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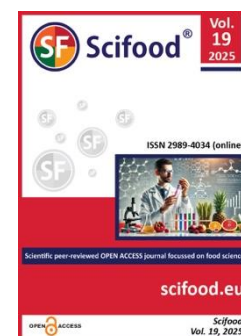
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The physicochemical and sensory profiles of spreadable dairy matrix obtained from kidney bean milk-like extract and cow milk blends

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ABSTRACT

As consumer interest in plant-based alternatives to traditional dairy products increases, incorporating legumes such as white beans into dairy products presents a promising opportunity for developing nutritious and palatable options. This study investigates the physicochemical and sensory properties of a dairy matrix (DM) created by mixing cow's milk with a milk-like extract of white kidney beans in various ratios. Dried white kidney beans (*Phaseolus vulgaris*) were sourced from a local market in Talas, Kyrgyzstan. The beans were soaked overnight, washed, ground with added water, and filtered to obtain a milk-like extract (BMLE), stored at 4°C. The DM was prepared by mixing BMLE with cow's milk in five proportions (0%:100%, 30%:70%, 50%:50%, 70%:30%, and 100%:0%) and pasteurizing at 95°C for 25 minutes. Adding 40% CaCl₂ at 95°C resulted in protein precipitation, forming a curd-like matrix, which was then self-pressed to a moisture content of 68–79% and stored at 4°C. A spreadable dairy matrix was formed in samples containing up to 50% BMLE. These experimental DMs exhibited significantly higher spreadability than the control (100% cow's milk). Higher BMLE content correlated with increased water holding capacity (WHC) and spreadability. Samples containing BMLE had higher acidity levels compared to the control. Color analysis revealed that samples with BMLE had a more pronounced reddish hue (a*), whereas the control exhibited stronger yellowish and bluish tones (b*). Sensory analysis indicated distinct taste differences between experimental and control samples, although overall acceptability remained comparable.

Keywords: cow's milk, white kidney bean, milk-like extract, dairy matrix, hybrid cheese

INTRODUCTION

The challenges of overpopulation and climate change have prompted research into alternative protein sources to address food insecurity. Alternative proteins, such as plant-based proteins, cultured meats, and single-cell proteins, require fewer natural resources and emit fewer greenhouse gases than traditional animal agriculture, offering a more sustainable approach to meet the growing global protein demand [1], and [2]. Sales of plant-based dairy alternatives (PBDAs) have grown over the last decade and are expected to continue rising [3]. The growing trend of reducing animal product consumption, particularly dairy, contributes to ecological sustainability and health benefits. Consequently, food technology research focuses on developing hybrid cheese analogues incorporating plant-based components while preserving sensory and functional qualities [4]. Cheese analogues can be prepared

from the milk of various animals, for example, from sheep's milk with the addition of 0.03% xanthan gum and quinoa seed flour [5].

Previous studies have explored cheese analogues made from sheep's milk with added xanthan gum and quinoa seed flour, demonstrating their viability as functional dairy alternatives.

In Western nations, shifting concerns about environmental sustainability and health, coupled with the desire to reduce animal protein consumption, are driving changes in dietary preferences. While alternative diets are gaining traction, many individuals in the Western population are hesitant to completely overhaul their dietary habits in favour of plant-based or alternative-protein-based foods. However, there is a growing demand for affordable and appealing alternative products that retain familiar tastes and have a positive environmental effect. For example, this study suggests that the legume burger has the lowest environmental impact compared with a beef-based burger [6]. It is worth noting that the market for plant-based dairy alternatives is experiencing rapid growth. Hybrid products, which incorporate alternative protein sources while partially replacing animal protein, present a promising solution to this challenge. By reducing the environmental impact of food production and offering affordable, familiar, healthy, and tasty options, hybrid foods can cater to both environmental and consumer needs. In this transition toward alternative-protein-based diets, hybrid foods containing dairy or egg components may emerge as a viable option, as these protein sources have a lower carbon footprint than meat products [6]. White kidney beans (*Phaseolus vulgaris*, Figure 1), also known as cannellini beans, are renowned for their nutritional value and versatility in culinary applications.



Figure 1 *Phaseolus vulgaris*.

Incorporating white beans into food products can enhance dietary fiber content and influence sensory and functional properties. For instance, adding white bean flour to lean pork burgers increased cooking yield and hardness, suggesting improved water holding capacity (WHC) and texture [7]. To address these challenges and make cheese production more sustainable, the concept of "hybrid cheeses" has emerged. This definition clarifies that hybrid cheese is a cheese product that incorporates dairy-derived components (milk) and plant-based ingredients, with both components present in the final product at varying concentrations. Hybrid cheeses involve incorporating plant-based components into the milk matrix to create products that combine the desirable attributes of both dairy and plant-based ingredients [5]. Manufacturers aim to achieve a fast and cost-efficient transition toward more sustainable cheese production by integrating new technological approaches into conventional cheese production methods. The concentrations of dairy and plant-based components can vary in the final product, allowing for flexibility in formulation to achieve desired characteristics such as texture, flavour, and nutritional profile.

The final product retains dairy-derived and plant-based ingredients, highlighting their contribution to the cheese's overall composition and characteristics [8], and [9].

Legumes are particularly suitable for incorporation into dairy matrices due to their high protein (20–24%, up to 35% for soy), fat (2–4%), starch (up to 38%), dietary fiber (6–12%), and iron content (3–11 mg%). White kidney

beans, widely cultivated in Kyrgyzstan, provide a rich source of high-quality plant protein, making them a suitable candidate for dairy-plant protein composites, including cheese spreads. It appears that kidney bean cultivation in Kyrgyzstan is a relatively recent development. Historically, peas and soybeans were cultivated in the southern regions of Kyrgyzstan. However, starting from the late 1990s, kidney bean production has seen a noticeable increase, particularly in the Talas region. This increase in kidney bean production in the Talas region seems to be driven by a specific focus on exporting to international markets. Overall, this shift in agricultural focus towards kidney bean production reflects changes in crop preferences and market dynamics in Kyrgyzstan, with the country leveraging its resources to meet demand in international markets such as Turkey [10]. Despite their high protein content and nutritional value, legumes are consumed in low quantities among the population of Kyrgyzstan, and they are often not included in the diet willingly. This discrepancy between the nutritional benefits of legumes and their low consumption may stem from several factors: traditional Kyrgyz cuisine prioritize other food items over legumes; cultural dietary habits and preferences play a significant role in determining food choices, and legumes are not having a prominent place in traditional dishes; some people have a negative perception of legumes or find their taste unappealing. The main variety of beans popular among farmers in the region is the white kidney bean (Figure 2).



