

ORIGINAL ARTICLE

## *Nutritional value of fermented products of Lupin in sports performance*

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### ABSTRACT

**Introduction:** This study aims to suggest alternative products and propose new horizons. In the study, a non-milk-based fermented product was obtained as a result of the enrichment of lupin milk (LM), which is obtained from lupin, with egg white protein powder (EWPP) and various disaccharides (lactose and maltose). **Material-Methods:** To promote the viability of starter cultures, lactose and maltose were separately added to lupine milk at a rate of 0.5% w/v. The lactose and maltose milk was then pasteurized at 85 °C for 20 minutes and cooled to 50-55 °C. Following cooling, the egg white protein powder (3% w/v) and the dry matter were adjusted to 12-13%. As a result, maltose and lactose were added to lupine milk at a rate of 0.5% (w/v) to obtain LMM and LML samples, and LM (K) sample was obtained without adding any ingredient to lupin milk. In this study, the effects of different disaccharides and egg white protein powder on starter cultures and physics-chemical, rheological, microbiological, and sensory properties of fermented products are determined on days 1, 7, 14, and 28 of storing.

**Results:** The rheological properties defined in ABSM and ABSL during storage were similar to LM but more acceptable. The LM sample was identical to the yogurt gel but was more watery than the other samples.

**Conclusions:** Regarding our study and extensive literature review, lupin milk can be offered as an alternative to animal milk-based products. The popularisation of lupin milk for athletes is strongly suggested.

*Keywords:* lupin milk, sports nutrition, sport, sports performance

## INTRODUCTION

### Energy Requirements and Nutrients

The need for energy is one of the main concerns for the human body, especially for athletes and people who are engaged in physical activities. For an athlete's sports field, exercise type, duration, and personal aspects affect the body's energy needs. The more athletes exercise, the more energy they require [1]. For instance, a regular person who takes part in fitness training less than three days a week and each exercise lasts about 40 minutes and follows a normal diet is likely to counterbalance their energy requirement. However, an athlete who exercises most days a week, and each exercise lasts about 3 hours, would require a more specific diet and balance their energy by nutritional intake [2,3]. Nutrition is a factor that is not to be underestimated. For instance, an approximate daily energy expenditure calculation of athletes who cycle the Tour de France is 12,000 kcal [4,5].

### Sports Nutrition

It is the primary goal of any professional athlete to achieve optimal performance. Many factors, such as training, nutrition, genetics, etc., influence that process. It is equally important for each athlete to maintain good health with minimum health problems. To reach maximum athletic performance and maintain good health condition as an athlete, nutrition is one of the critical factors. In planning an athlete's nutrition intake, many variables exist, such as age, gender, physical aspects, etc. [6].

Nutrition helps increase athletic performance and enhances resistance to

harmful factors. Therefore, it is crucial to be conscious of the type and adequacy of nutrition intake. Otherwise, it is expected quite possible for the athlete to lose their efficiency and duration during exercises, be exposed to stress, and develop health problems [7].

Sports nutrition includes both solid and liquid food. These also contain carbohydrates, proteins, lipids, etc. There are several categories, such as macronutrients, micronutrients, and isotonic solutions. Nutrition that includes various kinds of carbohydrates helps provide energy to the body by giving glycogen. Products that include protein promote the synthesis of protein in the human body. Micronutrients include minerals, vitamins, etc., which are necessary for the body. And lastly, some beverages help rehydrate the body [8].

### Protein

Protein is particularly essential for athletes. It is necessary to heal injuries and damaged tissues and produce powerful enzymes and hormones [9]. Besides, protein intake is a must for the body to make protein. 1.2 - 2.1 g/kg/d is the required protein intake suggested by sports guidelines [10]. However, this amount may range according to the athlete. For instance, athletes engaged in high-intensity power exercises and bodybuilders require more protein than the value mentioned above [11,12].

### Lupin Milk as a Nutrient for Athletes

In the past years, a new interest in the search for alternative non-animal milk products

occurred. Lupin is one of the recent discoveries that offer an alternative. Lupin (*Lupinus albus* L.) is a plant belonging to the *Lupinus* species of the *Papilionaceae* (*Leguminales*) family. Alternative raw materials are used worldwide for products such as bread, biscuits, cakes, pasta, confectionery, and soy sauce. Lupin is used in soy alternatives, high-quality vegetable oil with antioxidant content, gluten-free flour, emulsifier matter, and fermented products as an alternative to milk and snacks [13]. The composition of lupin grains includes moderate carotenoids (beta carotene, lutein, and zeaxanthin) [14], tocopherols [15], and other bioactive components. The lipid fraction of lupin contains a component called lupeol [16]. Lupeol is triterpene alcohol that improves epidermal tissue reconstitution and inhibits the cellular growth of melanoma cells [17,18]. In grains, different carbohydrate species (galactose and arabinose) are found at 2.83g / 100g [19] in non-starch dry matter [20-22].

