

ORIGINAL RESEARCH

Synbiotic yoghurt with walnut and cereal brittle added as a next-generation bioactive compound: Development and characteristics

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Abstract

The article presents a technology developed for the production of synbiotic Yoghurt with new bioactive filler based on natural components. The Yoghurt has prebiotic and sorption properties. A higher consumer appeal of the product developed has been substantiated; its characteristics compared with the Yoghurt of traditional production technology have been presented. The brittle, containing peeled walnuts, as well as barley, wheat, rye, oatmeal and buckwheat flakes, sugar, and water, was used as a filler. Optimum time and temperature regimes of boiling caramel mixtures and brewing raw walnut-cereal mass in the brittle have been selected. The formulation developed enables increasing the nutritional and biological values of the finished product. The research studies of the finished product involved an analysis of organoleptic, physicochemical, and microbiological points. When performing the tasks, the approved regulatory and technical documentation (GOST) was applied. Each measurement was carried out in triplicate. The physicochemical characteristics of the samples developed were compared with the requirements for the quality of fermented milk products. The nutritional and biological values were calculated. The increase in consumer properties, and nutritional and biological values of the finished dairy product was scientifically substantiated and experimentally confirmed.

KEYWORDS

bioavailability, meal plan models, micronutrient deficiency, nutrient intake, scaling up nutrition, supplementation

1 | INTRODUCTION

For millennia, microbes have been used in the production of food and beverages; the technology involves the fermentation process. In recent years, the attention of scientists has been attracted by

identifying fundamental mechanisms and influence patterns of food and beverages on the human body, and as a consequence, the options of their use in the food industry for preventive and therapeutic purposes. As a result, the world community has got a new term "probiotics." Lactic acid bacteria (LAB) are normal microflora in the

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intestines of mammals (Markowiak & Slizewska, 2018; Young, 1998). They play an important role in the process of vital activity and act as an immunomodulator. The LAB are widely used for prevention and treatment of gastrointestinal diseases, as well as for improvement of digestion and more complete absorption of nutrients. Probiotic microorganisms include lactic acid bacteria such as *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, and *L. rhamnosus*. These applied live bacteria promote the development of the immune response and distribution of the vaccine component in the body. Complexes of pathogenic and nonpathogenic food microbes are currently evaluated as candidates for oral vaccines. Intestinal microflora plays a crucial role in the metabolism of various nutrient substrates in the vital activity. There are numerous studies of pre-, pro-, and synbiotics, confirming clinical efficiency in maintaining the balance of the gastrointestinal microbiota to improve health (Nakagawa, Yabuuchi, Yasokawa, & Nagashima, 2005; Quigley, 2019; Singh, Amdekar, Yadav, Mishra, & Jain, 2011; Sukhikh, Krumlikov, Evsukova, & Asyakina, 2017; Vyas & Ranganathan, 2012). Prebiotics are known to be food components that are not digested and absorbed in the upper gastrointestinal tract, since gastric enzymes cannot act on them; but they are fermented by the microflora of the human large intestine and stimulate its growth and vital activity. They reach the colon intact and are selectively fermented to have a beneficial effect. Probiotics are live cultures of microorganisms and substances of microbial and other origin. Synbiotics are combined bacterial preparations containing prebiotics and probiotics and belong to the innovation group of biologics (Mohanty, Mishra, Mohapatra, & Sahu, 2018; Roberfroid, 2000; de Vrese & Schrezenmeir, 2008). A bioactive compound is a simple substance that has a biological activity associated with its ability to modulate one or more metabolic processes, which leads to improved health (Angiolillo, Nobile, & Conte, 2015; Watson & Preedy, 2015).

One of the most important tasks in addressing the food safety and nutrition issues and achieving the goals of sustainable development is the elimination of all forms of malnutrition (FAO et al., 2017). In 2016, the decade of the UN action on nutrition began (2016–2025).

