couverture **supplemented**, and as a comparison **non-supplemented** with LAB, stored at temperatures of 4, 18 and 30°C for 3 months.

Viability of LAB in a product

The biggest viability of *Lactobacillus* bacteria in products after 3 months of storage was observed when stored at a refrigeration temperature (4°C). The highest viability was observed in raisins coated with dark (88.9%) and white (88.0%) chocolate, slightly lower was noticed in raisins coated with milk chocolate (86.5%) (Table 31). In products stored at a temperature of 18°C amount of live bacterial cells was lower by two orders of magnitude, amounting 10^5 CFU · g^{-1}. When products were stored at a stress temperature (30°C) a severe decrease in an amount of live cells was observed, even just after one month of storage. The biggest drop in LAB viability was observed in raisins coated with white chocolate, to 58.6%, next in raisins coated with milk chocolate, to 59.7%, and finally in raisins coated with dark chocolate viability lowered to 61.3%.

Sample	**Storage temperature**		
	4°C	18°C	30°C
	Viability of bacteria (%)		
Raisins coated with milk chocolate	**88.0** ± 2.3	**73.1** ± 3.0	**59.7** ± 3.9
Raisins coated with dark chocolate	**88.9** ± 3.0	**72.7** ± 3.8	**61.3** ± 4.0
Raisins coated with white chocolate	**86.5** ± 2.7	**66.3** ± 2.0	**58.6** ± 4.0

Table 31. Viability of *Lactobacillus* bacteria in chocolate coated raisins after 3 months of storage.

Sample	**Storage temperature**		
	4°C	18°C	30°C
	The amount of live bacterial cells in the product (CFU · 80 g^{-1})		
Raisins coated with milk chocolate	3.4×10^8	2.5×10^7	2.5×10^6
Raisins coated with dark chocolate	3.8×10^8	2.3×10^7	3.2×10^6
Raisins coated with white chocolate	2.7×10^8	8.0×10^6	2.1×10^6

Table 32. The amount of live bacterial cells of *Lactobacillus* species in chocolate coated raisins, with a weight of 80g, after 3 months of storage.

The worst *Lactobacillus* bacteria viability, at all storage temperatures, was observed in raisins coated with white couverture. Storage of raisins coated with dark, white and milk couvertures supplemented with *Lactobacillus* bacteria, at a temperature of 4°C provides a maintenance of probiotic properties of these products. Temperature of 18°C, in case of raisins coated with dark and milk couvertures, also prevents them from loosing probiotic properties during storage. Storage of those products in this temperature allows to maintain high lactic bacteria viability during the whole storage period, namely 3 months.

Consuming a package of chocolate coated raisins, with a weight of 80 g, stored at 4°C provides a consumer with 10^8 CFU of probiotic bacteria. The same package of raisins coated with dark and milk chocolate stored at a temperature of 18°C contains 2.3×10^7 CFU · 80 g^{-1} and 2.5×10^7 CFU · 80 g^{-1}, respectively, while raisins coated with white chocolate an amount of 8.02×10^6 CFU · 80 g^{-1} of final product (Table 32). After storage of chocolate coated raisins at 30°C consumed amount of lactic acid bacteria would amount to a level of 10^6 CFU · 80 g^{-1} of final product, and would be below recommended level (10^7 CFU · g^{-1}) for functional food.

6. Summary

Proposed technology enables to introduce to dark, white and milk couvertures, live cultures of lactic acid bacteria (as a lyophilisate) and to use them for obtaining raisins coated with chocolate, characterized by soft consistency.

Results of research and development project indicated what follows:

- Addition of live cultures of lactic acid bacteria to dark and milk couvertures caused a slight increase of dry mass if products, and in raisins coated with white couverture a slight lowering of this parameter.
- LAB supplementation of couvertures used for coating of raisins, only mildly increased the value of water activity in products coated with dark and milk couverture. Bigger increase of a_w was noticed for raisins coated with white couverture.
- Supplementation with lactic acid bacteria of dark, white and milk couvertures didn't influence acidity of fresh products, and during storage this parameter decreased only slightly.
- Temperature and storage time of chocolate coated raisins influenced changes of polymorphic forms of fat from couvertures used for coating of raisins. Fat from supplemented and non-supplemented with LAB couvertures was characterized by similar ranges of melting temperatures of both, first and second polymorphic form.
- Raisins coated with dark and milk couvertures after supplementation with LAB had higher hardness values than raisins coated with analogous non-supplemented couvertures. While raisins coated with white couverture after LAB supplementation

became softer. During storage at temperatures of 4 and 18°C raisins coated with dark, milk and white couvertures supplemented with LAB gradual decrease in hardness was observed. After 2 months of storage products were softer than when they were fresh.

- LAB supplementation of couvertures used for coating raisins practically didn't affect organoleptic properties of received products. The best rating in a 5-point scale received raisins coated with white couverture, next notes belonged to products in milk and dark couverture.
- Raisins coated in chocolate supplemented with LAB can be stored and exhibited in a store at room temperature. Number of live bacterial cells in products during whole storage period remained at a functional level.

7. Conclusion

Viability of lactic acid bacteria in some confectionery products appears to be unexpectedly high. It is caused by low moisture content in products mentioned in this chapter, as well as reqiured water activity (below 0.6), high concentration of carbohydrates, mainly saccharose and limited access of oxygen. However, viability depends mainly on recipe of product (mainly the type of fat), technological processes used for obtaining products and the time of these processes, and finally storage conditions.

Many solutions for application of lactic acid bacteria to confectionery, pastry and other kinds of products, cited in this chapter, is the subject of patent protection.

Application of bacteria in a form of preserved preparation, in which live cells are put in a state of anabiosis, allows to maintain high viability of LAB in confectionery products during storage. LAB addition to confectionery products - a type of food often consumed by kids and youth, allows to enrich the diet of this group of consumers with probiotic products with taste similar to traditional products, which are also ready for distribution and sale analogous to products without addition of bacterial preparations, not requiring refrigeration temperatures and hence being always "within reach".

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