Lupin has long been found to benefit human health, including obesity, high blood pressure, insulin resistance, and cholesterol levels. Foods enriched with lupine grains provide satiety, provide energy balance [23], control glycemic index, and have beneficial effects on blood lipid levels [24], hypertension [25], and intestinal health.

Lupin protein can be obtained by peeling lupine shells to get grains and flouring the grains. Lupin protein contains high levels of lysine, low methionine, and cystine amino acids. Lupin, as well as having a profile very close to the amino acid profile of soy protein, is a good alternative because it contains a higher percentage of protein than soy and is described as a "new soy" [26]. Lupin protein-based ice creams were produced. Lupin protein in ice

cream has been reported to be easy to process and has excellent sensory properties [27]. After the lupin grains are correctly treated, lupin milk is obtained. In studies, the protein value of lupin milk was 4.90 g / 100g, fat content was 5.00g / 100g, the total dry matter ratio was 11.20g / 100g, and pH was reported as 6.30 [28]. In studies conducted on the production of fermented dairy products from lupin milk, it was determined that yogurt starter cultures could not use carbohydrates contained in lupin milk composition. Therefore the activity of starter cultures could be increased by adding various disaccharides [29].

In literature, it is stated that fermented products can be produced from lupin milk, fermentation time is shortened, and the probiotic number is increased [29,30]. On the other hand, some studies argue that fermented dairy products can be produced from lupine milk. Still, since yogurt starter cultures cannot use carbohydrates in the composition of lupin milk, it is necessary to enrich the milk by adding various disaccharides and thus increase the activity of starter cultures [29]. In the literature, although there are few studies on fermented product trials produced as a result of the enrichment of lupin milk with different sugars, no studies on the production of non-dairy fermented products from lupin milk by addition of EWPP, lactose, and maltose have been found. The study aimed to determine the possibilities of non-milk-based fermented product production by adding two different disaccharides (lactose and maltose) separately after adjusting the dry matter content of the lupin milk to 12-13% with EWPP. Besides, it aims to determine the effects of EWPP and different disaccharide use on starter culture activity and the effects of fermented products

on physicochemical, rheological, and sensory properties.

## MATERIALS AND METHODS

### Materials

*Raw Lupin, Egg White Protein Exposure (EWPP), Starter Culture, Lactose, and Maltose*

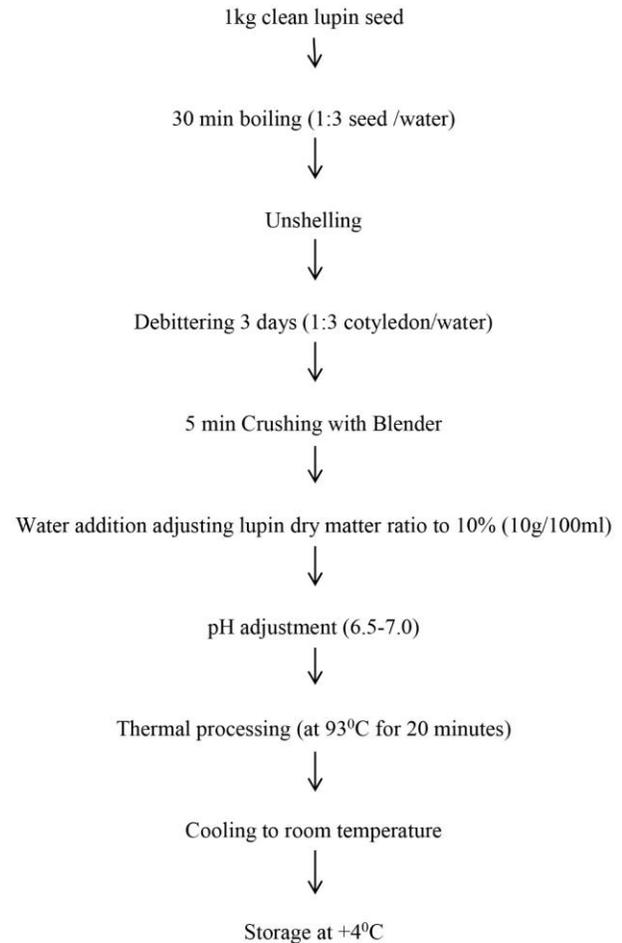
In our experiment, raw lupin (*Lupinus albus* L.) was obtained from Ödemiş (İzmir, Turkey), pasteurized Alfasol® brand egg white protein powder (EWPP) (Ph: 7.00; total microorganism <math>\leq 100\text{ CFU / g}</math>; Coliforms <math>\leq 10\text{ CFU / g}</math>, does not contain *Salmonella* and *Aureus*, humidity level 7.10%) from Kimbiotek Kimyaevi Maddeler San. Tic. A.Ş. (İstanbul-Turkey), JOINTEC VB530 lyophilized yogurt culture from CSL laboratory (Strade per Merlino, 3,26839, Italy), Sucrose and maltose from Sigma-Aldrich firm. Yogurt samples were produced at Ege University, Faculty of Agriculture, Dairy Technology Department Pilot Plant.