In Russia, in the framework of the Order "On basic principles of state policy in the field of healthy nutrition of the population of the Russian Federation for the period of up to 2020," there were some improvements noted in the area of nutrition of the population due to changes in the structure of food consumption, that

Highlights

- A higher consumer appeal of the product developed has been substantiated; its characteristics compared with the Yoghurt of traditional production technology have been presented.

is, increasing the share of specialized meat and dairy products (The order of the Government of the Russian Federation dated 25.10.10). So, obvious is the relevance of expanding the range of functional fermented milk products that improve digestion, quickly eliminate hunger, and are a source of micro- and macro-nutrients (McAuliffe, Kilcawley, & Stefanovic, 2019; Slozhenkina, Druker, Kryuchkova, & Zlobina, 2018; Slozhenkina, Serova, Vodolazkova, & Zlobina, 2017). Among the variety of dairy products, Yoghurts can be considered the most common and attractive for the consumer; their advantages, in particular, include light texture, pleasant organoleptic characteristics, and various fillers and functional ingredients that are able to be added to the production technology and increase the biological and nutritional value of the product (Mohammadi-Sartang et al., 2018; Sivieri et al., 2017). Nontraditional components such as inulin (Mazloomi, Shekarforoush, Ebrahimnejad, & Sajedianfard, 2011), fructooligosaccharides (Madhu, Amrutha, & Prapulla, 2012), oat/barley beta-glucan (Ladjevardi, Yarmand, Emam-Djomeh, & Niasari-Naslaji, 2016; Vasiljevic, Kealy, & Mishra, 2007), different kinds of rice (Kumari, Ranadheera, Prasanna, Senevirathne, & Vidanarachchi, 2015; Wattananapakasem, Valenberg, Fogliano, Costabile, & Suwannaporn, 2018), pineapple peel powder (Sah, Vasiljevic, McKechnie, & Donkor, 2016), Lesser Yam Tubers (*Dioscorea esculenta* L.) (Winarti & Saputro, 2015), probiotics and prebiotics (Hill, Ross, Arendt, & Stanton, 2017), red ginger extract (Larasati, Panunggal, Afifah, Anjani, & Rustanti, 2017), Binahong Leaf Extract (Lestari, Nissa, Afifah, Anjani, & Rustanti, 2017), and Purple Sweet Potato (Tari, Handayani, & Hartati, 2018) are known to be used as fillers for the production of synbiotic yoghurt.

The authors proposed a recipe and developed a technology for the production of a new synbiotic Yoghurt using a nontraditional filler, that is, brittle of walnut and five cereal flakes (barley, wheat, rye, oats, and buckwheat) as a prebiotic component and plant sorbent (Figure 1).



FIGURE 1 Raw materials for enriched product: (a) walnuts; (b) cereal flakes; (c) sugar; (d) water; and (e) milk

2 | MATERIALS AND METHODS

2.1 | The samples of raw materials and the production technology

The set yoghurt was produced in accordance with the traditional technological scheme (Tamime & Robinson, 2007) that includes the following steps: delivery of raw materials; filtering, cooling, and storage; heating, standardization, and homogenization; pasteurization and recooling; fermentation; filling, packaging, and labeling; souring and recooling; and storage (Figure 2 and Table 1).

At the fermentation stage, dry bacterial starter culture was used to produce dairy products based on *Lactobacillus bulgaricus*, *Lactobacillus casei*, and *Streptococcus thermophilus* (LbS 22.11 series,

"AiBi," OOO "Green Lines," Russia). AiBi direct application starters are highly concentrated lyophilized cultures containing a mixture of precisely defined strains that provide effective and reliable development of the acidity, good texture of the product, precisely defined production process, and exactly determined time of ripening. They are phage-alternative and phage-resistant for a long time.