Figure 2 Dried white kidney beans.

This variety comprises at least 70% of all beans cultivated in the region, indicating its significant dominance in local agriculture. White kidney beans are favoured by farmers, likely due to factors such as high yield potential, adaptability to local growing conditions, market demand, and agronomic characteristics suitable for cultivation in the region aligned in the value chain analysis made by Tilekeyev et al. [11]. No other comprehensive studies on this subject have been conducted in the Kyrgyz Republic to date. Its processing into valuable products or ready-to-use matrices for food applications is relevant and essential.

Scientific Hypothesis

Considering their rich protein content and other beneficial nutrients, white kidney beans present a promising possibility for incorporation into dairy-plant protein composites to form the basis for various food products, including cheese spreads. We believe such compositions can be called a dairy or food matrix (DM/FM). According to the decision of the members of the International Dairy Federation, which is the world's leading source of expertise and scientific knowledge for the dairy industry, the “dairy matrix” describes the unique structure of dairy food, its components (e.g., nutrients and non-nutrients), and how they interact [12]. Therefore, we have used this term (dairy matrix) and this abbreviation (DM) in the text of the article.

Hybrid cheese or spread, which combines traditional cheese-making techniques with alternative protein sources, could offer a novel approach to address the demand for sustainable and nutritious food options. In addition to the above properties, the modern informed consumer pays attention to the physiological functionality of the offered food products, i.e., the ability to provide health benefits by improving certain body functions and reducing the risk of diseases. This study confirms that milk proteins are suitable for delivering polyphenols to parts of the gastrointestinal tract [13]. In this regard, fresh prebiotic cheese spreads have been developed, enriched with agave inulin (AI), thyme (*Thymus callieri* Borbás ex Velen.), and hawthorn berries (*Crataegus monogyna* Jacq.) as plant sources of biologically active compounds [14]. Microgreens of red cabbage (*Brassica oleracea* var. *capitata* f.

rubra) and flax seeds (*Linum usitatissimum*) are also used as plant components in the composition of cheese spreads [15].

Whey proteins have also been used as a base for starch- and gelatin-stabilized spreads [16]. Spreads can also be made entirely plant-based. The main ingredients of vegan cheese spread are jackfruit seed powder (a close relative of breadfruit), cashew kernels, and sunflower oil [17].

Some plant proteins, such as soy and nuts' proteins, are often used to replace casein. However, despite the potential benefits, there is a lack of comprehensive knowledge and research on the production of DM incorporating some components of white kidney beans. Challenges may include achieving the desired texture and flavour while maintaining the nutritional integrity of the final product [18]. The scientific novelty of producing DM with white kidney beans lies in its potential to offer a sustainable and protein-rich alternative to traditional cheese, as well as to convert Kyrgyz white kidney beans into a significant raw material consumed by local processing.

Objectives

This study explores the feasibility of incorporating white kidney bean milk-like extract (BMLE) into dairy matrices for cheese spread applications. The research assesses the physicochemical and sensory attributes of the resulting matrices, contributing to the understanding of their functional properties. Additionally, the study seeks to address the lack of knowledge surrounding the production of such food matrix, substantiate scientific novelty, and assess practical challenges associated with this innovative approach.

MATERIALS AND METHODS

Samples

Sample description: We have used five samples of dairy matrix prepared from cow's milk, kidney bean milk-like extract, and their blends.

Samples collection: White kidney beans (*Phaseolus vulgaris*) were obtained from a local market in Talas, Kyrgyzstan (harvested in 2023; 18.2% moisture, 18.0% protein, 1.5% fat, 53.9% carbohydrate, 6.6% crude fiber). Cow's milk was sourced from a local Kaunas market (3.12% fat, 3.45% protein, 4.57% lactose, pH 6.70).

Samples preparation: *White kidney bean milk-like extract (BMLE) preparation.*

BMLE was prepared following a modified soymilk extraction method. White kidney beans (1.75 kg) were soaked overnight in tap water at room temperature (water-to-bean ratio of 1:1 v/w).

After draining, the soaked white kidney beans (3.5 kg) were rinsed and blended with fresh water, maintaining a water-to-soaked bean ratio of 3:1 (v/w), using an ErgoMixx 1000W hand blender (Bosch, Germany). The resulting slurry was filtered through muslin cloth to separate undissolved solids, yielding BMLE, which was used immediately.



Figure 3 BMLE preparation.

Number of samples analyzed: Five samples were initially prepared; however, only the last three were used for further analysis, as the first two did not yield curd.

Chemicals

A 40% - CaCl_2 solution was freshly prepared from anhydrous calcium chloride (Sigma Aldrich, Germany). All reagents used for laboratory analysis were of U.S.P. purity or higher. All solvents, including water, were used with the LC/MS label.

Animals, Plants, and Biological Materials

White kidney beans (*Phaseolus vulgaris*), Talas, Kyrgyzstan. Cow's milk, Kaunas, Lithuania.

Instruments

Ultrafast liquid chromatography (UFLC), UFLC instrument (Shimadzu, Japan), Automatic injector SIL-30AC (Shimadzu, Japan), pH meter (Sartorius Professional meter for pH measurements, Germany). Portable colorimeter PCE-CSM5 (PCE instruments, UK), Anton Paar rotational rheometer, model RheolabQC (Germany), "Mira3" scanning electron microscope (SEM, Tescan Orsay Holding, a.s., Brno-Kohoutovice, Czech Republic).

Laboratory Methods

Determination of DM composition

DM composition (dry matter, fat, protein, ash, and carbohydrate content) was determined on Day 1 according to established methods such as ISO 5534:2004 [19], ISO 1735:2004 [20], ISO 22662 [21], and ISO 8968-3:2004 [22]. Water-holding capacity (WHC) was calculated as the ratio of moisture to dry matter content.

Determination of amino acid profile

The amino acid (AA) compositions of the samples were analysed according to ISO ISO 23319:2022 [23], using ultrafast liquid chromatography (UFLC) with automated o-phthalaldehyde (OPA)/9-fluorenylmethyl chloroformate (FMOC)/Mercaptoethanol (MERC) derivatization. Standard solutions of the amino acids including alanine (ALA), aspartic acid (ASP), arginine (ARG), cystine (CYS), glycine (GLY), valine (VAL), leucine (LEU), isoleucine (ILE), threonine (THR), serine (SER), proline (PRO), methionine (MET), glutamic acid (GLU), phenylalanine (PHE), lysine (LYS), histidine (HIS), tyrosine (TYR), asparagine (ASP), and tryptophan (TRP) were used for this analysis (A9781 Sigma-Aldrich, Germany).