### Methods

#### *Preparation of Bacteria Cultures*

The reconstituted milk with 12% dry matter was sterilized for 10 minutes at 115 °C and cooled to 37 °C. Classical yogurt cultures to be inoculated into lupin milk were transferred to the milk and reconstituted with 12% lean dry matter. After the milk is mixed, it is left to incubate at 37 °C, and the incubation process is terminated when the pH of the working culture decreases to 4.6. Thus, the culture to be used in production was prepared.

*Lupin Milk Production (LM) – Figure 1.*



**Figure 1.** Lupin Milk Production

#### *Non-milk Based Fermented Product (FP) Production*

In the study, the dry matter of lupin milk (LM) was adjusted to 13.2% with the addition of EWPP (approximately 3% w / v). Starter culture, lactose (0.5% w / v) and maltose (0.5%

w / v) were added to produce non-milk based fermented products. Lactose and maltose were added to the milk before the pasteurization of the milk. The study added pasteurized EWPP after being pasteurized and cooled to 50-55 °C to avoid clotting.

Lupin milk was divided into three equal sections in the research's fermented product (FP) production. Lactose (0.5% w/v) was added to the first part, maltose (0.5% w/v) was added to the second part, and no ingredient was added to the third part. After every three parts were separately pasteurized at 85 °C for 20 minutes, they were cooled to 50-55 °C, and EWPP was added to each portion with a rate of 3% (w/v), and they were homogenized with Ultra Turrax Blender (40 seconds at 1200 rpm) (IKA, Merc, Germany) for 3-5 minutes. Then, the samples were cooled to 42-43 °C, and a 3% (w/v) starter culture was added. Thus, fermented product samples were prepared as lactose-added lupin milk fermented product (LML), maltose-added lupin milk fermented product (LMM), and plain lupin milk fermented product (K). Samples were divided into plastic containers (200g) and left to incubate (42-43 °C), and incubation was stopped at pH 4.60. Physicochemical, rheological, and sensory analyzes were performed on samples stored at 4 °C ± 1 for 21 days on days 1, 7, 14, 21, and 28. In the study, 21st-day analyses were not made for statistical evaluation because they were similar to 28th-day comments.

## Analysis of Lupin Milk and Fermented Products

### Physico-chemical Analysis

**Dry matter (%):** Dry weight was made by a gravimetric method in milk and yogurt samples [31].

**Fat (%):** Fat values of milk and yogurt samples were determined according to the Gerber method [31].

The pH value of milk and yogurt samples was determined by the SS-3 Zeromatic (Beckman Instruments Inc., California, USA) brand pH meter [31].

**Titration acidity (% LA):** Titration acidity of milk and yogurt samples was determined according to AOAC 2000 [31].

**Protein value (%):** Protein value was obtained by the Kjeldahl method [31].

**Viscosity (cP):** Viscosity value in yogurt samples was estimated with Brookfield's Digital Viscometer (Model DV-II+PRO, USA) (180 rpm, 10 °C), in milk and yogurt samples with LV2 spindle (23.47 g), between 13% and 42%] as cP [32].

**Syneresis (g):** Syneresis in yogurt samples was determined in g by weighing the amount of 25 g of yogurt sample at 4 ± 1 °C filtered through coarse filter paper in 120 minutes [33].

**Texture Profile Analysis (TPA):** TPA values of yogurt samples were determined by Texture Analyzer (Brookfield CT3 4500 model, USA / Shape Cylinder; Target 10 mm; Test speed 1 mm / sec).

**Sensory tests:** On the 1st, 7th, 10th, 14<sup>th</sup>, and 21st days of storage, the sensory analyzes on the yogurt samples were performed according to Uysal et al.[34].

