



Development and Techno-functional Characterization of Beyaz Cheese Fortified with Walnut (*Juglans regia* L.) Leaf Powder and *Lactobacillus acidophilus* LA-5

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Abstract

This study aimed to produce a new functional Beyaz cheese variety with the addition of different ratios (0.5% and 1%) of walnut leaf powder (WLP) and probiotic bacteria (*Lactobacillus acidophilus* LA-5.). The probiotic shelf-life and techno-functional properties of Beyaz cheeses were investigated at 30-day intervals during ripening in brine (120 days at +4 °C). The highest acidity, ash, salt, Fe, Cu, Mn, a^* , b^* , H° , and C^* values were found in the WLP-supplemented cheeses compared with those in the control without probiotic (C) and probiotic control (PC) cheeses ($p < 0.01$). The count of *L. acidophilus* LA-5 in probiotic cheeses decreased to less than 10^6 colony-forming units (CFU)/g after the 60th day of storage. Compared with PC (77.52%), WLP increased the viability rate (82.04–86.52%) over the 60-day shelf-life period. The total phenolic content (TPC), total flavonoid content (TFC), chlorophyll *a* and chlorophyll *b* contents, and total antioxidant activity (DPPH•, CUPRAC, and FRAP assays) of the cheeses significantly increased with the addition of 0.5 and 1% WLP ($p < 0.01$). The addition of WLP increased proteolysis (WSN, pH 4.6–SN, TCA–SN and PTA–SN) and lipolysis ($p < 0.01$). The general acceptability of cheese samples was $PC > C > PWL_{0.5\%} > PWL_{1\%}$. Consequently, a new variety of functional Beyaz cheese with a probiotic shelf life of 60 days that is rich in bioactive compounds and microminerals (Fe, Cu and Mn) and natural for healthy nutrition can be produced with the addition of *L. acidophilus* LA-5 and 0.5% WLP.

Keywords Beyaz cheese (Turkish White pickled cheese) · Walnut leaf powder · *Lactobacillus acidophilus* LA-5 · Probiotic shelf-life · Functional food · Bioactive compounds

Introduction

Consumers are becoming increasingly aware of the relationship between food and health; hence, functional foods are gaining popularity. Functional foods contain bioactive compounds (such as antioxidants, dietary fiber, minerals, and vitamins) and live probiotic microorganisms that positively affect the health of the consumer in addition to providing

basic nutrition [1, 2]. Milk and dairy products are widely used in the development of functional foods [3]. For this purpose, the fortification of dairy products with natural herbal sources [1, 4, 5] and probiotic bacteria [1, 2, 6–9] is a common application.

Cheese is the dairy product that is produced the most and has the most varieties among dairy products [10]. The addition of herbs and spices to cheese has been practiced since ancient times, usually in the production of traditional cheeses. There are numerous cheeses produced with natural herbs in many countries, such as Italy (Casoperuto, Marzolino, Romano pepato, and Piacentinu Ennese cheeses), Switzerland (Swissalp Panorama and Bellevue cheeses), France (Le Roule and Boulette d'Avesnes cheeses), the Netherlands (Kanterkass cheese), Egypt (Karish cheese), Syria (Shankalish cheese), Morocco (Jben and Raib cheeses), and Turkey (Van Otlı, Sürk, and Carra cheeses) [11]. Previous studies have shown that herbal sources such as red pepper, green pepper, thyme, ginger, basil, mint, black cumin,

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turmeric, dill, cress parsley, garlic, saffron, pomegranate peel, and *Moringa oleifera* leaf powder are added to the production of some cheeses [11–17].

With the introduction of modern technologies and “green chemistry” principles, new steps have been taken to transform waste and byproducts from the agriculture and food sector into high-value-added products [18]. Walnut leaf, which is an agricultural waste, is a medicinal and aromatic plant used in folk medicine for the treatment of various diseases. It is an important natural resource with a rich phytochemical profile [19]. Walnut (*Juglans regia* L.) is a tree belonging to the Juglandaceae family and is widely cultivated around the world, especially in Southeast Europe, East Asia, and North America [20, 21]. Walnut leaves are a rich source of antioxidants, including secondary bioactive compounds such as phenolic acids, flavonoids, organic acids, tocopherols, triterpenic acids, terpenes, terpenoids, tetralone derivatives, megastigman derivatives, and juglone [22]. Walnut leaves contain 15.03% protein, 3.41% fat, 9.04% ash, 23.46% carbohydrate, 7.43% tannin, 38.64 g/kg Ca, 11.79 g/kg K, 0.90 g/kg Na, 7.52 g/kg Mg, 190.53 mg/kg Fe, 24.30 mg/kg Zn, 148 mg/kg Mn, and 2.63 mg/kg Co [23]. It is also remarkable in terms of volatile aroma compounds (caryophyllene oxide, β -caryophyllene, germacrene, α -pinene, β -pinene, etc.) [20]. Walnut leaves are used in traditional medicine to treat venous insufficiency, hemorrhoids, hypoglycemia, diarrhea, and fungal and microbial infections [19, 20]. In in vitro and in vivo epidemiological studies, walnut leaves have been reported to have therapeutic effects, such as antioxidant, antimicrobial, antifungal, anti-inflammatory, antidiabetic, antihelminthic, antidiarrheal, antihistamine, antiulcer, antiasthmatic, immunomodulatory, antifertility, and hepatoprotective effects [21, 22]. Owing to their rich bioactive compounds and therapeutic properties, walnut leaves are promising for the production of functional foods and for extending the shelf-life of foods [19].

The principal aim of this research was to convert WLP, an agricultural waste rich in bioactive compounds, into added value in the production of Beyaz cheese (Turkish White pickled cheese) as a functional additive. Beyaz cheese is a type of cheese produced from pasteurized milk and ripened in brine. Cheese, which is manufactured via traditional and industrial methods, is the cheese type with the highest economic value in Turkey [10]. This cheese generally acquires its properties by maturing for 3 or longer periods.

In this study, we aimed to produce new functional Beyaz cheese variety(s) with the addition of different ratios (0.5% and 1%) of walnut leaf powder (WLP) and probiotic bacteria (*L. acidophilus* LA-5). The probiotic shelf-life and antioxidant, physicochemical, microbiological, biochemical, mineral, textural, and sensory qualities of Beyaz cheeses were investigated at 30-day intervals during ripening in brine (120 days at +4 °C). Thus, an alternative for converting walnut

leaves into added value as a functional additive was developed, and new and inexpensive probiotic Beyaz cheeses with nutritional and functional properties were produced.

Materials and Methods

Materials

Raw cow milk, calcium chloride (CaCl₂, Koruma Group of Companies, Denizli, Turkey), microbial-type liquid rennet (750 IMCU/mL, Fermento, BF Intermak, Konya, Turkey), and rock salt (Pınar Salt, Ankara, Turkey) were obtained from the Atatürk University Food and Livestock Application and Research Center, Pilot Factory of Dairy Products (Erzurum, Turkey). The probiotic strain of *Lactobacillus acidophilus* (Nu-trish LA-5) and starter culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) were obtained from Chr-Hansen (Istanbul, Turkey). Walnut (*J. regia* L.) leaves were collected from walnut trees belonging to a local genotype from a garden in Bayburt Province (Turkey) at the end of September 2021 and were washed and dried (5–6 days) in the shade.

Preparation of Walnut Leaf Powder (WLP) and Preliminary Trials

The dried walnut leaves were ground to a fine powder via a laboratory blender (Blender 8011ES, HGB2 WTS3 Model, USA), and the particle size was standardized by passing through a 500-micron mesh sieve. The WLP was subsequently transferred into glass jars, wrapped with aluminum foil, and pasteurized in an autoclave (WiseClave, WAC-47, Korea) set at 70 °C for 15 min. The WLP was maintained at +4 °C until cheese production and analysis.

Preliminary trials were performed to determine the WLP ratios and select probiotic bacteria for cheese production. Various WLP concentrations (0.5%, 1%, and 2%) were used to evaluate the flavor preference of the cheese, in accordance with previous research [15, 24, 25]. According to the organoleptic test results, the addition of 2% WLP to the cheese increased the foreign taste and masked the cheese flavor. The 0.5% and 1% WLP ratios were determined to be the most ideal ratios and did not cause bitterness in the cheese. In preliminary experiments in which *Bifidobacterium animalis* subsp. *lactis* (BB-12) and *L. acidophilus* LA-5 were added, the addition of WLP (0.5 and 1%) did not negatively affect the viability of either probiotic bacteria. The live bacteria count in the preliminary test cheeses produced with both probiotics was 10^{8–9} CFU/g. Previous studies have shown that *L. acidophilus* LA-5 maintains its viability at a relatively high rate in Beyaz cheese and is preferable [6, 8, 26]. Therefore, *L. acidophilus* LA-5 was used in this study.

Production of the Beyaz Cheese Samples

The production of Beyaz cheeses was carried out in two replicates at Atatürk University Food and Livestock Application and Research Center, Dairy Products Pilot Factory (Erzurum, Turkey), on the basis of the classical Beyaz cheese procedures reported by Erkaya and Sengul [6]. The raw milk was pasteurized at 70 °C for 12 min, cooled to 37 °C, and divided into four equal parts. After the addition of 0.002% (weight/volume, w/v) starter culture and 0.015% (w/v) CaCl₂, one group was separated as a control. The remaining three groups were inoculated with 0.075% (w/v) probiotic bacteria (*L. acidophilus* LA-5). All inoculated milk was pre-ripened (~ 60 min) at 37 °C until the pH reached 6.2. The milk was then coagulated with rennet (1.5 mL/100 L) for 60 min. The coagulum was cut into ~ 1 cm³ cubes and transferred to a cheesecloth to separate the whey. WLP was subsequently added to the remaining cheese curds separately at 0.5% and 1%, except for the control cheese (C) and probiotic-containing control cheese (PC) curds, and homogeneous mixing was ensured. The curds were pressed (~ 2.5 kg weight/1 kg curd) at room temperature for 2 h. After the removal of the whey, the cheese blocks were portioned into blocks of 7 × 7 × 7 cm, and the fresh cheeses were put into brine (12° Baume) for 5 h at room temperature. The brined blocks were then placed into plastic containers (500 mL), the package was filled with new brine (10° Baume), and the container was closed. Packaged cheeses were stored for 120 days at 4 °C ± 1. The analyses determined on the 1st, 30th, 60th, 90th, and 120th days of ripening were performed in duplicate (separately for each replicate).

Physicochemical Analysis

The dry matter (gravimetric method; AOAC 926.08), protein (micro-Kjeldahl method, total nitrogen % × 6.38; AOAC 920.123), and ash (gravimetric method; AOAC 935.42) contents of the cheese samples were determined according to AOAC [27]; the pH values (using a digital pH meter/Seven Compact TM pH/Ion S220 brand), titratable acidity (% lactic acid), fat (Gerber method), and salt (titration method with AgNO₃) contents were determined according to Kurt et al. [28].

Colorimetric Analysis

The color characteristics of the cheese samples were determined via colorimetry (Minolta CR-200, Minolta Chromometer, Japan) as *L** (100: white, 0: black), *a** (+ : red, - : green), *b** (+ : yellow, - : blue), *H*° (hue angle; 0°: red, 90°: yellow, 180°: green, 270°: blue, 360°: red) and *C** (color saturation). Measurements were recorded directly at different points on the cross-sectional surface of cheese slices

(5–10 mm thin) placed in optical glass Petri dishes (100 × 20 mm) on a white background [29].

