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Yogurt-like product from lupine (*Lupinus albus* L.) milk as an alternative to dairy products

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Abstract:
Recently, the number of people suffering from allergy to caw's milk has increased. Lupine, a plant rich in protein, can be a good alternative product for non-dairy products production. We aimed to obtain a yogurt-like product based on lupine milk and evaluate its properties.

Lupine milk was obtained from lupine seeds, egg white protein powder, disaccharides, and starter cultures were added to the milk to obtain yogurt-like products: samples with maltose, samples with lactose, and samples without sugars. Physico-chemical and microbiological characteristics of the products were determined by the standard techniques. Sensory attributes were evaluated by trained panelists.

In the study, the effects of egg white protein powder and disaccharides on the activities of starter cultures and the properties of the yogurt-like products obtained were investigated. The relationship between the addition of sugar and the growth of starter cultures was found to be significant ($P < 0.05$). In terms of physico-chemical, rheological, and microbiological properties, the yogurt-like products obtained from lupine milk with disaccharides demonstrated good results, especially the sample with maltose. Sensory analysis revealed high sensory properties of the yogurt-like products.

Yogurt-like products from lupine milk can be used as an alternative to cow's milk fermented products, but more detailed studies should be conducted on their formulations.

Keywords: Lupine milk, maltose, lactose, fermentation, non-dairy yogurt-like product, starter cultures

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INTRODUCTION

The increasing prevalence of protein allergenicity to cow's milk has driven the food industry towards the design, supply, and production of new plant-based milk alternatives. Studies on the creating of formulations with sensory acceptability that are suitable for vegetarian diets have rapidly increased in recent years. In addition, fermented products made from plant-derived milk instead of fermented products from animals' milk have become of interest [1].

One of the plant-based alternatives to cow's milk is lupine milk. Lupine (*Lupinus albus* L.) is a plant belonging to the *Lupinus* species of the *Papilioneceae* (*Legumineceae*; butterfly-flowered) family. Lupine is used as a soy alternative in such products as bread, biscuits, cakes, pasta, confectionery, and soy sauce. Besides, due to its antioxidant content, lupine is also used in high-quality vegetable oil, gluten-free flour, emulsifying agents, and alternative fermented products [2, 3].

Today, lupine attracts attention as functional food because it is rich in protein, minerals, vitamins, oleic acid, fiber and other valuable components, as well as because its antioxidant capacity. Lupine seeds contain significant amounts of polyphenols, carotenoids, phytosterols, tocopherols, and alkaloids, as well as peptides with antioxidant, antimicrobial, anti-carcinogenic, and anti-inflammatory activities [4].

Lupine milk is obtained from lupine grains. The protein value of lupine milk is 4.90 g/100 g, the fat content is 5.00 g/100 g, the total dry matter ratio is 11.20 g/100 g, and the pH is 6.30 [1, 2, 5]. Lupine milk characteristics make it suitable to produce dairy products such as set yogurt, probiotic yogurt, and cheese [6].