**Ash (%):** Ash was made by a gravimetric method in milk and yogurt samples [31].

**Carbohydrate level (g):** Carbohydrate value was measured with AtagoPolax x 2L (Japanese) model polarimeter [35].

### Statistics

Yogurt samples were examined with three parallel and two recurrence analyses. For this purpose, the SPSS version 15 (IBM SPSS Statistics) statistical analysis package program was used. According to Duncan's Multiple Comparison Test, important data obtained from variance analysis ANOVA were tested at  $p < 0.05$ .

### Fat Extraction and Preparation of Fatty Acid Methyl Esters

Each homogenized sample was extracted according to the Gerber method to obtain oil (ISO 11870: 2009 - ID 152: 2009), and fatty acid methyl esters were prepared according to AOCS [36] and examined in gas chromatography (GC). [Chromatography; Supelco SP-2380 is a fused silica capillary column (60m 0.25mm i.d., 0.2mm film thickness; Supelco Inc., Bellefonte, PA, USA) and flame ionized detector Hewlett-Packard GC (model 6890). The injection volume is 1 $\mu$ l, and the GC oven temperature is programmed at a rate of 4 °C/min from 100 °C to 220 °C. The injector and detector temperature are 300 °C, the carrier gas is Helium, and the flow rate is 1ml/min. Fatty acid methyl esters were detected on the day the 1st of storage in lupin milk and fermented samples.

### Microbiological Analysis

#### *Lb. bulgaricus* Counting

MRS-Agar (Merck, Germany) was used for *Lb. bulgaricus* counting. Irregular white colonies (30 - 300) formed after anaerobic incubation at 42°C for three days were counted, and the number of *Lb. bulgaricus* per gram was found to be cob-g [37].

#### *Str. thermophilus* Counting

M17 agar medium containing lactose was used for *str. thermophilus* count. The incubation of the sown petroleum was carried out at 37°C for 72 hours under aerobic conditions, and the typical colonies formed at the end of the pregnancy were counted [38].

## RESULTS AND DISCUSSION

### Physicochemical Properties

In the research, dry matter was determined as 10.02%, fat 3.58%, protein 5.05%, carbohydrate 2.59 g / 100g, titration acidity (° SH) 0.131%, pH 6.38, ash 1.3% and viscosity 3.52 cP (20 ° C) in lupin milk. The physicochemical properties of non-milk-based fermented samples are given in Table 1.

During storage, the relationship between acidity increase and sugar addiction/variety was significant ( $p < 0.05$ ). In the study, the increase in acidity of LM<sub>M</sub> and LM<sub>L</sub> samples was higher than in LM (K) samples, and it was associated with maltose and lactose content added to lupin milk at 0.5% (w / v) level. The results related to the increase in acidity were compatible with the studies [30,39], indicating the viability of lactic acid bacteria in plant-based fermented products and, thus increase in acidity.

Protein (%) values were decreased in all samples during storage, and examples with the highest protein hydrolysis were determined as

LM<sub>M</sub>, LM<sub>L</sub>, and LM (K), respectively. In this study, protein hydrolysis was associated with egg white protein powder added to lupin milk at 3% (w / v) to increase the protein level (5.08%) and dry matter in the lupin milk composition. In the study, protein hydrolysis

was associated with the sugar type and egg white protein powder added to milk (p <0.05).

