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Impact of Probiotic and Bioprotective Cultures on the Quality and Shelf Life of Butter and Buttermilk

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Abstract: In this study, butter and the corresponding buttermilk samples were produced with cream fermented by aromatic (A) or probiotic (P) cultures with or without complementary bioprotective culture (BC). The samples were characterised for their composition and colour parameters. Texture and rheological properties were evaluated at 10 and 20 °C. Microbiological (lactobacilli, lactococci, and yeast and mould counts) and sensory (aroma, taste, texture, and global evaluation) analyses were also performed. All butter sample characteristics were in accordance with the Portuguese standard. Regarding colour, the sample obtained with cream fermented by probiotics plus bioprotective culture (PBC) presented higher L* and b* values, indicating a slightly higher yellow chroma. However, colour differences (ΔEab*) in the butter samples were, in most cases, not detectable by a common observer. Butter samples P and PBC presented a significantly higher viscous modulus and consequently higher dynamic viscosity values (ca. log 6.5 Pa.s at 10 °C and log 5 Pa.s at 20 °C). Butter samples presented a pseudoplastic behaviour, and rheological parameters showed a high dependence on temperature. The counts of lactobacilli and lactococci in the butter samples were of the order of log 7–8 CFU/g, while yeast and mould counts were lower than log 2 CFU/g until the 30th day of storage, after which they showed a sharp increase to ca. log 5 CFU/g between the 30th and the 60th days of storage. Regarding sensory attributes of butter, sample P received the highest overall liking, followed by samples ABC and PBC. Sample A was the least appreciated. Buttermilk samples presented significant differences regarding their composition, viscosity, and colour parameters. In all cases, lactobacilli and lactococci counts exceeded log 7 CFU/mL after 30 days of storage, but yeast and mould counts were of the order of log 5–6 CFU/mL at the 15th day of storage. Samples P and PBC presented yeast and mould counts ca. 1–2 log cycles lower than samples A and ABC, indicating the potential of probiotic and bioprotective cultures to extend the shelf life of the product. Regarding the sensory attributes of buttermilk, samples P and PBC received the highest overall liking, followed by sample ABC. Sample A received the lowest scores, as had occurred with the butter samples. However, in all cases, the scores obtained by the buttermilk samples were lower compared to the ones of the corresponding butter. It can be concluded that both probiotic butter and buttermilk present high levels of lactobacilli and lactococci and can maintain their probiotic potential throughout the storage period.

Keywords: butter; buttermilk; aromatic starter culture; probiotics; bioprotective cultures

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1. Introduction

Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, have gained significant attention for their role in promoting gut health and overall well-being [1–3]. These beneficial bacteria are known to modulate the gut microbiota, leading to various health improvements, such as enhanced immunological responses, relief of lactose intolerance symptoms, reduced risk of cancer and heart diseases, reduced colitis, alleviation of diarrhoea, modulation of immunity, reduction in cholesterol levels, anti-inflammatory properties, maintenance of blood pressure, improvement in

irritable bowel disease, better oral and urogenital health, and even potential antidepressant effects [4–11].

The impact of starter cultures in enhancing the safety of foods through the production of metabolites that inhibit the growth of pathogenic and spoilage microorganisms has also been investigated by several authors. This emphasis on enhancing safety aligns with the development of bioprotective culture (BC), starters, and probiotics, which aim to provide foods with specific sensory attributes, confer nutritional benefits, and ensure food safety [12–14]. Particularly in the case of dairy products, extensive work has been conducted on such bioprotective cultures [15–22]. Vasiliauskaite and coworkers [19] explored the use of indigenous lactobacilli strains isolated from fermented cow milk for developing bioprotective coatings to maintain the quality of fresh cheese. The study identified *Lacticaseibacillus paracasei* A11 as a safe strain with strong antifungal properties, thereby extending the shelf life of the product. Shi and Maktabdar [20] discussed the use of LAB as biopreservatives in dairy products to combat spoilage moulds. The research highlighted the importance of BC in preventing food waste, economic losses, and potential health risks associated with mould contamination. Shi and Knøchel [21] investigated the growth potential of various moulds at different temperatures and their susceptibility to LAB cultures. The research highlighted the potential of LAB as a natural alternative to chemical preservatives in combating fungal spoilage in dairy products.

Probiotics are commonly used in various dairy products, either as starter cultures or incorporated post-fermentation, to enhance both the functional characteristics and health-promoting properties of the final products. Fermented dairy products containing probiotics have been shown to improve human health by both stimulating the growth of beneficial gut bacteria and increasing the production of metabolic by-products [11].

Probiotic butter is a novel concept that combines the benefits of probiotics with a popular dairy product. Only a few studies have explored the incorporation of probiotics into butter to create a functional food product with enhanced nutritional value and potential health benefits. Bellinazo et al. [23] investigated the viability of *Lactobacillus casei* strains in probiotic butter formulations. Ostadzadeh et al. [24] demonstrated that the addition of probiotic strains to butter can lead to a decrease in cholesterol levels, indicating the potential of such a product as a functional food. Furthermore, the addition of probiotics to butter has been shown to be an option to improve the income of small-to-medium dairy industries [25]. Previously, we evaluated the characteristics of sheep's butter and buttermilk produced with sweet cream and by cream fermented with an aromatic starter, as well as with commercial kefir and probiotic cultures [26]. So far, the research on probiotic butter highlights its potential as a functional food product that combines its creamy texture with the health benefits of probiotics. By enhancing the bioactivity, nutritional profile, and potential health benefits of butter, the incorporation of probiotics opens new avenues for dairy product innovation and the development of functional foods that promote both taste and health.

Buttermilk, the by-product of butter production, is a versatile ingredient with various applications in the food industry due to its unique physicochemical properties. The composition of buttermilk can vary depending on the method of butter production and the type of milk used. Generally, buttermilk is low in fat and calories compared to whole milk, making it a healthier alternative. Buttermilk is also a good source of calcium and other essential nutrients, such as riboflavin, phosphorus, and potassium. It is rich in proteins, with high nutritional value and functional properties that can enhance the quality of food products. Its multi-functionality is utilised in improving water-holding capacity, texture, and protection against lipid oxidation in bakery, confectionery, and sauce-type products [27,28]. The utilisation of buttermilk extends to the production of functional beverages, such as cheese and ice cream, enhancing the quality of the final products [29–34]. Additionally, buttermilk can be used in the production of kefir and quark cheese and as a marinating agent for meat, showcasing its versatility in different culinary applications [34–38]. Furthermore, the processing of buttermilk using innovative techniques like membrane technologies

allows for the extraction of high-quality milk protein concentrates with desirable physicochemical properties and structural parameters [39]. In addition, the addition of buttermilk or buttermilk powder to products like Cheddar-style cheese or reduced-fat cheeses can influence functionality, texture, and sensory attributes, enhancing the overall quality of the product [40,41].

2. Materials and Methods

2.1. Butter and Buttermilk Production

Sixty litres of cream (40% fat) produced by Lactogal SA were obtained from a local market (Makro, Coimbra, Portugal). Before churning, the cream was divided into four 15 L batches, which were subjected to the following conditions:

(1) Inoculated with 5% (*v*/*v*) aromatic culture (Flora Danica, FD-DVS™, CHR Hansen, supplied by Promolac, Lisbon, Portugal) containing *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc*, *Lactococcus lactis* subsp. *lactis*, and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*; kept for 24 h at 20 °C in defatted pasteurised milk; then maintained at 4 ± 2 °C for 24 h (A).

(2) Inoculated with 5% (*v*/*v*) of the aromatic culture plus bioprotective culture containing *Lactobacillus rhamnosus* and *Lactobacillus paracasei* (Fresh Q4, CHR Hansen, supplied by Promolac, Lisbon, Portugal) at a level of 1.5 U/15 L cream according to the manufacturer's instructions (ABC).

(3) Inoculated with 5% (*v*/*v*) probiotic culture containing *Lactobacillus acidophilus* (nutrish LA-5™, CHR Hansen), kept for 24 h at 20 °C in defatted pasteurised milk, then maintained at 4 ± 2 °C for 24 h (P).

(4) Inoculated with 5% (*v*/*v*) probiotic culture plus bioprotective culture containing *Lactobacillus rhamnosus* and *Lactobacillus paracasei* (Fresh Q4, CHR Hansen, supplied by Promolac, Lisbon, Portugal) at a level of 1.5 U/15 L cream according to the manufacturer's instructions (PBC).

Taking in account the manufacturer's indications regarding the number of viable cells in freeze-dried cultures, it can be estimated that the initial levels of the inoculated cultures were of the order of 5–7 log UFC/mL cream.

Butter samples were produced in a 30 L butter churner (Termoinox, Vale de Cambra, Portugal). Butter grains were washed with cold (4 °C) pasteurised water and salted (1%, *w*/*w*) prior to the final working step, in which the butter grains were pressed and squeezed to remove moisture.

Finally, the butter samples were packed into 500 mL polypropylene boxes (Standipack™, supplied by Makro, Coimbra, Portugal) and stored at <7 °C for 90 days. Samples for analysis were collected one day after production and after 30, 60, and 90 days.

After cream churning, the buttermilk was collected, packaged in 500 mL PET bottles (Eplas™, Marinha Grande, Portugal), and characterised and evaluated over 28 days of refrigerated storage (<7 °C). A flow diagram of the production process is presented in Figure 1.

2.2. Physicochemical Analysis

Butter samples and their respective buttermilk were evaluated for dry matter, fat, ash, colour, texture, rheology, and viscosity. The physicochemical analyses were performed at least in triplicate.

Analytical methods used for milk were adopted for the analyses of buttermilk. Moisture was removed by oven drying at 105 °C according to AOAC method 925.23 [42]. Total solids were calculated by difference. Ashes were determined by incineration of dry samples in an HD-23 Hobersal™ electric muffle furnace at 550 °C according to AOAC method 945.46 [43]. The titratable acidity of butter was evaluated according to the Portuguese standard NP-1712 [44]. The titratable acidity of buttermilk was evaluated according to NP 470 [45]. The pH was directly determined using a pH meter (Hanna Instruments, model HI9025, Póvoa de Varzim, Portugal) with an FC200 probe. The pH meter was previously

calibrated according to the manufacturer's instructions using Hanna Instruments HI7004 (pH 4.01) and HI 7007L (pH 7.01) buffer solutions. Fat content of butter and buttermilk samples was analysed by the Gerber method (SuperVario-N Funke Gerber™ centrifuge, Berlin, Germany) [46].