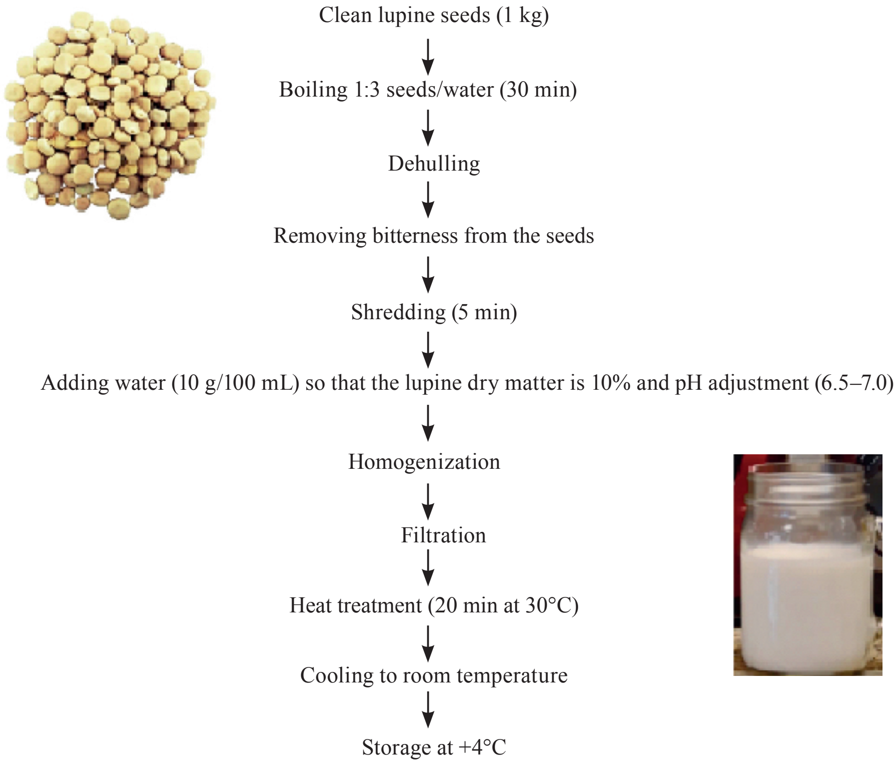

Figure 1 Preparation of lupin milk from the seeds of *Lupinus albus* L.

In addition to the use of lupine milk itself, there are studies on the use of its components in the production of dairy products. For example, lupine proteins were used in ice-cream production and yielded ice cream with high sensory properties [7].

Some studies, have reported that fermented products can be produced from lupine milk, but since yogurt starter cultures cannot use carbohydrates in the composition of lupine milk, it is necessary to enrich this milk by adding disaccharides, increasing thereby the activity of starter cultures [6].

The growth of lactic acid bacteria in artificial environments (other than milk) is difficult, but they can easily grow in most plant-based media/substrates. Additionally, lactic acid bacteria have been found to rapidly increase acidity (decrease in pH) in plant-derived environments to a point where other competitive organisms cannot thrive.

Krunglevičiūtė *et al.* have reported that acidity (hence the growth of lactic acid bacteria) in a fermented product made using lupine milk depends on the lupine variety and amino acid levels [8]. However, it has been stated that lupine milk has a unique nutritional composition and may support the increase in the number and survival of lactic acid bacteria in fermented products.

Egg white protein powder is obtained as a result of drying the egg white protein by the traditional drying method. Pasteurized egg white protein powder is produced by drying egg white protein by the conventional spraying method [9]. In our research, egg white protein powder was used to increase dry matter level.

The aim of this study was to obtain a yogurt-like product based on lupine milk as an alternative to cow's milk and evaluate its microbiological, physico-chemical, and sensory attributes.

STUDY OBJECTS AND METHODS

Raw lupine (*Lupinus albus* L.), pasteurized egg white protein powder (Alfasol®), JOINTEC VB530 lyophilized culture, lactose, and maltose were obtained from Ödemiş (Turkey-İzmir), Kimbiotek Chemical Substances Inc. (Istanbul-Turkey), CSL laboratory (Strade per Merlino, 3,26839, Italy), and Sigma-Aldrich, respectively.

Production of lupine milk. Lupine milk was extracted from seeds by the method illustrated in Fig. 1.

Production of non-dairy yogurt-like product. In the study, plant-based yogurt-like products were obtained by from lupine milk with functional properties, egg white protein powder, and disaccharides (lactose and maltose) with the following fermentation with *Lactobacillus bulgaricus* + *Streptecoccus thermophiles* (Table 1).

Lupine milk was aliquoted into three equal batches to obtain three samples, namely milk without saccharides (control), milk with lactose (0.5%, w/v), and milk with maltose (0.5%, w/v). Lactose and maltose were added into the milk, the batches were pasteurized separately at 85°C for 20 min, and cooled to 50–55°C.

Table 1. Experimental design

	Lactobacillus bulgaricus + *Streptecoccus thermophilus*	Egg white protein powder	Lactose	Maltose
Lupine milk (control)	3%	3%		
Lupine milk with lactose	3%	3%	0.5%	
Lupine milk with maltose	3%	3%		0.5%

After adding egg white protein powder (3%, w/v) to each batch, they were homogenized for 3–5 min with an Ultra Turrax Blender at 1200 rpm. Then, the samples were cooled to 42–43°C and the starter culture (3%, w/v) was added. Thus, we prepared three yogurt-like samples based on the lupine milk samples. The samples were incubated at 42–43°C and pH 4.60 for three days. On days 1, 7, 14, and 28 of storage at 4°C, we performed physico-chemical, rheological, microbiological, and sensory analyses.

Physico-chemical analyses. Dry matter was determined by the gravimetric method. The ash content was determined using AOAC methods. The fat content was determined by Gerber's method. pH value was detected using an SS-3 Zeromatic (Beckman Instruments Inc., California, USA) pH meter, and titration acidity (% lactic acid) was determined according to the alkali titration method. The protein value was determined by the Kjeldahl method according to AOAC in milk and yogurt-like product samples [10]. Viscosity was determined with a digital viscometer (V100003/FungiLabAlpha), and syneresis was determined as described by Kieserling *et al.* [11, 12]. The texture properties were determined with a texture analyzer (HDPL2/CEL5/TA-XT Plus), and the carbohydrate content was determined with an Atago Polax×2L polarimeter (Japanese).