	Storage Time	LM (K)	LM <sub>L</sub>	LM <sub>M</sub>
<b>Dry matter (%)</b>	Day 1	12.82±1.44 <sup>aA</sup>	13.00±1.14 <sup>aB</sup>	13.00±1.13 <sup>aB</sup>
	Day 7	12.25±1.26 <sup>aA</sup>	12.75±1.06 <sup>aB</sup>	12.85±1.69 <sup>aC</sup>
	Day 14	10.69±1.33 <sup>aA</sup>	11.36±1.46 <sup>aB</sup>	11.65±1.47 <sup>aC</sup>
	Day 28	10.44±1.67 <sup>aA</sup>	11.03±1.57 <sup>aB</sup>	11.26±1.33 <sup>aC</sup>
<b>Viscosity (cP)</b>	Day 1	899±5.11 <sup>aA</sup>	941±8.21 <sup>aB</sup>	1021±9.25 <sup>aC</sup>
	Day 7	1056±8.23 <sup>bA</sup>	1154±8.36 <sup>bB</sup>	1163±8.73 <sup>aC</sup>
	Day 14	1274±9.63 <sup>bA</sup>	1566±9.45 <sup>bB</sup>	1621±8.91 <sup>bC</sup>
	Day 28	1663±9.74 <sup>bA</sup>	1841±9.98 <sup>bB</sup>	2047±9.95 <sup>bC</sup>
<b>Syneresis (g)</b>	Day 1	9.52±1.01 <sup>aA</sup>	8.67±1.06 <sup>aB</sup>	8.55±1.02 <sup>aC</sup>
	Day 7	12.25±2.06 <sup>aA</sup>	11.36±1.03 <sup>AB</sup>	11.22±1.11 <sup>aC</sup>
	Day 14	13.49±1.12 <sup>aA</sup>	12.41±1.07 <sup>aB</sup>	12.10±1.53 <sup>aC</sup>
	Day 28	15.95±2.07 <sup>aA</sup>	14.65±2.06 <sup>aB</sup>	14.24±2.54 <sup>aC</sup>
<b>pH</b>	Day 1	4.60±1.22 <sup>aA</sup>	4.58±1.29 <sup>aB</sup>	4.56±1.11 <sup>aC</sup>
	Day 7	4.57±0.81 <sup>aA</sup>	4.42±1.06 <sup>aB</sup>	4.39±1.46 <sup>bC</sup>
	Day 14	4.45±0.63 <sup>aA</sup>	4.29±1.21 <sup>aB</sup>	4.25±1.89 <sup>bC</sup>
	Day 28	4.41±0.78 <sup>bA</sup>	4.19±1.63 <sup>bB</sup>	4.16±1.42 <sup>bC</sup>
<b>Titration acidity (%LA)</b>	Day 1	0.912±0.12 <sup>aA</sup>	0.938±0.10 <sup>aB</sup>	0.944±0.55 <sup>aC</sup>
	Day 7	0.988±0.22 <sup>aA</sup>	1.045±0.65 <sup>aB</sup>	1.095±0.63 <sup>bC</sup>
	Day 14	1.039±0.35 <sup>bA</sup>	1.121±0.75 <sup>bB</sup>	1.133±0.71 <sup>bC</sup>
	Day 28	1.044±0.63 <sup>bA</sup>	1.128±0.43 <sup>bB</sup>	1.139±0.39 <sup>bC</sup>
<b>Fat (%)</b>	Day 1	3.55±0.41 <sup>aA</sup>	3.57±0.66 <sup>aB</sup>	3.57±0.51 <sup>aB</sup>
	Day 7	3.12±0.96 <sup>bA</sup>	3.36±0.60 <sup>aB</sup>	3.38±0.62 <sup>bB</sup>
	Day 14	2.75±0.25 <sup>cA</sup>	3.19±0.82 <sup>bB</sup>	3.22±0.84 <sup>bB</sup>
	Day 28	2.35±0.57 <sup>cA</sup>	2.88±0.74 <sup>cB</sup>	2.93±0.78 <sup>cB</sup>

<b>Protein (%)</b>	Day 1	5.03±0.91 <sup>aA</sup>	5.00±0.82 <sup>aA</sup>	4.98±0.52 <sup>aB</sup>
	Day 7	4.62±0.93 <sup>aA</sup>	4.45±0.67 <sup>aA</sup>	4.33±0.87 <sup>aB</sup>
	Day 14	4.22±0.67 <sup>bA</sup>	4.16±0.88 <sup>aA</sup>	4.02±0.74 <sup>aB</sup>
	Day 28	4.06±0.76 <sup>bA</sup>	3.86±0.50 <sup>bA</sup>	3.75±0.46 <sup>bB</sup>
<b>Carbohydrate (%)</b>	Day 1	2.57±0.92 <sup>aA</sup>	3.51±0.80 <sup>aB</sup>	3.48±0.91 <sup>aB</sup>
	Day 7	2.54±0.82 <sup>aA</sup>	2.85±0.85 <sup>aB</sup>	2.44±0.72 <sup>aC</sup>
	Day 14	2.12±0.49 <sup>aA</sup>	1.57±0.63 <sup>aB</sup>	1.25±0.56 <sup>aC</sup>
	Day 28	1.95±0.57 <sup>aA</sup>	0.92±0.22 <sup>aB</sup>	0.84±0.21 <sup>aC</sup>
<b>Ash (%)</b>	Day 1	0.55±0.09 <sup>aA</sup>	0.57±0.09 <sup>aA</sup>	0.57±0.03 <sup>aA</sup>
	Day 7	0.31±0.08 <sup>aA</sup>	0.41±0.07 <sup>aA</sup>	0.43±0.07 <sup>aA</sup>
	Day 14	0.21±0.08 <sup>aA</sup>	0.33±0.09 <sup>aA</sup>	0.34±0.06 <sup>aA</sup>
	Day 28	0.16±0.07 <sup>aA</sup>	0.29±0.02 <sup>aA</sup>	0.30±0.02 <sup>aA</sup>