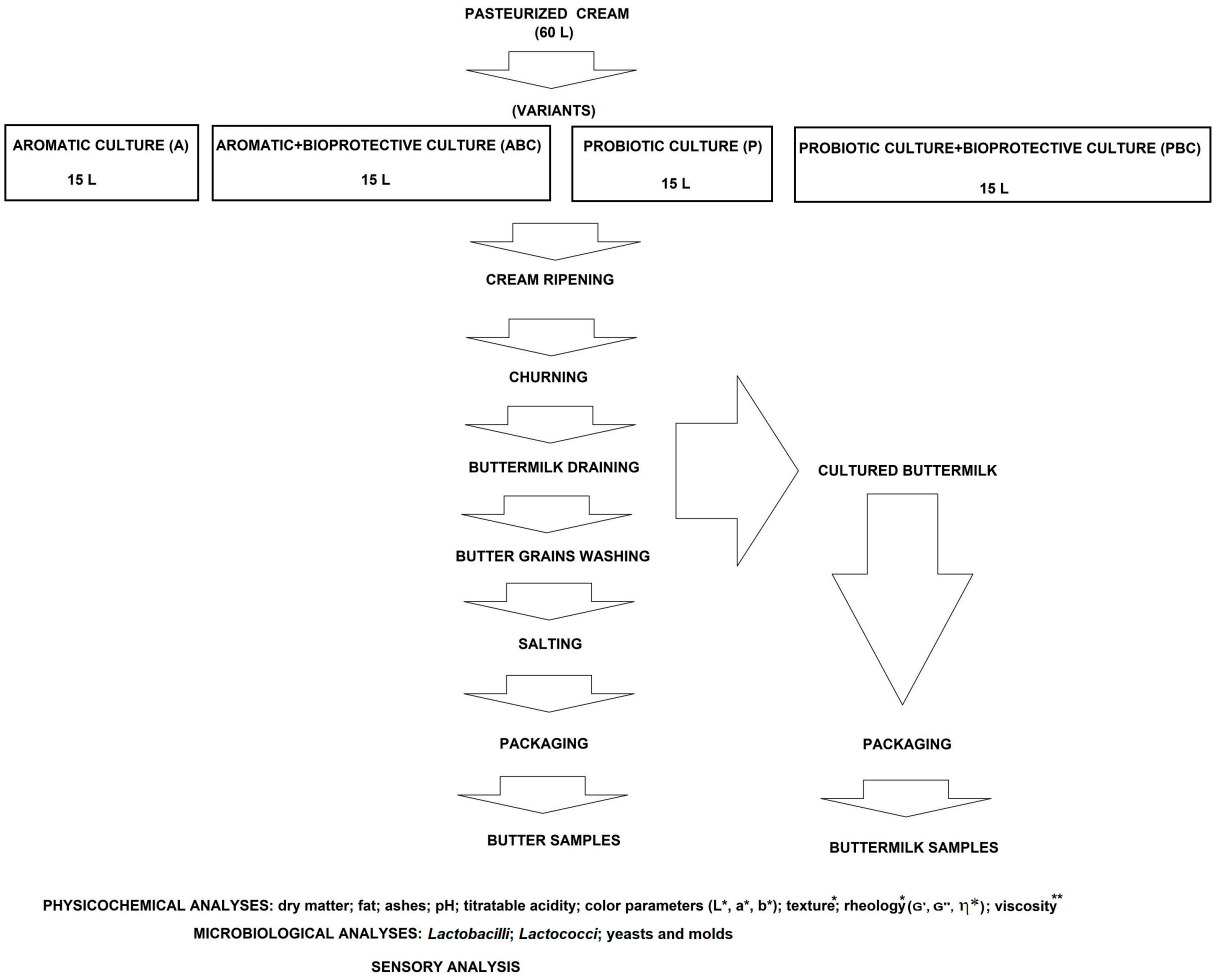

Figure 1. Diagram of butter and buttermilk production and tests performed (* tests conducted only with butter samples; ** tests conducted only with buttermilk samples).

The colour of butter and buttermilk samples was determined with a Minolta Chroma Meter colourimeter, model CR-200B, calibrated with a white standard (CR-A47: Y = 94.7; x 0.313; y 0.3204). The conditions used were as follows: illuminant C, 1 cm diameter aperture, and 10_ standard observer. The colour coordinates were measured in the CIEL*a*b* system.

The colour difference (ΔEab*) was calculated as follows [47]:

$$\Delta Eab^* = [(L^* - L0^*)^2 + (a^* - a0^*)^2 + (b^* - b0^*)^2]^{1/2} \quad (1)$$

where L0*, a0*, and b0* and L*, a*, and b* are the average values measured for the products under comparison.

A Stable Micro Systems texture analyser, model TA.XT Express Enhanced (Stable Micro Systems Ltd., Godalming, UK), was used for texture analysis of the butter samples one week after production, and the results were calculated using the Specific Expression PC software (version 6,1,11,0). Evaluation was performed at 10 and 20 °C. The tests were run with a penetration distance of 20 mm at 2 mm/s using an acrylic cylindrical probe with

a diameter of 6.4 mm and a height of 35 mm. Three measurements were taken for each block of butter, and the results were expressed as the mean value ± standard deviation.

Rheological tests were conducted at 10 and 20 °C using a Rheostress 6000 rheometer with a plate-to-plate geometry (TMP35 bottom plate and P35 TiL upper plate) equipped with the HAAKE RheoWin Job Manager software, version 4.82.0002, for data acquisition (ThermoHaake™, ThermoFisher Scientific, Waltham, MA, USA). Values of the elastic and viscous moduli (G′ and G″) and the complex viscosity (η*) were recorded at frequencies from 0.05 to 1.0 Hz.

The viscosity of buttermilk samples was evaluated by a Brookfield viscometer, model DV2T (Ametek, Harlow, UK), using spindle n° 1 and a speed of 100 rpm for 1 min at 7 ± 2 °C. Results are presented in centipoises (cPs).

2.3. Microbiological Analysis

Microbiological analysis allowed for the quantification of lactococci, lactobacilli, and yeasts and moulds. From each collected sample (10 g of butter, 15 mL of buttermilk), tenfold dilutions were prepared in Ringer solution with tween 80 (Merck Schuchardt, Hohenbrunn, Germany). For butter samples previously placed in a sterile Stomacher's bag with Ringer solution and tween 80, the first dilution was prepared by melting them at 42 °C in a water bath. Quantification of lactobacilli was performed according to ISO 15214 [48]. Briefly, the dilutions were inoculated by the pour plate method in MRS agar (De Man Rogosa and Sharp-Biokar Diagnostics, Allone, France) in a double layer and incubated at 37 °C for 72 h under anaerobic conditions. Lactococci were evaluated on M17 agar (Biokar Diagnostics, Allone, France) incubated at 37 °C for 48 h. Yeasts and moulds were evaluated in Coke Rose Bengal agar (Liofilchem Diagnostici, Roseto degli Abruzzi, Italy) according to ISO 6611 [49] and incubated at 25 °C for 7 days. All microbiological analyses were performed in triplicate.

2.4. Sensory Analysis

Sensory analysis was based on a laboratory acceptance sensory test conducted in isolated booths with the same lighting and temperature conditions. Evaluation was carried out using a non-trained panel consisting of 25 members [50]. Samples, identified by three-digit random numbers, were rated for aroma, taste, texture, and overall impression using a 5-point hedonic scale (1 = dislike extremely and 5 = like extremely). The members of the panel were informed of the objectives of the test and signed the informed consent form in use at the Polytechnic of Coimbra, giving their consent for the treatment and publication of results.

2.5. Statistical Analysis

The results were submitted to a one-way ANOVA using STATISTICA software V.12.0, 2013 (Statsoft, Tulsa, OK, USA). Mean values were compared using the Tukey's honestly significant difference (HSD) test. Differences were considered significant at $p < 0.05$. Significant correlations and principal component analysis (PCA) were also evaluated.

3. Results

3.1. Physicochemical Properties

3.1.1. Butter Samples

The gross composition of butter samples is displayed in Table 1. No significant differences were observed between samples regarding dry matter, fat, pH, and titratable acidity. Values are in accordance with Portuguese legal requirements for butter composition [51].

Concerning the colour parameters of the butter samples (Table 2), differences were observed regarding lightness (L*), with sample PBC presenting higher values. Differences were also observed regarding the blue-yellow hue (b*), with sample PBC presenting higher values. However, the calculated colour difference values (ΔEab*), which are a better indicator of the colour attributes of the samples, were lower than 1, except for sample A vs.

sample PBC (Table 3). ΔEab* values lower than 1 indicate that a common observer cannot detect differences in the colour of the products. Hence, one can conclude that the different treatments did not affect the perceived colour of butter samples.

Table 1. Physicochemical properties of butter samples (mean ± standard deviation; n = 3).

Product	Dry Matter (% *w*/*w*)	Fat (% *w*/*w*)	OSNF (% *w*/*w*)	Ashes. (% *w*/*w*)	pH	Titratable Acidity (mL NaOH 1 N/100 g)
A	84.66 ± 1.25	81.71 ± 1.27	2.95 ± 0.97	0.73 ± 0.06	4.73 ± 0.06	2.43 ± 0.40
ABC	85.33 ± 0.47	82.66 ± 0.53	2.67 ± 0.12	0.69 ± 0.03	4.73 ± 0.15	2.13 ± 0.45
P	84.67 ± 1.70	81.14 ± 1.70	3.52 ± 0.35	0.95 ± 0.04	4.63 ± 0.06	2.00 ± 0.26
PBC	85.67 ± 1.25	82.06 ± 0.93	3.60 ± 0.49	0.72 ± 0.03	4.72 ± 0.06	2.23 ± 0.35

OSNF = other solids not fat (including ashes).

Table 2. Colour parameters of butter samples (mean ± standard deviation; n = 3).

Product	L*	a*	b*
A	92.83 ± 0.05 ^{a}	−6.73 ± 0.12 ^{a}	18.77 ± 0.26 ^{ac}
ABC	93.73 ± 0.09 ^{bc}	−6.70 ± 0.08 ^{a}	18.97 ± 0.41 ^{ac}
P	93.80 ± 0.08 ^{bc}	−6.67 ± 0.05 ^{a}	17.93 ± 0.05 ^{b}
PBC	94.23 ± 0.05 ^{c}	−6.67 ± 0.09 ^{a}	19.23 ± 0.21 ^{c}

(L*) Lightness; (a*) green-red hue; (b*) blue-yellow hue. Different superscript letters in the same column indicate significant differences between samples ($p < 0.05$).

