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# Metabolic and microbial stability of protein-fortified fermented dairy drinks: Effect of base and storage

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ABSTRACT

Aging, particularly in the elderly, is marked by physiological changes that alter protein requirements and utilization, while higher protein intake helps maintain nutritional status and physical function. Fermented dairy products improve protein digestibility and calcium bioavailability, offering benefits for the elderly. Whey protein is a suitable fortifier due to its solubility, digestibility, and acceptance. For these reasons, this study aimed to formulate and analyse whey protein–fortified fermented dairy drinks suitable for home preparation and acceptable to elderly consumers in terms of nutritional, functional, and sensory properties. To facilitate self-care or caregiver-assisted intake, the drinks were designed to be prepared in advance and stored for consumption throughout the day. Changes in sensory attributes, microbial load, physicochemical properties (pH and color), organic acids, sugars, and free amino acids (FAA) were evaluated. Regarding sensory quality, all formulations were free of off-flavors, and notable differences in their profiles allowed for adaptation to different preferences. Microbial counts remained high (>8 log CFU $g^{-1}$) throughout storage, with quark and kefir-drinks showing the highest stability. The addition of whey protein decreased lightness and increased a* and b*, whereas storage at 24h20C maximized kefir acidification. Lactic acid was the predominant organic acid, and lactose and glucose levels decreased over time due to microbial activity. Finally, storage at 24h20C led to enhanced proteolytic activity; notably, kefir significantly doubled their essential FAA content. Overall, all formulations were shelf-stable under the tested conditions.

## 1. Introduction

Malnutrition is a widespread health issue in the elderly (individuals aged 65 or over), increasing morbidity and mortality, contributing to physical decline and geriatric syndromes (anorexia, dementia, frailty, sarcopenia) (Norman et al., 2021). Reduced appetite, impaired taste and smell, swallowing difficulties (dysphagia), decreased gastric flexibility are some of the physiological changes associated with advanced age that can lead to malnutrition (Norman et al., 2021; Norton et al., 2021). Clinical malnutrition mainly occurs in hospitals, care centres and nursing homes, but it is also common among older adults living in community (Van Den Broeke et al., 2018). The physiological changes associated with aging, especially in the elderly, can also alter protein requirements and utilization (Deutz et al., 2014; Norman et al., 2021). Other factors influencing malnutrition and protein deficiency include the presence of chronic diseases, pharmacological treatments, physical and cognitive disabilities that limit the ability to shop for and prepare food, and socio-economic aspects (Deutz et al., 2014; Norman et al., 2021). As a result of these conditions, the ability of the elderly to perform basic and instrumental activities of daily living is often limited, needing support and care from others (WHO, 2024).

A study conducted in five European countries (n = 1825) revealed that 37.7% of elderly were at considerable risk of inadequate protein intake (Hung et al., 2019). In fact, protein-energy malnutrition is highly prevalent in residential care centres, affecting an estimated 16% to 70% of elderly residents (Agarwal et al., 2013). Moreover, there is strong evidence that adequate protein intake is essential to prevent malnutrition and sarcopenia and that protein requirements increase with age (1.0–1.2 g/kg body weight), reaching 1.5 g/Kg/day in cases of acute or chronic illness, injury, or malnutrition (Norman et al., 2021; Weiler

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et al., 2023). These recommendations are based on results indicating that higher protein intake helps to maintain or to improve the nutritional status and the physical function, promotes muscle protein synthesis, and preserves lean body mass in elderly (Weiler et al., 2023; Carballo-Casla et al., 2024). However, increasing protein intake to reach the proposed recommendations could be a significant challenge for the elderly, as they already struggle to meet the current Recommended Daily Allowance (RDA).

Nowadays, a wide variety of protein-fortified foods is on the market for the general population. Among those, drinks may be preferable to solid foods as means of increasing protein intake in the elderly due to the age-related alterations in oral physiology (swallowing difficulties and reduced appetite). Milk stands out from other drinks due to its natural nutritional properties. A recent publication analysed milk/dairy drinks and yoghurts/fermented milk carrying protein claims and found that 58.1% and 41.1% were fortified with this nutrient (Beltrá et al., 2024). Regarding their nutritional quality, as many as 61.3% of milk/dairy drinks and 82.1% of yoghurt/fermented milk products with protein claims were considered "less healthy" (Beltrá et al., 2024). The main reasons were the presence of sweeteners and high levels of free sugar (Beltrá et al., 2024). This indicates that the healthiness of foods with proteins available in the market is far from guaranteed. In addition, these products are usually more expensive, which may be an additional obstacle to include them in the diet (Brooks et al., 2010). Consequently, home-preparation of protein-fortified foods could represent an opportunity to improve functionality and nutritional quality of already healthy foods, while achieving the desired sensory characteristics at a lower cost.

Lactic acid bacteria (LAB) fermented dairy foods may provide health benefits beyond basic nutrition compared to plain milk, largely due to the activity of LAB (Leewendaal et al., 2022; Marco et al., 2017). They also improve milk's nutritional quality through enhanced protein digestibility and increased calcium bioavailability (Leeuwendaal et al., 2022). These effects are particularly beneficial for elderly, who often exhibit impaired metabolism, as well as a decreased anabolic response to dietary protein (Bauer et al., 2013; Khalaf et al., 2025). In addition, a recent study suggested that high consumption of fermented dairy products was associated with a lower risk of esophageal and stomach cancer in elderly participants, while no significant association was found with milk consumption (Oncina et al., 2024).

In dairy product development, particularly concerning yoghurt formulations, casein and whey protein derivatives are extensively employed for their functional capacity to enhance key physicochemical parameters, to improve the overall sensory profile and to increase protein content (Żulewska et al., 2025). Due to the excellent emulsifying and solubility properties of whey protein (Norton et al., 2021; Zhao et al., 2025), it might be the preferred choice for fortifying fermented milk. These characteristics enable the production of a smooth, drinkable product suitable for the elderly, who often experience swallowing difficulties (dysphagia) or eating disorders. In addition, whey proteins present high leucine content, amino-acid availability and fast digestibility demonstrated also in the elderly (Zanini et al., 2020). It should be noted that consumers have a more positive perception of whey protein compared to casein, possibly due to greater familiarity (Rovai et al., 2024).

For the reasons outlined above, this study aims to formulate and analyse whey protein-fortified fermented dairy drinks, based on different fermented dairy foods of diverse sensory profile, suitable for home preparation that are nutritionally and functionally suitable to elderly consumers. To facilitate self-care or care provided by others, these products are prepared in advance and stored for consumption throughout the day. Changes may be ongoing during storage, which may affect the properties of these home-made drinks. Therefore, potential changes in physicochemical properties, chemical compounds, and microbial load will be evaluated.