**Table 1.** Physicochemical Properties of Non-milk-based Fermented Samples (n = 3).

a, b, c The difference between values with different expressions in the same column is statistically significant ( $p < 0.05$ )

A, B, C The difference between values with different expressions in the same row is statistically significant ( $p < 0.05$ )

In the study, an acidity increase was observed in a fermented product produced only from lupin milk (LM) due to the activities of starter cultures without adding any ingredient. However, the acidity of the products obtained by adding different sugars (maltose and lactose) to lupin milk at 0.5% (w/v) is higher than that of the K sample. Therefore, the viscosity is higher than the K sample, and the syneresis level was found to be low compared to the K sample. In the study, sugar type/level added to lupin milk was found to be related to viscosity and syneresis ( $p < 0.05$ ).

Our results are consistent with the studies indicating that the symbiotic

relationship between microorganisms is impaired, and the pH development is slowed down or stopped with the increase in syneresis [40]. Syneresis was low in all samples, so the symbiotic relationship was not disturbed, and the pH increased. Different studies have reported that developing lactic acid bacteria (LAB) in an artificial environment (except milk) is troublesome but may develop quickly

in most plant - derived environments /substrates. Researchers relate this situation with the fact that most of the prebiotics used as food auxiliaries are saccharide derivatives [oligosaccharides (fructo-, gluco-, galacto-, isomalto-, xylo- and soy-oligosaccharides),

inulin, lactulose, lactosucrose, guar gum, resistant starch, pectin, chitosan], and they are primarily obtained from plants [39]. The literature states that fermented products can be produced from lupin milk, the fermentation time is shorter, and the starter culture level (especially probiotic microorganisms) is increased [23,29]. In this respect, it is seen that our research results are consistent with the literature.

### Rheological Properties

Syneresis and viscosity values are given in Table 1, and texture analysis results are in Table 2. The consistency values of the samples were significant for sugar type × storage time interaction ( $p < 0.05$ ). Clot stability (hardness) was increased in all models during storage,

	Depolamazamani	ABS	ABS <sub>L</sub>	ABS <sub>M</sub>
<b>Hardness(g)</b>	Day 1	36.22±1.05 <sup>aA</sup>	45.05±1.22 <sup>aB</sup>	51.30±1.15 <sup>aC</sup>
	Day 7	42.03±1.09 <sup>aA</sup>	52.47±1.47 <sup>bB</sup>	60.42±2.63 <sup>bC</sup>
	Day 14	55.33±1.01 <sup>bA</sup>	58.75±1.36 <sup>bB</sup>	67.55±2.78 <sup>cC</sup>
	Day 28	62.42±1.02 <sup>cA</sup>	66.88±2.11 <sup>cB</sup>	71.96±2.90 <sup>cC</sup>
<b>Viscosity</b>	Day 1	0.04±0.02 <sup>aA</sup>	0.05±0.03 <sup>aA</sup>	0.05±0.01 <sup>aA</sup>
	Day 7	0.03 ±0.01 <sup>aA</sup>	0.03±0.01 <sup>aA</sup>	0.04±0.02 <sup>aA</sup>
	Day 14	0.02±0.01 <sup>aA</sup>	0.03±0.01 <sup>aA</sup>	0.03±0.01 <sup>aA</sup>
	Day 28	0.01±0.01 <sup>aA</sup>	0.02±0.01 <sup>aA</sup>	0.02±0.01 <sup>aA</sup>
<b>Flexibility(mm)</b>	Day 1	4.00±0.52 <sup>aA</sup>	4.20±0.94 <sup>aB</sup>	4.63±0.99 <sup>aC</sup>
	Day 7	4.11±0.83 <sup>aA</sup>	4.29±0.63 <sup>aA</sup>	5.16±0.66 <sup>aB</sup>
	Day 14	4.36±0.64 <sup>aA</sup>	4.62±0.80 <sup>bB</sup>	5.55±0.83 <sup>bC</sup>
	Day 28	4.58±0.84 <sup>bA</sup>	4.81±0.91 <sup>bA</sup>	5.87±0.80 <sup>bB</sup>
<b>Gumminess(g)</b>	Day 1	41.05±1.12 <sup>aA</sup>	72.56±1.27 <sup>aB</sup>	79.47±1.22 <sup>aC</sup>
	Day 7	45.63±1.23 <sup>aA</sup>	78.56±2.24 <sup>aB</sup>	83.47±2.88 <sup>aC</sup>
	Day 14	49.22±1.66 <sup>aA</sup>	82.10±1.96 <sup>bB</sup>	85.33±2.10 <sup>bC</sup>
	Day 28	52.11±1.35 <sup>bA</sup>	85.45±2.67 <sup>cB</sup>	87.22±2.66 <sup>cC</sup>
<b>Chewiness(mJ)</b>	Day 1	0.14±0.05 <sup>aA</sup>	0.86±0.11 <sup>aA</sup>	0.92±0.30 <sup>aA</sup>
	Day 7	0.16±0.04 <sup>aA</sup>	1.12±0.26 <sup>aA</sup>	1.21±0.14 <sup>aA</sup>
	Day 14	0.21±0.03 <sup>aA</sup>	1.82±0.49 <sup>aA</sup>	1.88±0.47 <sup>aA</sup>
	Day 28	0.35±0.13 <sup>aA</sup>	2.02±0.28 <sup>aA</sup>	2.09±0.66 <sup>aA</sup>