Determination of fatty acid composition. Each homogenized sample was extracted according to the Gerber method to obtain oil as described in [14], fatty acid methyl esters were prepared according to AOCS and investigated using gas chromatography [15]. We used a Supelco SP-2380 (Supelco Inc., Bellefonte, USA) fused a silica capillary column (60×0.25 mm i.d., 0.2 mm film thickness) and a Hewlett-Packard gas chromatographer (model 6890) with a flame ionizing detector. Injection volume: 1 µL; oven temperature: 4°C/min 100°C to 220°C; injector and detector temperature: 300°C; carrier gas: helium; and flow rate: 1 mL/min. Fatty acid methyl esters were detected in the lupine milk and yogurt-like samples on day 1 of storage.

Microbiological analysis. MRS-agar (Merck, Germany) was used to count *L. bulgaricus.* All the samples under study were subjected to anaerobic incubation at 42°C for 3 days on MRS-agar. *L. bulgaricus* count was determined as CFU/g [16]. An M_{17} agar medium containing lactose was used for *S. thermophiles* counting. The incubation of the planted Petri dishes was carried out under aerobic conditions at 37°C for 72 h. The typical colonies formed at the end of the incubation were counted [17].

Sensory tests. Sensory analysis was performed by 10 trained panelists on days 1, 7, 10, 14, and 21 according to Jovanović *et al.* [18].

Statistics. The samples were examined in three repetitions and two replications. SPSS version 15 (IBM SPSS Statistics) statistical analysis package program was used. The data considered significant according to the analysis of variance (ANOVA) was tested at the $P < 0.05$ level using the Duncan multiple comparison test.

RESULTS AND DISCUSSION

Physico-chemical properties. In the study, dry matter in lupine milk was determined as 10.02%, fat 3.58%, protein 5.05%, carbohydrates 2.59 g/100 g, titration acidity (°SH) 0.131, pH value 6.38, ash 1.3%, and viscosity 3.52 cP (20°C). The physico-chemical properties of the yogurt-like samples based on lupine milk are given in Table 2.

The acidity in the samples with lactose and maltose was higher than that in the samples without sugars during storage, which was associated with maltose and lactose added to the lupine milk. The acidity increase in the samples with maltose during storage was higher than that in the products with lactose. The relationship between the increase in acidity and the sugar addition/type added to the samples was significant ($P < 0.05$).

Bintsis has reported that lactic acid bacteria develop better especially in the presence of glucose and some other sugars (sucrose, maltose), which cause higher acidity increase [19]. Our research results were found to be compatible with the literature, and depending on the glucose ratio, the highest acidity increase was determined in the yogurt-like product with maltose. Additionally, the results regarding the increase in acidity were found to be compatible with studies by Ozcan *et al.* who stated that the viability of lactic acid bacteria in plant-based yogurt-like products increased, thus increasing the acidity of the product [20].

Dry matter in all the samples decreased by the end of storage. The highest decrease was determined in the samples without sugars, while the decrease in the samples with lactose and maltose was found to be close to each other. However, the dry matter decrease in the products with maltose was found to be lower than that in the samples with lactose. The relationship between the type of sugar used in the production of non-dairy

Table 2 Physicochemical properties in yogurt-like samples based on lupine milk (n = 3)