## 2. Materials and methods

### 2.1. Experimental design

A total of five protein-fortified dairy drink formulations were prepared using three commercial dairy ingredients (Commercial Products, CP): i) a fat-free fermented dairy product [Y: yoghurt, BY: bifidus yoghurt, K: kefir, FW: fresh whipped cheese, QU: quark]; ii) UHT whole milk (3.6 % fat content) (M); and iii) unflavoured whey protein concentrate powder (WPC, 80% protein, 2.0; HSN®, Spain). This WPC was obtained through ultrafiltration and spray-drying processes and contains sunflower lecithin as an emulsifier to ensure optimal dispersibility (Fig. 1).

To determine an acceptable ratio between fermented dairy products and whole milk (60:40 vs. 70:30), a descriptive analysis focusing on viscosity, in order to develop drinkable products, was conducted by a panel of experts ($n = 7$) from the research group. Based on their evaluation, a 60% fermented dairy base and 40% whole milk ratio was selected to achieve a drinkable product. Subsequently, the final protein content of each mixture was calculated, and whey protein was added as needed to reach a target of 15 g of protein per 100 g of each dairy-blend base. To ensure a homogeneous dispersion, the mixtures were subjected to mechanical stirring for 10 minutes at 20 °C. The absence of discernible lumps or protein aggregates was visually confirmed immediately after preparation and remained consistent throughout the storage period. The listed values in Table 1 (14.23, 13.40, 14.23, 11.53, and 10.78 g) denote the amount of whey protein added to 100g of the Y, BY, K, FW, and QU blends, respectively.

All formulations were prepared under aseptic conditions inside a laminar flow hood, using autoclaved equipment and disinfecting work surfaces with 70% alcohol. Additionally, the use of CP guaranteed the initial microbiological safety of the ingredients. Two independent batches of each of the five formulations ($n = 10$) were produced, and all analyses were performed in duplicate or triplicate. The formulations were prepared and stored under different controlled time and temperature conditions to mimic real home conditions. Potential changes in nutritional properties and fermentation metabolites under these conditions were evaluated: 0 (freshly prepared sample used as a baseline control), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C), and 24h4C (24 h at 4 °C).

Samples were coded according to the fermented CP and storage condition:

- Protein-fortified fermented dairy drinks based on yoghurt (Y): Y0h, Y8h20C, Y24h20C and Y24h4C.
- Protein-fortified fermented dairy drinks based on bifidus yoghurt (BY): BY0h, BY8h20C, BY24h20C and BY24h4C.
- Protein-fortified fermented dairy drinks based on kefir (K): K0h, K8h20C, K24h20C and K24h4C.
- Protein-fortified fermented dairy drinks based on fresh whipped cheese (FW): FW0h, FW8h20C, FW24h20C and FW24h4C.
- Protein-fortified fermented dairy drinks based on quark (QU): QU0h, QU8h20C, QU24h20C and QU24h4C.

### 2.2. Sensory analysis

Seven trained panellists performed the sensory analysis. It was divided in two sessions. In the first, the panel selected the attributes from previous lexicon developed for drinkable and/or dairy products (Muelas et al., 2018; Cano-Lamadrid et al., 2020; Clemente et al., 2021; Issa-Issa et al., 2020). In the second session samples were scored for the selected attributes: appearance (viscosity and uniformity); odor (acetaldehyde, buttery, cooked, lactic and protein powder); flavour (animal, cooked, protein powder, sourness, sweetness and aftertaste); and texture (creamy and smoothness). Prior to serving, all samples were shaken to ensure proper homogenization, and a volume of 40 mL was dispensed in

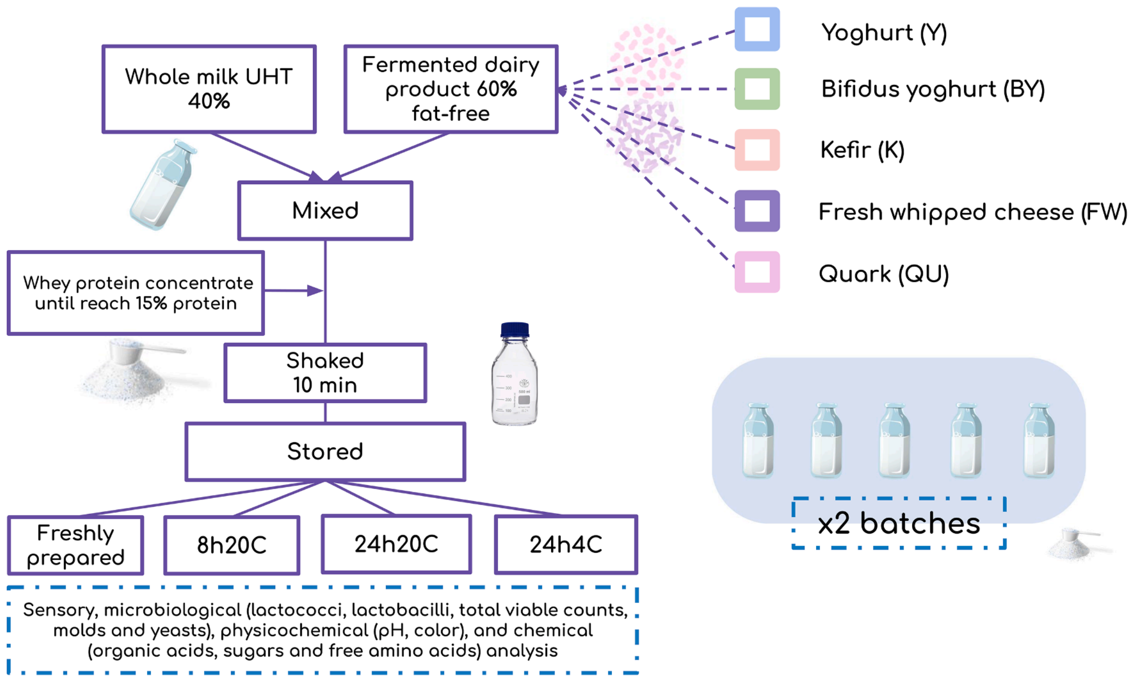


**Fig. 1.** Experimental design of protein-fortified fermented dairy drinks produced by consumers. (UHT: ultra-high temperature, 8h20C: 8 hours at 20°C, 24h20C: 24 hours at 20 °C, 24h4C: 24 hours at 4 °C).