Table 3. Colour differences (ΔEab*) between butter samples.

	ABC	P	PBC
A	0.43	0.82	1.09
ABC		0.54	0.16
P			0.94

Texture measurements were carried out at temperatures intended to replicate the usual conditions of butter consumption. The lower temperature (10 °C) corresponds to butter after removal from the refrigerator, while 20 °C corresponds to butter kept out of the refrigerator. Figure 2 presents typical force/time (A,C) and force/distance (B,D) graphs recorded at 10 and 20 °C of a representative butter sample (ABC). Differences between samples regarding the maximal force registered were clearly observed at both temperatures, indicating a nearly four times higher firmness (positive peak) of samples evaluated at 10 °C when compared to the same sample at 20 °C. The data in Table 4 provide a more detailed indication of the differences observed. Besides the higher firmness recorded at 10 °C, positive peak areas (softness) were clearly higher at 10 °C when compared to 20 °C, indicating a harder texture. These differences were less pronounced regarding the negative peak areas (adhesiveness). Sample P presented the highest softness and adhesiveness, both at 10 and 20 °C. The values for firmness observed at 10 °C were higher than the ones reported by Ferreira et al. [25], who reported values of ca. 6.9 and 15.7 N for butter produced with cream fermented by probiotics and with an aromatic starter culture, respectively. The hardness of sheep's butter samples reported by Silva et al. [26] was higher than the ones observed at 10 °C in the present work. However, the texture of sheep's butter samples was determined at ca. 7 °C, and the lower temperature may have had the largest influence.

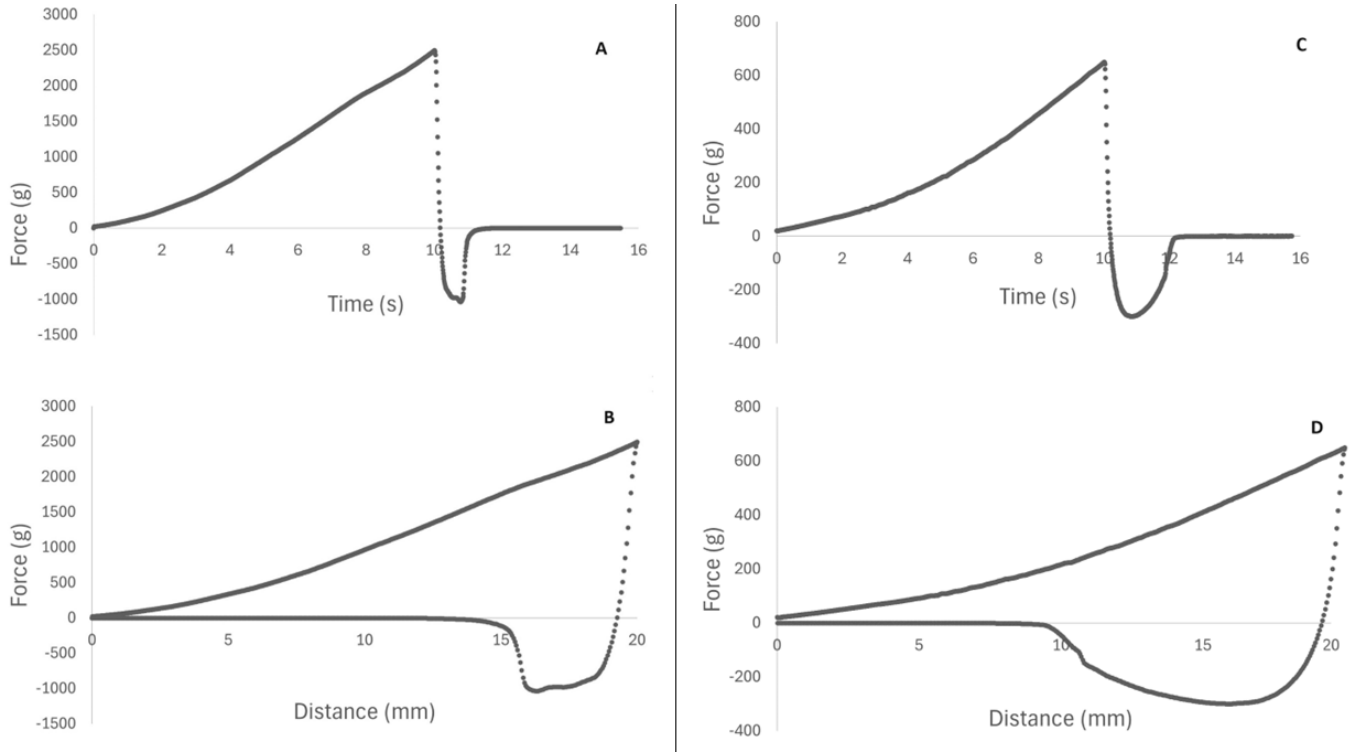

Figure 2. Representative force–time and forcedistance graphs of a representative butter sample (**A**–**C**) measured at 10 °C (**A**,**B**) and at 20 °C (**C**,**D**).

Table 4. Texture parameters of butter samples at different temperatures (mean ± standard deviation; $n = 3$).

Product	Firmness (N)	Softness (N.s)	Adhesiveness (N.s)
A:10 °C	26.50 ± 1.77 ^{a}	116.83 ± 7.47 ^{a}	−66.72 ± 0.57 ^{a}
A:20 °C	7.07 ± 0.87 ^{c}	29.91 ± 4.05 ^{c}	−46.39 ± 3.86 ^{d}
ABC:10 °C	24.92 ± 0.97 ^{a}	108.84 ± 3.91 ^{a}	−78.66 ± 8.14 ^{b}
ABC:20 °C	6.94 ± 0.35 ^{c}	29.84 ± 1.33 ^{c}	−51.37 ± 4.76 ^{d}
P:10 °C	36.17 ± 1.79 ^{b}	157.47 ± 7.76 ^{b}	−116.56 ± 11.69 ^{c}
P:20 °C	9.13 ± 0.48 ^{d}	38.31 ± 1.64 ^{d}	−60.79 ± 12.41 ^{d}
PBC:10 °C	27.79 ± 1.42 ^{a}	118.90 ± 6.20 ^{a}	−82.46 ± 5.91 ^{b}
PBC:20 °C	7.65 ± 0.46 ^{c}	33.69 ± 1.88 ^{c}	−58.35 ± 2.29 ^{e}

Different superscript letters in the same column indicate significant differences between samples ($p < 0.05$).

The rheological parameters of butter samples evaluated at 10 and 20 °C are presented in Figures 3 and 4. All butter samples presented a pseudoplastic behaviour with a high dependence on temperature. Figure 3 presents the elastic (G′) and viscous (G″) moduli of the butter samples. In all cases, the elastic modulus was higher than the viscous modulus, indicating the solid-like characteristics of the products. As had occurred with softness, at 10 °C, samples P and PBC (Figure 3B,D) presented higher G′ values when compared to samples A and ABC. This was reflected in the higher dynamic viscosity (η*) values observed for these samples (Figure 4B,D). The same pattern was observed at 20 °C. The dynamic viscosity values observed at 20 °C were, in all cases, more than one log cycle below the ones recorded at 10 °C, indicating the very high dependency of the texture of butter with temperature.

Significant ($p < 0.05$) correlations were observed between several parameters of butter samples. Ashes presented positive correlations with firmness (0.90) and softness (0.92) and a negative correlation with adhesiveness (−0.86). The titratable acidity presented a

negative correlation with green-red hue (a*) (−0.69) and blue-yellow hue (b*) (−0.67). As expected, firmness presented a high positive correlation with softness (0.90) and a negative correlation with adhesiveness (−0.91).

Figure 5 presents the results of the principal component analysis based on the physicochemical properties of butter samples. Figure 5A presents the projection of the physicochemical attributes on principal components 1 and 2, which represent 60.42% of the explained variance. Figure 5B presents the projection of samples over both planes. The PCA allowed discrimination of butter sample P from all other samples. This differentiation most probably resulted from the significantly higher firmness, softness, and adhesiveness presented by this sample when compared to all others. However, the statistical tool was unable to clearly discriminate the remaining samples.