Microbiological Analysis

A 10 g sample (cheese, WLP and milk) was mixed with 90 mL of sterile salt solution (0.85% NaCl) and homogenized with a Stomacher (Interscience Bagmixer 400 P, France) for 2 min. A tenfold dilution was made from the initial dilution until a 10⁻⁸ dilution was reached. *L. acidophilus* LA-5 enumeration in the probiotic cheeses was performed at 37 °C for 72 h in anaerobic jars by MRS-Bile agar (0.15%, w/v; Bile Salt-Oxoid, Basingstoke, Hampshire, UK). Colonies were counted under a Leica DM500 microscope via a Leica ICC50 high-definition (HD) camera (Leica Microsystems, Wetzlar, Germany) [30]. The percentage of viable *L. acidophilus* LA-5 was calculated via the formula [% viability = (CFU at 2 months of storage/initial CFU) × 100] given by Shin et al. [31]. The enumeration of total aerobic mesophilic bacteria (TAMB) (Plate Count Agar; Merck Darmstadt, Germany) was performed at 30–32 °C for 48 h [32], *Lactobacilli* (MRS Agar; Merck) at 30 ± 1 °C for 72 h and *Lactococci* (M-17 Agar; Merck) at 30 ± 1 °C for 24 h [33], total coliform bacteria (Violet Red Bile Agar; Merck) at 35–37 °C for 48 h [32], *Escherichia coli* (Chromocult Tryptone Bile X Glucuronide Agar; Merck) at 44 °C for 24 h [34], and yeasts and molds (Dichloran Rose Bengal Chloramphenicol Agar; Merck) at 25 °C for 3–5 days [35]. The results are given as log colony forming units (CFU)/g cheese.

Texture Profile Analysis (TPA)

TPA of the cheese samples was determined by texture analyzer (Brookfield CT3 Model, Brookfield Engineering Laboratories, Middleboro, MA, USA) assisted by a 50 mm cylindrical probe (TA 25/1000) according to Liu et al. [36], with slight modifications. The cheese samples were cut into cubes (2 cm³) and kept at room temperature for 30 min before the test. The analysis conditions were as follows: pretest speed, 1 mm/s; test speed, 1 mm/s; posttest speed, 2 mm/s; surface trigger, 5 g; hold time, 5 s; and compression ratio, 50%. The hardness, adhesiveness, cohesiveness, springiness, chewiness, gumminess, and resilience values of the samples were calculated from the force–time curves. TPA analysis was performed on four replicates for each cheese sample.

Mineral Analysis

The mineral composition of the cheese samples (macro: Ca, K, P, Na, and Mg; micro: Cu, Fe, Mn, Zn, and Se) was determined according to Bertin et al. [37], with slight modifications. First, cheese samples were dried at 70 °C in an oven until dry weight. A 70 mg sample was mixed with

8 mL nitric acid (HNO_3 , 65%, Merck) and 2 mL hydrogen peroxide (H_2O_2 , 30%, Merck) and digested (30 min at 200 °C) in a microwave oven (Milestone Ethos Up). The samples were subsequently diluted (15-fold) with ultrapure water, filtered (0.45 μm) and analyzed via inductively coupled plasma–mass spectrometry (ICP–MS–Agilent 7800 series, Agilent Technologies, Japan). Before starting the analysis, the instrument was purged with helium gas for 45 min. Measurements were performed at 1200 W RF power, 1 L/min carrier gas (argon) flow, and 0.30 rps nebulizer pump speed. The mineral content of the samples was calculated from the calibration curves of the multielement standard solution VIII (24 elements in dilute nitric acid, Merck) prepared in the range of 10–500 ppb ($\mu\text{g}/\text{kg}$) using the “Mass Hunter 4.4 Workstation Software 7800 ICP–MS Top C.01.04” software. The results are given in mg/100 g dry weight for macro minerals and $\mu\text{g}/100$ g dry weight for microminerals. In addition, the mineral contribution (%) of a serving portion of fresh Beyaz cheese (100 g) to daily nutrition was approximated on the basis of the reference limits reported by Nabrzyski [38].

Bioactivity Analysis

Preparation of Extracts from Cheese Samples for Determination of Antioxidant Activity

Extraction (two replicates) of the samples was carried out according to the method proposed by Vukić et al. [29], with slight modifications. For this purpose, 4 g of cheese was transferred separately to Falcon tubes, 16 mL of ethanol (80%, v/v) was added, and the mixture was extracted in an orbital shaker (IKA KS 130, IKA) for 12 h (25 °C, 400 rpm), followed by centrifugation at 10,000 rpm (Allegra X-30R Centrifuge, Germany) for 10 min at 4 °C. The supernatants were filtered through Whatman No. 1 filter papers, and the ethanolic extracts were stored at –20 °C in the dark until analysis. The prepared extracts were analyzed for total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity (DPPH•, CUPRAC, and FRAP assays).

Determination of Total Phenolic Content (TPC)

The TPC of the WLP and cheese extract was determined colorimetrically at 765 nm using a Folin-Ciocalteu reagent (Merck) as described by Altin et al. [39]. Gallic acid (Merck) was used as a standard to prepare the calibration curve ($y = 0.0072x + 0.0010$, $R^2 = 0.9979$). The results are reported as mg gallic acid equivalent (GAE)/100 g sample.

Determination of Total Flavonoid Content (TFC)

The TPC of the WLP and cheese extracts was determined at 510 nm via the aluminum chloride method reported by Dewanto et al. [40]. Quercetin (Sigma–Aldrich, Germany) was used as a standard to prepare the calibration curve ($y = 0.0008x + 0.0038$, $R^2 = 0.9990$). The results are reported as mg quercetin equivalent (QE)/g sample.

Determination of Total Antioxidant Activity

Three methods were employed to measure the total antioxidant activity of the WLP and cheese sample extracts. The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging capacity of the extracts was determined by measuring the absorbance at 517 nm as reported by Brand-Williams et al. [41] and Gülçin et al. [42]. The cupric ion-reducing antioxidant capacity (CUPRAC) of the extracts was determined at 450 nm via the method of Apak et al. [43]. The ferric-reducing antioxidant power (FRAP) of the extracts was determined at 593 nm via the method of Benzie and Strain [44]. Standard Trolox solutions were prepared at six concentrations for the calibration curve ($y = 0.0049x + 0.0136$, $R^2 = 0.9987$ for the DPPH• assay; $y = 0.0012x - 0.0860$, $R^2 = 0.9951$ for the CUPRAC assay; $y = 0.0174x + 0.0232$, $R^2 = 0.9993$ for the FRAP assay). All antioxidant activities were expressed as mg Trolox (Sigma–Aldrich) equivalent (TE) per 100 g sample.

Chlorophyll (Chl) Pigment Analysis

The Chl *a* and Chl *b* contents of the WLP and cheese samples were analyzed via the spectrophotometric method reported by Barros et al. [45], with slight modifications. For this purpose, 2 g of cheese sample or 0.1 g of WLP was homogenized by adding 8 mL of an acetone ($\text{C}_3\text{H}_6\text{O}$, Merck)–hexane (C_6H_{14} , Merck) mixture (40:60, v/v), followed by centrifugation at 5000 rpm for 5 min. The supernatants were filtered through Whatman No. 1 filter papers, and the absorbance was recorded at 645 nm and 663 nm. The contents of Chl *a* and Chl *b* were calculated according to the following equation: Chl *a* (mg/100 mL) = $(0.999 \times A_{663}) - (0.0989 \times A_{645})$; Chl *b* (mg/100 mL) = $(-0.328 \times A_{663}) + (1.77 \times A_{645})$, and the results are expressed in μg per g sample.

Proteolysis and Lipolysis Analysis

Water-soluble nitrogen (WSN), 12% trichloroacetic acid-soluble nitrogen (TCA–SN), 5% phosphotungstic acid-soluble nitrogen (PTA–SN), and pH 4.6-soluble nitrogen (pH 4.6–SN) fractions of the cheese samples were prepared according to the methods of Hayaloglu et al. [46]. The

nitrogen content of the fractions was determined via the micro-Kjeldahl method [47] and expressed as a percentage of total cheese nitrogen. Lipolysis (acid degree value: meq KOH/100 g fat) analysis of the cheese samples was performed according to the BDI (Bureau of Dairy Industries) method [48].

Sensory Analysis

For the sensory evaluation of the cheese samples, the color, texture and appearance, odor, flavor, foreign taste, salinity, and overall acceptability criteria were scored as 1–9. Cheese samples ($\sim 2 \times 2 \times 2 \text{ cm}^3$) were presented at 6–8 °C after being placed on plates. The samples were evaluated on a score scale of 1 (poor/reject) to 9 (very good/ideal) by a group of 20 semitrained panelists (nonsmokers, aged between 25 and 60 years, informed by us about the product and sensory analysis methods and with the same panel group as possible). Water and bread were given to the panelists to separate the flavors among the different samples.

Statistical Analysis

All the data were statistically analyzed via SPSS 20.0 (SPSS, Inc., Chicago, IL, USA), which is licensed software. The factors that were significant according to the analysis of variance (ANOVA) performed on all the variables were evaluated via the Duncan multiple comparison test at the 95% confidence level. The correlations among the determined variables (physicochemical, microbiological, and textural characteristics; proteolysis; and bioactivity) were calculated via Pearson's correlation test. The correlation coefficients were categorized according to the classification proposed by Schober et al. [49]. Principal component analysis (PCA) was

performed via SIMCA 14.1 software (MKS UMETRICS, Umea, Sweden), which focuses on the bioactivity and ripening properties of cheese samples.

Results and Discussion

Characteristics of the Raw Materials Used in Cheese Production

The chemical composition and microbiological properties of cow milk were as follows: pH 6.55, titration acidity (lactic acid) 0.17%, dry matter 12.84%, milk fat 4.30%, protein 3.21%, ash 0.74%, TAMB 4.21 log CFU/g, yeast-mold < 2 log CFU/g, and coliform bacteria < 1 log CFU/g. The results demonstrate that the milk is within the expected composition limits. Composition and properties of WLP: pH 5.51, dry matter 94.02%, protein 10.23%, ash 10.46%, Chl *a* 1612 µg/g and Chl *b* 570 µg/g; color characteristics: L^* 46.19, a^* −9.37, b^* 21.87, H° 113.10, and C^* 23.78; and microbiological characteristics: yeast-mold < 2 log CFU/g and coliform bacteria < 1 log CFU/g. The results revealed that pasteurization applied to both milk and WLP for cheese production was effective and that the samples did not pose a food safety risk to indicator microorganisms.