**Table 1**
Nutritional information of commercial products and protein-fortified fermented dairy drinks.

| Dairy Products | Energy (Kcal) | Carbohydrates (=sugars) | Total fat | Saturated fat | Protein | Salt |
|---|---|---|---|---|---|---|
| Commercial Products* | | | | | | |
| M | 64 | 4.60 | 3.60 | 2.50 | 3.20 | 0.12 |
| WPC | 370 | 5.10 | 3.30 | 2.40 | 80.00 | 0.35 |
| Y | 39 | 5.60 | 0.10 | 0.10 | 3.90 | 0.15 |
| BY | 42 | 3.80 | 0.40 | 0.30 | 5.00 | 0.11 |
| K | 34 | 4.50 | 0.10 | 0.10 | 3.90 | 0.13 |
| FW | 46 | 4.00 | 0.00 | 0.00 | 7.50 | 0.10 |
| QU | 52 | 4.60 | 0.50 | 0.20 | 8.50 | 0.09 |
| Protein-fortified fermented dairy drinks** | | | | | | |
| Y0h | 102 | 5.93 | 1.97 | 1.40 | 15.00 | 0.19 |
| BY0h | 100 | 4.80 | 2.12 | 1.50 | 15.00 | 0.16 |
| K0h | 99 | 5.27 | 1.97 | 1.40 | 15.00 | 0.18 |
| FW0h | 96 | 4.83 | 1.82 | 1.28 | 15.00 | 0.15 |
| QU0h | 97 | 5.15 | 2.10 | 1.38 | 15.00 | 0.14 |

* : g/100 g or 100 mL according to the information provided by the manufacturer.
** : g/100 g of the 60:40 dairy-blend base plus whey protein (see material and methods). M: UHT whole milk; WPC: Unflavoured whey protein concentrate; Y: Yoghurt; BY: Bifidus Yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark; Y0h: freshly prepared based on yoghurt; BY0h: freshly prepared based on bifidus Yoghurt; K0h: freshly prepared based on kefir; FW0h: freshly prepared based on fresh whipped cheese; and, QU0h: freshly prepared based on Quark.

odor-free plastic containers labelled with random three-digit codes. Panellists evaluated each attribute on a 0–10 scale (0 = none; 10 = extremely high) with 0.5-point increments, except for viscosity and aftertaste attributes, which were measured in time (seconds).

### 2.3. Microbial load and physicochemical parameters (pH and color)

MRS Agar was used to enumerate lactobacilli counts (Lab) at 37 °C under microaerophilic conditions for 48 h, whereas M17 Agar was employed to determine lactococci counts (Lac) at 37 °C under aerobic conditions for 24 h (Jiménez-Redondo et al., 2022). Plate Count Agar (Petrifilm™) was used to determine total viable counts (TVC) at 37 °C under aerobic conditions for 48-72 h and Rose Bengal Agar was used for the detection of moulds and yeasts at 25 °C under aerobic conditions for 3-5 days, respectively. Each sample was analysed twice (n = 4).

The color properties of protein-fortified fermented samples and CP were assessed in the CIEL*a*b* color space (Konica Minolta CM-5) and pH was measured using a pH-meter (pH 60DHS, XS Instruments) (Teruel-Andreu et al. (2024a). Three measurements were taken for both determinations of the two batches of each formulation (n = 6).

### 2.4. Organic acid and sugar identification and quantification

The determination of organic acids and sugars was carried out by High-Performance Liquid Chromatography (HPLC) according to the methodology of Teruel-Andreu et al. (2024b), with minor modifications. Briefly, 2 g of sample were homogenized in 4 mL of ultrapure water acidified with 0.1% $H_3PO_4$, shaken vigorously for 20 s at 11,000 rpm, and centrifuged at 15,000 rpm for 20 min at 4°C. The analysis was conducted in isocratic mode using 0.1% $H_3PO_4$ as the mobile phase at a flow rate of 0.5 mL/min, with an injection volume of 20 μL and a total run time of 35 min. Each sample was analysed in duplicate in each batch ($n$ = 4).

### 2.5. Free amino acids

Free amino acids (FAA) were determined using an LC-MS/MS system (8050, Shimadzu, Kyoto, Japan) following a modification of the method by Shimadzu Corporation (2016). Briefly, 0.2 g of sample were mixed with 400 μL of ethanol and homogenized for 10 min. The mixture was centrifuged at 12,000 rpm for 15 min. The resulting supernatant was diluted (1:5, v/v) in ethanol, filtered through a 0.22 μm membrane, and collected in amber vials. Samples (3 μL injection volume) were analyzed using a 30 min chromatographic run. Identification and quantification were performed using external standards, and results were expressed as mg per 100 g of product. The analysis was carried out in duplicate in each batch (n = 4). Data processing included the identification of 22 FAA; however, for the comparative analysis and discussion, only major components contributing > 1% to the total FAA profile were included.

Minor amino acids, such as alanine, aspartic acid, glycine, histidine, 4-hydroxyproline, methionine, and methionine sulfoxide, were excluded.

### 2.6. Statistical analysis

A One-way ANOVA test and a Tukey test ($p < 0.05$) were performed to compare the means and determine whether there were significant differences between them (XLSTAT). The statistical analysis was carried out per product type. Principal component analysis (PCA regression map) was conducted for the sensory descriptors.

## 3. Results and discussion

### 3.1. Preparation of the protein-fortified drinks and nutritional value

Five fermented dairy products were selected based on their ingredient lists, which consisted solely of milk and diverse lactic cultures. Their declared nutrient composition, as provided by the manufacturers, is presented in Table 1. The combination of the fat-free fermented products and whole milk was used to develop drinkable beverages with fat content. From a nutritional standpoint, the inclusion of whole milk was intended to provide a matrix naturally containing fat-soluble vitamins (A, D, E, and K). According to the literature, the presence of milk fat is associated with a more efficient absorption of these vitamins (Melse-Boonstra, 2020; Sanjulián et al., 2025). Furthermore, vitamin D is well-documented to enhance calcium bioavailability (Melse-Boonstra, 2020; Sanjulián et al., 2025), a key factor considering the high calcium requirements of the target elderly population.

The protein content of the original fermented products ranged from 3.2 to 8.5 g/100 g or 100 ml (Table 1). To produce the protein-fortified drink, whey protein was added to a 100 g portion of the 60:40 mixture to achieve a total protein content of 15 g. The nutritional composition of the final drinks is shown in Table 1.

### 3.2. Sensory Profile of protein-fortified fermented dairy drinks

Aging is known to alter sensory perception (particularly taste and smell) often leading older adults to perceive foods as less flavourful (Methven et al., 2012). In addition, individual differences in physiology, social factors, and preferences can significantly influence sensory experience (Norton et al., 2021). Age-related differences in texture perception have also been observed, with older adults commonly perceiving foods as less creamy or fatty and more mouth-dry. However, some attributes, such as thickness and mouthcoating, appear unaffected by age, suggesting that these changes are both product- and attribute-specific (Kremer et al., 2005; Kremer et al., 2007a; Kremer et al., 2007b; Withers et al., 2013). These sensory alterations can reduce the enjoyment of certain foods, influence dietary choices, and therefore compromise nutritional status (Norton et al., 2021). That is why it is important to determine the sensory profile of each product, helping consumers to select the most suitable dairy base for the formulation of protein-rich beverages.

Fig. 2 shows the principal component analysis (PCA) where the data from the descriptive analysis of the freshly made drinks is shown (Table 1S). The F1 axis explained 39.96% while the axis F2 explained 37.90% of the total data variance. It should be noted that the type of fermented dairy base (Y, BY, K, FW and QU) significantly influenced the sensory profile of high-protein drinks formulated for elderly.