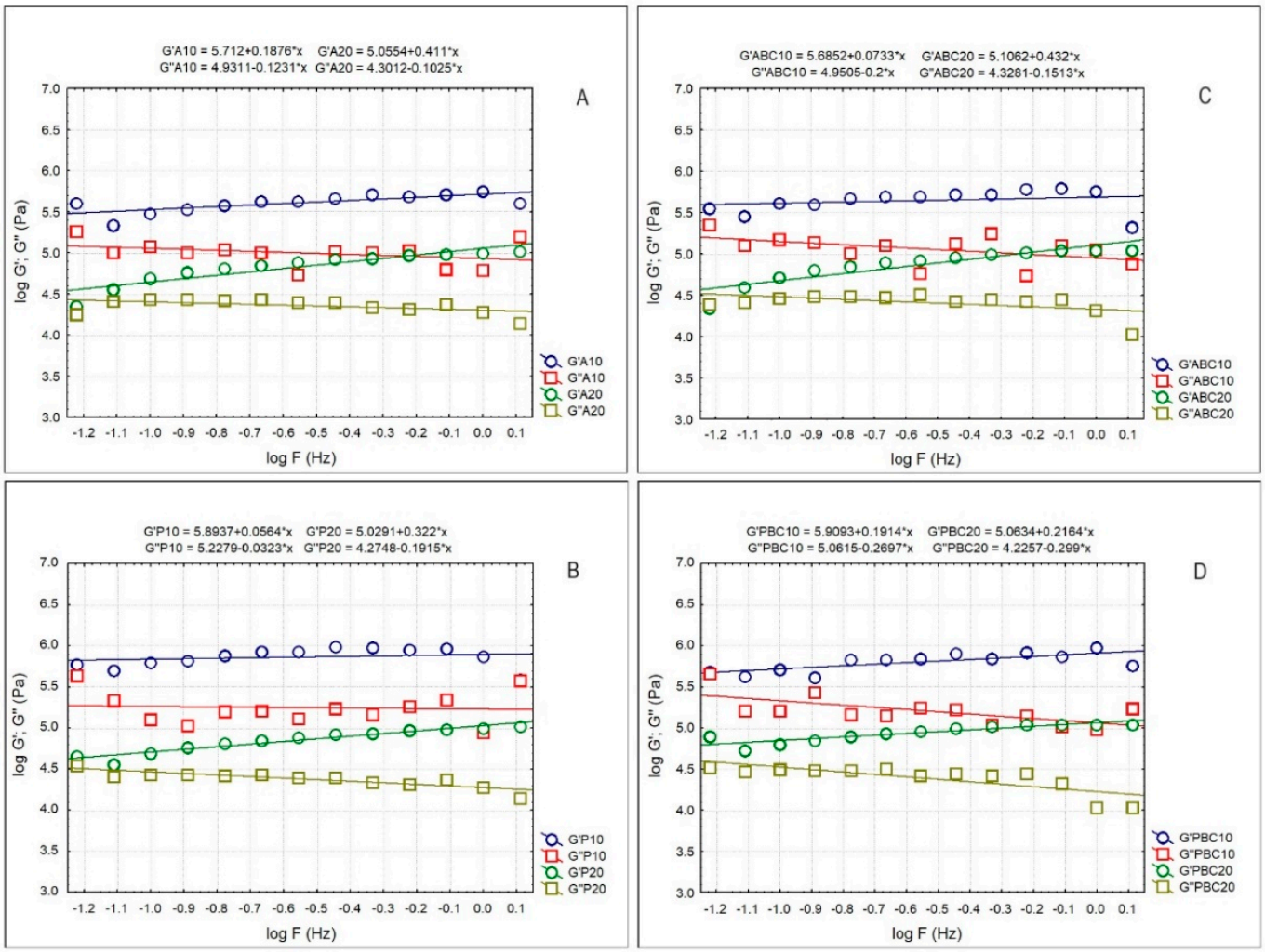

Figure 3. Elastic (G′) and viscous (G″) moduli of butter samples at 10 and 20 °C (n = 3). (**A**) Butter with aromatic culture; (**B**) butter with aromatic plus bioprotective culture; (**C**) butter with probiotic culture; (**D**) butter with probiotic plus bioprotective culture.

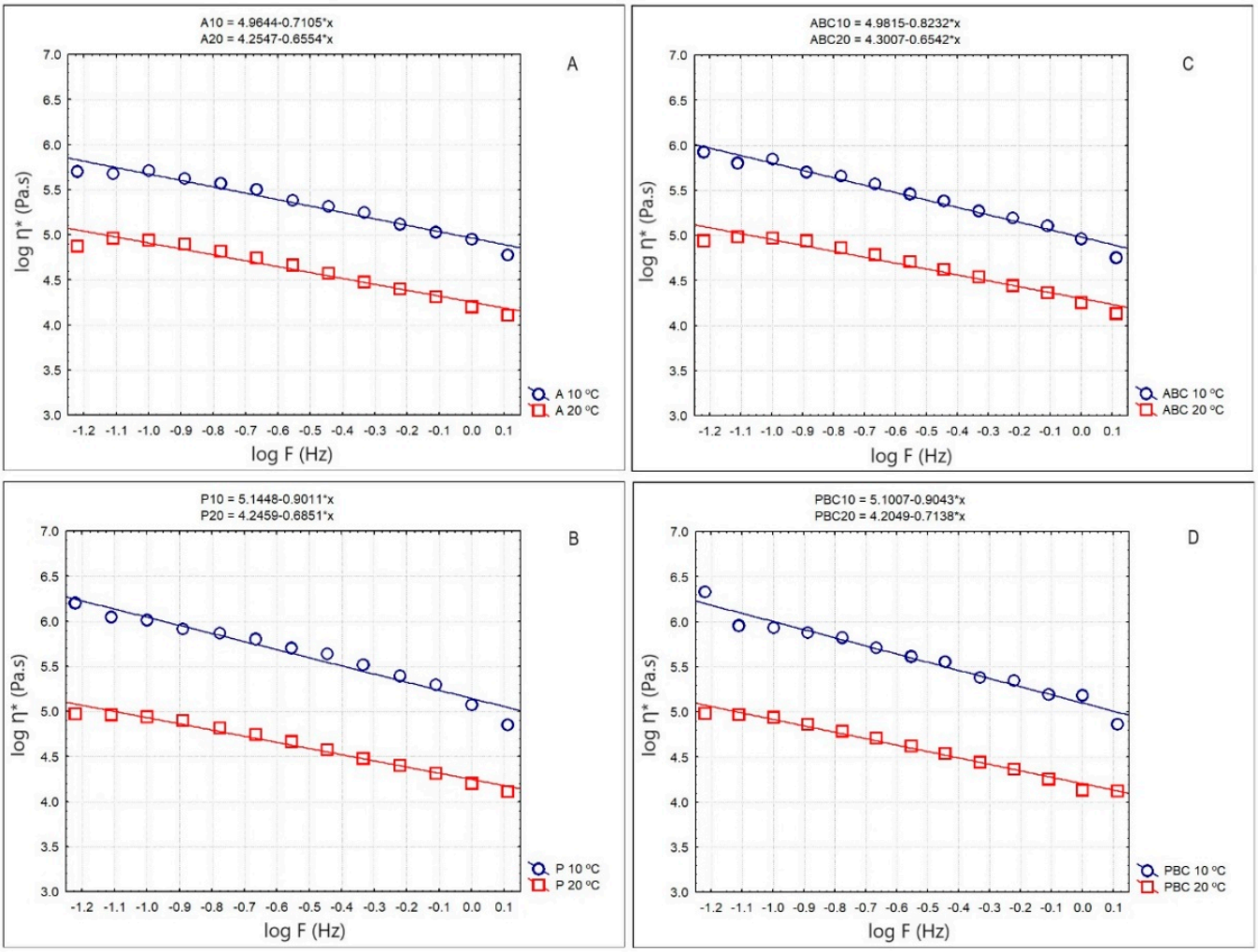

Figure 4. Dynamic viscosity (η*) of butter samples at 10 and 20 °C (n = 3). (**A**) Butter with aromatic culture; (**B**) butter with aromatic plus bioprotective culture; (**C**) butter with probiotic culture; (**D**) butter with probiotic plus bioprotective culture.

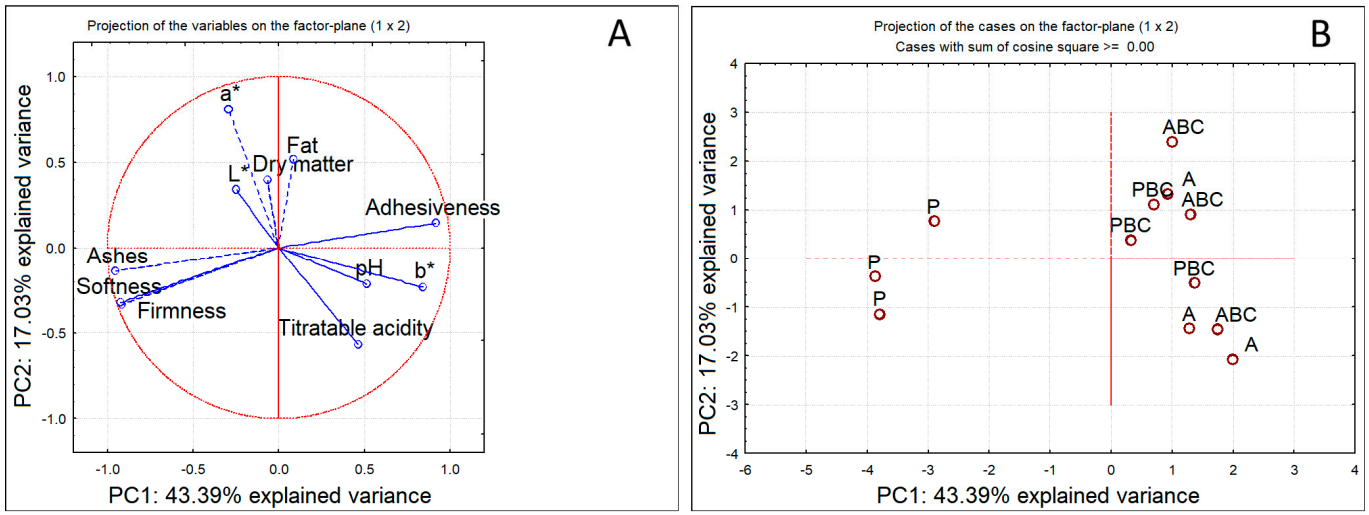

Figure 5. Projection of physicochemical attributes (**A**) and of butter samples (**B**) on principal components 1 and 2.

3.1.2. Buttermilk Samples

Table 5 presents the gross composition of buttermilk samples. Differences were observed regarding most of the parameters evaluated.

Table 5. Physicochemical properties of buttermilk samples (mean ± standard deviation; n = 3).

Product	Dry Matter (% *w*/*w*)	Fat (% *w*/*w*)	Ashes (% *w*/*w*)	pH	Titratable Acidity (% Lactic Acid)
A	10.16 ± 0.18 [a]	1.53 ± 0.06 [a]	0.77 ± 0.03 [a]	4.40 ± 0.10 [a]	0.88 ± 0.05 [a]
ABC	11.15 ± 0.05 [b]	0.63 ± 0.15 [b]	0.77 ± 0.02 [a]	4.30 ± 0.00 [a]	0.97 ± 0.11 [a]
P	9.79 ± 0.25 [a]	0.37 ± 0.23 [b]	0.75 ± 0.02 [a]	4.30 ± 0.02 [a]	1.01 ± 0.08 [a]
PBC	10.14 ± 0.03 [a]	1.20 ± 0.52 [a]	0.75 ± 0.00 [a]	4.20 ± 0.03 [b]	0.99 ± 0.14 [a]

Different superscript letters in the same column indicate significant differences between samples ($p < 0.05$).

The viscosity of the buttermilk samples is presented in Table 6. Sample PBC presented the highest viscosity, followed by samples A and P. This observation aligns with the higher dynamic viscosity observed in PBC butter samples. Sample ABC had significantly lower viscosity values. Hence, it can be considered that these differences may have resulted from the activity of the cultures used, namely the impact of the cultures on the pH of the samples, as a significant negative correlation was observed between the viscosity and the pH (−0.70) of the buttermilk samples.

Table 6. Viscosity of buttermilk samples (mean ± standard deviation; n = 3).

Product	Viscosity (cPs)
A	65.63 ± 0.06 [a]
ABC	61.10 ± 0.17 [b]
P	65.77 ± 0.15 [a]
PBC	78.70± 0.17 [c]

Different superscript letters in the same column indicate significant differences between samples ($p < 0.05$).

Regarding colour parameters of the buttermilk samples (Table 7), sample ABC presented the highest lightness values, while sample PBC presented a significantly higher b* value, indicating its higher yellowness, as had occurred with the corresponding butter sample. Sample A presented a significantly lower b* value, indicating a less pronounced yellow chroma. Table 8 presents the ΔEab* values between buttermilk samples. Except for sample A vs. sample AB and sample A vs. sample P, all other comparisons presented values higher than 1, indicating that colour differences between buttermilk samples could be perceived by a common observer.