Brittle was chosen as bioactive filler with prebiotic and adsorbing properties; there was a developed formulation that included peeled walnuts, flakes of wheat, rye, oat, barley and buckwheat, sugar, and water. All brittle samples were produced according to one recipe, except for Test I sample added with citric acid (5 g per 100 g of the mixture). Three brittle samples were developed, that is, Control with low melting point; Test I with citric acid, and Test II with high melting point.

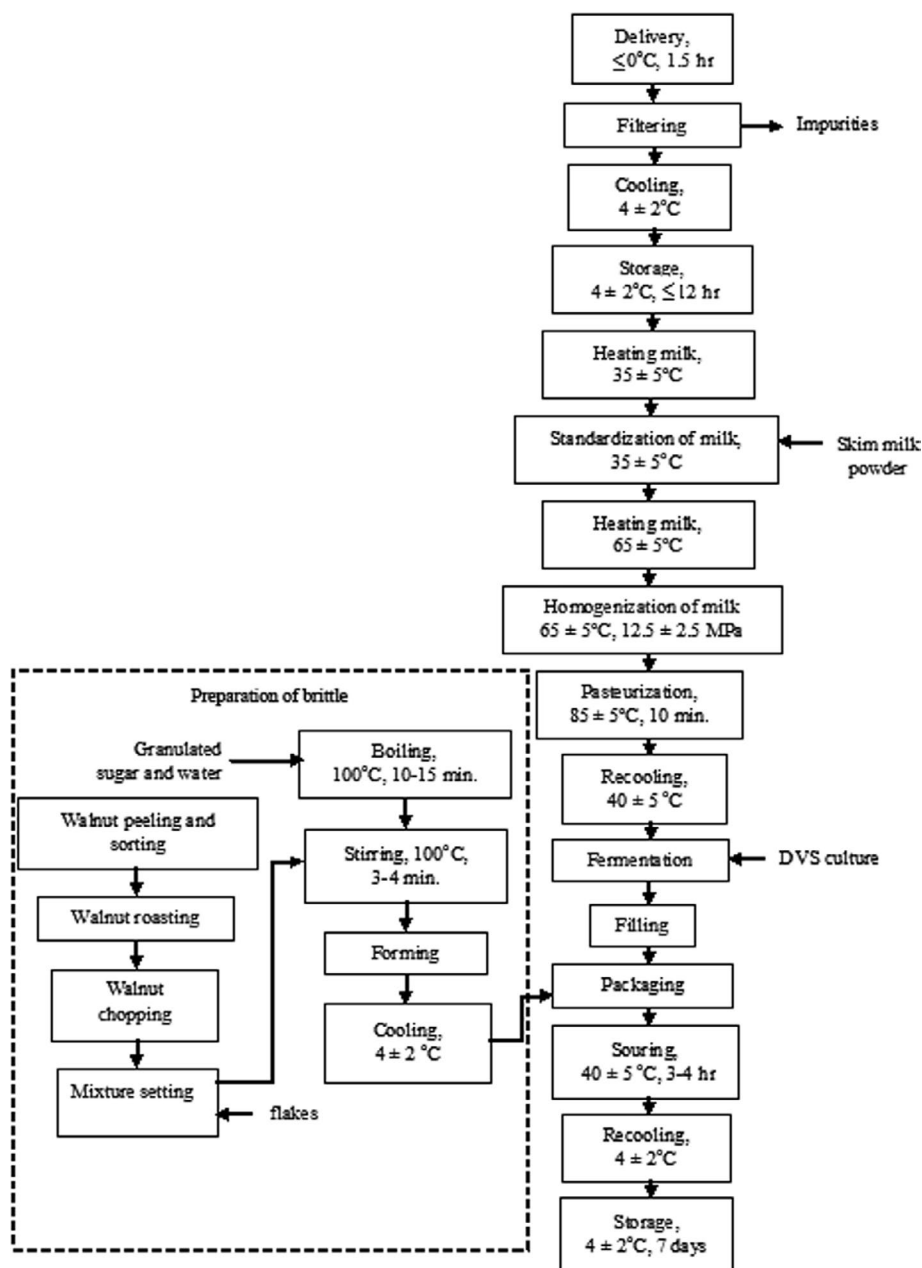


FIGURE 2 Technological scheme for the product enriched (thermostatic method)