In the following lines, a summary of the main sensory attributes identified in the developed products were described. FW0h and QU0h were the most viscous, creamy, and sourness ones, with an animal aroma and longer aftertaste than the others. BY0h was associated with a lactic (milk) and acetaldehyde aroma while Y0h was the sweetest with a

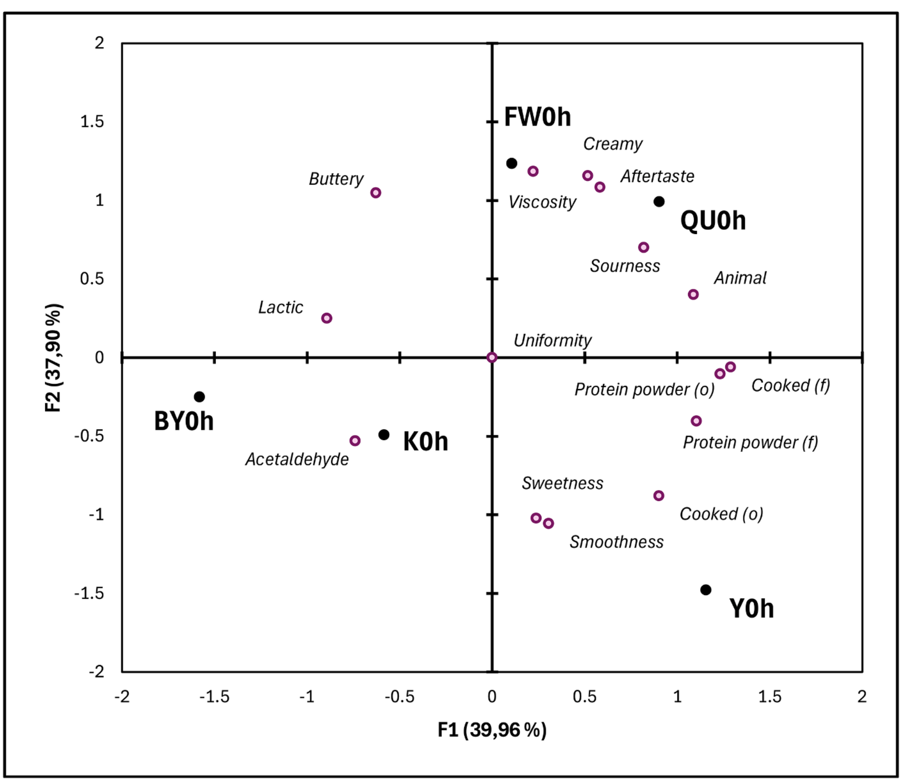


**Fig. 2.** Principal Component Analysis (PCA) scores plot showing the descriptive sensory analysis data of protein-fortified fermented dairy drinks produced by consumers (Y0h: freshly prepared based on yoghurt; BY0h: freshly prepared based on bifidus Yoghurt; K0h: freshly prepared based on kefir; FW0h: freshly prepared based on fresh whipped cheese; and, QU0h: freshly prepared based on Quark); o: odor; f: flavour. The F1 axis explained 39.96% of the total data variance; while the axis F2 explained 37.90% of the total variance.

cooked aroma. Finally, K0h was the least viscous, creamy, and sour one, presenting the greatest smoothness along with Y0h. Both QU0h and Y0h presented the strongest protein powder aroma and flavour. The sensory descriptors “acetaldehyde”, previously associated with the characteristic fresh taste of yoghurt (Chen et al., 2017), and “lactic” were identified as predominant in BY0h, Y0h, and K0h. On the other hand, the descriptor 'buttery', commonly associated with the flavors of butter, cream, and vanilla (Li et al., 2024), was linked to FW0h and QU0h, which exhibited the highest levels of sourness, creaminess and viscosity. Not only the protein type and fat, but starter cultures also contribute to thickness and mouthfeel, greasiness, and smoothness (Buldo et al., 2021).

The type of whey protein and its protein composition can also affect the lubrication properties of the dairy system (Lian et al., 2026). The sensory profile of whey protein is characterized by descriptors such as sweet, aromatic, cooked, and milky (Bull et al., 2017). Some of those attributes could be detected in some drinks, such as yoghurt-based products, as evidenced by the results of the PCA (Fig. 2). Y0h showed the highest level of sweetness, possibly because of the higher addition of whey protein in their formulation (see Materials and methods). In this sense, the release of specific FAA during fermentation also plays a crucial role in taste formation and aftertaste persistence (Zhumakadyrova & Mazhitova, 2024). The descriptors grainy, astringent, dry, chalky, thick, mouth-coating, and mouth-dryness have also been reported for whey proteins (Bull et al., 2017; Norton et al., 2021). Mouth-dryness has been attributed to electrostatic interactions between whey proteins and salivary proteins (particularly in acidified matrices such as fermented dairy products), muco-adhesion, and the intrinsic astringency of acidic environments (Giles et al., 2024). However, none of these sensory attributes were detected by the trained panellists in the present study in the developed drinks, as the main component of the mix was the fermented milk.

### 3.3. Microbial counts

Commercial fermented products usually only indicate in labelling information ‘lactic acid fermented cultures’ without specifying the genus or species, so we assume that they will be mostly lactobacilli and lactococci. Fig. 3 and Table 2S show the counts of lactococci (Lac, Fig. 3A), lactobacilli (Lab, Fig. 3B), and total viable counts (TVC, Fig. 3C) in freshly prepared drinks and after different storage conditions. The estimated microbial load of ingredients was also shown in Table 2S. All drinks exhibited microbial Lac counts exceeding 8 log CFU $g^{-1}$, except for FW8h20C. Y and BY-drinks showed no statistically significant differences ($p > 0.05$) in Lac counts among storage conditions (0h, 8h20C, 24h20C and 24h4C) and compared to CP (8.94-9.66 log CFU $g^{-1}$ and 8.84-9.06 log CFU $g^{-1}$, respectively). Common yoghurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subesp. bulgaricus*) are thermophilic microorganisms (Codex Alimentarius Commission, 2011), with an optimal growth temperature of 43°C (Chopde et al., 2025). Despite being in a nutrient-rich medium such as milk, it can be said that the microorganisms survived or grown very slowly due to the storage conditions (4 and 20 °C). As to K-drinks, no significant differences were detected among samples (8.59–9.15 log CFU $g^{-1}$), except for K24h20C, which exhibited the highest Lac counts. These findings align with the optimal growth conditions of mesophilic cultures typically employed in kefir production (optimal temperature condition around 25°C) (Meral-Aktaş et al., 2025b). According to the Codex Alimentarius Commission, 2011, kefir grains containing *Lactobacillus kefiri*, species of the genera *Leuconostoc, Lactococcus*, and *Acetobacter* are used in association with both lactose- and non-lactose-fermenting yeasts. Although not the primary aim of this research, further examination of the remaining yeast and bacterial communities would be valuable to assess potential variations under the tested storage conditions. Regarding the FW-drinks, Lac counts generally showed no significant differences among samples, indicating overall stability of the microbial population during storage (7.57–8.27 log CFU $g^{-1}$), except for FW8h20C, which exhibited a significant decline in viable cells (p <