Physicochemical Characteristics

The results of the analysis of the physicochemical properties of the experimental Beyaz cheese samples are given in Table 1. The addition of WLP and *L. acidophilus* LA-5 significantly affected the physicochemical properties of the cheese samples during the ripening period ($p < 0.01$). The pH of the cheese samples ranged from 5.00–5.12, the acidity

Table 1 Physicochemical properties of experimental Beyaz cheese samples

Samples	<i>n</i>	pH	Acidity (%LA)	Dry matter (%)	Fat (%)	Protein (%)	Ash (%)	Salt (%)
C	10	5.12 ± 0.13 ^a	0.78 ± 0.12 ^c	36.33 ± 2.64 ^b	14.93 ± 1.38 ^b	14.41 ± 1.75 ^a	5.87 ± 0.15 ^b	4.57 ± 0.35 ^b
PC	10	5.00 ± 0.05 ^b	0.86 ± 0.08 ^b	37.66 ± 1.65 ^a	15.85 ± 0.75 ^a	14.58 ± 0.96 ^a	5.74 ± 0.22 ^c	4.57 ± 0.24 ^b
PWL _{0.5%}	10	5.01 ± 0.10 ^b	0.90 ± 0.18 ^a	35.65 ± 1.36 ^c	14.68 ± 1.34 ^b	13.83 ± 0.76 ^b	5.71 ± 0.18 ^c	4.77 ± 0.31 ^b
PWL _{1%}	10	5.03 ± 0.08 ^b	0.91 ± 0.15 ^a	35.45 ± 0.68 ^d	13.95 ± 1.26 ^c	13.64 ± 1.04 ^b	6.10 ± 0.27 ^a	5.03 ± 0.47 ^a
	<i>p</i>	**	**	**	**	**	**	**
Ripening (Day)	<i>n</i>	pH	Acidity (%LA)	Dry matter (%)	Fat (%)	Protein (%)	Ash (%)	Salt (%)
1	8	5.18 ± 0.10 ^a	0.66 ± 0.07 ^c	38.82 ± 2.13 ^a	16.22 ± 0.88 ^a	15.79 ± 1.48 ^a	5.87 ± 0.24 ^a	4.58 ± 0.51 ^b
30	8	5.06 ± 0.09 ^b	0.80 ± 0.07 ^b	36.70 ± 0.87 ^b	15.41 ± 0.74 ^b	13.95 ± 0.75 ^b	5.83 ± 0.17 ^b	4.71 ± 0.36 ^{ab}
60	8	5.00 ± 0.05 ^c	0.93 ± 0.08 ^a	35.27 ± 0.65 ^d	14.66 ± 1.00 ^c	13.80 ± 0.66 ^b	5.76 ± 0.23 ^b	4.60 ± 0.35 ^b
90	8	4.98 ± 0.07 ^c	0.96 ± 0.11 ^a	35.92 ± 0.93 ^c	14.22 ± 1.13 ^{cd}	13.78 ± 0.68 ^b	5.93 ± 0.38 ^a	4.90 ± 0.36 ^a
120	8	4.99 ± 0.10 ^c	0.97 ± 0.09 ^a	34.66 ± 1.23 ^e	13.75 ± 1.48 ^d	13.27 ± 0.55 ^c	5.89 ± 0.18 ^a	4.90 ± 0.28 ^a
	<i>p</i>	**	**	**	**	**	**	**

^{a–d}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. **Cheeses; C:** Control without probiotic, **PC:** Probiotic control, **PWL_{0.5%}:** Probiotic and 0.5% walnut leaf powder, **PWL_{1%}:** Probiotic and 1% walnut leaf powder

ranged from 0.78 to 0.91%, the dry matter content ranged from 35.45 to 37.66%, the fat content ranged from 13.95 to 15.85%, the ash content ranged from 5.71 to 6.10%, and the salt content ranged from 4.57 to 5.03%. As the WLP ratio increased, the amounts of dry matter, fat, and protein in the cheese decreased relatively, whereas the amounts of ash and salt increased ($p < 0.01$) compared with those in the control samples. This may be because WLP added in powder form increased the moisture content of the cheese mass because of its water-binding properties (3.44 g water/g sample). Similarly, Mousavi et al. [50] and Kamel et al. [9] reported that the dry matter content of cheese decreased with the addition of mushroom (*Ganoderma lucidum*) extracts (0.5–2.0%) and carrot powder (0.2–0.6%), respectively. In accordance with the Turkish Food Codex Communiqué on Cheese [51], the cheese samples (average 78%) were classified as soft cheese ($70\% \geq \text{WFCM}$) in terms of the amount of water in the fat-free cheese mass (WFCM) and as semifat cheese ($25\% \leq \text{milk fat} < 45\%$) in terms of the amount of fat in dry mass content (39.32–42.11%). The increasing effect of WLP on ash and salt content in cheese may be due to the saline water binding from the cheese brine due to the water retention properties of WLP or its high ash content (10.46%). Similarly, Yerlikaya and Karagözlü [52] reported that the addition of capers (8%), Eid et al. [15], galangal (*Alpinia officinarum*) powder (0.25–1%), and Kamel et al. [9] carrot powder increased the salt content of cheese. El Hatmi et al. [53] reported that the addition of rosy garlic (*Allium roseum*) powder (0.5%), El-Sayed [25] spinach powder (0.5–2%), and El-Sayed and Ibrahim [54] red radish powder (1–3%) increased the ash content of cheese.

The pH value, dry matter, fat, and protein contents decreased, whereas the salt and acidity contents increased in all cheese samples during the ripening period ($p < 0.01$). Similarly, previous studies reported that the dry matter, protein, and fat contents of Beyaz cheese decreased [8, 14, 52] and that the ash content [55] increased during ripening. This was due to the decrease in dry matter amount during ripening, which decreased the relative amounts of fat and protein. This decrease is also known to be due to the osmotic equilibrium between the cheese and the brine [6]. Indeed, the dry matter amount was significantly ($p < 0.01$) strongly positively correlated with the fat ($r = 0.86$) and protein ($r = 0.75$) amounts (Fig. 1a).

Color Characteristics

The color of food is an important criterion for consumer acceptance. The color characteristics of the experimental Beyaz cheese samples are given in Table 2. WLP addition and ripening period significantly affected color characteristics ($p < 0.01$). In the Beyaz cheese samples, the L^* value ranged between 63.24 and 84.66, the a^* value ranged

between -3.24 and -6.07 , the b^* value ranged between 14.29 and 18.18, the H° value ranged between 102.37 and 108.37, and the C^* value ranged between 14.84 and 19.06. As the WLP ratio increased, the L^* value decreased, whereas the a^* , b^* , H° , and C^* values increased ($p < 0.01$). Similarly, Mazinani et al. [56] reported that the L^* value varied between 26.39 and 58.76, the a^* value between -1.17 and -6.16 , and the b^* value between 4.66 and 12.17 during ripening (45 days) in UF-Feta cheeses with *Spirulina platensis* (0.3–0.8%) and mint (0.5–1%) addition. Researchers reported that brightness (100: white, 0: black) decreased as the plant ratio increased, whereas the green ($-a^*$) and yellow ($+b^*$) color values increased. El-Sayed [25] reported that L^* (51.85–70.05) and b^* (14.09–21.49) values decreased and that a^* values (-1.17 – 2.68) increased as the ratio of spinach in cheese increased. The ability of WLP to increase the green color value is related to the composition of Chl. Indeed, strong positive correlations were found between the a^* value and the Chl a ($r = 0.83$), Chl b ($r = 0.87$), b^* ($r = 0.85$), H° ($r = 0.94$), and C^* ($r = 0.91$) values at significant levels ($p < 0.01$) (Fig. 1b). During ripening, the L^* value generally decreased, the a^* value increased until day 60, and the b^* and C^* values did not change after day 30. Similarly, Mazinani et al. [56] reported that the brightness of cheeses decreased and the green color value increased during ripening.

Microbiological Properties and Probiotic Viability

The results of the analysis of the microbiological properties of the experimental Beyaz cheese samples are given in Table 3. The WLP addition and ripening periods significantly affected the probiotic shelf-life and microbiological properties of the cheese samples ($p < 0.01$). The lowest count of TAMB was determined in the C sample (7.42 log CFU/g), whereas the highest count was determined in the PWL_{1%} sample (7.88 log CFU/g). The addition of WLP increased the number of lactobacilli (7.03–7.13 log CFU/g) compared with that in the control group (5.00–6.67 log CFU/g) ($p < 0.01$). The counts of lactococci were between 6.62 and 7.31 log CFU/g. TAMB, lactobacilli, and lactococci counts decreased in cheese samples during ripening ($p < 0.01$). This situation may be due to differences in cheese composition after biochemical reactions occur in the cheese during ripening or changes in the cheese microbiota. Similarly, Mazlum and Atasever [55] and Uzkuç and Karagül Yüceer [57] reported that TAMB, lactobacilli, and lactococci counts decreased in Beyaz cheese during the ripening period. Indeed, the TAMB count was strongly correlated ($p < 0.01$) with lactobacilli ($r = 0.91$), lactococci ($r = 0.93$), and *L. acidophilus* LA-5 ($r = 0.95$) counts (Fig. 1c).

As the WLP ratio increased, the counts of *L. acidophilus* LA-5 (6.35–6.50 log CFU/g) increased significantly ($p < 0.01$)

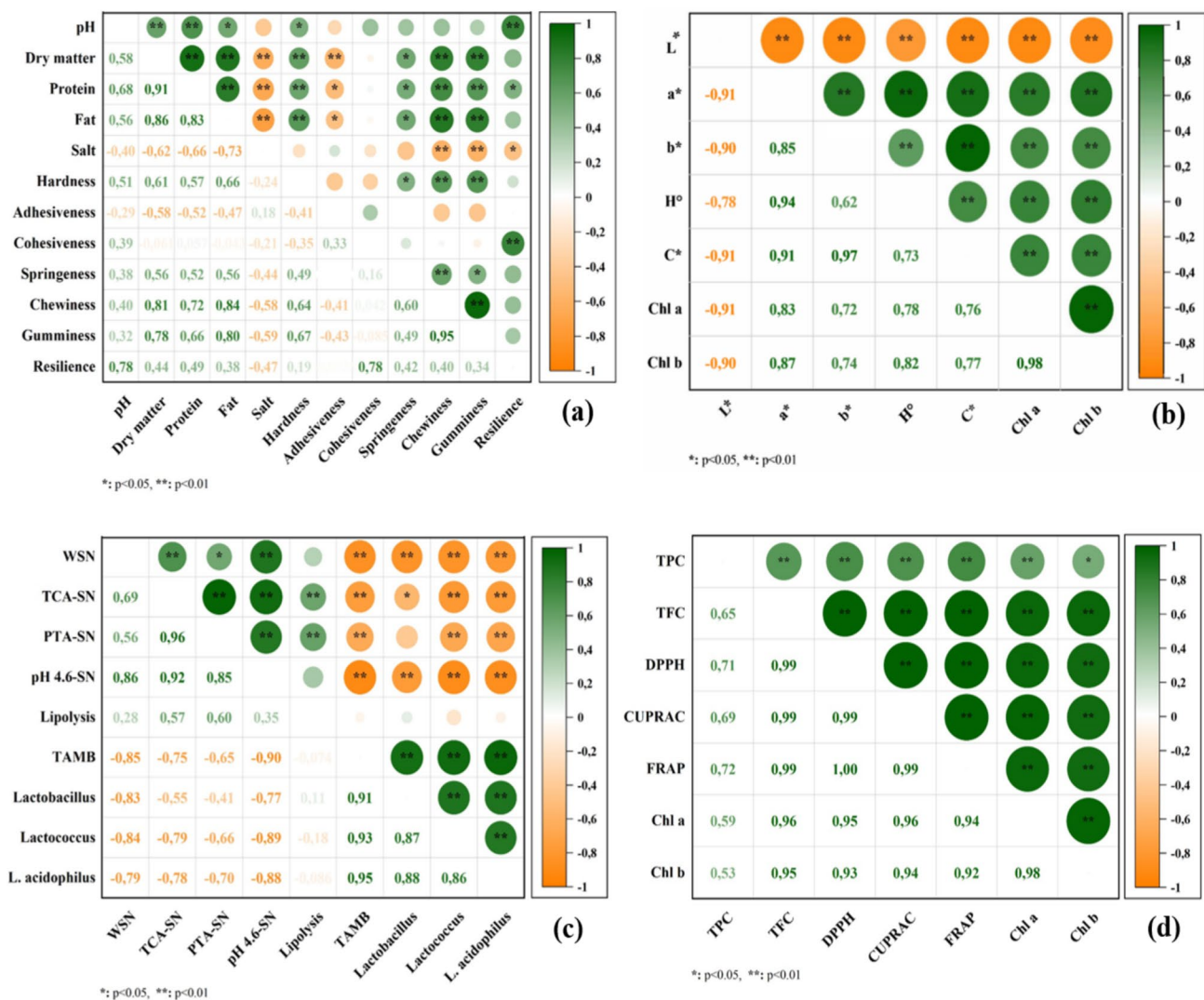


Fig. 1 Pearson's correlation coefficients between some analysis parameters of experimental Beyaz cheese samples. * $p < 0.05$ and ** $p < 0.01$. The color scale bar indicates a positive (white to green) or negative (white to orange) correlation