To commence the analysis, each sample (approx. 0.4 g) underwent hydrolysis with 25 mL of 6 M HCl for 24 hours at 103 °C. The results were quantitatively transferred into a 250 mL beaker using a 150–200 mL solution of 0.2 mol Na⁺/L, pH 2.20 trisodium citrate dehydrate. The resulting hydrolysate was partially neutralized by gradually adding 17 mL of 7.5 N sodium hydroxide solution while stirring continuously, ensuring the temperature remained below 40 °C (in a cold water bath). The pH was adjusted to 2.20 at room temperature using sodium hydroxide solution (7.5 N).

Before injection, all samples were filtered through 0.45-μm filters. The amino acids were separated using a UHPLC column YMC-Triart C18 (1.9 μm, YMC co. Ltd.) on a UFLC instrument (Shimadzu, Japan), which was equipped with a fluorescence detector RF-20AXs and a pre-treatment function equipped automatic injector SIL-30AC (Shimadzu, Japan). The analytical conditions were as follows: mobile phase consisting of solvent A (20 mmol/L potassium phosphate buffer, pH 6.5) and solvent B (45/40/15 acetonitrile/methanol/water); flow rate set at 0.5 mL/min; column temperature maintained at 45 °C; detection wavelengths: RF-20AXs Ex. at 350 nm, Em. at 450 nm to Ex. at 266 nm, Em. at 305 nm (9.0 min). A calibration set comprising five levels was utilized, covering a concentration range of 9.375–150.00 μmol/L, except cysteine, covering a concentration range of 8.08–75.00 μmol/L.

Determination of pH

Dairy matrix samples were analysed in duplicate over 30 days to monitor changes in pH. pH levels were directly measured using a pH meter (Sartorius Professional meter for pH measurements, Germany).

Color determination

The color parameters of the DM were assessed using a portable colorimeter PCE-CSM5 (PCE instruments, UK) equipped with a CIE L*a*b* CH option. In this context, L* represents the measure of lightness (0 indicating black and 100 indicating white), a* signifies the measure of redness (or negative a* for greenness), b* denotes the measure of yellowness (or negative b* for blueness), h* (hue angle) indicates the degree of the dominant spectral component (red, green, and blue) ranging from 0° to 360°, and c* (chroma) represents the saturation of a color.

The overall color difference (ΔE) during sample storage was calculated using the following formula [24]: $\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$, where L₀, a₀, and b₀ represent the values on day 1, and L, a, and b denote the values measured throughout the storage period.

Determination of rheological properties (spreadability)

The rheological evaluation of DM samples was conducted using an Anton Paar rotational rheometer, model RheolabQC (Germany), equipped with a flexible cup holder and probe type CC17. Shear rate measurements were performed in the range of 0.1–100/s at 10-second intervals. The temperature of the cheese samples was maintained at 4±2 °C, while the measurements were carried out at 20±2 °C. Viscosity and torque (%) values for each shear rate were recorded during the measurements using RheoCompass software.

Determination of sensory profile

A Quantitative Descriptive Analysis (QDA) was conducted to assess the sensory characteristics of spreadable DM samples. An 8-member partially trained panel underwent a re-training process to acquaint them with using a 1–10 intensity scale and evaluating sensory traits. They outlined the descriptors of spreadable DM, pinpointing nine attributes through collaborative discussion. Throughout the QDA assessment, each panellist evaluated the samples,

rating the attributes of each sample using a 1–10 intensity scale. The samples were presented randomly and labelled with unique three-digit identifiers. Two tasting sessions were conducted as replicates [25].

Determination of microstructure

Microstructural analysis was conducted according to the procedure mentioned by [17]. Microstructural analysis of DM samples was performed using a "Mira3" scanning electron microscope (SEM, Tescan Orsay Holding, a.s, Brno-Kohoutovice, Czech Republic). Samples were lyophilized using a freeze-dryer, mounted on a 51 mm diameter silicon wafer (MicrotoNano, Haarlem, The Netherlands) without double-sided adhesive carbon discs. Silicon was selected as the substrate due to the prevalence of CHON elements in organic samples, minimizing elements that could skew the results and enhance the signal-to-noise ratio. The SEM operated in high vacuum mode utilizing backscattered electron (BSE) and secondary electron (SE) detectors. Magnification was increased to 1.0 kx for precise dimensional measurements and element composition analysis at 5 kV acceleration voltage.

Description of the Experiment

Preparation of dairy matrix

The five samples from cow's milk (CM) and white kidney bean milk-like extract (BMLE) were prepared in different proportions: DM1 (30% CM and 70% BMLE), DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), BC (100% BMLE), and CC (100% CM). The blends were pasteurized at 95°C for 25 min. Curd was obtained by adding a 40% CaCl₂ solution to a hot milk blend. The curd was formed during 10–15 min and separated from whey using a muslin cloth, and self-pressed for 1 hour at room temperature to 68–79 % moisture content. The weight of each sample was checked to calculate the yield. Each sample was packed in plastic containers and kept refrigerated at 4 °C for further analysis. The composition of the matrix, its microstructure, amino acids, and sensory profiles were evaluated on day 1. Curd color, pH, rheology properties, and overall acceptability were determined on days 1, 10, 20, and 30.

Quality Assurance

Number of repeated analyses: All instrument measurements were performed in triplicate.

Number of experiment replications: Each experiment was duplicated to determine a single value.

Reference materials: Standard solutions of the amino acids including alanine (ALA), aspartic acid (ASP), arginine (ARG), cystine (CYS), glycine (GLY), valine (VAL), leucine (LEU), isoleucine (ILE), threonine (THR), serine (SER), proline (PRO), methionine (MET), glutamic acid (GLU), phenylalanine (PHE), lysine (LYS), histidine (HIS), tyrosine (TYR), asparagine (ASP), and tryptophan (TRP), (A9781 Sigma-Aldrich, Germany). A calibration set (a concentration range of 9.375–150.00 µmol/L, except for cysteine, which covers a concentration range of 8.08–75.00 µmol/L).

Calibration: Instruments were calibrated at regular intervals using traceable standards, and calibration certificates were maintained following ISO 17025 guidelines.

Laboratory accreditation: The experiments were performed in a laboratory accredited according to the International standard ISO 17025.

Data Access

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Statistical Analysis

The data processing and analysis were conducted using the SPSS statistical package (SPSS Inc., version 27, Chicago, IL, USA). Descriptive statistics (Explore), one-way analysis of variance (ANOVA), and General linear models were employed for data analysis. Differences between means were evaluated using the Tukey test. Statistical analysis was performed at a significance level of 95%.