**Table 2.** Texture Change During Storage in Non-Milk Based Fermented Products.

a, b, c The difference between values with different expressions in the same column is statistically significant ( $p < 0.05$ )

A, B, C The difference between values with different expressions in the same row is statistically significant ( $p < 0.05$ )

and the effect of storage was significant ( $p < 0.05$ ). Hardness, flexibility, gumminess, and chewiness increased, and viscosity decreased in all samples during storage. The hardness determined in LM<sub>M</sub> during storage was higher than in other examples. Between days 1 and 28, the hardness value of LM<sub>L</sub> samples was more elevated than LM.

In the study, the texture properties of the samples showed similar changes during storage. The rheological properties determined in LM<sub>M</sub> and LM<sub>L</sub> during storage were identical to LM but more acceptable. It was found that the LM sample was similar to the yogurt gel but had a more aqueous (fermented) consistency than the other samples. During storage, the increase in acidity was lower, syneresis was higher, and the viscosity was lower in LM samples.

### Fatty Acid Composition in Lupin Milk and Fermented Products

Saturated fatty acids (SFA) were determined at 14.17g / 100g, monounsaturated fatty acids (MUFA) at 52.3g / 100g and polyunsaturated fatty acids (PUFA) at 9.86g / 100g. The proportions of saturated and unsaturated fatty acids in lupine milk were oleic acid (C18: 1) (n-9) 49%, linoleic acid (C18: 2) (n-6) 23.41%, palmitic acid (C16: 0)% 7.33, Gadoleic acid (C20: 1) (n-9) 3.46%, stearic acid (C18: 0)

1.62%, arachidic acid (20: 0) 2.85%, erucic acid 1.38%, Myristic acid (C14: 0)% 0.49, Pentadecanoic acid (C15: 0) 0.21% and Lauric acid (C12: 0) 0.05%. The research determined that the fatty acid profile of fermented products made with different sugars added to lupin milk was very close to the lupin milk fat profile. However, both the lupin milk and the fatty acid profile in the fermented products produced from this milk were examined, and it was found that the fatty acids in the prophylaxis of circulatory system diseases are important in terms of containing important groups of fatty acids [41].

### Microbiologic Analysis

According to the microbiological counting results, the starter culture level of LM samples decreased from the beginning to the storage end. Accordingly, on the 1st day of storage, *Str. thermophilus* level was determined as 6.45 Log<sub>10</sub>kob<sup>-ml</sup> and 4.27 Log<sub>10</sub>kob<sup>-ml</sup> on the 28th day. In LM samples, *Lb. bulgaricus* story was 6.38 Log<sub>10</sub>kob<sup>-ml</sup> on the 1st day of storage and 5.12 Log<sub>10</sub>kob<sup>-ml</sup> on the 28th day.

In LM samples, *Lb. bulgaricus* and *Str. thermophilus* development was found to be weaker than LM<sub>L</sub> and LM<sub>M</sub> samples. However, this situation did not appear to be a problem producing a fermented product. On the

contrary, it strengthened the opinion that the lupin milk composition is suitable for non-dairy fermented product production. In the study, the determination of starter culture levels between 7 Log<sub>10</sub>kob<sup>-g</sup> and 8 Log<sub>10</sub>kob<sup>-g</sup> groups until the 14th day of storage was associated with protein level (5.08%) and protective properties of lupin milk on cultures.