compared with those of the probiotic control (PC: 6.27 log CFU/g). Similarly, counts of *L. acidophilus* LA-5 during ripening were found to be in the range of 6.95–9.0 log CFU/g in probiotic UF-Feta cheeses with *S. platensis* and mint addition by Mazinani et al. [56] and in the range of 6.48–7.91 log CFU/g in Beyaz cheese with the addition of microalgae by Suna and Yilmaz-Ersan [8]. Similarly, Özer et al. [26], Sadeghi et al. [58], and Mazinani et al. [56] reported that the counts of *L. acidophilus* decreased in probiotic Beyaz cheese during ripening. The counts of *L. acidophilus* LA-5 in probiotic cheeses decreased to less than 10^6 CFU/g after day 60 (Table 3). Thus, the probiotic shelf-life of the cheeses was determined to be 60 days under the conditions of this research. This decrease may be due to the relatively low pH and high salt level in Beyaz brine cheeses, which affect the tolerances (acid, salt, etc.) of

freely added probiotic strains [26]. In a previous study, vacuum packaging improved probiotic viability and reduced salt levels in Beyaz cheese with *L. acidophilus* [6]. The lowest viability rate was detected in the PC sample (77.52%), whereas the highest viability rate was detected in the sample with 1% WLP addition (86.52%) ($p < 0.01$) (Table 3). The results revealed that the addition of WLP had no negative effect on the probiotic count and increased the viability rate during the 60-day shelf-life. This may be due to the growth-promoting effect of WLP on bacterial growth, as it contains significant amounts of carbon, nitrogen, and mineral sources [20, 23]. Similarly, Mazinani et al. [56] reported that the addition of *S. platensis* (6.95–9.00 log CFU/g) positively affected the viability of *L. acidophilus* LA-5 in the probiotic Beyaz cheese. On the other hand, Sadeghi et al. [58] reported that increasing the amount

Table 2 Color properties of experimental Beyaz cheese samples

Samples	n	L*	a*	b*	H°	C*
C	10	84.66 ± 1.47 ^a	−3.63 ± 0.37 ^b	14.29 ± 0.84 ^c	104.17 ± 1.27 ^b	14.84 ± 0.87 ^c
PC	10	85.69 ± 1.28 ^a	−3.24 ± 0.31 ^a	14.58 ± 1.27 ^c	102.37 ± 1.09 ^c	14.94 ± 1.27 ^c
PWL _{0.5%}	10	71.59 ± 2.77 ^b	−5.54 ± 0.69 ^c	17.03 ± 1.28 ^b	107.93 ± 2.37 ^a	17.47 ± 0.78 ^b
PWL _{1%}	10	63.24 ± 3.32 ^c	−6.07 ± 0.67 ^d	18.18 ± 1.43 ^a	108.37 ± 1.48 ^a	19.06 ± 1.57 ^a
<i>p</i>		**	**	**	**	**
Ripening (Day)	n	L*	a*	b*	H°	C*
1	8	77.97 ± 9.45 ^a	−4.60 ± 1.51 ^{bc}	14.94 ± 1.68 ^b	106.63 ± 3.89 ^a	15.59 ± 1.93 ^b
30	8	77.19 ± 9.89 ^{ab}	−4.76 ± 1.48 ^{cd}	16.29 ± 2.21 ^a	105.89 ± 2.93 ^b	16.99 ± 2.52 ^a
60	8	76.27 ± 10.03 ^b	−5.00 ± 1.52 ^d	16.32 ± 2.46 ^a	106.56 ± 2.75 ^a	16.99 ± 2.70 ^a
90	8	73.71 ± 10.41 ^c	−4.49 ± 1.00 ^b	16.26 ± 2.17 ^a	105.09 ± 2.22 ^c	16.47 ± 1.63 ^a
120	8	76.32 ± 9.45 ^b	−4.23 ± 1.04 ^a	16.28 ± 1.34 ^a	104.37 ± 2.68 ^d	16.83 ± 1.50 ^a
<i>p</i>		**	**	**	**	**

^{a–d}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. **Cheeses**; **C**: Control without probiotic, **PC**: Probiotic control, **PWL_{0.5%}**: Probiotic and 0.5% WLP, **PWL_{1%}**: Probiotic and 1% WLP

Table 3 Microbiological properties and *L. acidophilus* LA-5 count (log CFU/g) of experimental Beyaz cheese samples

Samples	n	TAMB	Lactobacillus	Lactococcus	<i>L. acidophilus</i> LA-5	Viability (%)
C	10	7.42 ± 1.30 ^d	5.00 ± 2.53 ^c	6.62 ± 1.50 ^d	-	-
PC	10	7.77 ± 1.16 ^b	6.67 ± 2.28 ^b	7.31 ± 1.30 ^a	6.27 ± 1.76 ^b	77.52 ± 3.71 ^c
PWL _{0.5%}	10	7.59 ± 1.13 ^c	7.03 ± 1.84 ^a	6.85 ± 1.63 ^c	6.35 ± 1.72 ^{ab}	82.04 ± 2.62 ^b
PWL _{1%}	10	7.88 ± 1.11 ^a	7.13 ± 1.75 ^a	7.09 ± 1.58 ^b	6.50 ± 1.91 ^a	86.52 ± 3.26 ^a
<i>p</i>		**	**	**	**	**
Ripening (Day)	n	TAMB	Lactobacillus	Lactococcus	<i>L. acidophilus</i> LA-5	
1	8	9.39 ± 0.24 ^a	9.42 ± 0.39 ^a	9.39 ± 0.32 ^a	8.48 ± 0.31 ^a	
30	8	8.22 ± 0.27 ^b	7.45 ± 0.99 ^b	7.13 ± 0.96 ^b	7.63 ± 0.38 ^b	
60	8	7.83 ± 0.30 ^c	6.62 ± 1.90 ^c	7.03 ± 0.55 ^b	6.85 ± 0.35 ^c	
90	8	6.65 ± 0.37 ^d	5.12 ± 0.73 ^d	6.00 ± 0.20 ^c	4.95 ± 0.40 ^d	
120	8	6.25 ± 0.17 ^e	3.69 ± 1.02 ^e	5.29 ± 0.48 ^d	3.83 ± 0.31 ^e	
<i>p</i>		**	**	**	**	

^{a–e}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. **TAMB**: Total aerobic mesophilic bacteria. **Cheeses**; **C**: Control without probiotic, **PC**: Probiotic control, **PWL_{0.5%}**: Probiotic and 0.5% WLP, **PWL_{1%}**: Probiotic and 1% WLP

of cumin essential oil (7.5–30 µL/100 mL) added to Iranian Beyaz cheese decreased the number of *L. acidophilus* LA-5 (4.49–8.87 log CFU/g) in the range of 0.40–1.58 log CFU/g.

The counts of coliform bacteria (< 1 log CFU/g), yeast and mold (< 2 log CFU/g), and *E. coli* (< 1 log CFU/g) in the cheese samples were below the detectable limits during ripening (120 days) (data not shown). The results revealed no contamination during the production and storage of the cheeses and that the cheeses did not pose a food safety risk in terms of indicator microorganisms.

Texture Profile Analysis Results

The results of the texture profile analysis of the experimental Beyaz cheese samples are given in Table 4. The addition of WLP and *L. acidophilus* LA-5 significantly affected the

textural properties of the cheese samples during the ripening period ($p < 0.01$). The hardness value indicates the maximum amount of force required to compress the cheese or damage the structure [12, 59]. Compared with that of the PC cheese, the hardness value of the cheese was not affected by the addition of WLP ($p > 0.05$). However, the addition of *L. acidophilus* LA-5 increased the hardness value compared with that of C ($p < 0.01$). This may be due to the decrease in pH because of the lactic acid fermentation of *L. acidophilus* LA-5. El Hatmi et al. [53] reported that the strong bonds formed between protein–protein interactions with a decrease in pH increased the firmness and hardness of cheese. Similarly, Pamuksuz et al. [60] reported that the textural properties of Beyaz cheese are affected by packaging pH values (pH 5.3, 5.0, and 4.7). Researchers have determined that cheese has a hard and brittle structure as

Table 4 Textural properties of experimental Beyaz cheese samples

Samples	<i>n</i>	Hardness (g)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Chewiness (mJ)	Gumminess (g)	Resilience
C	10	454.50 ± 253.17 ^b	1.56 ± 0.68 ^a	0.38 ± 0.07 ^a	6.27 ± 0.93 ^{ab}	11.60 ± 6.02 ^b	155.88 ± 73.61 ^b	0.110 ± 0.016 ^a
PC	10	656.38 ± 255.15 ^a	1.24 ± 0.43 ^c	0.24 ± 0.05 ^b	6.54 ± 0.68 ^a	20.44 ± 8.83 ^a	265.93 ± 120.42 ^a	0.077 ± 0.017 ^b
PWL _{0.5}	10	690.05 ± 230.26 ^a	1.38 ± 0.37 ^{bc}	0.20 ± 0.04 ^c	5.91 ± 1.27 ^{bc}	8.83 ± 3.48 ^c	148.63 ± 53.94 ^b	0.066 ± 0.021 ^c
PWL ₁	10	675.05 ± 318.91 ^a	1.42 ± 0.41 ^{ab}	0.24 ± 0.04 ^b	5.43 ± 1.31 ^c	9.10 ± 6.27 ^c	159.34 ± 65.87 ^b	0.074 ± 0.013 ^{bc}
<i>p</i>		**	**	**	**	**	**	**
Ripening (Day)	<i>n</i>	Hardness (g)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Chewiness (mJ)	Gumminess (g)	Resilience
1	8	1005.03 ± 222.10 ^a	0.94 ± 0.46 ^b	0.28 ± 0.06 ^a	7.14 ± 1.13 ^a	21.29 ± 8.62 ^a	270.58 ± 126.73 ^a	0.106 ± 0.026 ^a
30	8	772.38 ± 118.84 ^b	1.61 ± 0.23 ^a	0.25 ± 0.06 ^c	6.35 ± 0.84 ^b	16.09 ± 7.17 ^b	246.35 ± 66.12 ^a	0.084 ± 0.024 ^b
60	8	508.65 ± 123.81 ^c	1.53 ± 0.42 ^a	0.26 ± 0.08 ^{ab}	5.79 ± 1.51 ^b	8.83 ± 4.13 ^c	160.67 ± 40.71 ^b	0.074 ± 0.024 ^{bc}
90	8	428.65 ± 119.29 ^d	1.40 ± 0.28 ^a	0.27 ± 0.10 ^{ab}	6.06 ± 1.10 ^b	9.18 ± 4.24 ^c	122.66 ± 32.19 ^c	0.070 ± 0.026 ^c
120	8	380.28 ± 137.89 ^d	1.53 ± 0.67 ^a	0.28 ± 0.10 ^a	4.85 ± 1.16 ^c	7.07 ± 3.72 ^d	111.95 ± 38.68 ^c	0.074 ± 0.025 ^{bc}
<i>p</i>		**	**	*	**	**	**	**