	Storage, days	Milk without sugars	Milk with lactose	Milk with maltose
Dry matter, %	1	12.82 ± 1.44^{aA}	13.00 ± 1.14^{aB}	13.00 ± 1.13^{aB}
	7	12.25 ± 1.26^{aA}	12.75 ± 1.06^{aB}	12.85 ± 1.69^{aC}
	14	10.69 ± 1.33^{aA}	11.36 ± 1.46^{aB}	11.65 ± 1.47^{aC}
	28	10.44 ± 1.67^{aA}	11.03 ± 1.57^{aB}	11.26 ± 1.33^{aC}
Viscosity, cP	1	899.00 ± 5.11^{aA}	941.00 ± 8.21^{aB}	1021.00 ± 9.25^{aC}
	7	1056.00 ± 8.23^{bA}	1154.00 ± 8.36^{bB}	1163.00 ± 8.73^{aC}
	14	1274.00 ± 9.63^{bA}	1566.00 ± 9.45^{bB}	1621.00 ± 8.91^{bC}
	28	1663.00 ± 9.74^{bA}	1841.00 ± 9.98^{bB}	2047.00 ± 9.95^{bC}
Syneresis, g	1	9.52 ± 1.01^{aA}	8.67 ± 1.06^{aB}	8.55 ± 1.02^{aC}
	7	12.25 ± 2.06^{aA}	11.36 ± 1.03^{AB}	11.22 ± 1.11^{aC}
	14	13.49 ± 1.12^{aA}	12.41 ± 1.07^{aB}	12.10 ± 1.53^{aC}
	28	15.95 ± 2.07^{aA}	14.65 ± 2.06^{aB}	14.24 ± 2.54^{aC}
pH	1	4.60 ± 1.22^{aA}	4.58 ± 1.29^{aB}	4.56 ± 1.11^{aC}
	7	4.57 ± 0.81^{aA}	4.42 ± 1.06^{aB}	4.39 ± 1.46^{bC}
	14	4.45 ± 0.63^{aA}	4.29 ± 1.21^{aB}	4.25 ± 1.89^{bC}
	28	4.41 ± 0.78^{bA}	4.19 ± 1.63^{bB}	4.16 ± 1.42^{bC}
Acidity (%LA), °SH	1	0.912 ± 0.120^{aA}	0.938 ± 0.100^{aB}	0.944 ± 0.550^{aC}
	7	0.988 ± 0.220^{aA}	1.045 ± 0.650^{aB}	1.095 ± 0.630^{bC}
	14	1.039 ± 0.350^{bA}	1.121 ± 0.750^{bB}	1.133 ± 0.710^{bC}
	28	1.044 ± 0.630^{bA}	1.128 ± 0.430^{bB}	1.139 ± 0.390^{bC}
Fat, %	1	3.55 ± 0.41^{aA}	3.57 ± 0.66^{aB}	3.57 ± 0.51^{aB}
	7	3.12 ± 0.96^{aA}	3.36 ± 0.60^{aB}	3.38 ± 0.62^{aB}
	14	2.75 ± 0.25^{aA}	3.19 ± 0.82^{aB}	3.22 ± 0.84^{aB}
	28	2.35 ± 0.57^{aA}	2.88 ± 0.74^{aB}	2.93 ± 0.78^{aB}
Protein, %	1	5.03 ± 0.91^{aA}	5.00 ± 0.82^{aA}	4.98 ± 0.52^{aB}
	7	4.62 ± 0.93^{aA}	4.45 ± 0.67^{aA}	4.33 ± 0.87^{aB}
	14	4.22 ± 0.67^{bA}	4.16 ± 0.88^{aA}	4.02 ± 0.74^{aB}
	28	4.06 ± 0.76^{bA}	3.86 ± 0.50^{bA}	3.75 ± 0.46^{bB}
Carbohydrates, %	1	2.57 ± 0.92^{aA}	3.51 ± 0.80^{aB}	3.48 ± 0.91^{aB}
	7	2.54 ± 0.82^{aA}	2.85 ± 0.85^{aB}	2.44 ± 0.72^{aC}
	14	2.12 ± 0.49^{aA}	1.57 ± 0.63^{aB}	1.25 ± 0.56^{aC}
	28	1.95 ± 0.57^{aA}	0.92 ± 0.22^{aB}	0.84 ± 0.21^{aC}
Ash, %	1	0.55 ± 0.09^{aA}	0.57 ± 0.09^{aA}	0.57 ± 0.03^{aA}
	7	0.31 ± 0.08^{aA}	0.41 ± 0.07^{aA}	0.43 ± 0.07^{aA}
	14	0.21 ± 0.08^{aA}	0.33 ± 0.09^{aA}	0.34 ± 0.06^{aA}
	28	0.16 ± 0.07^{aA}	0.29 ± 0.02^{aA}	0.30 ± 0.02^{aA}

^{a,b,c} different letters on the same column are statistically significant ($P < 0.05$)

^{A,B,C} different letters on the same line are statistically significant ($P < 0.05$)

yogurt-like products from lupine milk and dry matter was significant ($P < 0.05$).

The fat content decreased in all the samples by the end of storage, but this decrease was not significant ($P > 0.05$). We revealed that during storage fat amounts in the samples with maltose and lactose were higher than in the samples without sugars. This situation is associated with a more pronounced syneresis in the yogurt-like products without sugars compared to the other samples. In this study, the relationship between the fat content and the type of sugar added to the samples and the amount of egg white protein powder was found to be significant ($P < 0.05$).