RESULTS AND DISCUSSION

Cow's milk contained 3.45% protein and the freshly prepared white kidney bean milk-like extract contained 2.05% protein, consistent with previous studies on legume-based milk alternatives. Variations in protein content can be attributed to differences in bean variety, extraction methods, and processing conditions [27], [2]. A suitable spreadable DM was produced only with 50% or less BMLE blends. Samples BC (100%:0%) and DM1 (70%:30%) did not exhibit protein precipitation upon the addition of calcium chloride, a traditional coagulant widely used in tofu production from soybean milk [28]. Figure 5 illustrates the coagulation behaviour of various mixtures of milk and bean milk-like extract (BMLE) with calcium chloride. As the proportion of BMLE increases, coagulation becomes less pronounced. No typical coagulation occurs in the 100% bean extract sample (BC). This result highlights the role of casein in cow's milk, which readily coagulates in the presence of calcium chloride due to its micellar structure. In contrast, bean extract primarily contains plant proteins, such as globulins and albumins, which lack casein-like micelles and exhibit different coagulation properties [29].

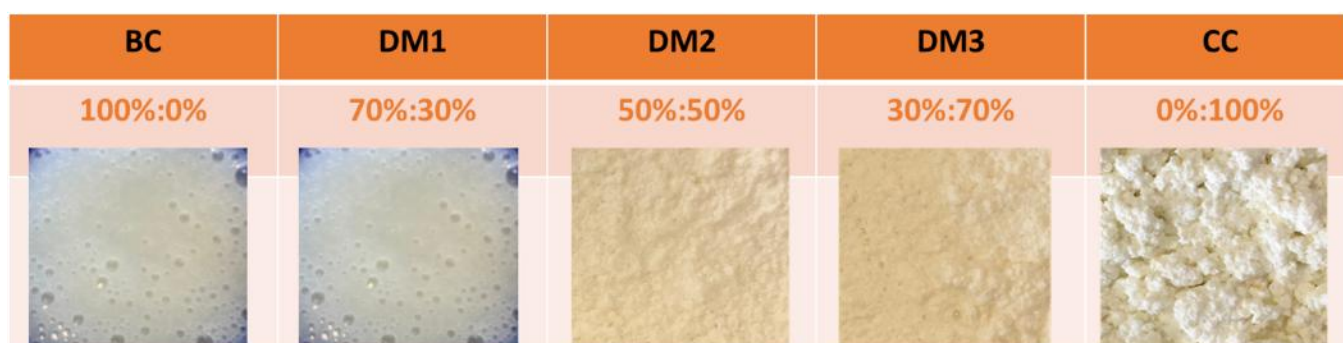


Figure 1 Dairy Matrices obtained.

Note: DM1 (30% CM and 70% BMLE), DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), BC (100% BMLE), and CC (100% CM).

White kidney bean proteins do not possess this micellar organization, making them less responsive to calcium chloride, leading to weak or no coagulation. A previous study confirmed that a protein mixture predominantly composed of plant proteins (46% soy, 32% pea, 16% casein, and 6% whey) exhibited non-coagulating behaviour after in vitro gastric digestion, similar to a protein mixture dominated by dairy proteins [30].

The blend with the highest proportion of BMLE (50%, DM2) resulted in the lowest yield of fresh cheese (18.63 ± 0.25 %). Slightly better yield was observed in the blend made with 30% BMLE and 70% cow's milk (DM3, 21.01 ± 0.09 %), while cheeses made solely from cow's milk had the highest yield (CC, 24.21 ± 0.08 %). This is due to the increase in the proportion of cow's milk in the mixture with a corresponding increase in the amount of precipitated milk proteins. Our further investigation was based on DM2, DM3, and CC samples.

Dairy Matrix composition

Table 1 presents the nutritional composition of three selected DM samples: DM2, DM3, and CC. Including BMLE resulted in lower fat, protein, ash, and carbohydrate content compared to the control (100% cow's milk).

Table 1 Nutritional composition of DM samples.

DM type	Fat content, %	Protein, %	Ash, %	Carbohydrates, %	WHC
DM2	7.50 ± 0.07^a	7.78 ± 0.06^a	1.47 ± 0.01^a	20.75 ± 0.01^a	3.82
DM3	9.05 ± 0.14^b	10.57 ± 0.16^b	1.91 ± 0.02^b	24.84 ± 0.01^b	3.03
CC	12.54 ± 0.02^c	12.87 ± 0.05^c	2.34 ± 0.02^c	31.79 ± 0.01^c	2.15

Note: The values are presented as mean $\pm$ standard deviation. Different letters (a, b, c) next to the values indicate statistically significant differences ($p < 0.05$) among the samples for each respective parameter.

The DM3 sample, formulated with the highest proportion of cow's milk (CC), exhibits the highest fat, protein, ash, and carbohydrate content. In contrast, the DM2 sample with the highest BMLE proportion shows lower values for these macronutrients. Since BMLE lowers fat content, it can be used in diets aimed at weight management or heart health. Products like low-fat yogurts, cheese alternatives, and dairy beverages with BMLE can cater to individuals seeking reduced fat intake. Incorporating white kidney bean extracts into dairy products may contribute to heart health by reducing overall fat content and providing bioactive compounds. A study demonstrated that white kidney beans have protective effects against high-fat diet-induced hepatic steatosis, evidenced by decreased obesity phenotype and improved serum lipid profiles [7]. White kidney bean extract contains alpha-amylase inhibitors that can reduce carbohydrate digestion and absorption, aiding in weight management by decreasing calorie intake from complex carbohydrates [31], and [32].

Amino acid profile

The composition of replaceable and essential amino acids in the three studied samples of dairy matrix is given in Table 2. A total of 17 amino acids were found in all samples, with asparagine and tryptophan being absent.

Table 2 Amino acid ($\mu\text{mol/L}$) composition in DM.