TABLE 1 Recipe of synbiotic Yoghurt with filler

Raw material	Amount, kg per 1,000 kg
Fermented milk base	
Standardized milk	923.0
Skim milk powder	76.9
Culture	0.1
Total	1,000.0
Filler	
Walnuts	277.0
Five cereal flakes	277.0
Granulated sugar	277.0
Water	169.0
Total	1,000.0
Fermented milk base/filler, %	70/30

The basic scheme of the brittle production included the operations of raw material preparation, syrup boiling, walnut–cereal mass brewing, forming, cooling, and storage (Figure 2 and Table 1).

Optimum time and temperature conditions for boiling caramel mixtures and brewing raw walnut–cereal masses in caramel were determined using a single-block logic programmable controller OVEN PLK 150–220.I-M (OOO Production Association OVEN, Russia) and the CODESYS v. 2 programming system (CODESYS Group, 3S-Smart Software Solutions GmbH, Germany).

The experimental study to establish the optimum temperature and time of melting the caramel mixture was carried out as follows. The sugar syrup was stirred and boiled to a moisture content of 4%–6% at a temperature of 100–180°C (Table 2). Then, premixed walnuts and cereals were poured into the syrup and mixed. The walnut–cereal mass was molded and cooled to 20°C. In the metal container, there was a temperature sensor preinstalled and completely immersed in the area of the caramel mixture during the experiment. The sensor was connected to a monoblock controller with discrete and analogue inputs/outputs on board for the OVEN PLK 150 small systems automation; the temperature and time regimes were recorded continuously throughout all the preparation stages. The controller was

programmed by the professional programming system CODESYS v.2. This program helped output the information to a computer screen; all processes were presented in a temperature–time curve (Figure 3a–c).

2.2 | Analysis of the finished product characteristics

The research studies of the finished product involved an analysis of organoleptic, physicochemical, and microbiological points. When performing the tasks, the approved regulatory and technical documentation (GOST) was applied. Each measurement was carried out in triplicate. The physicochemical characteristics of the samples developed were compared with the requirements for the quality of fermented milk products.

Technical regulations of the Customs Union “On safety of milk and dairy products” (TR TS 033/2013) and GOST 31981–2013 “Yoghurts. General specifications.” Sensory analysis of the developed enriched products was carried out in accordance with GOST R ISO 22935–2–2011 “Milk and milk products” and GOST R ISO 22935–3–2011 “Milk and milk products. Sensory analysis. Part 3. Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring” (a five-point scale applied). Microbiological characteristics of the developed milk products were determined in accordance with the requirements of GOST 32901–2014 “Milk and milk products. Methods of microbiological analysis.” The amino acid composition was analyzed by a HPLC analyzer for physiological fluids with postcolumn ninhydrin derivatization in accordance with the manufacturer's instruction manual (ARACUS, PMA GmbH, Germany). Photographs of the brittle samples structures were taken using the Mikmed-5 light microscope (AO LOMO, Russia), 10× magnification.

2.3 | The calculation of the nutritional and biological values

The nutritional value (NV) was evaluated in accordance with the following formula:

$$\frac{NV(kcal)}{100g} = 4 \cdot P + 9 \cdot F + 4 \cdot C$$

where *P* is the percentage of protein, %; *F* is the percentage of fat, %; *C* is the percentage of carbohydrates, %.