Protein values decreased in all the samples during storage, and the highest protein hydrolysis had the samples with maltose, followed by the samples with lactose and the samples without sugars. Among the samples, protein hydrolysis is associated with egg white protein powder added to lupine milk to increase the protein content and dry matter in lupine milk. The relationship between the sugar type added to milk and the addition of egg white protein powder and protein hydrolysis was significant ($P < 0.05$). In the study, the relationship between the type of sugar added to lupine milk and viscosity and syneresis was found to be significant ($P < 0.05$).

Al-Saedi *et al.* stated that yogurt-like products can be produced from lupine milk, with shortened fermentation time and the increased amount of the starter culture (especially probiotic microorganisms) [6]. In this respect, our research results are compatible with the literature.

Table 3 Fatty acid composition in yogurt-like products based on lupine milk

Fatty acid, g/100 g	Milk without sugars	Milk with maltose	Milk with lactose
Oleic Acid (C18:1)	49.00 ± 1.13	49.10 ± 1.05	49.10 ± 1.05
Linoleic Acid (C18:2)	23.41 ± 1.21	23.40 ± 1.07	23.40 ± 1.05
Palmitic Acid (C16:0)	7.33 ± 1.03	7.35 ± 1.13	7.34 ± 1.05
Gadoleic Acid (C20:1)	3.46 ± 1.12	3.47 ± 1.15	3.46 ± 1.05
Stearic Acid (C18:0)	1.62 ± 0.26	1.62 ± 0.04	1.62 ± 0.13
Arachidic Acid (C20:0)	2.85 ± 0.65	2.86 ± 0.70	2.85 ± 0.55
Miristic Acid (C14:0)	0.49 ± 0.01	0.48 ± 0.03	0.48 ± 0.06
Pentadecanoic Acid (C15:0)	0.21 ± 0.02	0.20 ± 0.01	0.21 ± 0.02
Lauric Acid (C12:0)	0.050 ± 0.001	0.050 ± 0.004	0.050 ± 0.003

Table 4 Texture changes in yogurt-like products based on lupine milk during storage

Indicator	Storage, days	Milk without sugars	Milk with lactose	Milk with maltose
Hardness, N	1	0.32 ± 0.01^{aA}	0.33 ± 0.02^{aB}	0.34 ± 0.05^{aC}
	7	0.36 ± 0.09^{aA}	0.37 ± 0.07^{bB}	0.40 ± 0.03^{bC}
	14	0.37 ± 0.01^{bA}	0.40 ± 0.06^{bB}	0.43 ± 0.08^{cC}
	28	0.38 ± 0.02^{cA}	0.42 ± 0.01^{cB}	0.48 ± 0.05^{cC}
Adhesiveness	1	0.04 ± 0.02^{aA}	0.05 ± 0.03^{aA}	0.05 ± 0.01^{aA}
	7	0.03 ± 0.01^{aA}	0.03 ± 0.01^{aA}	0.04 ± 0.02^{aA}
	14	0.02 ± 0.01^{aA}	0.03 ± 0.01^{aA}	0.03 ± 0.01^{aA}
	28	0.01 ± 0.01^{aA}	0.02 ± 0.01^{aA}	0.02 ± 0.01^{aA}
Springiness, mm	1	4.00 ± 0.52^{aA}	4.20 ± 0.94^{aB}	4.63 ± 0.99^{aC}
	7	4.11 ± 0.83^{aA}	4.29 ± 0.63^{aA}	5.16 ± 0.66^{aB}
	14	4.36 ± 0.64^{aA}	4.62 ± 0.80^{bB}	5.55 ± 0.83^{bC}
	28	4.58 ± 0.84^{bA}	4.81 ± 0.91^{bA}	5.87 ± 0.80^{bB}
Gumminess, g	1	41.05 ± 1.12^{aA}	72.56 ± 1.27^{aB}	79.47 ± 1.22^{aC}
	7	45.63 ± 1.23^{aA}	78.56 ± 2.24^{aB}	83.47 ± 2.88^{aC}
	14	49.22 ± 1.66^{aA}	82.10 ± 1.96^{bB}	85.33 ± 2.10^{bC}
	28	52.11 ± 1.35^{bA}	85.45 ± 2.67^{cB}	87.22 ± 2.66^{cC}
Chewiness, mJ	1	0.14 ± 0.05^{aA}	0.86 ± 0.11^{aA}	0.92 ± 0.30^{aA}
	7	0.16 ± 0.04^{aA}	1.12 ± 0.26^{aA}	1.21 ± 0.14^{aA}
	14	0.21 ± 0.03^{aA}	1.82 ± 0.49^{aA}	1.88 ± 0.47^{aA}
	28	0.35 ± 0.13^{aA}	2.02 ± 0.28^{aA}	2.09 ± 0.66^{aA}

^{a,b,c} different letters on the same column are statistically significant ($P < 0.05$)

^{A,B,C} different letters on the same line are statistically significant ($P < 0.05$)

In this study, the carbohydrate content decreased in all the samples during storage. The highest decrease was detected in the yogurt-like products with maltose, following by the samples with lactose. The relationship between the acidity increase and sugar addition/type and carbohydrate content was found to be significant ($P < 0.05$).