^{a–d}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. *: $p < 0.05$. **Cheeses**; **C**: Control without probiotic, **PC**: Probiotic control, **PWL_{0.5}**: Probiotic and 0.5% WLP, **PWL₁**: Probiotic and 1% WLP

the pH approaches the isoelectric point. El Hatmi et al. [53] reported that the addition of pink garlic powder increased the hardness value of full-fat cheese and decreased it in skim cheese. Researchers attributed this result to the interaction of proteins with water and other nonprotein compounds and the high fat content of the cheese [53]. In contrast, Tarakçı and Deveci [12] reported that the addition of spices (3%; black cumin, mint, thyme, oregano, paprika and isot pepper), Golmakani et al. [24], *S. platensis* (0.5–1.5%), and El-Sayed and Ibrahim [54] red radish powder decreased the hardness value of cheese. The hardness values (1005.03–380.28 g) of all the samples decreased significantly during ripening ($p < 0.01$). This result may be due to the increase in the content of WSN during ripening, hydrophobic interactions of the weakened protein matrix, increased moisture content of the cheese, and dissolution of calcium phosphate in the casein matrix, as stated by Türkmen and Güler [61] and Celile Oluk [62]. In addition, the amount of fat and salt in cheese and the use of CaCl_2 in brine affect hardness [61, 62]. Indeed, hardness was significantly and moderately positively correlated with pH ($r = 0.51$, $p < 0.01$), dry matter ($r = 0.61$, $p < 0.01$), protein ($r = 0.57$, $p < 0.01$), and fat ($r = 0.66$, $p < 0.01$) (Fig. 1a).

The adhesiveness value indicates the force required to pull the cheese away from the texture analyzer probe [59, 63]. The adhesiveness value increased with the addition of WLP and decreased with the addition of *L. acidophilus* LA-5 ($p < 0.01$). During ripening, the adhesiveness value (0.94–1.61 mJ) increased on the other days compared with that on the 1st day ($p < 0.01$), but there was no difference between the samples after the 30th day. Similarly, Golmakani et al. [24] and Eren Vapur et al. [63] reported that the adhesiveness value increased during ripening.

The cohesiveness value indicates the strength of internal bonds in cheese [59, 63]. The cohesiveness value varied between 0.20 and 0.38 in the samples and between 0.25 and 0.28 during ripening. The cohesiveness value of the cheese was not affected by the addition of 1% WLP, whereas the addition of *L. acidophilus* LA-5 decreased the cohesiveness value. Similarly, the cohesiveness of cheese has been reported not to be affected by the addition of *S. platensis* [24] or rosy garlic powder [53].

The springiness value indicates the degree to which the cheese returns to its original shape after compression [59, 63]. The addition of WLP decreased the springiness value of cheese (5.43–5.91 mm) compared with that of the control samples ($p < 0.01$). Similarly, Golmakani et al. [24] reported that the addition of 1.5% *S. platensis* decreased the springiness value of cheese. In contrast, El Hatmi et al. [53] reported that the addition of rosy garlic powder and El-Sayed and Ibrahim [54] red radish powder increased the springiness value of cheese. During ripening, the springiness value (7.14–4.85 mm) decreased compared with that on the 1st day ($p < 0.01$), but there was no difference among the 30th, 60th, and 90th days. Similarly, Golmakani et al. [24] and Celile Oluk [62] reported that the springiness value of cheese decreased during ripening.

The chewiness value indicates the energy required to make a solid food ready to swallow [12, 59]. The addition of WLP decreased the chewiness value of the cheeses (8.83–9.10 mJ) compared with that of the control samples ($p < 0.01$). Similarly, Golmakani et al. [24] reported that the addition of *S. platensis* decreased the chewiness value of cheese. In contrast, Tarakçı and Deveci [12] reported that the addition of spices and El-Sayed and Ibrahim [54] red radish powder increased the chewiness value of cheese. During

ripening, the chewiness value (21.29–7.07 mJ) decreased ($p < 0.01$), and there was no difference between the 60th and 90th days. Similarly, Golmakani et al. [24], El-Sayed and Ibrahim [54], and Celile Oluk [62] reported that the chewiness value of Beyaz cheese decreased during ripening. There is a linear relationship between hardness and chewiness in cheese. The chewiness value was positively correlated with hardness ($r = 0.64$, $p < 0.05$), elasticity ($r = 0.60$, $p < 0.05$), and gumminess ($r = 0.95$, $p < 0.01$) (Fig. 1a).

The gumminess value indicates the force required to make a semisolid food ready to swallow [63]. The addition of WLP decreased the gumminess value (148.63–159.34 g) compared with that of the PC sample (265.93 g) ($p < 0.01$). Similarly, Golmakani et al. [24] reported that the addition of *S. platensis* reduced the gumminess value of cheese. In contrast, Tarakçı and Deveci [12] reported that the addition of spices and El-Sayed and Ibrahim [54] red radish powder increased the gumminess value of cheese. During ripening, the gumminess value (270.58–111.95 g) decreased ($p < 0.01$), and there was no difference between the 1st and 30th days or between the 90th and 120th days. Similarly, Golmakani et al. [24], El-Sayed and Ibrahim [54], and Celile Oluk [62] reported that the gumminess value decreased in Beyaz cheese during ripening. This may be due to the decrease in the hardness value of the cheeses during ripening (Table 4). To maintain and/or further improve the textural properties

of Beyaz cheeses during ripening, preservation and ripening in vacuum packaging instead of brine can be recommended.

The resilience value indicates the recovery of a sample during deformation [63]. Compared with the control, the addition of WLP decreased the resilience value (0.066–0.074) ($p < 0.01$). Tarakçı and Deveci [12] reported that the addition of spices did not affect the resilience value of cheese. During ripening, the resilience value (0.106–0.070) decreased ($p < 0.01$), and there was no difference between the 60th and 120th days. Similar results were reported for Beyaz cheese by Eren Vapur et al. [63] and Türkmen and Güler [61].

Mineral Composition

Minerals are inorganic compounds with various functions in the body's metabolism and homeostasis. Different plants and animal foods are important sources of minerals for a good and balanced diet [64]. The macro- and micromineral compositions of the experimental Beyaz cheese samples are given in Table 5. The addition of WLP significantly affected the P, K, Fe, Cu, Mn, and Zn contents of the cheese samples ($p < 0.01$). The Ca, Na, and Mg contents of the cheese samples ranged from 958–1107 mg/100 g, 3601–3891 mg/100 g, and 34–37 mg/100 g, respectively ($p > 0.05$). The ripening period significantly affected the Ca, P, K, Mg, and

Table 5 Makro and mikro-mineral composition of experimental Beyaz cheese samples

Samples	n	Macro minerals (mg/100 g dw)				
		Ca	P	K	Na	Mg
C	4	1107 ± 136 ^a	639 ± 80 ^a	136 ± 36 ^b	3672 ± 467 ^a	37 ± 5 ^a
PC	4	993 ± 124 ^a	608 ± 65 ^{ab}	138 ± 17 ^b	3601 ± 124 ^a	34 ± 2 ^a
PWL _{0.5}	4	970 ± 60 ^a	579 ± 27 ^b	164 ± 18 ^a	3703 ± 104 ^a	37 ± 3 ^a
PWL ₁	4	958 ± 36 ^a	554 ± 14 ^b	142 ± 14 ^b	3891 ± 208 ^a	36 ± 1 ^a
	<i>p</i>	ns	*	*	ns	ns
Ripening (Day)	n	Ca	P	K	Na	Mg
1	8	949 ± 67 ^b	565 ± 32 ^b	129 ± 20 ^b	3705 ± 257	34 ± 1 ^b
120	8	1065 ± 111 ^a	625 ± 65 ^a	161 ± 14 ^a	3728 ± 288	37 ± 3 ^a
	<i>p</i>	*	**	**	ns	**
Samples	n	Micro minerals (µg/100 g dw)				
		Fe	Cu	Mn	Zn	Se
C	4	187 ± 35 ^b	25 ± 11 ^b	25 ± 4 ^c	4520 ± 170 ^a	19 ± 4 ^a
PC	4	194 ± 21 ^b	27 ± 9 ^b	37 ± 7 ^c	4036 ± 153 ^b	16 ± 3 ^a
PWL _{0.5}	4	515 ± 85 ^a	31 ± 5 ^b	273 ± 16 ^b	4042 ± 197 ^b	14 ± 2 ^a
PWL ₁	4	524 ± 38 ^a	45 ± 8 ^a	392 ± 9 ^a	3936 ± 368 ^b	20 ± 5 ^a
	<i>p</i>	**	**	**	**	ns
Ripening (Day)	n	Fe	Cu	Mn	Zn	Se
1	8	342 ± 180 ^a	33 ± 13 ^a	180 ± 168 ^a	4251 ± 291 ^a	19 ± 4 ^a
120	8	368 ± 183 ^a	31 ± 10 ^a	184 ± 167 ^a	4016 ± 315 ^b	16 ± 4 ^a
	<i>p</i>	ns	ns	ns	**	ns

^{a–c}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. *: $p < 0.05$. ns: no significant. **Cheeses**; C: Control without probiotic, PC: Probiotic control, PWL_{0.5%}: Probiotic and 0.5% WLP, PWL_{1%}: Probiotic and 1% WLP

Zn contents of the cheese samples ($p < 0.01$). The highest macromineral content was detected on day 120. The highest amount of Zn among the microminerals was detected on day 1. The differences observed throughout the ripening period may be due to mineral transitions between cheese and brine or variations in sampling methods.

Milk is a liquid food rich in Ca and P [64]. Compared with the control treatment, the addition of WLP decreased Ca and P contents and increased K content ($p < 0.05$). This may be because WLP added in powder form increased the moisture content of the cheese mass because of its water-binding properties. Similarly, Yerlikaya and Karagözlü [52] reported that the addition of capers decreased Ca (508–789 mg/100 g) and P contents (3.47–12.3 mg/100 g) and increased K content (178–266 mg/100 g) in Beyaz cheese. El-Sayed [25] reported that the addition of spinach powder increased the amount of Ca (35,475–74,891 mg/100 g) and K (1373–19,206 mg/100 g) in UF-Feta cheese.

Mineral deficiency (especially of Fe, Zn, and I) causes serious public health problems in many developing countries. Thus, mineral-enriched products are very important [65]. In this context, milk and milk products (except buttermilk) are good sources of Ca and P [38] but are deficient in microminerals (Fe, Cu, Mn, Se, etc.) [66]. Fe has many important functions, such as hemoglobin and myoglobin production, energy metabolism, and intracellular electron transport [64, 67]. The lowest content of Fe in the cheese samples (187–194 µg/100 g) was detected in the control samples, whereas the highest Fe content was detected in the samples with WLP addition (515–524 µg/100 g) ($p < 0.01$). The results showed that the addition of WLP increased the amount of Fe in cheese by an average of 173%. Similarly, El-Sayed [25] reported that the addition of spinach powder (42,760–84,900 µg/100 g), Metry et al. [68] pumpkin seed powder (220–2780 µg/100 g), and Suna and Yilmaz-Ersan [8] microalgae (2370–4680 µg/100 g) increased the Fe content in Beyaz cheese.