In the study, the level of *Str. thermophilus* was determined as 7.32 Log<sub>10</sub>kob<sup>-ml</sup> on day 1 and 8.56 Log<sub>10</sub>kob<sup>-ml</sup> on maltose-added LM<sub>M</sub> samples prepared by adding 0.5% (w / v) to lupin milk. *Lb. bulgaricus* level was determined as 7.03 Log<sub>10</sub>kob<sup>-ml</sup> on day 1 and 7.85 Log<sub>10</sub>kob<sup>-ml</sup> on day 7. *Str. thermophilus* level was 8.74 Log<sub>10</sub>kob<sup>-ml</sup> and *Lb. bulgaricus* level was 8.42 Log<sub>10</sub>kob<sup>-ml</sup> in LM<sub>M</sub> samples on the 14th day of storage. *Str. thermophilus* level 6.36 Log<sub>10</sub>kob<sup>-ml</sup>, *Lb. bulgaricus* level was 7.49 Log<sub>10</sub>kob<sup>-ml</sup>.

Accordingly, the *Str. thermophilus* level in LM samples was determined as 7.88 Log<sub>10</sub>kob<sup>-ml</sup> on day 1 and 8.89 Log<sub>10</sub>kob<sup>-ml</sup> on day 7 of storage. *Lb. bulgaricus* level was 7.21 Log<sub>10</sub>kob<sup>-ml</sup> on day 1 and 8.29 Log<sub>10</sub>kob<sup>-ml</sup> on day 7 of storage. In ABSL samples, *Str. thermophilus* level at day 14 was 6.81 Log<sub>10</sub>kob<sup>-ml</sup>, *Lb. bulgaricus* level was determined as 7.76 Log<sub>10</sub>kob<sup>-ml</sup>. On the 28th day of storage, *Str. thermophilus* level 6.26 Log<sub>10</sub>kob<sup>-ml</sup>, *Lb. bulgaricus* level was 7.16 Log<sub>10</sub>kob<sup>-ml</sup>.

### Sensory Analysis

In the sensory evaluation results, LM<sub>M</sub> and LM<sub>L</sub> samples were appreciated in terms of textural properties. In the evaluation during storage, LM<sub>M</sub> and LM<sub>L</sub> pieces obtained similar scores in terms of structure consistency.

It was associated with increased syneresis, acidity, viscosity, and hardness during storage. The relationship between an increase in storage time and structure consistency was significant ( $p < 0.05$ ). Two other sugar additions were found to be closer in terms of structural properties of the conventional fermented product, yogurt, and longer storage time in LM<sub>M</sub> and LM<sub>L</sub> samples. It has been suggested that the structure and consistency of the fermented models produced by adding different sugars to the lupin milk with the increase of storage time became more similar to the conventional fermented product and that the aroma of lupin milk was not felt (or felt low). It was continued particularly until the end of storage.

The structure consistency in LM samples was found to be less dense, and panelists concluded that it could be considered a fermented milk drink. Fat levels in sugar-added samples during storage were also thought to affect the taste. However, with the addition of two different sugars to lupin milk, no discoloration was detected in LM<sub>M</sub> and LM<sub>L</sub> samples, and it was accepted as acceptable by the panelists.

In the research, it was determined that adding 0.5% (w/v) maltose and lactose to lupine milk, products that are not milk-based and have similar properties to yogurt made from milk in terms of physicochemical, sensory, rheological, and microbiological properties can be produced.

### CONCLUSION

The study resulted from the development of starter cultures, egg white protein powder (EWPP), and various

disaccharides (lactose and maltose). Microbially are hydrolyzed, and a non-milk-based fermented product was obtained from the enrichment of lupin milk (LM) with bioactive peptides. In addition, the relationship between classical yogurt cultures and two different disaccharides and YBPT (antagonistic, synergist, stimulant effect) was demonstrated. At the same time, the rheological and sensory properties of fermented products produced with lupine milk were determined. A product that consumers can choose from has emerged. As a result of the research, a fermented product type that is not milk based has been presented to the consumer with the production of the required standards. Further studies should be carried out on the industrial-scale production of fermented dairy products from lupine milk and introduced to consumers. In summary, lupin milk is an innovative and promising nutritional product for sports nutrition. Thus, it is the statement of this study that lupin milk is a product that should be popularised and commonly used, particularly by athletes.

#### Authors' Contributions

Kavas N. and Oral O. designed the study; data were collected and analyzed by Kavas N., Kavas G., and Oral O.; data interpretation and manuscript preparation were undertaken by Kavas N., Kavas, G., and Oral, O. All authors approved the final version of the paper.

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#### Conflict Of Interest

The author certifies that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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