Table 7. Colour parameters of buttermilk samples (mean ± standard deviation; n = 3).

Product	L*	a*	b*
A	79.70 ± 0.14 [a]	−2.63 ± 0.12 [a]	0.57 ± 0.09 [a]
ABC	80.97 ± 0.12 [b]	−2.53 ± 0.33 [a]	1.13 ± 0.31 [a]
P	78.73 ± 0.05 [c]	−3.33 ± 0.05 [b]	1.17 ± 0.47 [a]
PBC	79.63 ± 0.52 [a]	−3.77 ± 0.09 [b]	2.63 ± 0.12 [b]

(L*) Lightness; (a*) green-red hue; (b*) blue-yellow hue. Different superscript letters in the same column indicate significant differences between samples ($p < 0.05$).

Table 8. Colour differences (ΔEab*) between buttermilk samples.

	ABC	P	PBC
A	0.97	0.89	2.78
ABC		2.81	2.77
P			1.57

Significant correlations ($p < 0.05$) were also observed between several parameters of buttermilk samples. Ashes had a positive correlation with green-red hue (a*) (0.62), while the pH had a negative correlation with blue-yellow hue (b*) (−0.89). As noted, the viscosity of the samples presented a significant negative correlation with pH. The impact of pH on protein interactions may be the reason for such behaviour.

Figure 6 presents the results of the principal component analysis of buttermilk samples. Figure 6A presents the projection of the physicochemical attributes on principal components 1 and 2, which represent 68.41% of the explained variance. Figure 6B represents the projection of samples over both planes. The PCA allowed discrimination of all buttermilk samples. Viscosity and colour parameters were most probably the main factors influencing such differentiation.

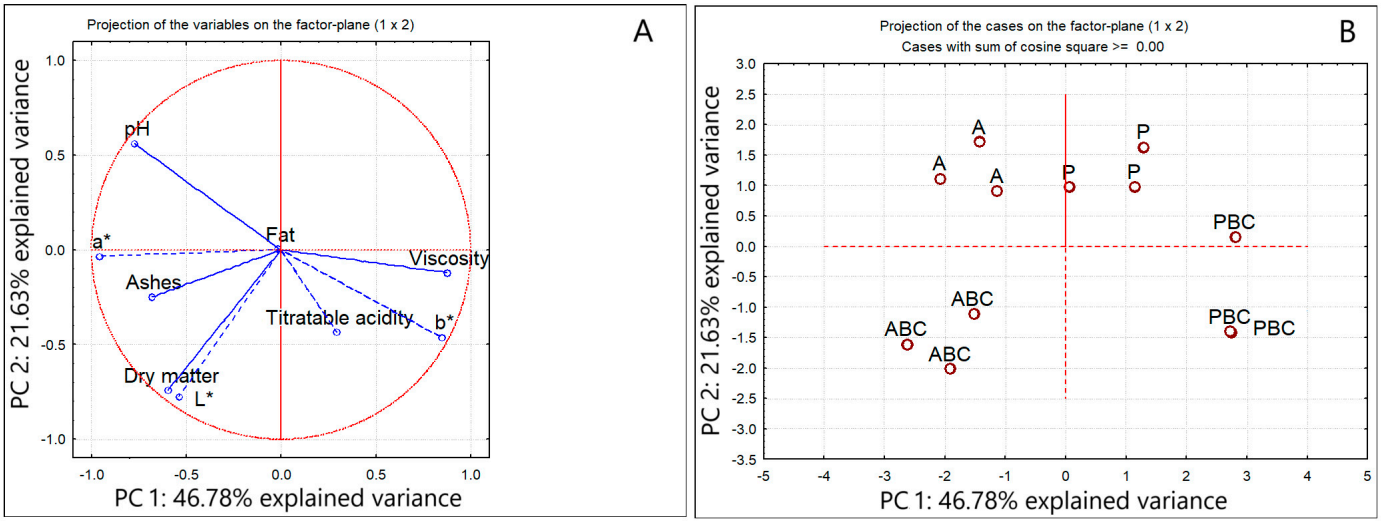

Figure 6. Projection of physicochemical attributes (**A**) and of buttermilk samples (**B**) on principal components 1 and 2.

3.2. Microbiological Characteristics of Butter and Buttermilk Samples

Figure 7 presents the microbial characteristics of butter samples and the corresponding buttermilk. Lower counts of lactobacilli and lactococci were observed on the butter samples produced with cream fermented by aromatic starter (A) in both the butter and buttermilk samples. The samples of butter and buttermilk containing probiotics and bioprotective culture (PBC) presented the highest amounts of lactobacilli and lactococci (ca. log 7–9 CFU/g or mL) throughout the storage period, indicating that probiotic cultures were not affected by storage at refrigeration temperatures. The observed counts were above the values indicated as necessary to claim the probiotic characteristics of a food product (>log 7 CFU/g) [52]. These results are particularly interesting in the case of butter, which could maintain its probiotic potential for at least three months.

Regarding yeast and mould counts, a sharp increase was observed between the 30th and the 60th day of storage of butter samples. Counts increased from ca. log 1–2 CFU/g at the first month of storage to ca. log 5 CFU/g after 2 months and were maintained at these levels until the 90th day.

In the case of buttermilk samples, yeast and mould counts increased steadily from the 1st to the 15th days of storage, reaching values of ca. log 5–6 CFU/mL, then decreased slightly to ca. log 3.5–5 CFU/mL. It must be pointed out that samples P and PBC presented the lowest levels of yeasts and moulds by the end of the storage period (<log 4 CFU/mL). Hence, it can be concluded that probiotics and the bioprotective culture reduced the growth of yeasts and moulds, and this had a positive impact on the shelf life of these samples.

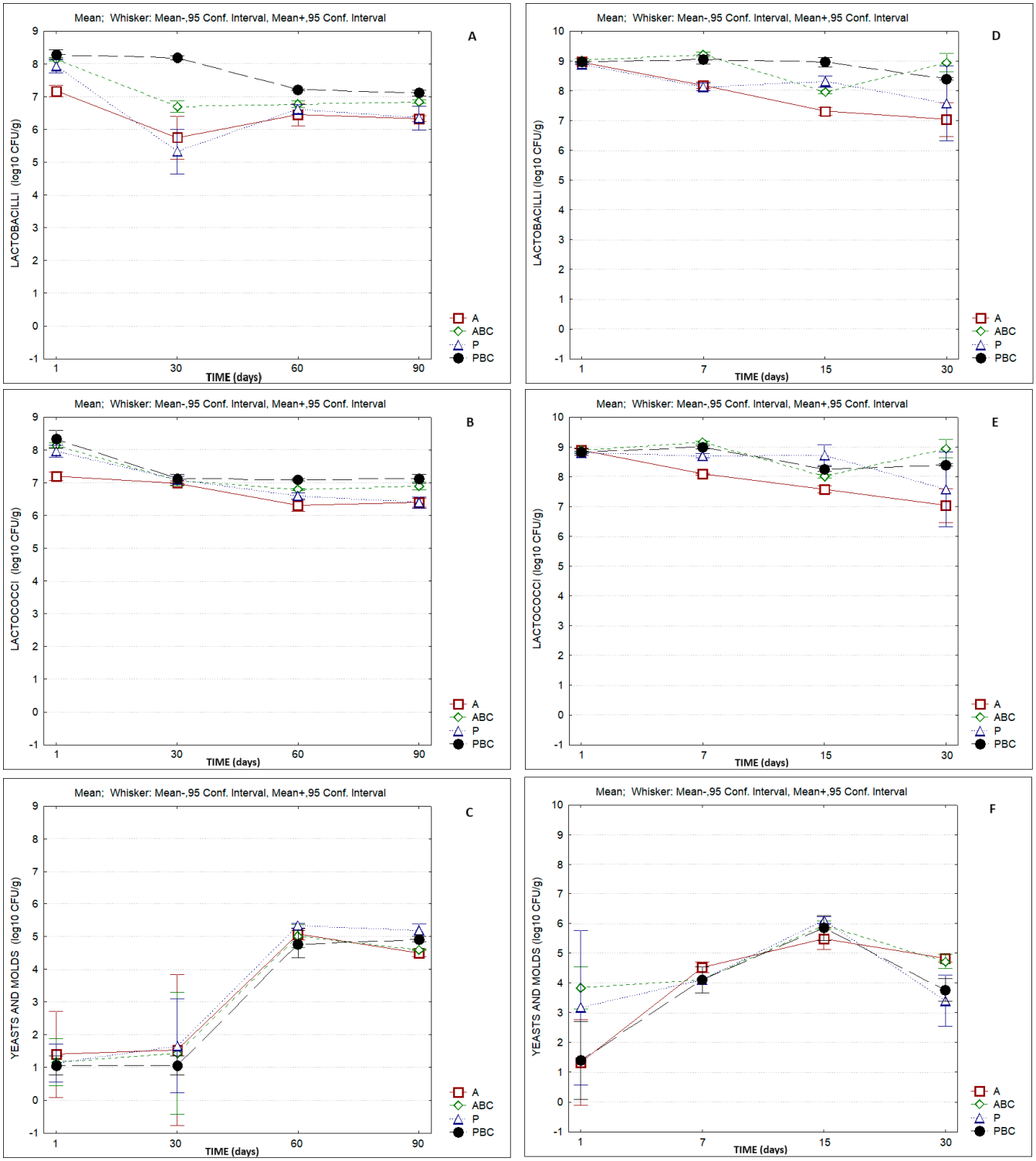

Figure 7. Microbial counts of butter ((**A**) lactobacilli; (**B**) lactococci; (**C**) yeasts and moulds) and corresponding buttermilk samples ((**D**) lactobacilli; (**E**) lactococci; (**F**) yeasts and moulds) (mean ± confidence interval; $n = 3$).

3.3. Sensory Characteristics of Butter and Buttermilk Samples

Regarding the sensory properties of butter and buttermilk samples, better scores were obtained by the P butter sample (Figure 8A). However, ABC butter obtained almost the same score. Sample A received the lowest scores for overall liking. Texture and taste of butter samples correlated positively with global evaluation (0.60 and 0.84, respectively).