Cu is an essential mineral with many important functions, such as iron utilization, hemoglobin formation, enzyme structure, fighting infections, stimulating the immune system, neutralizing free radicals, and promoting bone and elastic tissue development [64, 67]. The addition of 1% WLP increased the content (45 µg/100 g) of Cu in the cheese ($p < 0.01$). Similarly, El-Sayed [25] reported that the addition of spinach powder increased the Cu content (3860–11,240 µg/100 g) in UF-Feta cheese.

Mn has many important functions in protein, fat, and carbohydrate metabolism; enzyme structure; bone and cartilage development; tissue repair; brain function; and nerve conduction [64, 67]. The lowest content of Mn in cheese samples (25–37 µg/100 g) was detected in the control samples, whereas the highest Mn content was detected in the

sample with 1% WLP addition (392 µg/100 g) ($p < 0.01$). The results showed that the addition of WLP increased the amount of Mn in cheese by an average of 965%. Similarly, Yerlikaya and Karagözlü [52] reported that the addition of capers (9999–28,363 µg/100 g), El-Sayed [25] spinach powder (4350–19,600 µg/100 g), and Metry et al. [68] pumpkin seed powder (40–837 µg/100 g) increased the content of Mn in Beyaz cheese.

Zn, which is important for the structure of many enzymes, is an essential mineral with many functions in human physiology, such as growth and development, protein synthesis, tissue repair, digestion, and the immune system [64, 67]. The Zn content of the cheese samples varied between 3936 and 4520 µg/100 g. Compared with the C sample, the addition of WLP decreased the Zn content ($p < 0.01$). Similarly, Yerlikaya and Karagözlü [52] reported that the addition of capers decreased the content of Zn in Beyaz cheese (2483–3141 mg/100 g).

Se, which is the main component of the enzyme glutathione peroxidase found in many tissue types, functions as an antioxidant in combination with vitamin E, catalase, and superoxide dismutase [66]. The Se content of the cheese samples varied between 14 and 20 µg/100 g, and the addition of WLP did not affect the Se content ($p > 0.05$).

As a result of the consumption of 100 g of fresh Beyaz cheese, the individual Ca (800 mg), P (800 mg), K (2000 mg), Mg (280 mg), Fe (10 mg), Cu (1.5 mg), Mn (2 mg), Zn (12 mg) and Se (0.055 mg) consumption levels [38] were 42.40–52.21%, 25.31–30.15%, 2.57–2.87%, 4.50–4.97%, 0.70–1.92%, 0.64–1.10%, 0.47–7.17%, 11.78–14.21%, and 9.09–13.24%, respectively. Considering the functions of minerals in human physiology, this study revealed that WLP has significant potential for mineral fortification of foods and further enhances the health benefits of dairy products, especially those lacking microminerals (Fe, Cu, Mn, etc.).

Antioxidant Activity and Chlorophyll Contents

Cheese contains compounds with antioxidant activity, including compounds from animal feed via milk, compounds formed in cheese through bacterial metabolism, and bioactive peptides formed by the hydrolysis of milk proteins [4]. The results of the TPC, TFC, and total antioxidant activity (DPPH•, CUPRAC, and FRAP assays) analyses of the experimental Beyaz cheese samples are given in Table 6.

As the WLP ratio increased, the TPC, TFC, and total antioxidant activity of the cheese increased significantly ($p < 0.01$). The lowest TPC (121.95–130.30 mg GAE/100 g), TFC (29.94–30.69 mg QE/100 g), DPPH• (1.85–3.76 mg TE/100 g), CUPRAC (46.83–49.52 mg TE/100 g), and FRAP (0.51–0.91 mg TE/100 g) antioxidant activities were detected in the control group samples (C and PC, respectively). The highest TPC (220.10 mg GAE/100 g),

Table 6 Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity (DPPH•, CUPRAC and FRAP assays) of experimental Beyaz cheese samples and WLP

Samples	n	TPC	TFC	DPPH•	CUPRAC	FRAP
WLP	2	4830 ± 133	13,638 ± 515	4716 ± 224	16,594 ± 440	4861 ± 206
C	10	121.95 ± 36.26 ^c	30.69 ± 6.88 ^c	1.85 ± 2.10 ^c	46.83 ± 8.58 ^c	0.51 ± 0.37 ^c
PC	10	130.30 ± 36.53 ^c	29.94 ± 4.05 ^c	3.76 ± 1.66 ^c	49.52 ± 4.60 ^c	0.91 ± 0.41 ^c
PWL _{0.5%}	10	187.24 ± 47.21 ^b	112.39 ± 7.14 ^b	41.72 ± 4.03 ^b	142.08 ± 13.28 ^b	42.47 ± 2.52 ^b
PWL _{1%}	10	220.10 ± 50.31 ^a	196.31 ± 18.38 ^a	71.13 ± 5.95 ^a	237.90 ± 15.03 ^a	74.56 ± 8.62 ^a
	<i>p</i>	**	**	**	**	**
Ripening (Day)	n	TPC	TFC	DPPH•	CUPRAC	FRAP
1	12	98.36 ± 40.17 ^d	101.25 ± 75.27 ^a	30.04 ± 29.04 ^a	127.32 ± 81.07 ^a	28.87 ± 31.19 ^a
30	12	150.19 ± 29.56 ^c	92.15 ± 70.19 ^b	29.95 ± 29.31 ^a	115.23 ± 82.47 ^{cd}	29.25 ± 31.72 ^a
60	12	170.43 ± 46.58 ^b	92.75 ± 71.93 ^b	28.94 ± 29.60 ^a	110.58 ± 76.61 ^d	30.05 ± 32.77 ^a
90	12	199.17 ± 52.55 ^a	92.41 ± 72.62 ^b	29.22 ± 30.61 ^a	119.72 ± 79.95 ^{bc}	29.47 ± 32.32 ^a
120	12	206.34 ± 51.78 ^a	83.09 ± 67.58 ^c	29.94 ± 31.17 ^a	122.55 ± 88.07 ^{ab}	30.41 ± 33.85 ^a
	<i>p</i>	**	**	ns	**	ns

Results; TPC: as mg GAE/100 g; **TFC:** as mg QE/100 g; **DPPH•, CUPRAC and FRAP:** as mg TE/100 g. **: $p < 0.01$. ns: no significant. **Cheeses;** C: Control without probiotic, PC: Probiotic control, PWL_{0.5%}: Probiotic and 0.5% WLP, PWL_{1%}: Probiotic and 1% WLP

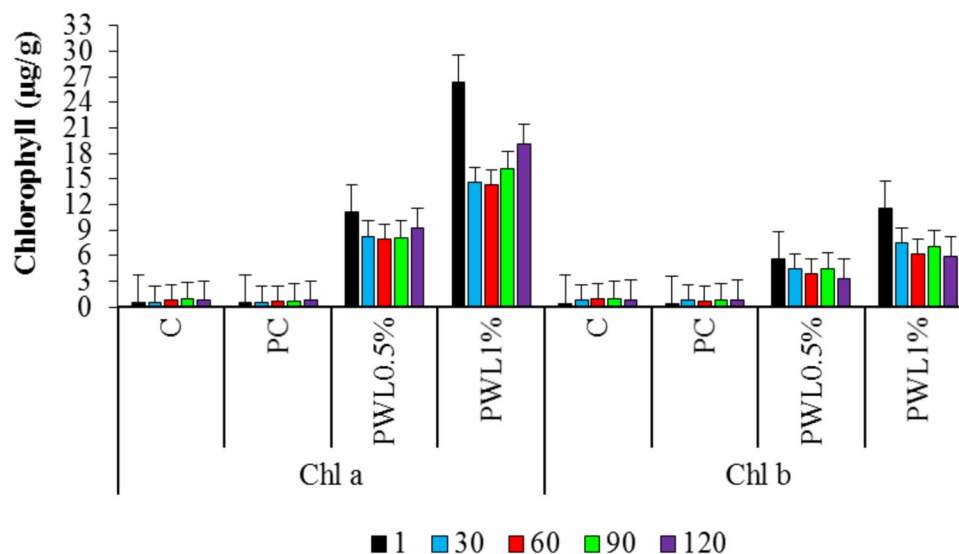
TFC (196.31 mg QE/100 g), DPPH• (71.13 mg TE/100 g), CUPRAC (237.90 mg TE/100 g), and FRAP (74.56 mg TE/100 g) were detected in cheese enriched with 1% WLP. This was associated with the high antioxidant activity of WLP (Table 6). This occurred because WLP contains 40-fold more phenolic compounds and 400-fold more flavonoid compounds than an equal quantity of plain cheese. The results of the present research indicated that WLP can be used as a good source of antioxidants in the fortification of dairy products. Similarly, del Olmo et al. [4] reported the addition of seaweed (1%), El Hatmi et al. [53] reported garlic powder, El-Sayed [25] reported spinach powder, Sulejmani et al. [13] reported mint, El-Sayed and Ibrahim [54] reported red radish powder, Eid et al. [15] reported galangal powder, Suna and Yilmaz-Ersan [8] reported microalgae, Mousavi et al. [50] reported mushroom extract, Aziz et al. [16] reported pomegranate peel powder (0.5–1%), and Garofalo et al. [17] reported that *Moringa oleifera* leaf powder (1–2%) increased the antioxidant activity of cheese. The antioxidant activity-enhancing effect of WLP may also be due to the composition of Chl. Indeed, the Chl content was significantly ($p < 0.01$) strongly positively correlated ($r = > 0.90$) with the TFC, DPPH•, CUPRAC, and FRAP antioxidant activities (Fig. 1d). The antioxidant activity was relatively greater in the PC sample than in the C sample, but the difference between the samples was insignificant.

The ripening period significantly affected the TPC, TFC, and CUPRAC antioxidant activity of the cheese samples ($p < 0.01$). Accordingly, the TPC (98.36–206.34 mg GAE/100 g) increased during ripening, whereas the TFC (101.25–83.09 mg QE/100 g) and CUPRAC (127.32–110.58 mg TE/100 g) relatively decreased on the other days compared with those on the 1st day ($p < 0.01$). Previous studies have shown that

the total phenolic content of cheese increases during ripening [4, 8, 53]. This situation can be explained by the catabolism of peptides and aromatic amino acids due to fermentation and proteolysis during ripening, as stated by del Olmo et al. [4]. The changes in the DPPH• and FRAP antioxidant activities of the cheeses during ripening were not statistically significant ($p > 0.05$). In the literature, studies have reported that the DPPH• radical scavenging capacity of cheese during ripening decreases [16, 50, 54] and increases [8, 53]. del Olmo et al. [4] reported that FRAP antioxidant activity increased in cheese during ripening.

Chlorophyll positively affects health because of its therapeutic properties, such as antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic effects [69]. The Chl contents of the Beyaz cheese samples during ripening are shown in Fig. 2. Accordingly, the Chl content of cheese samples increased significantly as the WLP ratio increased ($p < 0.01$). The lowest amounts of Chl *a* (0.54 µg/g) and Chl *b* (0.34 µg/g) were detected in the PC sample on the 1st day, whereas the highest amounts of Chl *a* (26.32 µg/g) and Chl *b* (11.56 µg/g) were detected in the WLP_{1%} sample on the 1st day. This was associated with the high Chl content (total 2182 µg/g) of WLP. This occurred because WLP contains 2000-fold more Chl compounds than an equal quantity of plain cheese. Although the amounts of Chl *a* and Chl *b* in cheese samples varied during ripening, they decreased on the other days compared with those on the 1st day ($p < 0.01$). Generally, the decrease in Chl content was more stable after the 30th day. Considering the health benefits of antioxidant compounds, this study revealed that WLP has significant potential for enhancing the bioactivity, enriching, and further increasing the health benefits of dairy products, especially those lacking these compounds.