Amino acid	DM2	DM3	CC
Aspartic Acid	0.72 $\pm$ 0.01 ^a	0.97 $\pm$ 0.05 ^b	1.19 $\pm$ 0.11 ^c
Glutamic acid	1.55 $\pm$ 0.04 ^a	2.20 $\pm$ 0.04 ^b	2.92 $\pm$ 0.25 ^c
Asparagine	nd	nd	nd
Serine	0.41 $\pm$ 0.01 ^a	0.59 $\pm$ 0.01 ^b	0.73 $\pm$ 0.06 ^c
Histidine	0.18$\pm$0.01^a	0.25$\pm$0.00^b	0.31$\pm$0.05^b
Glycine	0.15 $\pm$ 0.00 ^a	0.20 $\pm$ 0.00 ^b	0.22 $\pm$ 0.02 ^b
Threonine	0.34$\pm$0.01^a	0.47$\pm$0.00^a	0.60$\pm$0.05^a
Arginine	0.26 $\pm$ 0.01 ^a	0.36 $\pm$ 0.01 ^b	0.42 $\pm$ 0.04 ^c
Alanine	0.24 $\pm$ 0.01 ^a	0.34 $\pm$ 0.01 ^b	0.41 $\pm$ 0.03 ^c
Tyrosine	0.36 $\pm$ 0.01 ^a	0.51 $\pm$ 0.01 ^b	0.66 $\pm$ 0.05 ^c
Cystine	0.21 $\pm$ 0.01 ^a	0.32 $\pm$ 0.01 ^b	0.37 $\pm$ 0.01 ^c
Valine	0.40$\pm$0.01^a	0.58$\pm$0.03^b	0.83$\pm$0.11^c
Methionine	0.17$\pm$0.01^a	0.27$\pm$0.01^b	0.36$\pm$0.02^c
Tryptophan	nd	nd	nd
Phenylalanine	0.38$\pm$0.01^a	0.54$\pm$0.03^b	0.72$\pm$0.09^c
Isoleucine	0.36$\pm$0.00^a	0.52$\pm$0.03^b	0.72$\pm$0.08^c
Leucine	0.68$\pm$0.01^a	1.03$\pm$0.06^b	1.42$\pm$0.15^c
Lysine	0.66$\pm$0.02^a	1.02$\pm$0.08^b	1.39$\pm$0.18^c
Proline	0.72 $\pm$ 0.13 ^a	0.90 $\pm$ 0.16 ^a	1.38 $\pm$ 0.16 ^b

Note: Values labeled with different letters a, b, c, d within the same row indicate significant differences ($p \leq 0.05$). Essential amino acids are presented in bold. DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

Table 2 shows that as the dose of cow's milk in DM increases, the amount of all amino acids also increases, for some by an insignificant amount (from 0.02 $\mu\text{mol/L}$ for glycine), for others by a rather significant amount (up to 0.72 $\mu\text{mol/L}$ for glutamic acid). This is because cow's milk contains more protein than the milk-like extract from white beans (BMLE). Table 2 shows that Aspartic Acid and Glutamic Acid significantly increase from DM2 to CC, while Serine, Tyrosine, and Valine also increase substantially across the three cheese types. Histidine and Glycine increase from DM2 to DM3 and then plateau or slightly increase in CC. Proline was detected in all samples, increasing from DM2 to CC, while proline was below the detection level in the fermented drinks obtained from white kidney bean extract and cow's milk, as samples were not fermented by LABs [26]. Tryptophan and asparagine were not detected (nd) in any of the samples. This is likely due to degradation and conversion during acid hydrolysis: tryptophan is destroyed under acidic conditions, and asparagine is typically deaminated to aspartic acid, making them undetectable by this method. These results highlight the potential of incorporating BMLE to modify the amino acid profile of dairy matrices. While cow's milk contributes to higher overall amino acid levels, the of BMLE can provide a more balanced composition, potentially influencing both the nutritional and functional properties of the product and can be suggested in LPDs for the individuals with health disorders [33], and [34] instead of beans or cow's milk containing higher protein.

pH Changes During Storage

As pH is a dynamic parameter that continuously fluctuates during milk processing due to temperature shifts, pressure variations, water removal, and microbial activity, impacting protein interactions and overall product stability [35] the pH values of three spreadable DM samples (DM2, DM3, and CC) presented in the Table 3 were measured on days 1, 10, 20, and 30.

Table 3 pH changes in DM samples during the storage of 30 days.

Day	DM2	DM3	CC
1	5.27 $\pm$ 0.02 ^{Aa}	5.68 $\pm$ 0.02 ^{Ba}	5.40 $\pm$ 0.09 ^{Aa}
10	5.65 $\pm$ 0.02 ^{Ab}	5.95 $\pm$ 0.05 ^{Bb}	5.77 $\pm$ 0.01 ^{Bb}
20	5.65 $\pm$ 0.01 ^{Ab}	5.83 $\pm$ 0.02 ^{Ba}	5.74 $\pm$ 0.02 ^{Cb}
30	5.50 $\pm$ 0.03 ^{Ac}	5.77 $\pm$ 0.02 ^{Ba}	5.69 $\pm$ 0.01 ^{Bb}

Note: The pH values are presented as mean $\pm$ standard deviation. Different uppercase letters (A, B, C) indicate statistically significant differences ($p < 0.05$) among the samples for each respective day, and different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) among the days for each respective sample. DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

The pH changes during refrigeration storage show a sharp increase in the first 10 days, followed by slight acidification by day 30. The initial pH rise likely results from biochemical processes, including the activity of thermostable enzymes that produce alkaline by-products [36]. The slight decrease in pH after 10 days could be due to the presence of white bean extract (pH 7, 16), which likely alters the acid-base balance, contributing to these pH trends. The higher pH stability in DM3, compared to DM2 and CC, is linked to differences in protein composition and buffering capacity, which should be considered when designing plant-dairy hybrid products [37] as well as starch properties, which determine the stability of white kidney bean milk [38].

Color Analysis

The color of a cheese matrix is a critical quality attribute, influencing both consumer perception and product acceptance [39], and [40]. It is affected by various factors, including composition, processing conditions, and ingredient interactions [13]. In our study, the chromaticity parameters (L^* , a^* , b^* , c^* , h^*) determined by The CIE $L^*a^*b^*$ coordinate system what is suggested as the best color space for food color quantification [41] were monitored during refrigeration period of 30 days and the obtained data are presented in Table 4.

Table 4 Chromaticity parameters of DM samples during the refrigerated storage (30 days).