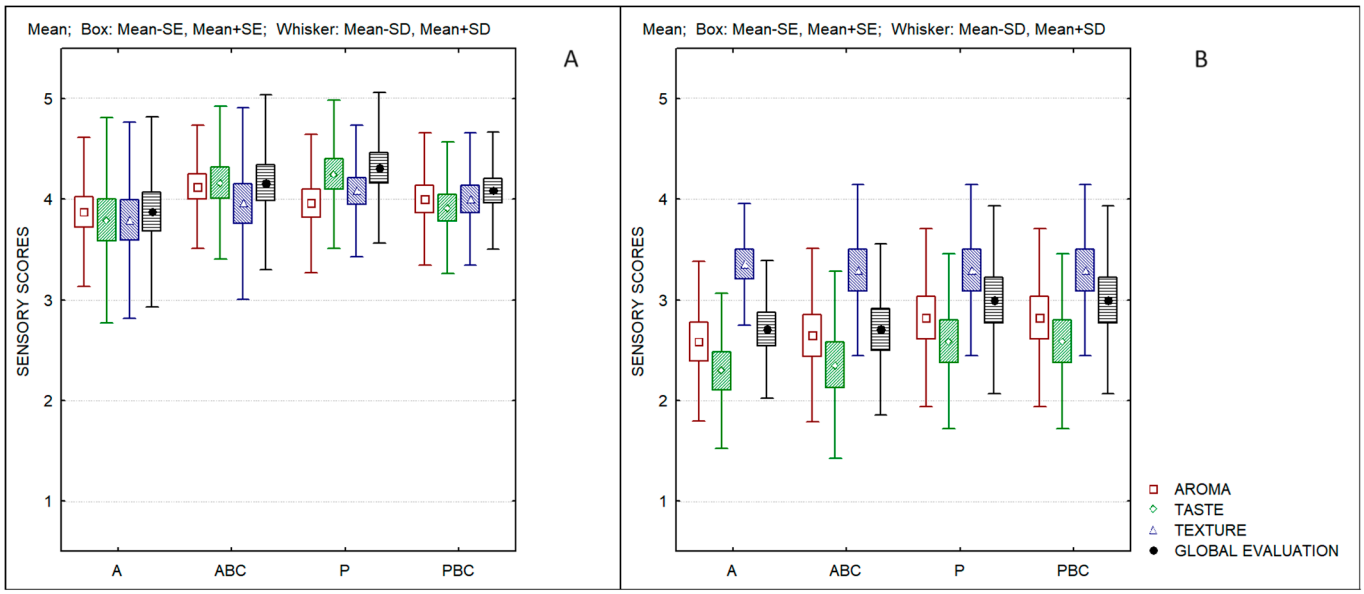

Figure 8. Comparison of sensory scores obtained for butter (**A**) and buttermilk samples (**B**).

Buttermilk samples received lower scores compared to the ones obtained by the corresponding butter samples. This may have resulted from the fact that the members of the panel were not familiar with fermented buttermilk. Sample PBC received the highest scores, followed by sample P. Samples A and ABC obtained the lowest scores for taste. However, the addition of sugar or flavourings may contribute to improving the sensory quality of such products. Significant ($p < 0.05$) negative correlations were found between the dry matter of buttermilk samples with the sensory attributes taste (−0.58) and global evaluation (−0.73).

4. Discussion

Butter is a water-in-oil emulsion containing saturated and unsaturated fatty acids, which can influence its stability and oxidative behaviour [53]. Cream, as the primary raw material for butter production, significantly impacts the quality and properties of butter [54]. Additionally, factors like fat globule size, emulsifiers, and cream ageing play a vital role in determining the microstructure and physical characteristics of butter [55,56]. The moisture content of butter, the water droplet size, and the melting behaviour have also been identified as key determinants of butter quality [57]. The fatty acid profile of butter also impacts its melting behaviour and textural properties [25,58,59]. The quality and functionality of butter are also affected by the seasonality of pasture-based production systems, where the C16:0/C18:1 ratio has been linked to changes in texture, hardness, and spreadability. The type of packaging materials can also impact the aroma compounds and overall quality of butter during storage [60,61]. One of the key challenges associated with butter is its poor spreadability at refrigeration temperatures. The physical properties of butter, including texture and mechanical attributes, are intricately linked to the interactions between fat crystals, the globular fat portion, and the solid/liquid fat fraction [55]. Sahin and coworkers [62] discussed the impact of hardness on butter spreadability, indicating that, as expected, increased hardness diminishes spreadability. Additionally, Lopes et al. [63] highlighted the typically poor spreadability of butter at refrigeration temperatures, prompting research efforts to enhance this characteristic. Musayeva et al. [64] introduced a spreadability index based on fatty acid profiles. Magan et al. [65] suggested that fatty acids with a lower melting point could potentially improve spreadability.

According to Lis et al. [66], the area under the force–time curve is an indicator of sample softness. Smaller areas under the curve indicate softer samples. This parameter can be a reliable indicator of a product's consistency. These authors also observed a

highly significant positive correlation between the firmness and softness of butter samples measured with the TA.XT. Plus texture analyser. The resistance to probe withdrawal (adhesiveness) is also an important textural parameter to be evaluated. The mass of the sample adhering to the probe after its withdrawal is responsible for the negative area under the force–time curve. Adhesiveness is expressed as the product of the force necessary to withdraw the probe from the sample and withdrawal time [N·s]. In the present study, firmness and softness presented strong negative correlations with adhesiveness (−0.91 and −0.88, respectively). Lactic acid bacteria may also play a role in releasing free fatty acids from triglycerides, influencing the texture of butter [26]. Ziarno and coworkers [67] reported values of 17 N for the hardness of pasteurised cream butter at 4 °C and 6.13 N 30 min after removing the samples from the refrigerator. Vioque-Amor and coworkers [59] tested the texture properties of sheep's, goat's, and cow's butter at 4, 10 and 20 °C. The values reported by these authors were of the order of 4–5 N at 4 °C, 1–3 N at 10 °C, and 0.1–0.5 N at 20 °C, clearly lower than the ones observed in the present study. However, these authors used a wire probe, which may explain the lower values recorded. Lower values for butter hardness were also reported by other authors [68,69]. Considering the reported data, it becomes evident that it is very difficult to compare the texture results obtained in different studies because the raw materials and the conditions of butter production have a strong impact on butter's textural properties. Moreover, the conditions for the measurement of texture properties also vary, which further makes comparisons difficult.

Butter typically has a pale-yellow colour, ranging from a light creamy hue to a deeper golden shade. The primary pigment responsible for the yellow colour of butter is β-carotene, which is naturally present in the grass that cows feed on. Cows that graze on fresh grass produce milk with higher levels of β-carotene, resulting in butter with a more intense yellow colour. In contrast, butter made from the milk of cows fed on silage or grains may have a paler colour due to the lower β-carotene content in their diet. The colour parameters obtained for butter samples in the present study showed higher lightness and blue-yellow hue (b*) values than the ones obtained by Ferreira et al. [25]. L* values of butter samples obtained in the present study are of the same order of magnitude as the ones reported by Silva et al. [26] for sheep's butter produced with sweet cream, cream fermented by the same aromatic starter, or a commercial kefir culture and a probiotic culture. Values for parameter a* obtained in the present study were lower, while b* values were higher than the ones reported by those authors. The difference in the blue-yellow hue indicates a much more pronounced yellow colour for cow's butter when compared to the ivory colour of sheep's butter. The results for the colour parameters obtained in the present work showed higher lightness values than the ones reported by Brożek and coworkers for milk butter and whey butter resulting from Gouda cheese (89.13 and 88.96, respectively) [58]. In addition, the a* values obtained by those authors (−3.41 and −3.27, respectively) and the b* values (32.08 and 29.84, respectively) were also different from the ones obtained in the present study. Our results for the L* and a* parameters were like the ones obtained by Pădureț for 84% fat butter, but the b* value reported by this author (33.05) was clearly higher than the ones observed in the present study, indicating a less intense yellow hue of our butter samples. Lower results for the L* parameter and higher results for the b* parameter were also obtained by Ziarno and coworkers [67]. The processing of butter can also affect its colour. Churning the cream to make butter leads to incorporation of air and can cause fat globules to scatter light, giving the butter a lighter appearance. Additionally, the temperature at which the butter is churned and the speed of the churning process can impact its final colour. Some manufacturers may add colouring agents to butter to achieve a consistent and appealing colour. Annatto, a natural dye, is commonly used to enhance the yellow colour of butter. This practice is more prevalent in regions where consumer preferences favour a rich, golden hue in butter. The fat content and fatty acid composition of butter also play a significant role in determining the colour of butter [70]. Hence, the differences between studies regarding colour parameters may result from one or several of these factors, which makes comparisons difficult between different studies.

The maturation of cream with appropriate starter cultures is crucial for ensuring the quality, shelf life, and sensory properties of butter [54]. The microbial composition of butter can vary based on different factors, such as the production method, storage conditions, and added ingredients. In a previous work, we highlighted the transition from traditional butter to probiotic butter, emphasising the importance of probiotic strains like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in enhancing the probiotic characteristics of butter [25]. Moreover, the use of innovative approaches like incorporating probiotics or plant-based additives such as garlic powder can impact the sensory properties and microbial quality of butter [26,68].

The results of microbial counts in the present work were of the same order as the ones observed in the study by Ferreira et al. [25]. However, in the present work, high levels of LAB were maintained for at least 90 days, indicating a good adaptation of the microorganisms, both in the butter and buttermilk samples. The lactobacilli and lactococci counts were like the ones reported by Silva and coworkers for sheep's butter and buttermilk [26]. These values were clearly higher than the ones reported by Bellinazo et al. [22] for *Lactobacillus casei* in butter with the addition of bixin as an antioxidant, which presented a clear decrease in microbial counts after the 45th day of storage. The counts of the LAB present in butter samples produced in this study were also clearly higher than the ones reported by Gaba et al. [71].

Regarding buttermilk, El-Hameed and coworkers [72] tested the addition of barley and oat flour to improve the viscosity and sensory characteristics of synbiotic buttermilk. In all cases, *S. thermophilus, L. acidophilus*, and *Bifidobacterium bifidus* counts exceeded log 7 CFU/g over 15 days of storage. The present work clearly indicates that probiotics and bioprotective culture are well adapted to both butter and buttermilk and maintain high cell counts throughout the tested storage periods.