The ash values of all the samples decreased during storage, with the highest decrease in the sample without sugars, while the values for the products with lactose and maltose did not differ significantly. There was no significant difference between the samples in terms of the ash level ($P > 0.05$).

Fatty acid composition. Saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in the yogurt-like products made from lupine milk were detected as 14.17, 52.3, and 9.86 g/100 g, respectively (Table 3).

Rheological properties. Syneresis and viscosity values are given in Table 2, and the texture characteristic are given in Table 4. Consistency values of the samples were found to be significant in terms of sugar type and storage time interaction ($P < 0.05$). During storage, the stability (hardness) of the curd increased and the effect of storage was significant ($P < 0.05$). Hardness, flexibility, gumminess, and chewiness increased, while stickiness decreased in all the samples. The hardness determined in the sample with maltose was higher than that in the other samples. This situation is associated with lower syneresis, higher viscosity increase, lower dry matter decrease compared to the other samples, and higher increase in acidity (decrease in pH value) during storage. The hardness of the samples with lactose was higher than that in the samples without sugars. The difference between the samples in terms of syneresis and the relationship between syneresis and sugar addition/type was significant ($P < 0.05$).

During storage, syneresis and dry matter decrease in the yogurt-like products with maltose were lower than in the samples without sugars, while those in the samples with lactose were lower than in the samples without sugars. The relationship between increased acidity and syneresis was found to be significant ($P < 0.05$).

The relationships between the viscosity, sugar ratio, egg white protein powder, and acidity increase in the samples under study were significant ($P < 0.05$). We revealed that the viscosity increased in all the samples during storage, with the highest increase in the product with maltose, while the lowest increase was in the samples without sugars. Egg white protein powder in the amount of 3% increased the viscosity of all the samples during storage.

The highest acidity and viscosity increase was determined in the samples with maltose, followed by the samples with lactose and the samples without sugars. The acidity (4.56 pH) and viscosity (1021 cP) values determined in the product with maltose on day 1 of storage were higher than those in the other samples. In the following days of storage, the increase in acidity was higher than in the other samples (Table 2). Accordingly, hardness and viscosity also increased.

The texture properties of the samples showed similar changes during storage. We determined that the rheological properties determined in the samples with maltose and lactose during storage were similar to those for the product without sugars but more acceptable. It was observed that the yogurt-like products without sugars were similar to the yogurt gel but had a more watery (yogurt-like beverage) consistency compared to the other samples. During storage, the increase in acidity was lower, the syneresis was higher, and viscosity was lower in the samples without sugars.

The rheological properties of curd in fermented milk products develop depending on the composition of milk, applied temperature, pH, soluble Ca^{++} ratio, and other factors (such as casein micelle size, various interactions, etc.). The increase in acidity decreases syneresis and increases protein hydrolysis, hardness, more soluble calcium, and in turn, viscosity [21].

In this study, we detected good physico-chemical and rheological properties of the fermented products produced with the addition of maltose and lactose. It was associated with high protein content and saccharide derivatives in the composition of lupine milk. Krunglevičiūtė *et al.* reported that the acidity of the fermented product made using lupine milk and the development of lactic acid bacteria are related to the lupine variety and amino acid level [8].

Sensory evaluation. Sensory evaluation revealed good sensory properties of the yogurt-like products with maltose and lactose (Fig. 2.) During storage, the samples with the disaccharides got close scores in terms of structure and consistency. This situation was associated with the scantiness in syneresis and an increase in acidity, viscosity, and hardness during storage. The relationship between the increase of storage time and structure and consistency was found to be significant ($P < 0.05$).