TABLE 2 Temperature and time regimes of melting and brewing caramel, walnut, and cereal mass

Process stage	Temperature, °C			Time, min			Description
	Control	Test I	Test II	Control	Test I	Test II	
Boiling the caramel mass	70	73	75	4–5	8	5–6	Start of melting
	110	90	90	7–8	10–11	7	Start of boiling
	119	115	125	13	15	12	Boiling the mixture
	126	142	158	17	25	15	Homogeneous mixture
	127	161	176	21–22	27	17	Darkening of the mass
Brewing the nut–cereal mass	127	170	180	22	28	18	Brewing
	80	110	110	23	29	19	Homogeneous mixture

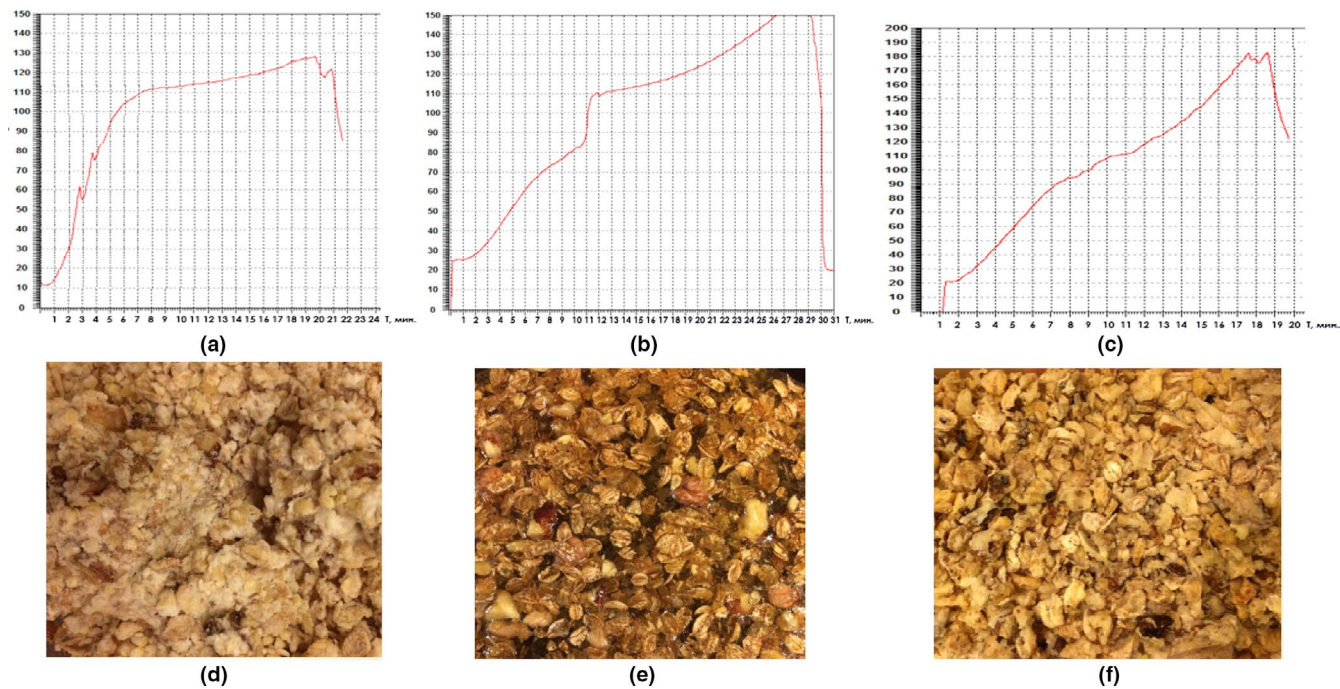


FIGURE 3 Temperature and time curves of the boiling process of caramel mixture and the structure of the brittle samples: (a, d) Control; (b, e) Test I; (c, f) Test II

TABLE 3 Characteristics of the brittle samples (mean ± SEM)

Parameter	Control	Test I	Test II
Dry matter, %	91.17 ± 0.74	96.16 ± 0.62**	94.14 ± 0.68*
Protein, %	6.95 ± 0.02	7.08 ± 0.03*	7.03 ± 0.02*
Fat, %	17.21 ± 0.13	18.02 ± 0.17*	17.62 ± 0.09 ^{ns}
Carbohydrates, %	48.94 ± 0.32	50.43 ± 0.26*	49.81 ± 0.23 ^{ns}
Nutritional value, kcal/100 g	378.5	392.2	385.9

Abbreviation: ns, not significant at $p > 0.05$ compared with data on the Control sample. *** $p < .001$.
* $p < .05$.
** $p < .01$.