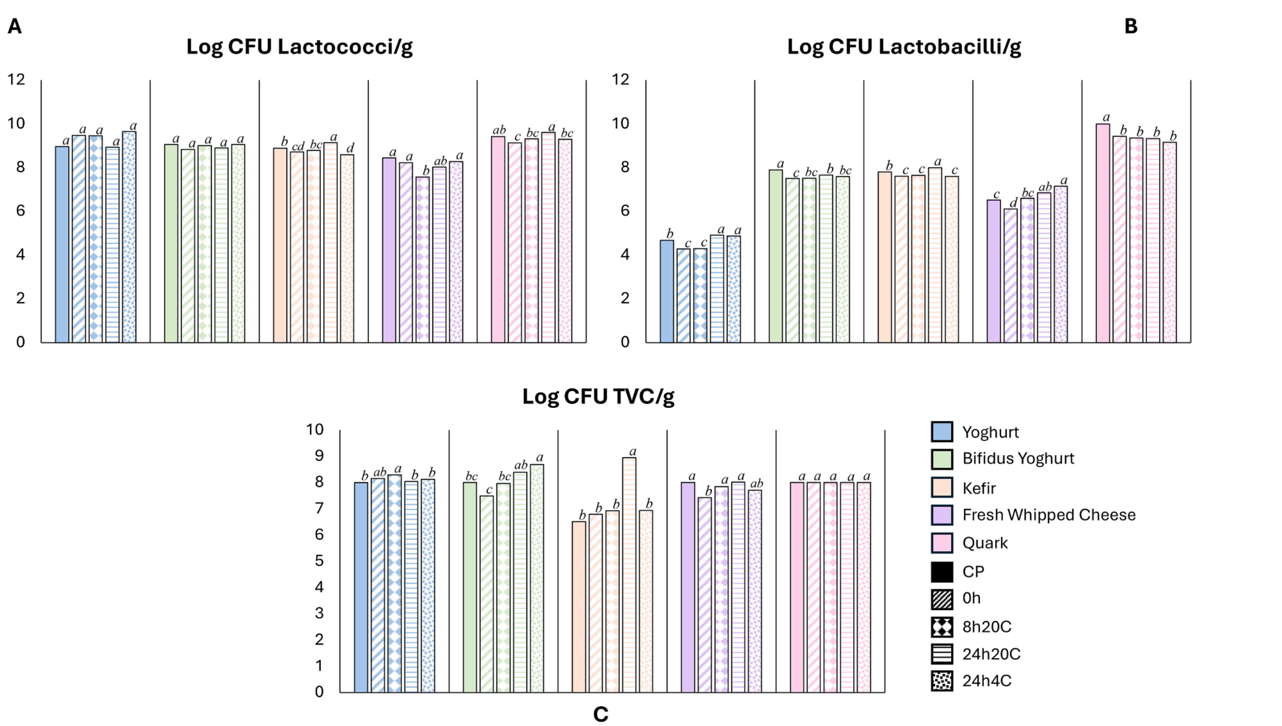


**Fig. 3.** Microbial load of lactococci (A), lactobacilli (B) and total viable counts (TVC) (C) in protein-fortified fermented dairy drinks stored under different conditions: CP (commercial products), 0h (freshly prepared sample), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C) and 24h4C (24 h at 4 °C). Values followed by a different letter within the same column are significantly different ($p < 0.05$) for the Tukey test. Y: Yoghurt; BY: Bifidus Yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark.

**Table 2**
Organic acid and sugar content (g/100 mL) of protein-fortified fermented dairy drinks.

| Samples | Acetic acid | Citric acid | Lactic acid | Lactose | Glucose | Galactose | Glycerol |
|---|---|---|---|---|---|---|---|
| Y0h | 0.07 ± 0.03 a | 0.18 ± 0.01 a | 0.36 ± 0.02 c | 3.86 ± 0.04 a | 0.09 ± 0.02 b | 0.37 ± 0.06 c | 0.32 ± 0.02 d |
| Y8h20C | 0.08 ± 0.01 a | 0.18 ± 0.00 ab | 0.51 ± 0.03 b | 3.57 ± 0.08 b | 0.14 ± 0.01 a | 0.56 ± 0.05 b | 0.47 ± 0.01 b |
| Y24h20C | 0.08 ± 0.01 a | 0.18 ± 0.00 ab | 0.70 ± 0.02 a | 3.26 ± 0.05 c | 0.15 ± 0.02 a | 0.75 ± 0.04 a | 0.61 ± 0.03 a |
| Y24h4C | 0.09 ± 0.01 a | 0.17 ± 0.00 b | 0.40 ± 0.03 c | 3.69 ± 0.04 b | 0.17 ± 0.02 a | 0.52 ± 0.02 b | 0.37 ± 0.01 c |
| BY0h | 0.08 ± 0.01 a | 0.17 ± 0.00 a | 0.58 ± 0.07 a | 3.41 ± 0.11 a | 0.10 ± 0.03 a | 0.57 ± 0.10 a | 0.52 ± 0.06 a |
| BY8h20C | 0.08 ± 0.00 a | 0.17 ± 0.00 a | 0.59 ± 0.01 a | 3.34 ± 0.05 ab | 0.07 ± 0.00 ab | 0.54 ± 0.00 a | 0.54 ± 0.00 a |
| BY24h20C | 0.09 ± 0.00 a | 0.17 ± 0.00 a | 0.69 ± 0.01 a | 3.17 ± 0.04 b | 0.07 ± 0.00 ab | 0.63 ± 0.01 a | 0.65 ± 0.01 a |
| BY24h4C | 0.07 ± 0.02 a | 0.17 ± 0.01 a | 0.73 ± 0.19 a | 2.51 ± 0.06 c | 0.05 ± 0.00 b | 0.45 ± 0.38 a | 0.44 ± 0.40 a |
| K0h | 0.09 ± 0.00 a | 0.17 ± 0.00 a | 0.80 ± 0.42 a | 3.25 ± 0.38 a | nd | 0.31 ± 0.00 b | 0.73 ± 0.38 a |
| K8h20C | 0.09 ± 0.01 a | 0.17 ± 0.00 a | 0.62 ± 0.31 a | 3.39 ± 0.28 a | nd | 0.38 ± 0.01 a | 0.57 ± 0.29 a |
| K24h20C | 0.10 ± 0.01 a | 0.13 ± 0.07 a | 0.83 ± 0.18 a | 3.04 ± 0.15 a | nd | 0.40 ± 0.01 a | 0.77 ± 0.17 a |
| K24h4C | 0.10 ± 0.02 a | 0.17 ± 0.00 a | 0.72 ± 0.36 a | 3.20 ± 0.30 a | nd | 0.34 ± 0.02 b | 0.66 ± 0.33 a |
| FW0h | 0.08 ± 0.01 a | 0.16 ± 0.00 a | 0.36 ± 0.01 b | 3.70 ± 0.06 a | nd | 0.15 ± 0.00 c | 0.33 ± 0.01 b |
| FW8h20C | 0.08 ± 0.00 a | 0.16 ± 0.00 a | 0.37 ± 0.01 b | 3.54 ± 0.06 ab | nd | 0.18 ± 0.01 b | 0.34 ± 0.01 b |
| FW24h20C | 0.09 ± 0.00 a | 0.16 ± 0.01 a | 0.45 ± 0.02 a | 3.46 ± 0.12 b | nd | 0.27 ± 0.01 a | 0.41 ± 0.02 a |
| FW24h4C | 0.09 ± 0.00 a | 0.17 ± 0.00 a | 0.37 ± 0.01 b | 3.74 ± 0.13 a | nd | 0.16 ± 0.01 bc | 0.34 ± 0.01 b |
| QU0h | 0.09 ± 0.01 a | 0.17 ± 0.01 a | 0.39 ± 0.01 b | 3.38 ± 0.14 ab | nd | 0.35 ± 0.01 c | 0.36 ± 0.00 b |
| QU8h20C | 0.08 ± 0.01 a | 0.12 ± 0.06 a | 0.40 ± 0.02 b | 3.17 ± 0.18 ab | nd | 0.40 ± 0.01 b | 0.37 ± 0.01 b |
| QU24h20C | 0.07 ± 0.01 a | 0.12 ± 0.06 a | 0.45 ± 0.02 a | 2.91 ± 0.12 b | 0.16± 0.00 | 0.50 ± 0.02 a | 0.41 ± 0.02 a |
| QU24h4C | 0.09 ± 0.00 a | 0.17 ± 0.01 a | 0.39 ± 0.03 b | 3.49 ± 0.34 a | nd | 0.37 ± 0.02 bc | 0.37 ± 0.03 b |