Parameter	Day	DM2	DM3	CC
L^*	1	55.30±1.39 ^{Aa}	55.94±0.79 ^{Aa}	57.88±1.16 ^{Aa}
	10	46.47±1.99 ^{Aa}	50.01±1.56 ^{Aa}	53.61±1.25 ^{Aa}
	20	56.83±2.78 ^{Aa}	57.31±1.07 ^{Aa}	61.53±1.77 ^{Ab}
	30	51.18±0.49 ^{Aa}	52.36±1.57 ^{Aa}	54.66±0.94 ^{Aa}
a^*	1	1.32±0.09 ^{Aa}	1.26±0.10 ^{Aa}	0.57±0.15 ^{Ba}
	10	0.98±0.22 ^{Aa}	0.97±0.04 ^{Aa}	0.35±0.06 ^{Ba}
	20	2.03±0.23 ^{Ab}	1.66±0.24 ^{Aa}	0.78±0.12 ^{Ba}
	30	1.44±0.09 ^{Aa}	1.49±0.30 ^{Aa}	1.29±0.19 ^{Ab}
b^*	1	6.35±0.12 ^{Aa}	6.32±0.15 ^{Aa}	6.90±0.15 ^{Aa}
	10	6.00±0.11 ^{Aa}	6.44±0.49 ^{Aa}	6.70±0.39 ^{Aa}
	20	7.66±0.36 ^{Ab}	7.11±0.24 ^{Aa}	8.20±0.42 ^{Aa}
	30	6.62±0.18 ^{Aa}	6.97±0.50 ^{Aa}	8.24±0.50 ^{Aa}
c^*	1	6.49±0.12 ^{Aa}	6.44±0.16 ^{Aa}	6.92±0.14 ^{Aa}
	10	6.08±0.12 ^{Aa}	6.51±0.47 ^{Aa}	6.71±0.39 ^{Aa}
	20	7.92±0.40 ^{Ab}	7.30±0.27 ^{Aa}	8.23±0.43 ^{Ab}
	30	6.78±0.19 ^{Aa}	7.13±0.44 ^{Aa}	8.35±0.52 ^{Ab}
h^*	1	78.23±0.90 ^{Aa}	78.71±0.67 ^{Aa}	85.24±1.28 ^{Ba}
	10	80.75±1.96 ^{Aa}	81.39±0.95 ^{Aa}	86.98±0.32 ^{Aa}
	20	75.18±0.95 ^{Aa}	76.91±1.44 ^{Aa}	84.55±0.69 ^{Aa}
	30	77.77±0.53 ^{Aa}	77.87±2.98 ^{Aa}	81.11±0.79 ^{Ab}
ΔL	1	42.59±1.37 ^{Aa}	41.95±0.80 ^{Aa}	40.08±1.14 ^{Aa}
	10	51.33±1.98 ^{Ab}	47.84±1.59 ^{Ab}	44.29±1.21 ^{Bb}
	20	41.27±2.81 ^{Aa}	40.70±1.09 ^{Aa}	36.68±1.68 ^{Ac}
	30	46.72±0.50 ^{Aa}	45.58±1.53 ^{Ab}	43.47±0.86 ^{Bb}

Note: The values are presented as mean ± standard deviation. Different uppercase letters (A, B) indicate statistically significant differences ($p < 0.05$) among the samples for each respective day, and different lowercase letters (a, b) indicate statistically significant differences ($p < 0.05$) among the days for each respective sample. DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

Our study found that the L^* values, indicating brightness, throughout the entire observation period (30 days) follow the same pattern for all samples: a decrease in brightness on the tenth day of storage, especially sharp for sample DM2, followed by an increase on the twentieth day, also more pronounced for DM2, and finally a repeated decrease in brightness values by the thirtieth day of storage of the samples. Throughout the study, the L^* values for sample CC remained higher compared to samples DM2 and DM3.

The values of a^* , b^* and c^* , indicating respectively the redness, yellowness and saturation of a color, for DM2 and DM3 changed in the same pattern as L^* , i.e. they decreased slightly by the tenth day of storage, increased more noticeably by the twentieth day and again reduced by the thirtieth day of observation. Moreover, for DM2, the changes in b^* and c^* are more pronounced and by the end of the observation period are close to the values for DM2. Sample CC behaved somewhat differently, for which the values of b^* and c^* noticeably increased by the twentieth

day, then remained almost unchanged. While the value of a^* , after some decrease by the tenth day, increased uniformly by the thirtieth day of storage, reaching values close to DM2 and DM3.

The values of h^* (hue angle) for DM2 and DM3 demonstrate a slight increase by the tenth day, followed by a decrease (by the twentieth day) and some increase by the thirtieth day of storage. Here, the CC sample also behaves somewhat differently, for which the h^* values after a small increase by the tenth day gradually decrease towards the end of the observation period.

The variable ΔL shows color change across days and samples. At Day 1, there are no significant differences between samples. At Day 10, DM2 has the highest values, while CC shows the lowest. At Day 20, DM2 and DM3 are similar, but CC is significantly lower. At Day 30, CC remains the lowest, while DM2 and DM3 are comparable. This indicates that the samples influence ΔL differently depending on the days.

One of the possible reasons for the observed variability in the L^* values of the samples during storage is the occurrence of biochemical processes in such complex milk-vegetable matrices, formed by the polyphenols interacting with milk proteins, particularly caseins, that influence the color [13]. The studies have highlighted that plant proteins typically exhibit a darker color than dairy proteins, which can explain the higher L^* value in dairy products due to the oxidation of phenolic compounds and the natural pigmentation of beans [42].

Rheological properties, spreadability

The functional and technological properties of such structures as multicomponent food matrices are of particular interest from the point of view of their behavior during production, storage, and use. The rheological and physical characteristics of a pizza cheese analogue containing rennet casein and vegetable oil were determined using a rotational rheometer [43]. The measurements showed that all samples followed the Herschel-Buckley viscoplastic model with different yield stresses. The highest apparent viscosity and shear stress values were obtained at pH 6.4. Similar studies for the milk-vegetable matrices we obtained were conducted for the first time. Cream cheese and similar spreadable foods are considered viscoplastic materials, meaning their texture can change under stress. One of the crucial aspects influencing consumer satisfaction is their spreadability, which determines how easily and evenly they can be spread.

Our study found that experimental DM2 incorporating bean milk-like extract (BMLE) had significantly lower yield stresses than control DM made solely from cow milk (CC). Among the samples with BMLE added, we observed that spreadability increased with higher amounts of BMLE incorporated into the mixture. Suitable spreadable DM was produced only when blends containing 50% or less BMLE were used. The graph in Figure 5 displays viscosity vs. shear rate for different samples during storage, obtained using the Anton Paar RheoCompass software.