Fig. 2 Chlorophyll contents of experimental Beyaz cheese samples during ripening (cheeses; C: control without probiotic, PC: probiotic control, PWL_{0.5%}: probiotic and 0.5% WLP, and PWL_{1%}: probiotic and 1% WLP; ** $p < 0.01$)



Proteolysis and Lipolysis

Cheese ripening plays an important role in the development of flavor characteristics. “Proteolysis,” “lipolysis,” and “glycolysis” are the most complex biochemical reactions that occur during cheese production and ripening [13, 57, 70]. The results of the proteolysis analysis of the experimental Beyaz cheese samples are given in Table 7. The addition of WLP and *L. acidophilus* LA-5 and the ripening period significantly affected the nitrogen fractions due to proteolysis in cheese samples ($p < 0.01$).

The WSN and pH 4.6–SN fractions refer to the total number of small molecular weight nitrogenous compounds, such as proteins, except for casein, all peptides, amino acids,

amines, urea, and ammonia [46]. The WSN (%TN) ratio varied between 17.44% and 22.46%, and the pH 4.6–SN ratio ranged between 14.30% and 17.43% ($p < 0.01$). The WSN (%TN) ratio in WLP-treated cheeses was not different from that in the C sample. These results show that WLP does not affect the WSN ripening index because of primary proteolysis in cheese. On the other hand, there was no difference between the plant ratios in terms of pH 4.6–SN (%TN), but the addition of WLP increased the pH 4.6–SN ripening index (17.24–17.43%) compared with that of the control group (14.30–14.75%). Similarly, Eid et al. [15] reported that the WSN (%TN) ratio (12.97–36.11%) in soft cheeses with the addition of galangal powder was close to that of the control group. In contrast, Yerlikaya and Karagözlü [52]

Table 7 Nitrogen fractions and lipolysis of experimental Beyaz cheese samples

Cheese samples	n	WSN (% TN)	pH 4.6-SN (% TN)	TCA-SN (% TN)	PTA-SN (% TN)	Lipolysis (ADV)
C	10	22.46 ± 10.31 ^a	14.75 ± 6.60 ^b	5.64 ± 2.19 ^c	1.48 ± 0.52 ^c	1.53 ± 0.18 ^c
PC	10	17.44 ± 5.57 ^b	14.30 ± 5.62 ^b	6.57 ± 2.26 ^b	2.29 ± 0.67 ^b	1.51 ± 0.17 ^c
PWL _{0.5%}	10	20.89 ± 4.71 ^a	17.24 ± 7.79 ^a	9.03 ± 4.74 ^a	2.96 ± 1.18 ^a	2.25 ± 0.28 ^b
PWL _{1%}	10	21.99 ± 6.18 ^a	17.43 ± 7.75 ^a	9.82 ± 4.65 ^a	2.97 ± 1.07 ^a	2.61 ± 0.30 ^a
	<i>p</i>	**	**	**	**	**
Ripening (Day)	n	WSN (% TN)	pH 4.6-SN (% TN)	TCA-SN (% TN)	PTA-SN (% TN)	Lipolysis (ADV)
1	8	10.17 ± 4.20 ^d	4.92 ± 0.95 ^e	2.76 ± 0.58 ^e	1.24 ± 0.37 ^e	1.76 ± 0.37 ^c
30	8	19.98 ± 2.98 ^c	12.85 ± 1.89 ^d	5.95 ± 1.04 ^d	1.90 ± 0.44 ^d	2.17 ± 0.55 ^a
60	8	21.71 ± 2.85 ^{bc}	17.72 ± 2.81 ^c	8.29 ± 2.54 ^c	2.57 ± 0.78 ^c	1.90 ± 0.49 ^b
90	8	23.01 ± 2.99 ^b	20.67 ± 3.59 ^b	10.08 ± 3.23 ^b	3.01 ± 1.00 ^b	1.89 ± 0.54 ^b
120	8	28.59 ± 5.96 ^a	23.50 ± 2.37 ^a	11.76 ± 3.54 ^a	3.39 ± 1.01 ^a	2.16 ± 0.61 ^a
	<i>p</i>	**	**	**	**	**

^{a–e}: Means shown with different exponential letters in the same column (↓) are statistically different from each other. **: $p < 0.01$. **Cheeses**; C: Control without probiotic, PC: Probiotic control, PWL_{0.5%}: Probiotic and 0.5% WLP, PWL_{1%}: Probiotic and 1% WLP

and Mousavi et al. [50] reported that the addition of caper (7.56–28.73%) and mushroom extracts (19.41–31.16%) increased the ratio of WSN (%TN) in cheese.

The TCA–SN fraction refers to the total number of compounds, such as medium–small peptides, amino acids, amines, urea, and ammonia [46]. The TCA–SN (%TN) ratio of the cheeses ranged from 5.64 to 9.82%. There was no difference in the TCA–SN (%TN) ratio among the plant levels, but the addition of WLP increased the TCA–SN ripening index (9.03–9.82%) compared with that of the control group (5.64–6.57%) ($p < 0.01$). Similarly, Çelik et al. [14] reported that the addition of cress (*Lepidium sativum* L.) (2.81–12.84%) and Mousavi et al. [50] mushroom extracts (6.81–21.42%) increased the ratio of TCA–SN (%TN) in UF-White cheese.

The PTA–SN fraction refers to the sum of very small peptides, amino acids, and smaller nitrogenous substances (except ammonia) [46]. The lowest PTA–SN (%TN) ratio was detected in the C sample (1.48%), whereas the highest values were detected in the cheese samples with WLP addition (2.96–2.97%). However, there was no difference in the PTA–SN (%TN) ratio at the plant level. Similarly, Tarakçı and Deveci [12] reported that the addition of mint and thyme increased the PTA–SN (%TN) ratio (0.48–2.66%) in Beyaz cheese.

During ripening, the WSN (10.17–28.17–28.59%), pH 4.6–SN (4.92–23.92–23.50%), TCA–SN (2.76–11.76–11.76%), and PTA–SN (1.24–3.24–3.39%) fractions increased significantly due to proteolysis ($p < 0.01$). This can be explained by the fact that proteolytic enzymes pass into the cheese mass and continue their activity as a result of the lysis of starter and probiotic bacteria. There may also have been increases in proteolysis due to the activity of the microflora in the cheese. In previous studies, the WSN [6, 12, 14, 15, 50, 52, 55, 57], TCA–SN [6, 12, 14, 50] and PTA–SN [6, 12] ripening index ratios of Beyaz cheese were reported to increase during storage.

L. acidophilus LA-5 significantly affected secondary proteolysis and increased the amounts of TCA–SN and PTA–SN ($p < 0.01$). Similarly, Bergamini et al. [71] investigated the effects of *L. acidophilus*, *Lactobacillus paracasei*, and *Bifidobacterium lactis* probiotic strains as single and mixed cultures on proteolysis in semihard cheeses. *B. lactis* did not affect proteolysis, whereas *L. paracasei* had a limited effect at the end of ripening. However, *L. acidophilus* significantly affects secondary proteolysis from the beginning of ripening and increases the amounts of small nitrogenous compounds and free amino acids [71]. Erkaya and Şengül [6] reported that proteolysis was greater in Beyaz cheeses produced with *L. acidophilus* than in cheeses made with *Bifidobacterium bifidum*. The greater proteolysis in the 1st month of ripening than in the other days may be related to the proteolytic activity of *L. acidophilus*. Indeed, the counts

of viable bacteria were $> 10^8$ CFU/g on the 1st day, and the count decreased as ripening progressed. Thus, limitations/decreases in the activity of the cheese microflora may have caused a more stable increase in proteolysis in the later stages of ripening.

Lipolysis is one of the most important biochemical reactions that contributes to the flavor of cheese and occurs during ripening [57, 70]. The lipolysis results (acid degree values) of the experimental Beyaz cheese samples are given in Table 7. The WLP addition and ripening periods significantly affected the degree of lipolysis in the cheese samples ($p < 0.01$). As the WLP ratio increased, lipolysis increased (2.25–2.61%) compared with that in the control group (1.51–1.53% ADV) ($p < 0.01$). During the ripening period, lipolysis (1.76–2.17 ADV) varied and increased on the other days compared with that on the 1st day ($p < 0.01$). Similarly, Yerlikaya and Karagözlü [52] reported that lipolysis increased during ripening in Beyaz cheeses supplemented with capers (1.22–1.54 ADV) and Aziz et al. [16] with pomegranate peel powder (0.42–1.38 ADV).

Sensory Evaluation

In the food industry, it is important to evaluate the sensory properties of foods to develop new products or increase product diversity. The results of the sensory analysis of the experimental Beyaz cheese samples are shown in Fig. 3. The addition of WLP and *L. acidophilus* LA-5 during the ripening period significantly affected the sensory characteristics of the cheese samples ($p < 0.01$). The cheese samples had scores ranging from 5.88–8.44 for color, 5.74–8.23 for texture and appearance, 5.45–8.25 for odor, 5.11–7.76 for flavor, 5.91–8.72 for foreign taste, 4.32–6.36 for salinity and 5.35–7.88 for overall acceptability during the storage period (Fig. 3). The ripening period significantly affected the sensory scores of the cheeses ($p < 0.01$). The sensory parameters of the cheeses generally ranged from 7 to 9 points between the 1st and 60th days of storage. In particular, in the cheeses enriched with WLP, the sensory scores clearly decreased with ripening after the 60th day. These results revealed that WLP can be used successfully as an additive in freshly consumed cheeses. However, significant changes in sensory quality after the 60th day revealed the use of WLP in short-term ripened brine Beyaz cheese. To maintain and/or further improve the sensory quality of Beyaz cheeses with WLP addition, preservation and ripening in vacuum packaging instead of brine can be recommended.