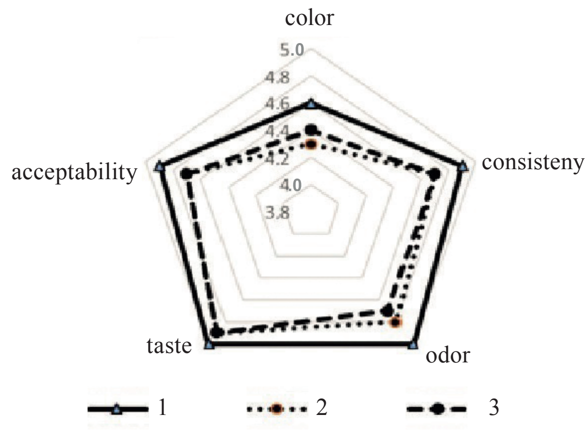

Figure 2 Sensory properties of yogurt-like products based on lupine milk (1), lupine milk with maltose (2), and lupine milk with lactose (3)

In the study, the samples with maltose and lactose were found to be close to yogurt with a prolonged storage time, which is a classical fermented product, in terms of structural properties. Additionally, the structure and consistency of these samples were more similar to the classical fermented product (yogurt) with no lupine flavor or with weak one during storage. The texture and consistency in the samples without sugars were found to be less viscous and the panelists concluded that they could be considered as yogurt. Apparently, the level of fat in the samples with disaccharides during storage also influenced the taste of the product.

Microbiological analysis. Changes in *L. bulgaricus* and *S. thermophilus* amounts in the yogurt-like products obtained from lupine milk are given in Fig. 3. The samples with maltose and lactose demonstrated the increased growth and activity of starter cultures.

In the production of non-dairy yogurt-like products from lupine milk, the relationship between the addition of sugar and the growth of starter cultures was found to be significant ($P < 0.05$). In the samples with maltose and lactose, syneresis decreased during storage which had a positive effect on the development of starter cultures.

In the products without sugars, the *L. bulgaricus* and *S. thermophilus* growth was weaker compared to those with sugars. However, this situation did not appear as a problem in the production of yogurt-like products in the study. On the contrary, it strengthened the opinion that the composition of lupine milk is a suitable raw material to produce non-dairy yogurt-like products. Different studies have reported that lupine proteins effectively maintain the viability of starter cultures in different