nd: values below the detection limit. Values followed by a different letter within the same column and sample are significantly different ($p < 0.05$) for the Tukey test. 0h (freshly prepared sample), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C) and 24h4C (24 h at 4 °C). Y: Yoghurt; BY: Bifidus yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark.

0.05). This reduction may be attributed to physiological stress associated with the transition to post-processing conditions, such as nutrient limitation (Sionek et al., 2024). Meanwhile, Lac counts in QU-drinks remained largely stable despite minor fluctuations (9.13–9.61 log CFU $g^{-1}$), showing slight growth after 24 hours of storage at 20°C. This stability likely reflects the comparatively lower fermentative activity of these cultures during storage, which can be attributed to the type of coagulation used in the production of these cheeses (acid, hot, 40–45°C) with thermophilic cultures (Schulz-Collins and Senge, 2004).

Lab counts differed depending on the dairy base used, with Y-drinks exhibiting the lowest counts. Lab counts in Y-drinks decreased compared to CP immediately after production and remained relatively stable, with no significant differences between 0h and 8h20C; however, a significant increase was observed after 24 h (from 4.28 to 4.92 log CFU $g^{-1}$). Similar tendency was observed in BY-drinks (7.51 - 7.66 log CFU $g^{-1}$). In line with the observations regarding Lac counts, the limited growth of Lab is also due to the thermophilic nature of the yoghurt starter culture (Chopde et al., 2025). Regarding K-drinks, Lab counts showed a slight but significant reduction compared to CP. No changes were observed after both 8h20C and 24h4C; however, a significant increase (7.60 to 8 log CFU $g^{-1}$) was detected in 24h20C. Our findings are in accordance with previous observations on mesophilic cultures (Meral-Aktaş et al., 2025b), which require longer storage at moderate temperatures to achieve significant growth. As for FW-drinks, Lab counts decreased immediately after preparation of the mixture, but an increase was observed during storage under any condition (6.11 - 7.14 log CFU $g^{-1}$). In QU-drinks, counts were highest, exceeding 9 log CFU $g^{-1}$. They were significantly lower than in CP, although no significant differences were observed among products during storage (9.16 - 9.43 log CFU $g^{-1}$). Further studies are needed to understand what happens during mixing, with osmotic stress from high whey protein concentration being a possible reason. However, it should also be noted that some studies have shown that whey protein can protect probiotics against oxidative stress, stabilise pH, and promote their survival in dairy matrices (Dinkçi et al., 2023). For example, Cordeiro et al. (2018) observed that the viability of *Lactobacillus casei* BL23 remained virtually unchanged in milk supplemented with 30% (w/v) whey protein isolate after 90 days of cold storage. In coherence with Lab and Lac counts, TVC counts in protein-fermented drinks ranged from 6.79 to 8.95 log CFU $g^{-1}$ with statistically significant but minor differences among products, except for K-drinks, where K24h20C showed a significant increase (2.44 log CFU $g^{-1}$). Meral-Aktas et al. (2025b) reported similar TVC counts in kefir from different animal milks, ranging from 7.23 to 8.89 log CFU $mL^{-1}$. Lactic acid bacteria represent the predominant microbial group in fermented dairy products, outcompeting other aerobes through microbial competition. However, some of them grow more efficiently on selective media and under microaerophilic conditions and may exhibit reduced growth on general agar under anaerobic conditions (Byrd et al., 2022). Consequently, TVC generally approximates lactic acid bacteria counts in these products, as reflected in this study (Fig. 3C and Table S2).

Yeast counts were undetectable in most samples (data not shown). European Commission, 2005 on microbiological criteria in food does not set a yeast limit for yoghurt, but it does require counts below 100 CFU $g^{-1}$ in butter, which in this case was used as the reference dairy product. Additionally, molds and yeasts were determined as indicators of contamination. The BY-drinks showed yeast counts. Average yeast counts of 1.57, 2.01, and 0.59 log CFU $g^{-1}$ were obtained in both batches of BY8h20C and BY24h20C and in one batch of BY24h4C, respectively. Commercial bifidus yoghurt showed undetectable counts of yeasts, they were probably present at an acceptable level and grew in the prepared drink during storage. Yeasts counts were also undetectable in all other prepared drinks. In the case of kefir, the absence of yeast is consistent with the cultures included in the commercial product, as it did not contain kefir grains (source of yeast), and only lactic acid bacteria are indicated in the label (*Lactococcus lactis, Lactococcus mesenteroides, Lactococcus cremoris, Streptococcus thermophilus, Leuconostoc sp.*, and *Lactobacillus sp.*). The Codex Alimentarius Standard stipulates that there must be a minimum count of $10^4$ CFU $g^{-1}$ of yeast in kefir (Codex Alimentarius Commission, 2011), which is not fulfilled in the present product. However, Lab and Lac populations remained at optimal levels after all storage conditions tested, and for all dairy bases tested.

Despite high LAB viability at 24 h, domestic cross-contamination remains a risk in high-protein media lacking a final cooking step. Caregivers should follow strict hygiene, especially for blending equipment (U.S. Food and Drug Administration, 2023; Food Standards Agency, n.d.). To ensure safety, drinks must be kept in closed containers at pH $< 4.6$, utilizing the synergy between acidity and competitive inhibition against opportunistic pathogens in non-sterile settings. Following the '4-hour rule' for cumulative time outside refrigeration, labelling prepared batches, and discarding leftovers are essential practices to minimize risks during advance preparation (European Food Safety Authority, 2025; Food Standards Australia New Zealand, 2025).