The sensory properties of new dairy products pose challenges that need to be addressed to meet consumers' preferences [73]. Sensory characteristics of butter are influenced by various factors, such as composition, feed systems, processing methods, and additives. Specific volatile aromatic compounds, like aldehydes and ketones, play a significant role in the sensory attributes of butter and can vary based on the feed system used [74]. Pasture-based butter has been found to exhibit enhanced sensorial properties, particularly in flavour and appearance [75]. The presence of specific volatile organic compounds contributes to the aroma of butter, with over 230 volatile compounds identified in dairy fat ingredients [59]. Moreover, the addition of certain ingredients can impact the oxidative stability and sensory properties of butter [76,77]. Synbiotic buttermilk samples with added barley and oat flour were highly rated by a sensory panel (>7 on a 9-point hedonic scale). Hence, as previously indicated, the addition of other ingredients like sugar, flavourings, or fruit preparations to buttermilk formulations can enhance their sensory properties, and these options must be evaluated in future work.

5. Conclusions

Overall, it can be concluded that the fermentation of cream with probiotic and bioprotective cultures represents an interesting opportunity to enhance the functional properties of butter and obtain the corresponding probiotic buttermilk. In this study, both butter and buttermilk samples containing probiotics exceeded the cell counts required for a product to be labelled as containing probiotics. Moreover, high probiotic cell counts were maintained throughout the storage period (90 days for butter and 28 days for buttermilk). Furthermore, sensory properties of butter and buttermilk samples were improved by fermenting cream with probiotic and bioprotective cultures, and this feature can be interesting from an industrial point of view. However, further work is needed to improve the sensory properties of buttermilk samples.

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supervision, C.P.; project administration, C.P.; funding acquisition, C.P. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Sensory analysis is not a procedure that needs to be submitted to the ethics commission (EC) of the Polytechnic of Coimbra. Most of the panellists are members of the staff of the institution and frequently perform such types of tests. However, when consumer sensory tests were carried out, the panellists were informed about the objectives of the work and signed the informed consent form provided by the EC (CIEIPC_CILE_02). Hence, this research did not necessitate formal ethical approval as per the situation at that time. In the course of the implementation of this study, no experiments violating human, or animal laws were performed. This research follows Law No. 58/2019 of 8 August, the GDPR, the Declaration of Helsinki, and the Oviedo Convention.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Further enquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Natt, S.; Katyal, P. Current trends in non-dairy probiotics and their acceptance among consumers: A review. *Agr. Rev.* **2021**, *43*, 450–456. [CrossRef]
2. Dahiya, D.; Nigam, P. The gut microbiota influenced by the intake of probiotics and functional foods with prebiotics can sustain wellness and alleviate certain ailments like gut-inflammation and colon-cancer. *Microorganisms* **2022**, *10*, 665. [CrossRef] [PubMed]
3. Siciliano, R.; Reale, A.; Mazzeo, M.; Morandi, S.; Silvetti, T.; Brasca, M. Paraprobiotics: A new perspective for functional foods and nutraceuticals. *Nutrients* **2021**, *13*, 1225. [CrossRef]
4. Sherwani, K.; Bukhari, D. Probiotics in processed dairy products and their role in gut microbiota health. In *Effect of Microbiota on Health and Disease*; El-Sayed, H., Ed.; Intechopen: London, UK, 2022. [CrossRef]
5. Vargas-Ramella, M.; Pateiro, M.; Maggiolino, A.; Faccia, M.; Franco, D.; Palo, P. Buffalo milk as a source of probiotic functional products. *Microorganisms* **2021**, *9*, 2303. [CrossRef]
6. Hammam, A.; Ahmed, M. Technological aspects, health benefits, and sensory properties of probiotic cheese. *Appl. Sci.* **2019**, *1*, 1113. [CrossRef]
7. Akter, S.; Park, J.; Jung, H. Potential health-promoting benefits of paraprobiotics, inactivated probiotic cells. *J. Microb. Biotech.* **2020**, *30*, 477–481. [CrossRef] [PubMed]
8. Syiemlieh, I.; Morya, S. Dairy and non-dairy based probiotics: A review. *Pharma Innov.* **2022**, *11*, 2956–2964. [CrossRef]
9. Agamennone, V.; Krul, C.; Rijkers, G.; Kort, R. A practical guide for probiotics applied to the case of antibiotic-associated diarrhea in the netherlands. *BMC Gastroent.* **2018**, *18*, 103. [CrossRef]
10. Colombo, M.; Todorov, S.; Eller, M.; Nero, L. The potential use of probiotic and beneficial bacteria in the brazilian dairy industry. *J. Dairy Res.* **2018**, *85*, 487–496. [CrossRef] [PubMed]
11. Wojciechowska-Alwin, N.; Sikora, D.; Lipski, A.; Skrypnik, D. Influence of natural dairy probiotics on health. *J. Pre-Clin. Clin. Res.* **2019**, *13*, 153–161. [CrossRef]
12. García-Díez, J.; Saraiva, C. Use of starter cultures in foods from animal origin to improve their safety. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2544. [CrossRef]
13. Austrich-Comas, A.; Serra-Castelló, C.; Jofré, A.; Gou, P.; Bover-Cid, S. Control of *Listeria monocytogenes* in chicken dry-fermented sausages with bioprotective starter culture and high-pressure processing. *Front. Microbiol.* **2022**, *13*, 983265. [CrossRef] [PubMed]
14. Bhattacharya, D.; Nanda, P.; Pateiro, M.; Lorenzo, J.; Dhar, P.; Das, A. Lactic acid bacteria and bacteriocins: Novel biotechnological approach for biopreservation of meat and meat products. *Microorganisms* **2022**, *10*, 2058. [CrossRef]
15. Gao, J.; Li, X.; Zhang, G.; Sadiq, F.; Simal-Gándara, J.; Xiao, J.; Sang, Y. Probiotics in the dairy industry—Advances and opportunities. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 3937–3982. [CrossRef]
16. Kaur, H.; Kaur, G.; Ali, S. Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties. *Fermentation* **2022**, *8*, 425. [CrossRef]
17. González, F.; Delgado, S.; Ruiz, L.; Margolles, A.; Ruas-Madiedo, P. Functional bacterial cultures for dairy applications: Towards improving safety, quality, nutritional and health benefit aspects. *J. Appl. Microb.* **2022**, *133*, 212–229. [CrossRef]

18. Meloni, M.; Piras, F.; Siddi, G.; Cabras, D.; Comassi, E.; Lai, R.; Scarano, C. Comparison of activity of commercial protective cultures and thermophilic lactic acid bacteria against listeria monocytogenes: A new perspective to improve the safety of sardinian pdo cheeses. *Foods* **2023**, *12*, 1182. [CrossRef] [PubMed]
19. Vasiliauskaite, A.; Milerienė, J.; Kasparaviciene, B.; Aleksandrovas, E.; Songisepp, E.; Rud, I.; Šernienė, L. Screening for antifungal *Indigenous lactobacilli* strains isolated from local fermented milk for developing bioprotective fermentates and coatings based on acid whey protein concentrate for fresh cheese quality maintenance. *Microorganisms* **2023**, *11*, 557. [CrossRef] [PubMed]
20. Shi, C.; Maktabdar, M. Lactic acid bacteria as biopreservation against spoilage molds in dairy products—A review. *Front. Microbiol.* **2022**, *12*, 819684. [CrossRef]
21. Shi, C.; Knøchel, S. Sensitivity of molds from spoiled dairy products towards bioprotective lactic acid bacteria cultures. *Front. Microbiol.* **2021**, *12*, 631730. [CrossRef]
22. Farrokh, A.; Ehsani, M.; Moayednia, N.; Nezhad, R. Lactic acid fermentation of synbiotic cream: Effects on physicochemical characteristics and formation of l (+), and d (–)-lactic acid isomers. *Appl. Ecol. Environ. Res.* **2018**, *16*, 7447–7466. [CrossRef]
23. Bellinazo, P.; Vitola, H.; Cruxen, C.; Braun, C.; Hackbart, H.; Silva, W.; Fiorentini, Â. Probiotic butter: Viability of *Lactobacillus casei* strains and bixin antioxidant effect (*Bixa orellana* L.). *J. Food Process. Preserv.* **2019**, *43*, e14088. [CrossRef]
24. Ostadzadeh, M.; Najafi, M.; Ehsani, M. Lactic acid bacteria isolated from traditional iranian butter with probiotic and cholesterol-lowering properties: In vitro and in situ activity. *Food Sci. Nutr.* **2022**, *11*, 350–363. [CrossRef] [PubMed]
25. Ferreira, L.; Borges, A.; Gomes, D.; Dias, S.; Pereira, C.; Henriques, M. Adding value and innovation in dairy smes: From butter to probiotic butter and buttermilk. *J. Food Process. Pres.* **2020**, *46*, e14867. [CrossRef]
26. Silva, T.; Pires, A.; Gomes, D.; Viegas, J.; Pereira-Dias, S.; Pintado, M.; Pereira, C. Sheep's butter and correspondent buttermilk produced with sweet cream and cream fermented by aromatic starter, kefir and probiotic culture. *Foods* **2023**, *12*, 331. [CrossRef] [PubMed]
27. Prasad, A.; Kothari, N. Cow products: Boon to human health and food security. *Trop. Anim. Health Prod.* **2021**, *54*, 12. [CrossRef]
28. Vlasenko, I.; Bandura, V.; Semko, T.; Fialkovska, L.; Ivanishcheva, O.; Palamarchuk, V. Innovative approaches to the development of a new sour milk product. *Potravin. Slovak J. Food Sci.* **2021**, *15*, 970–981. [CrossRef]
29. Sakkas, L.; Evageliou, V.; Igoumenidis, P.; Moatsou, G. Properties of sweet buttermilk released from the churning of cream separated from sheep or cow milk or sheep cheese whey: Effect of heat treatment and storage of cream. *Foods* **2022**, *11*, 465. [CrossRef]
30. Barukčić, I.; Božanić, R. Valorisation of whey and buttermilk for production of functional beverages—An overview of current possibilities. *Food Tech. Biotech.* **2019**, *57*, 448–460. [CrossRef]
31. Skryplonek, K.; Dmytrów, I.; Mituniewicz-Małek, A. The use of buttermilk as a raw material for cheese production. *Int. J. Dairy Tech.* **2019**, *72*, 610–616. [CrossRef]
32. Szkolnicka, K.; Dmytrów, I.; Mituniewicz-Małek, A. Buttermilk ice cream—New method for buttermilk utilization. *Food Sci. Nutr.* **2020**, *8*, 1461–1470. [CrossRef] [PubMed]
33. Zhao, L.; Feng, R.; Mao, X. Addition of buttermilk powder improved the rheological and storage properties of low-fat yogurt. *Food Sci. Nutr.* **2020**, *8*, 3061–3069. [CrossRef]
34. Monteiro, P.; Gomes, P. Production of kefir as an alternative for utilization of buttermilk. *Rev. Inst. Lat. Cândido Tostes* **2019**, *73*, 162–171. [CrossRef]
35. Ozturkoglu-Budak, S.; Akal, H.; Turkmen, N. Use of kefir and buttermilk to produce an innovative quark cheese. *J. Food Sci. Tech.* **2020**, *58*, 74–84. [CrossRef] [PubMed]
36. Latoch, A.; Libera, J.; Stasiak, D. Physicochemical properties of pork loin marinated in kefir, yoghurt or buttermilk and cooked sous vide. *Acta Sci. Pol. Tech. Aliment.* **2019**, *18*, 163–171. [CrossRef]
37. Mahmoudi, I.; Moussa, O.; Chouaibi, M.; Telmoudi, A.; Boukari, W.; Hassouna, M. Development and characterisation of functional cultured buttermilk fortified with flaxseed. *Acta Aliment.* **2022**, *51*, 218–227. [CrossRef]
38. Rose, H.; Bakshi, S.; Kanetkar, P.; Lukose, S.; Felix, J.; Yadav, S.; Paswan, V. Development and characterization of cultured buttermilk fortified with *Spirulina plantensis* and its physico-chemical and functional characteristics. *Dairy* **2023**, *4*, 271–284. [CrossRef]
39. Deinychenko, G.; Huzenko, V.; Dmytrevskyi, D.; Zoltukhina, I.; Chervonyi, V.; Horielkov, D. A new method of elimination the polarizing layer during the membrane processing of buttermilk. *Acta Period. Tech.* **2022**, *53*, 1–10. [CrossRef]
40. Hickey, C.; O'Sullivan, M.; Davis, J.; Scholz, D.; Kilcawley, K.; Wilkinson, M.; Sheehan, J. The effect of buttermilk or buttermilk powder addition on functionality, textural, sensory and volatile characteristics of cheddar-style cheese. *Food Res. Int.* **2018**, *103*, 468–477. [CrossRef]
41. Borges, A.R.; Pires, A.F.; Marnotes, N.G.; Gomes, D.G.; Henriques, M.F.; Pereira, C.D. Dairy by-products concentrated by ultrafiltration used as ingredients in the production of reduced fat washed curd cheese. *Foods* **2018**, *9*, 1020. [CrossRef]
42. AOAC International. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC Official Method 925.23: Washington, DC, USA, 1995.
43. AOAC International. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC Official Method 945.46: Washington, DC, USA, 1995.
44. *Portuguese Standard No.1712*; Butter—Determination of Total Acidity (1981). Instituto Português da Qualidade (NP): Setubal, Portugal, 1981. (In Portuguese)