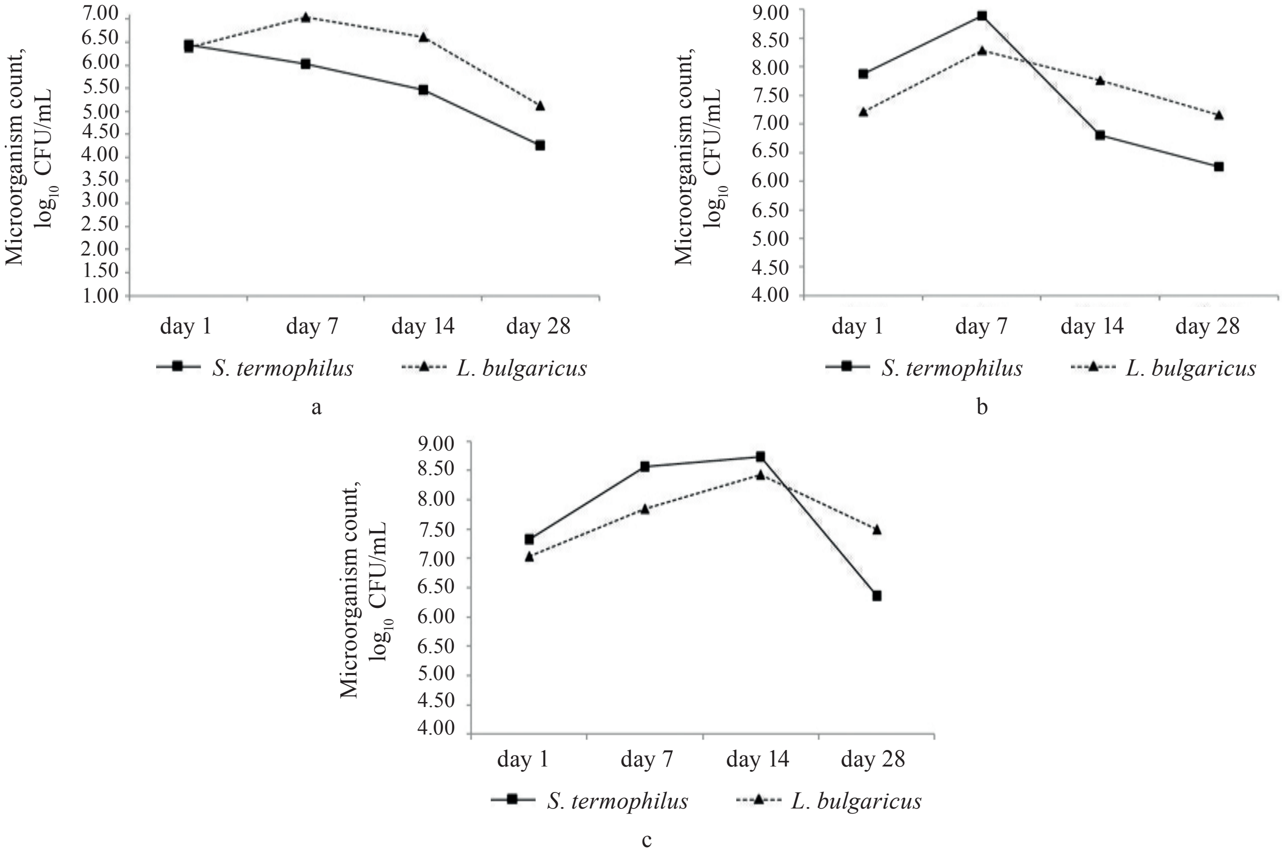

Figure 3 Number of microorganisms in yogurt-like products based on: lupine milk during storage (a), lupine milk with lactose during storage (b), and lupine milk with maltose during storage (c)

products based on lupine milk and can protect the starter cultures by wrapping them like a capsule [22].

Our research results were found to be compatible with studies that stated that starter cultures show better growth in the presence of some sugars (such as glucose and maltose) [19]. It has been reported that lupine milk contains carbohydrates at the level of 2.83 g/100 g, including different types of carbohydrates (galactose and arabinose) [23, 24]. In this work, the maximum growth of *L. bulgaricus* and *S. thermophilus* (8 $\log_{10}$ CFU/mL) in the starter cultures on day 14 in the samples with maltose was associated with the adaptation (prolongation of the lag^{+} phase) and growth phase of starter cultures in the presence of maltose.

However, this effect could not be detected in the samples with lactose added. On the contrary, the development of starter cultures in the samples with lactose was particularly high (7–8 $\log_{10}$ CFU/mL) until day 7 of storage, and decreased to 6–7 $\log_{10}$ CFU/mL after day 14 of storage.

L. bulgaricus levels in the products with maltose were found to be lower than those in the samples with lactose between days 1 and 7 of storage. However, at days 14 and 28 of storage, *L. bulgaricus* levels in the samples with maltose were found to be higher than those in the samples with lactose. *S. thermophilus* levels, on the other hand, were found to be lower in the yogurt-like product with maltose until day 7 of storage (including day 7) and higher after day 7 than those in the products with lactose.

These results show that *S. thermophilus* was effective, also it is associated with an increased glucose concentration in the medium as a result of reaching a higher level of *S. thermophilus* than of *L. bulgaricus*. We determined relationships between the glucose ratio and bacterial growth, between the bacterial growth and acidity increase, and between acidity increase and hardness, viscosity, and syneresis. These results were found to be compatible with Bintsis [19]. *L. bulgaricus* and *S. thermophilus* were determined in the samples without sugars during storage, as well as the slower growth of acidity on the same storage days was attributed to the increase in syneresis observed in those samples. With the increase in syneresis in the yogurt-like products, the symbiotic relationship between microorganisms was disrupted, pH development slowed down or stopped [25].

Elsamani determined that the levels of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were preserved and increased in probiotic ice creams produced from lupine milk on day 30 of storage [26]. The author associated it with the protection of the proteins found in high levels in the composition of lupine milk that wrap the probiotics like a capsule.

CONCLUSION

In our study, lupine milk was obtained from lupine with functional properties and egg white protein powder. Different concentrations of lactose and maltose were added to lupine milk to obtain a non-dairy yogurt-like product.

The growth of *Lactobacillus bulgaricus* and *Streptecoccus thermophiles* was weaker in the disaccharide-free products compared to the samples with maltose and lactose. The increase in acidity in the samples with disaccharides during storage (28 days) was higher than that in the samples without sugars, and this was associated with maltose and lactose added to lupine milk.

It has been concluded that yogurt-like samples produced from lupine milk can be produced due to their similarity to fermented products (especially yogurt) of animal origin in terms of physico-chemical and rheological properties. However, with time, the samples with maltose and lactose were found to be closer to classical yogurt in terms of all properties. Sensory evaluation revealed that the smell and aroma of lupine were not pronounced. Thus, lupine yogurt-like products had high sensory properties.

Consequently, yogurt-like products based on lupine milk can be used as an alternative to fermented products produced from cow's milk, and more detailed studies should be conducted to formulate and optimize lupine fermented milk products.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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