### 3.4. pH and color parameters

It is important to highlight that according to previous studies, pH is a parameter that has a significant impact on the quality of fermented products (Aktas et al., 2023). Fig. 4A shows the evolution of the pH of the samples as a function of the storage time. It was observed that a significant difference in pH between CP and the developed drinks storage conditions in each drink ($p < 0.05$). The pH values of CPs were below 4.53, being higher in freshly prepared samples Y0h, K0h, FW0h, BY0h, and QU0h (5.72, 5.62, 5.49, 5.43, and 5.35, respectively), likely due to the addition of milk. Overall, pH decreased significantly during storage at 20°C. After 24 h, the reduction of pH compared to freshly ones was 0.69, 0.61, 0.27, 0.18, and 0.11 in K24h20C, Y24h20C, BY24h20C, FW24h20C, and QU24h20C, respectively. This tendency was in accordance with the variation of microbial load explained in the above section (Fig. 3). The pH of fermented products generally remains stable or decreases during storage because lactic acid bacteria convert lactose into lactic acid, which leads to a further decrease, influencing the texture, flavour, and shelf life of the product (Meral-Aktaș et al., 2025a). Nevertheless, refrigerated samples exhibited a slight increase in pH compared to freshly prepared drinks. This may be due to the buffering activity of dairy products, especially milk and fermented milks, which is related to the sum of the individual activities of different acid-base groups in substances such as inorganic phosphate, citrate, organic acids, amino acids, and milk proteins (caseins and whey proteins) (Walstra and Jenness, 1984; Wang et al., 2023).

Color is a key factor in food quality, strongly influencing consumer acceptability (Meral-Aktas et al., 2025a). Fig. 4B, C, and 4D represent the CIEL*a*b* coordinates for the drinks, corresponding to lightness (L*), redness (a*), and yellowness (b*), respectively. The incorporation of whey protein (WPC) significantly reduced the L* parameter, leading to lower values in all freshly prepared drinks compared to CP. Y-, BY-, and K-drinks followed the same trend throughout storage, with a significant increase in L* observed at 24h20C. However, after 24h4C, a significant decrease in L* was observed for all drinks, probably due to changes in particle size distribution as the powder was integrated into the matrix (Coggins et al., 2010). L* is related to the particle size of fat and protein globules, which affects their reflectance and light scattering capacity (Grasso et al., 2020). The addition of whey protein significantly increased the a* coordinate, although it did not reach red in all cases (Fig. 4C). Likewise, the b* coordinate showed the same behavior, with a significant increase observed in all drinks. These color changes can be attributed to the intrinsic yellowish tone of whey and the formation of Maillard reaction products during drying, which promote browning and reduce lightness (Milovanovic et al., 2020).

### 3.5. Organic acids and sugars identified and quantified by HPLC

Table 2 presents the organic acid and sugar content of the developed drinks, as well as their changes under the evaluated storage conditions (see Table 3S for the organic acid and sugar content of the individual ingredients). The following organic acids were identified in the drinks: lactic, acetic, and citric acids in all CP and other drinks, whereas only citric acid was detected in whey protein (0.03 g/mL). The presence of acetic acid is generally associated with the heterofermentative pathway of lactose, produced for example, by bifidobacteria strains, and imparts a vinegary taste (Li et al., 2024). No significant differences were observed in acetic acid content over the storage period for any of the formulations ($p > 0.05$), which is consistent with the study by Vénica et al. (2025) on the production of functional protein yoghurts. Citric acid is a component of the tricarboxylic acid (TCA) cycle (Lu et al., 2018). No significant differences were found in citric acid content among the formulations, and our values agree with Barros et al. (2019), who reported values between 0.08 and 0.15 g/mL in yoghurt. Citric acid can also be found naturally in milk, and it was detected in the commercial milk analysed in the present study (see Table 3S) (Moiseenko et al., 2023). Regarding lactic acid, it represents the main organic acid in protein-fermented dairy drinks, which imparts their characteristic sour and refreshing flavour (Li et al., 2024). In general, lactic acid content in CP was higher than in freshly prepared drinks, consistent with the low pH values and microbial counts observed (Fig. 3 and 4). This observation may also be partly attributed to a dilution effect due to the incorporation of milk. Notably, the sample exhibiting the highest lactic acid concentration did not correspond to the sample perceived as the sourest in the sensory analysis (Fig. 2). Among the samples, K24h20C, Y24h20C, and BY24h20C exhibited the highest lactic acid concentrations throughout 24h20C storage (0.83, 0.70, and 0.69 g/ mL, respectively).

In addition to organic acids, sugar content was also evaluated to determine whether there were any metabolic changes during storage.

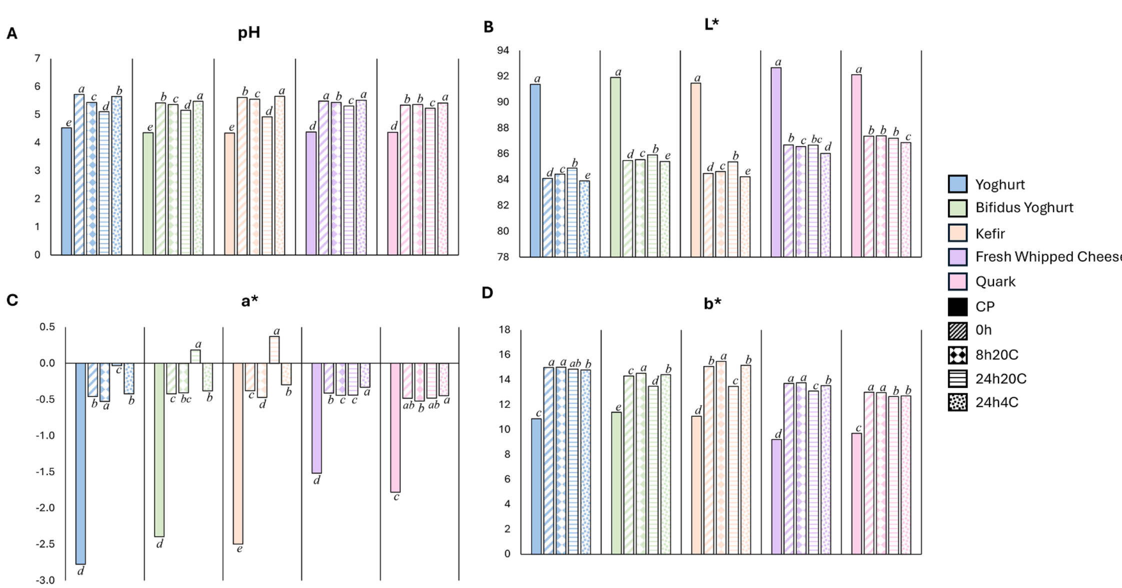


**Fig. 4.** pH (A) and color coordinates L* (B), a* (C), and b* (D) of protein-fortified fermented dairy drinks stored under different conditions: CP (commercial products), 0h (freshly prepared sample), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C) and 24h4C (24 h at 4 °C). Values followed by a different letter within the same column are significantly different ($p < 0.05$) for the Tukey test. Y: Yoghurt; BY: Bifidus Yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark.