The amino acid score (AAS) was calculated as follows:

$$\text{AAS (\%)} = \frac{\text{Evaluated protein amino acid content (g/100 g protein)}}{\text{FAO scoring model amino acid content (g/100 g protein)}} \times 100$$

The coefficient of amino acid score difference (CAAS) was calculated by the following formula:

$$\text{CAAS (\%)} = \frac{\sum (C_i - C_{\min})}{n}$$

where C_i is the excess of amino acid score, %; $C_{\min}$ is the minimum score of essential amino acids of evaluated protein relative to the physiological norm (FAO scoring model amino acid content, %)

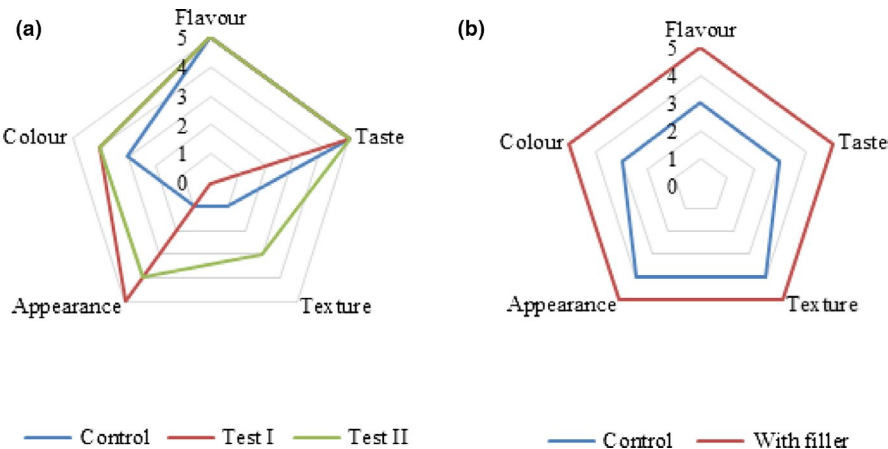


FIGURE 4 Profilograms of the samples: (a) brittle; (b) finished product

TABLE 4 Comparative characteristics of the Yoghurt samples

Parameter	Control (conventional technology)	Test (with filler)
Dry matter, %	13.44 ± 0.06	37.65 ± 2.44*
Protein, %	3.29 ± 0.05	4.41 ± 0.08*
Fat, %	3.69 ± 0.03	7.87 ± 0.04*
Carbohydrates, %	4.72 ± 0.04	18.25 ± 0.11*
Nutritional value, kcal/100 g	65.3	161.5

Abbreviation: ns, not significant at $p > .05$ compared with data on the Control sample. ** $p < .01$; *** $p < .05$.

* $p < .001$.

$$\sum (C_i - C_{\min}) = \Delta AAS$$

n is the number of essential amino acids.

The biological value (BV) was calculated by the following formula:

$$BV(\%) = 100 - CAAS$$

To assess the balance of essential amino acids with respect to the reference protein, the utility K_i coefficient was calculated.

$$K_i = \frac{AS_{\min}}{AS_i}$$

where AS_i is the amino acid score of the i -indispensable amino acid in the product; $AS_{\min}$ is the amino acid score of the first limiting amino acid in this product.

The utility coefficient of the i -indispensable amino acid (A_i) was used to calculate the utility coefficient of the amino acid composition (U), which was a numerical characteristic that reflected the balance of essential amino acids with respect to the standard (A_{st}):

$$U = \frac{\sum_{i=1}^n (A_i K_i)}{\sum_{i=1}^n A_i \text{st}}$$

2.4 | Statistical analysis

The experimental data were analyzed by the Statistica 10.0 package using conventional techniques. The threshold of 0.05 was considered a minimum threshold of the significance of differences (Johnson & Bhattacharyya, 2010). The MS Office 2010 was used for the graphical interpretation of the digital data.