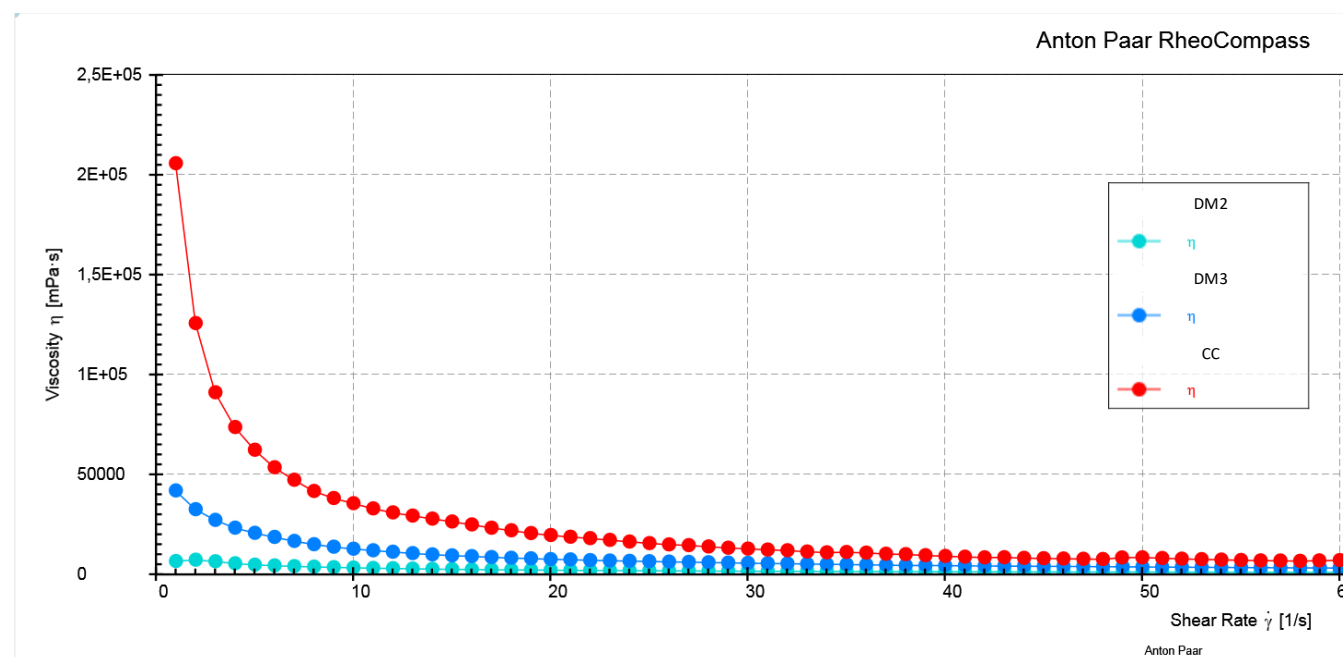


Figure 2 Flow curves (apparent viscosity and shear rate relationship) of DM samples on Day 1. Note: DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

Similar data were obtained for DM samples that were stored for 10 and 20 days from the date of production. All three data series show a decreasing trend in viscosity as the shear rate increases. The red series (CC) starts with the highest initial viscosity and shows the most significant drop. The blue (DM2) and green (DM3) series have similar trends with slightly different viscosities. Viscosity-shear rate relationships in Figure 5 indicated that DM samples with BMLE exhibited lower yield stress, enhancing spreadability compared to the control. Due to the high starch content in the BMLE, the authors suggest that starch's content and physicochemical properties may play an important role in the stability of the final product [38].

Sensory profile

The sensory profile is crucial in product formulation, ensuring consumer acceptance, which largely depends on taste, texture, aroma, and appearance. Even if a product has excellent nutritional or functional properties, it may fail in the market if it does not meet consumer expectations in sensory attributes. This is especially important in dairy and plant-based alternatives, where familiarity and preference for certain textures and flavours influence purchasing decisions [44]. In our study, we conducted QDA on Day 1 and acceptability testing every 10 days. All samples had low sourness with no significant differences among samples. DM2 has the highest off-flavour rating, followed by DM3, with CC significantly lower. CC has a higher sweetness rating compared to DM2 and DM3. CC has a significantly higher taste intensity rating (Table 5). Cow milk cheese (CC) has the highest acceptability scores at all-time points compared to DM2 and DM3 (Table 6).

Table 5 Sensory profile.

Descriptors	DM2	DM3	CC
Even, consistent color	7.84±3.30 ^a	8.56±1.16 ^a	8.30±2.24 ^a
White to yellow color	2.58±2.08 ^a	5.46±3.03 ^a	2.22±2.56 ^a
Sourness	0.62±0.69 ^a	1.84±3.45 ^a	1.86±3.44 ^a
Off-flavour	7.32±1.36 ^a	5.20±0.57 ^a	1.50±1.87 ^b
Sweetness	4.00±3.72 ^a	4.40±2.58 ^a	6.70±1.79 ^a
Taste intensity	3.16±2.44 ^a	3.92±2.75 ^a	7.18±1.81 ^b
Homogenous taste	9.06±0.70 ^a	8.06±1.07 ^a	5.92±2.87 ^b
Firmness	3.62±4.09 ^a	4.36±2.63 ^a	6.30±2.59 ^a
Clean, spotless surface	6.60±3.13 ^a	6.66±2.13 ^a	5.38±3.60 ^a
Smooth surface	9.36±0.78 ^a	9.30±0.80 ^a	9.36±0.87 ^a

Note: The values are presented as mean ± standard deviation. Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) among the samples for each respective descriptor. DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

Table 6 Overall acceptability of DM samples during refrigerated storage.

Day	DM2	DM3	CC
1	5.48±3.73 ^{Aa}	6.96±2.74 ^{Aa}	8.86±1.35 ^{Aa}
10	4.18±1.48 ^{Aa}	6.70±2.25 ^{Aa}	7.38±0.85 ^{Aa}
20	4.88±3.25 ^{Aa}	5.75±3.50 ^{Aa}	6.95±2.39 ^{Aa}
30	5.63±1.11 ^{Aa}	7.13±1.18 ^{Aa}	7.75±0.65 ^{Ba}

Note: The values are presented as mean ± standard deviation. Different uppercase letters (A, B, C) indicate statistically significant differences ($p < 0.05$) among the samples for each respective day, and different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) among the days for each respective sample. DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), and CC (100% CM).

Despite the low scores in Table 6, the panellists still considered the DMs acceptable. This can be attributed to the unique flavour of the bean milk, a characteristic inherent to legumes, which may be unfamiliar to consumers. These findings align with previously reported sensory profile studies [38], [2], and [33]. White beans contain lipoxygenase (LOX), an enzyme that catalyzes the oxidation of unsaturated fatty acids, resulting in volatile compounds responsible for off-flavours such as grassy, beany, and rancid notes. Studies have shown that eliminating LOX isozymes in soybeans reduces the amounts of these volatile off-flavour compounds [45]. Several hypotheses suggest that non-volatile compounds, such as saponins, phenolic compounds, and alkaloids, contribute to the bitterness and astringency of pulses [46].