45. *Portuguese Standard No. 47*; Milk—Determination of Total Acidity (1981). Instituto Português da Qualidade (NP): Setubal, Portugal, 1981. (In Portuguese)
46. Funke-Guerber (Ed.) *Laboratory Catalogue for Milk Analysis*; Funke-Dr. N. Gerber Labortechnik GmbH: Berlin, Germany, 2018.
47. A Simple Review of CIE ΔE* (Colour Difference) Equations. Available online: https://techkonusa.com/a-simple-review-of-cie-%CE%B4e-color-difference-equations/ (accessed on 22 July 2024).
48. *ISO Standard, No. 15214*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria—Colony-Count Technique at 30 Degrees °C. International Organization for Standardization: Geneva, Switzerland, 1998.
49. *ISO Standard No 6611 | IDF 94:2004*; Milk and Milk Products—Enumeration of Colony-Forming Units of Yeasts and/or Moulds—Colony-Count Technique at 25 °C. International Organization for Standardization: Geneva, Switzerland, 2004.
50. Stone, H.; Sidel, J. *Sensory Evaluation Practices*, 3rd ed.; Food Science and Technology; Academic Press: New York, NY, USA, 2004; pp. 247–277.
51. *Portuguese Standard, No. 1711*; Pasteurized Butter-Definition, Classification, Characteristics, Packaging and Labelling. Instituto Português da Qualidade (NP): Setubal, Portugal, 1986. (In Portuguese)
52. Guarner, F.; Sanders, M.H.; Al, E. *World Gastroenterology Organisation Global Guidelines, Probiotics and Prebiotics*; World Gastroenterology Organisation: Milwaukee, WI, USA, 2023.
53. Seki, H.; Sugimoto, R. Evaluation of a colourimetric method for the measurement of oxidation in butter. *Food Res.* **2022**, *6*, 303–308. [CrossRef] [PubMed]
54. Karaca, Y. Production and quality of kefir cultured butter. *Mljekarstvo* **2018**, *68*, 64–72. [CrossRef]
55. Panchal, B.; Truong, T.; Prakash, S. Influence of emulsifiers and dairy ingredients on manufacturing, microstructure, and physical properties of butter. *Foods* **2021**, *10*, 1140. [CrossRef]
56. Panchal, B.; Truong, T.; Prakash, S. Influence of fat globule size, emulsifiers, and cream-aging on microstructure and physical properties of butter. *Int. Dairy J.* **2021**, *117*, 105003. [CrossRef]
57. Jones, A.; Martini, S. Relationship between the physical properties of butter and water loss during lamination. *J. American Oil Chem. Soc.* **2022**, *99*, 585–597. [CrossRef]
58. Brożek, O.; Kiełczewska, K.; Bohdziewicz, K. Physicochemical and sensory properties with special emphasis on thermal characteristics of whey butter from gouda cheese production compared to milk butter. *Pol. J. Food Nutr. Sci.* **2022**, *72*, 407–419. [CrossRef]
59. Vioque-Amor, M. Butter from different species: Composition and quality parameters of products commercialized in the south of spain. *Animals* **2023**, *13*, 3559. [CrossRef]
60. Pedrotti, M.; Khomenko, I.; Cappellin, L.; Fontana, M.; Marco, S.; Falchero, L.; Biasioli, F. Rapid and noninvasive quality control of anhydrous milk fat by ptr-ms: The effect of storage time and packaging. *Biol. Mass Spec.* **2018**, *53*, 753–762. [CrossRef]
61. Topnikova, E. Effect of packaging method on butter quality preservation. *Dairy Ind.* **2024**, *2*, 53–60. [CrossRef]
62. Sahin, E.; Erem, E.; Güzey, M.; Kesen, M.; Icyer, N.; Ozmen, D.; Cakmak, H. High potential food wastes: Evaluation of melon seeds as spreadable butter. *J. Food Process. Pres.* **2022**, *46*, e16841. [CrossRef]
63. Lopes, F.; Ribeiro, C.; Rodriguez, N.; Gama, M.; Morenz, M.; Antoniassi, R.; Bizzo, H. Butter fatty acid composition as a function of soybean oil supplementation and time of milking, and performance of holstein x gyr cows fed with chopped elephant grass-based diets. *Semin. Ciênc. Agrárias* **2019**, *40*, 2025. [CrossRef]
64. Musayeva, K.; Sederevičius, A.; Monkevičienė, I.; Žymantienė, J.; Oberauskas, V.; Kerzienė, S.; Želvytė, R. Milk fatty acid profile in cows as influenced by diet supplementation with rapeseed pomace and extruded full-fat soya in different feeding periods. *Acta Vet. Brno* **2021**, *90*, 27–34. [CrossRef]
65. Magan, J.; O'Callaghan, T.; Kelly, A.; McCarthy, N. Compositional and functional properties of milk and dairy products derived from cows fed pasture or concentrate-based diets. *Compr. Rev. Food Sci. Food Safety* **2021**, *20*, 2769–2800. [CrossRef] [PubMed]
66. Lis, A.; Staniewski, B.; Ziajka, J. A comparison of butter texture measurements with the AP 4/2 penetrometer and TA.XT. Plus texture analyzer. *Int. J. Food Prop.* **2021**, *24*, 1744–1757. [CrossRef]
67. Ziarno, M.; Derewiaka, D.; Florowska, A.; Szymańska, I. Comparison of the spreadability of butter and butter substitutes. *Appl. Sci.* **2023**, *13*, 2600. [CrossRef]
68. Pădureț, S. The effect of fat content and fatty acids composition on colour and textural properties of butter. *Molecules* **2021**, *26*, 4565. [CrossRef]
69. Nayanangi, W.; Pagthinathan, M.; Gunathilaka, S. Evaluation of chemical, physical, microbial and sensory properties of garlic butter by using cow milk. *Eur. J. Agr. Food Sci.* **2022**, *4*, 81–86. [CrossRef]
70. Pourfarzad, A.; Kisomi, R. Effect of lecithin and mono- and di-glyceride on quality and shelf life of hazelnut butter: Chemometric approach. *Pol. J. Food Nutr. Sci.* **2020**, *70*, 399–408. [CrossRef]
71. Gaba, K.; Anand, S.; Syamala, A. Development of value-added butter by incorporating whey protein hydrolysate-encapsulated probiotics. *Microorganisms* **2023**, *11*, 1139. [CrossRef]
72. El-Hameed, M.; El-Sattar, E.; Khalifa, S.; El-Neshawy, A. An attempt to produce synbiotic buttermilk beverages supplemented with barley and oat flour. *Zagazig J. Agr. Res.* **2019**, *46*, 1985–1994. [CrossRef]
73. Iriondo-DeHond, M.; Miguel, E.; Castillo, M. Food byproducts as sustainable ingredients for innovative and healthy dairy foods. *Nutrients* **2018**, *10*, 1358. [CrossRef] [PubMed]

74. Garvey, E.; Sander, T.; O'Callaghan, T.; Drake, M.; Fox, S.; O'Sullivan, M.; Kilcawley, K. A cross-cultural evaluation of liking and perception of salted butter produced from different feed systems. *Foods* **2020**, *9*, 1767. [CrossRef] [PubMed]
75. Stephenson, R.; Ross, R.; Stanton, C. Carotenoids in milk and the potential for dairy based functional foods. *Foods* **2021**, *10*, 1263. [CrossRef] [PubMed]
76. Aydin, S.; Kahyaoğlu, D. Antioxidant effect potential of garlic in vitro and real food system: Effects of garlic supplementation on oxidation stability and sensory properties of butter. *Eu. J. Lipid Sci. Tech.* **2020**, *122*, 1900261. [CrossRef]
77. Mehdizadeh, T.; Mohammadipour, N.; Langroodi, A.; Raeisi, M. Effect of walnut kernel septum membranes hydroalcoholic extract on the shelf life of traditional butter. *Heliyon* **2019**, *5*, e01296. [CrossRef]