3 | RESULTS AND DISCUSSION

3.1 | Comparative quality evaluation of the brittle samples

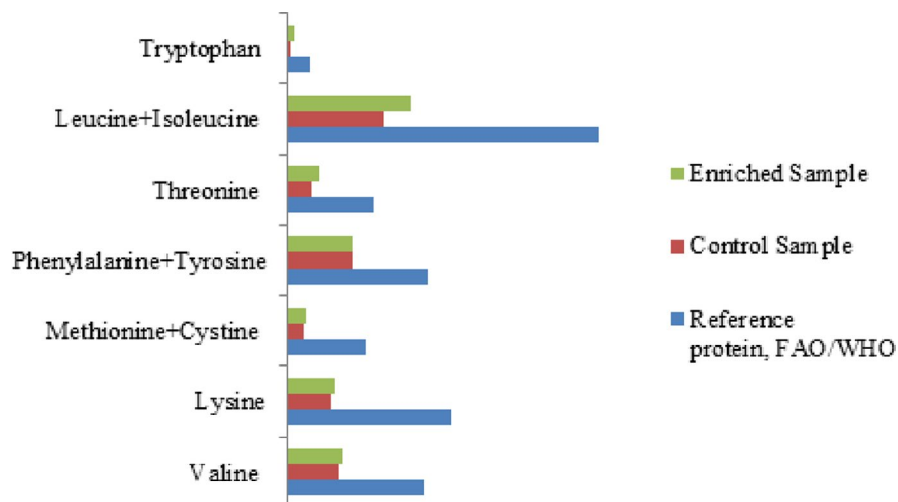
The Control brittle sample was made at low melting point of the caramel mass (up to 127°C); the moisture content in the finished

TABLE 5 Indices of the biological value of the Yoghurt samples

Amino acids, mg per 1 g of protein	Control Sample					Enriched Sample					Reference protein, FAO/WHO						
	A _i	AAS,%	ΔAAS	CAAS,%	BV	K _i	A _i *K _i	U	A _i	AAS,%		ΔAAS	CAAS,%	BV	K _i	A _i *K _i	U
Valine	14.8	37.03	21.87			0.41	6.06		15.90	39.75	15.40			0.61	9.74		40.0
Lysine	12.9	26.88	11.72			0.56	7.27		13.60	28.33	3.99			0.86	11.69		48.0
Methionine + Cystine	4.9	21.35	6.20			0.71	3.48		5.60	24.35	0.00			1.00	5.60		23.0
Phenylalanine + Tyrosine	18.8	45.88	30.73	14.31	85.69	0.33	6.21	0.475	19.10	46.59	22.24	10.63	89.37	0.52	9.98	0.659	41.0
Threonine	7.3	29.36	14.21			0.52	3.79		9.40	37.60	13.25			0.65	6.09		25.0
Leucine + Isoleucine	27.8	30.56	15.41			0.50	13.79		35.90	39.45	15.10			0.62	22.16		91.0
Tryptophan	1.0	15.15	0.00			1.00	1.00		1.90	28.79	4.44			0.85	1.61		6.6

Abbreviations: AAS, an amino acid score; BV, a biological value; CAAS, a coefficient of amino acid score difference; K_p , an utility coefficient; U_i , an utility coefficient of the amino acid composition.