DM microstructure

The structural features of the protein matrix were determined using microstructural analysis of samples of milk-plant clots, the results of which are presented in Figure 6.

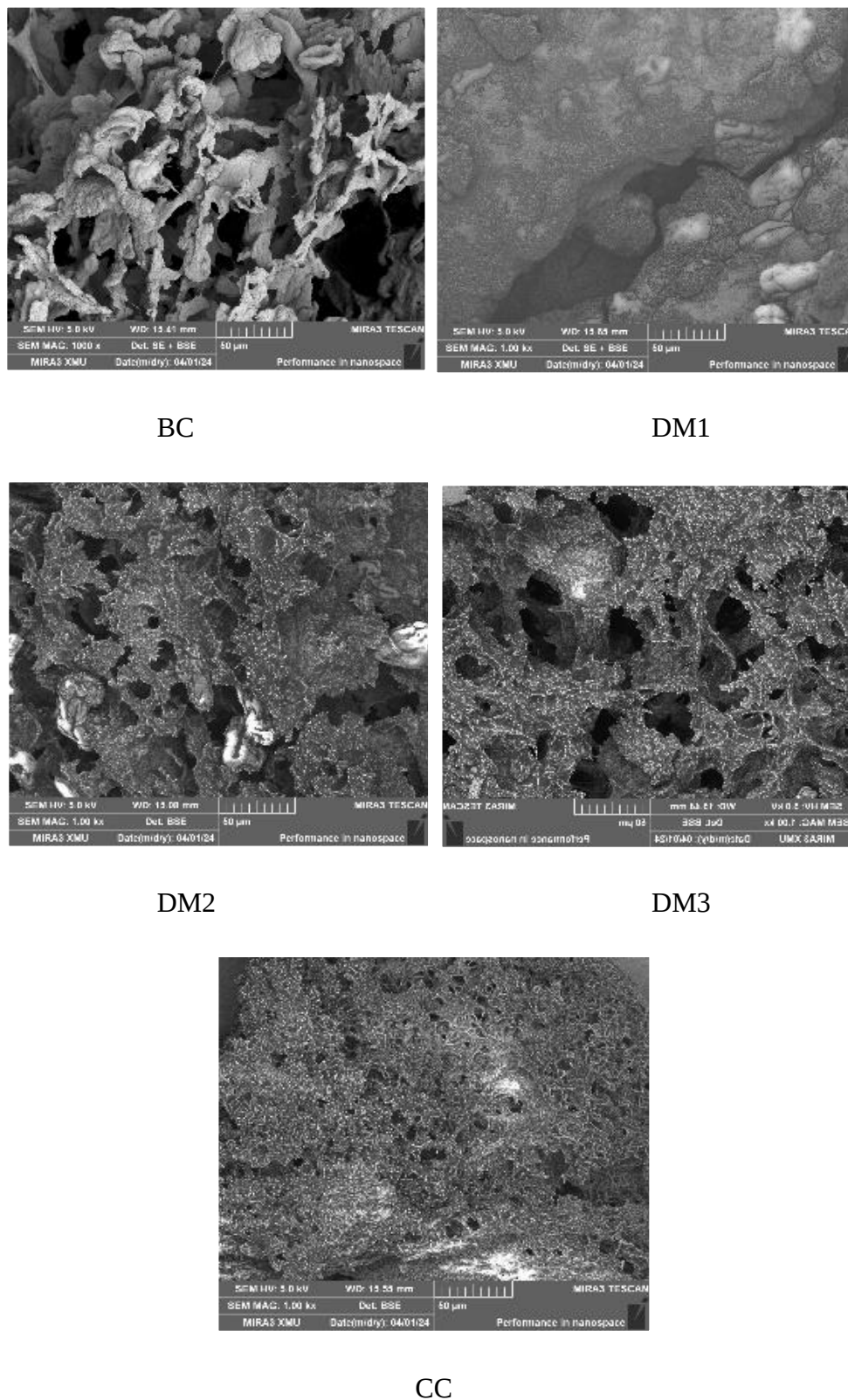


Figure 3 Scanning electron micrographs of the samples.

Note: DM1 (30% CM and 70% BMLE), DM2 (50% CM and 50% BMLE), DM3 (70% CM and 30% BMLE), BC (100% BMLE), and CC (100% CM). SEM HV: 5.0 kV. SEM MAG: 1.00 kx., bar = 50 µm.

The micrographs in Figure 6 illustrate the structural differences among various DM samples: BC, DM1, DM2, DM3, and CC. The BC sample has a highly porous, fibrous structure, resembling soy milk yogurt with large pores, indicating an interconnected protein-fat network [42]. The DM1 sample appears to have a more compact and less porous structure than BC, suggesting a denser matrix with fewer air pockets. The micrograph of DM2 reveals a porous structure with larger voids and a somewhat loose network, likely due to the incorporation of BMLE, which alters the protein matrix formation. The DM3 sample shows an even more pronounced porous structure than DM2, with a higher number of large voids, indicating an increased BMLE content, which impacts the texture and density of the curd. The micrograph of CC presents a dense and relatively uniform structure with fewer and smaller voids compared to the other samples, reflecting a curd made purely from cow's milk without any additions.

The variations in the microstructure are due to differences in the formulation, particularly the ratios of cow's milk to white kidney bean milk-like extract. Incorporating BMLE results in a more open and porous structure, which can affect the curd's texture, spreadability, and moisture content. Plant proteins and fiber can influence dairy matrices' viscosity, spreadability, and mouthfeel. While some formulations may require stabilizers or emulsifiers to improve sensory properties, adding legumes has enhanced structural integrity in low-fat dairy products [47].

CONCLUSIONS

Incorporating white kidney bean milk-like extract (BMLE) into dairy matrices offers a promising avenue for developing cream-based dairy products with enhanced nutritional profiles. Our study successfully formulated spreadable dairy matrices containing up to 50% BMLE, achieving improved spreadability and water holding capacity (WHC), which can be offered in the cream cheese and dairy paste production. However, higher BMLE concentrations introduced a noticeable beany flavour, indicating the need for further optimization to enhance sensory acceptance. To address these challenges, innovative dairy processing technologies such as membrane filtration and microfluidics can be employed. These methods may help refine the texture and flavour profiles of BMLE-infused dairy products, thereby improving their functionality and consumer appeal. In summary, while the inclusion of BMLE in dairy matrices enhances certain physicochemical properties, optimizing processing techniques is essential to mitigate undesirable flavours and achieve products that meet consumer sensory expectations. DMs provide a good food composition for further development of spreadable cheese analogues with different additives.

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