The panelists reported that the addition of WLP at both rates did not cause a bitter taste in cheese. Under the conditions of this study, the cheeses enriched with 0.5% WLP were the most liked. The overall acceptability scores of the cheese samples were in the order of PC > C > PWL_{0.5%} > PWL_{1%}. In the scoring criteria, 9–8 points indicate that

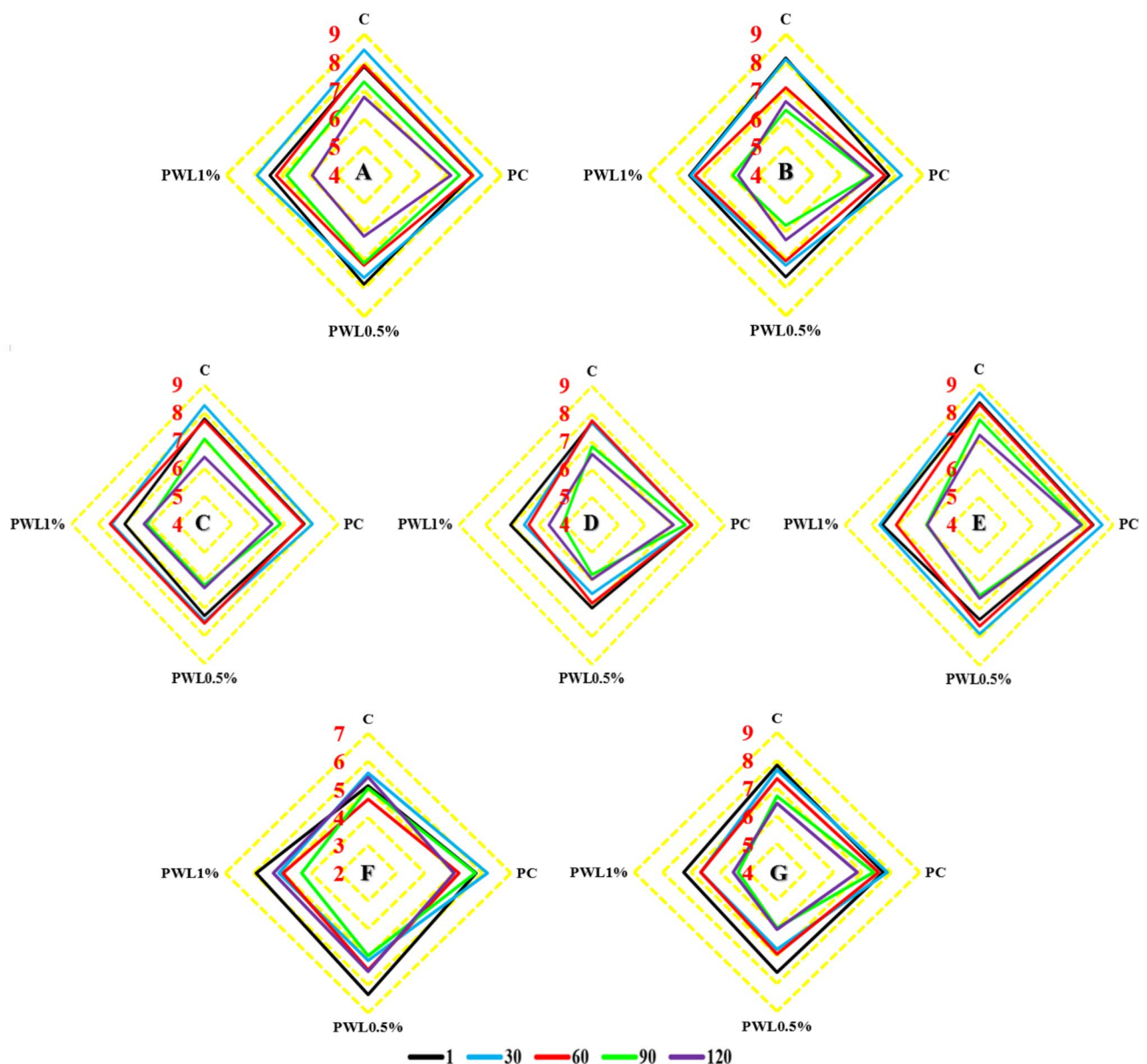


Fig. 3 Sensory characteristics (A: color, B: texture and appearance, C: odor, D: flavor, E: foreign taste, F: salinity, and G: overall acceptability) of experimental Beyaz cheese samples during rip-

ening (cheeses; C: control without probiotic, PC: probiotic control, PWL_{0.5%}: probiotic and 0.5% WLP, and PWL_{1%}: probiotic and 1% WLP)

there is no foreign taste, and 7–6 points indicate that there is very little. As the addition of WLP increased, the scores of the sensory parameters (except salinity) decreased. This may be because consumers generally prefer plain cheese and are not accustomed to different flavors. In addition, WLP, which was added as a functional additive in this study, is not as widely used as spices, and the unknown taste may have caused the perception of foreign taste. Indeed, del Olmo et al. [4] reported that the addition of seaweed and Golmakani et al. [24] reported that *S. platen-*

seaweed taste, etc.) in cheese. Researchers reported that seaweed flavor caused a decrease in sensory score [4, 24]. There was a general decrease in the sensory score during ripening ($p < 0.01$). The cheese samples were generally accepted in terms of all the sensory parameters and were liked by the consumer panel. However, since the cheeses were preserved in brine, they were found to be acceptably salty. Similarly, Golmakani et al. [24] reported that the addition of *S. platen-*

Deveci [12] mint, thyme, paprika, and isot, Golmakani et al. [24] *S. platensis*, El Hatmi et al. [53] rosy garlic powder, El-Sayed [25] spinach powder, Mousavi et al. [50] mushroom extract, Suna and Yilmaz-Ersan [8] microalgae, and Kamel et al. [9] carrot powder addition caused a decrease in sensory scores compared with those of the control cheese samples.

Principal Component Analysis (PCA)

PCA was applied to evaluate the relationships between the bioactive and ripening properties of cheese samples by reducing them to a smaller number of principal dimensions. Figure 4a–d shows the hierarchical clustering, score scatter plot, loading scatter plot, and biplot of the PCA for Beyaz cheeses. The first two principal components (PC1 = 66.8% and PC2 = 27%) explained 93.8% of the variance, and this percentage is sufficient for qualitative purposes.

As a result of PCA, the cheese samples could be divided into five main groups (Fig. 4a–b). In PC1, the samples containing 0.5% and 1% WLP are located on the right side of the axis, whereas the control group samples are located on the left side. The control cheeses on the 1st day of ripening were located in a distinctly different group from those on the other days. Therefore, the change after the 30th day of ripening was similar to that in the control groups. Among the cheeses containing WLP, samples from the 1st day and 30th day were different from those from the other days of ripening. In PC2, samples from the 1st and 30th days of ripening are located on the right side of the axis, whereas samples from the 60th, 90th, and 120th days are located on the left side (Fig. 4b). As shown in Fig. 4c, positive correlations were observed between DPPH•, CUPRAC, and FRAP and chlorophyll contents; WSN and pH 4.6–SN; and TCA–SN and PTA–SN. This classification revealed that the addition of WLP affected

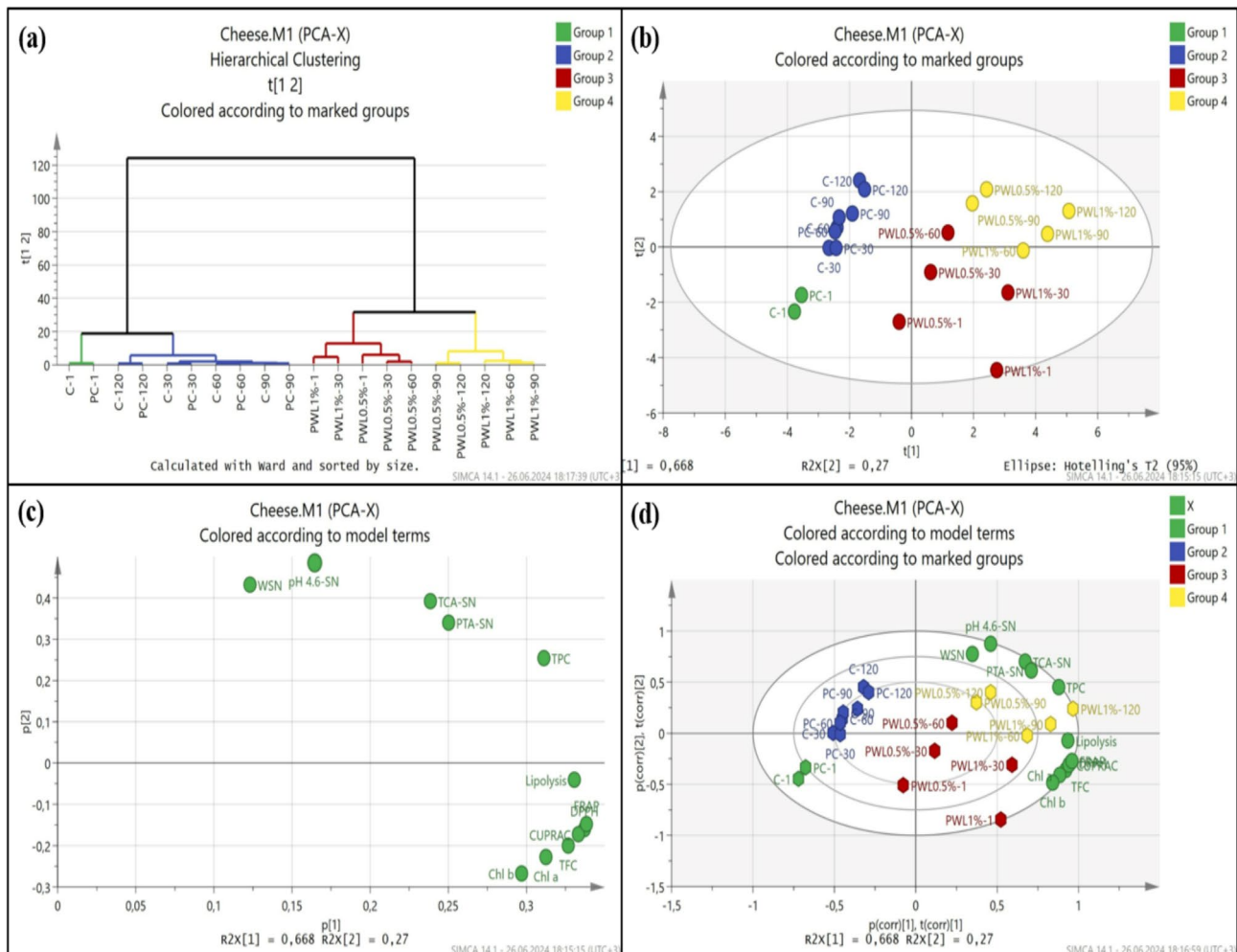


Fig. 4 Dendrogram (a), score scatter plot (b), loading scatter plot (c), and biplot (d) of the principal component analysis (PC1 vs. PC2) on bioactive, proteolysis, and lipolysis of experimental Beyaz cheese samples

the antioxidant activity, chlorophyll content, and ripening characteristics of the samples (Fig. 4d). Thus, WLP-treated samples presented high bioactivity and ripening index values at the 90th and 120th days of ripening.

Conclusions

In the present study, the possibility of using walnut leaves, a valuable agricultural waste, with *L. acidophilus* LA-5 in the production of Beyaz cheese as a functional additive and/or nutraceutical was investigated. As the WLP ratio increased, the counts of *L. acidophilus* LA-5 increased significantly compared with those in the C and PC control samples. The probiotic shelf-life ($> 10^6$ CFU/g) of functional Beyaz cheeses was determined to be 60 days under the conditions of this research. The addition of WLP increased proteolysis and lipolysis. Compared with the control, WLP addition increased the TPC, TFC, antioxidant capacity, Chl *a* content, Chl *b* content, and mineral (Fe, Cu, and Mn) content. The addition of WLP decreased the textural properties (cohesiveness, springiness, chewiness, gumminess, and resilience) and sensory scores of the cheese. Nevertheless, all samples were sensory scored between 5 (average) and 9 (very good/ideal) out of 9 points during the ripening period. The general acceptability of cheese samples was $PC > C > PWL_{0.5\%} > PWL_{1\%}$. As a result, WLP has the potential to be used as a functional additive in Beyaz cheese production. The results revealed that a new variety of Beyaz cheese with a probiotic shelf life of 60 days that is rich in bioactive compounds and microminerals (Fe, Cu, and Mn), which are natural for healthy nutrition, can be produced with the addition of *L. acidophilus* LA-5 and 0.5% WLP. Considering the high bioactivity and rich mineral composition of WLP, further studies are needed to increase the number of healthy and functional cheese varieties.

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Author Contribution MAS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Resources, Writing – original draft, Writing – review & editing, SÇ: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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