FIGURE 5 Amino acid composition of the samples in comparison with the reference protein (FAO/WHO), mg per 1 g of protein



product exceeded the similar value in the Test ones by 4.99% ($p < .01$) and 2.97% ($p < .05$), respectively. The Control sample was inferior to the Test ones (Table 3). So, the consistency of the Control sample was too short-brittle due to the insufficient caramelization degree (Figure 3d). The higher moisture content in the Control sample led to a lower content of macronutrients. So, the protein content was lower by 0.13 ($p < .05$) and 0.08% ($p < .05$); fat content by 0.81 ($p < .05$) and 0.41% (ns); and carbohydrate content by 1.49 ($p < .05$) and 0.87% (ns), respectively. In this regard, the Control was characterized by a minimum nutritional value in comparison with its analogues (by 3.62 and 1.96%, respectively). Taking into account the defects of the Control sample caused by insufficient heating, the caramelization temperature of the Test I sample was brought to 170°C. However, despite the fact that this sample was characterized by the highest content of macronutrients, by organoleptic characteristics it was inferior to the Control and Test II samples in too hard and dry consistency and unpleasant dark brown color (Figure 3e). Moreover, citric acid in its composition caused sour taste. The Test II sample was prepared at higher melting point of the caramel mass (up to 180°C), without citric acid added, which made it possible to obtain a sample with the most attractive organoleptic and physicochemical characteristics (Figure 3f). Thus, the most attractive brittle characteristics were found to be achieved without citric acid, at the melting temperature of the caramel mixture of 100–180°C and in-process time of 19 min (Table 2 and Figures 3 and 4).

3.2 | Comparative quality evaluation the of finished product samples

To evaluate the quality of the finished product, a brittle Yoghurt sample (brittle was precrushed and mixed with Yoghurt) was compared with the Yoghurt sample produced according to conventional technology (Tamime & Robinson, 2007). Comparative analysis of the Control sample and the enriched

one is given in Table 4. So, the introduction of a filler in Yoghurt allowed increasing the content of dry substances in it by 24.21% ($p < .001$); protein by 1.12% ($p < .001$); fat by 4.18% ($p < .001$); and carbohydrates by 13.53% ($p < .001$). The nutritional value of the product developed was 161.5 kcal per 100 g. According to the microbiological points, the samples developed met the requirements of the Technical Regulations of the Customs Union “On the safety of milk and dairy products” (TR CU 033/2013). The organoleptic analysis made it possible to design a profilogram (Figure 4). This analysis also revealed the superiority of the enriched product over the Control sample. So, this study found a positive effect of the filler developed on the amino acid composition of Yoghurt and its biological value (Table 5 and Figure 5).

As the evaluation showed (Table 6), the formation of lactic acid microorganisms was different in yoghurt samples enriched with bioactive filler (Test) and not enriched (Control). The brittle in Yoghurt influenced the growth and reproduction of lactic microflora.

In the industrial Yoghurt production according to the technology developed, the finished product was proposed to be packed in two-section plastic cups. Yoghurt and filler should be mixed before eating by the consumer.

4 | CONCLUSIONS

The technology was developed for the production of a new synbiotic Yoghurt with a plant filler in form of brittle with prebiotic and sorption properties. The brittle formulation was developed; in the course of laboratory studies using specialized hardware support, the temperature and time regimes of its production were optimized. The increase in consumer properties, and nutritional and biological values of the finished dairy product was scientifically substantiated and experimentally confirmed.

TABLE 6 Microbiological indicators of Yoghurt samples

Indicator	Technical regulations of the customs union 033/2013	Storage period, days											
		1		3		5		7		9			
		Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
CFU/cm ³ (g)	Lactic acid microorganisms, not less than 1×10^7	1.3×10^9	1.1×10^9	3.9×10^9	1.3×10^9	2.3×10^9	1.9×10^9	1.4×10^9	4.3×10^8	1.5×10^8	1.2×10^7		
Weight of the product that does not allow, g:													
Coliforms	0.01	Not determined											
Yeast, CFU/cm ³ (g), not more than	50	Not determined											
Mold, CFU/cm ³ (g), not more than	50	Not determined											

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing.

INFORMED CONSENT

Written informed consent was obtained from all study participants.

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