**Table 3**
Essential free amino acids content (mg/100 g) of protein-fortified fermented dairy drinks.

| Codification | Ile | Leu | Lys | Phe | Thr | Trp | Val | Total |
|---|---|---|---|---|---|---|---|---|
| Y0h | 204.63 ± 21.52 ab | 218.88 ± 56.94 b | 32.71 ± 22.20 a | 471.75 ± 69.344 b | 30.43 ± 19.11 a | 75.28 ± 18.35 a | 92.68 ± 32.16 a | 1135.06 ± 227.12 a |
| Y8h20C | 174.79 ± 24.77 b | 266.36 ± 41.21 b | 27.55 ± 11.96 a | 477.47 ± 69.52 b | 31.92 ± 19.44 a | 80.27 ± 17.57 a | 85.95 ± 23.66 a | 1152.10 ± 202.96 a |
| Y24h20C | 170.18 ± 14.83 b | 372.53 ± 17.28 a | 13.25 ± 6.86 a | 599.89 ± 19.75 a | 30.15 ± 21.27 a | 87.95 ± 7.43 a | 127.37 ± 23.78 a | 1408.82 ± 49.48 a |
| Y24h4C | 217.86 ± 24.72 a | 297.98 ± 88.49 ab | 17.30 ± 10.07 a | 440.52 ± 82.99 b | 26.69 ± 19.35 a | 78.92 ± 9.73 a | 84.13 ± 18.34 a | 1171.55 ± 49.70 a |
| BY0h | 217.11 ± 36.65 a | 493.09 ± 53.67 a | 20.48 ± 8.65 a | 275.59 ± 23.93 a | 32.10 ± 20.94 a | 96.01 ± 5.13 b | 94.52 ± 10.42 ab | 1238.54 ± 87.15 a |
| BY8h20C | 173.92 ± 17.95 b | 405.50 ± 22.14 b | 17.41 ± 8.73 a | 336.23 ± 150.41 a | 26.15 ± 18.04 a | 89.92 ± 3.10 b | 88.58 ± 20.72 b | 1146.15 ± 160.52 a |
| BY24h20C | 168.32 ± 9.73 bc | 471.10 ± 36.87 ab | 27.26 ± 1.39 a | 254.66 ± 5.89 a | 53.14 ± 3.73 a | 111.39 ± 2.42 a | 123.02 ± 11.34 a | 1217.42 ± 58.28 a |
| BY24h4C | 117.54 ± 4.57 c | 276.39 ± 9.20 c | 18.53 ± 1.48 a | 168.43 ± 10.21 a | 36.89 ± 1.28 a | 67.45 ± 2.86 c | 70.37 ± 6.08 b | 763.86 ± 32.57 b |
| K0h | 38.90 ± 4.53 c | 97.67 ± 11.23 c | 5.14 ± 0.93 a | 390.15 ± 7.62 b | 13.06 ± 0.51 b | 30.46 ± 1.32 b | 25.54 ± 1.54 b | 606.28 ± 24.81 c |
| K8h20C | 82.05 ± 6.55 b | 198.91 ± 10.71 b | 3.47 ± 0.26 a | 447.29 ± 18.12 a | 14.07 ± 1.82 b | 40.79 ± 1.58 a | 44.16 ± 5.95 b | 836.94 ± 40.23 b |
| K24h20C | 128.08 ± 6.28 a | 458.09 ± 25.58 a | 3.88 ± 0.07 a | 390.50 ± 5.01 b | 78.12 ± 5.82 a | 20.91 ± 1.01 c | 262.41 ± 14.50 a | 1348.71 ± 52.22 a |
| K24h4C | 47.80 ± 5.25 c | 111.44 ± 11.35 c | 6.31 ± 3.65 a | 352.23 ± 36.91 b | 11.14 ± 0.97 b | 27.96 ± 2.79 b | 27.37 ± 3.15 b | 590.43 ± 61.02 c |
| FW0h | 67.29 ± 6.46 a | 185.71 ± 7.46 a | 3.47 ± 0.23 bc | 276.85 ± 14.54 bc | 20.23 ± 3.60 a | 47.40 ± 3.05 a | 82.88 ± 14.65 a | 693.24 ± 37.53 a |
| FW8h20C | 61.39 ± 10.28 a | 187.93 ± 23.75 a | 2.94 ± 0.61 c | 301.80 ± 19.78 ab | 22.04 ± 2.43 a | 47.99 ± 5.91 a | 76.54 ± 7.81 a | 710.86 ± 68.49 a |
| FW24h20C | 30.09 ± 0.13 b | 110.95 ± 0.01 b | 5.42 ± 0.05 a | 314.49 ± 1.08 a | 19.36 ± 0.27 a | 41.11 ± 0.12 a | 42.57 ± 0.57 b | 572.97 ± 1.06 b |
| FW24h4C | 55.75 ± 4.04 a | 160.44 ± 6.42 a | 3.76 ± 0.19 b | 251.71 ± 6.90 c | 18.16 ± 0.52 a | 42.07 ± 1.47 a | 71.09 ± 2.78 a | 612.04 ± 20.15 ab |
| QU0h | 173.63 ± 6.54 a | 296.22 ± 9.64 a | 10.14 ± 2.39 b | 303.42 ± 12.91 a | 33.24 ± 1.88 a | 53.26 ± 2.19 b | 135.75 ± 5.69 a | 1016.44 ± 40.24 a |
| QU8h20C | 162.84 ± 5.01 a | 286.25 ± 11.01 a | 20.66 ± 2.13 a | 303.02 ± 16.04 a | 29.60 ± 2.80 ab | 55.15 ± 2.74 b | 124.48 ± 5.49 a | 992.84 ± 44.31 a |
| QU24h20C | 167.13 ± 13.47 a | 303.89 ± 24.14 a | 24.94 ± 4.00 a | 329.26 ± 26.11 a | 29.82 ± 3.73 ab | 62.63 ± 3.29 a | 126.81 ± 12.48 a | 1055.92 ± 85.93 a |
| QU24h4C | 181.56 ± 10.07 a | 313.50 ± 19.54 a | 11.17 ± 1.40 b | 314.19 ± 20.83 a | 23.36 ± 3.61 b | 57.79 ± 3.42 ab | 146.00 ± 11.65 a | 1060.51 ± 68.92 a |

Values followed by a different letter within the same column and sample are significantly different ($p < 0.05$) for the Tukey test. 0h (freshly prepared sample), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C) and 24h4C (24 h at 4 °C). Y: Yoghurt; BY: Bifidus yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark. Ile: isoleucine; Leu: leucine; Lys: lysine; Phe: phenylalanine; Thr: threonine; Trp: tryptophan; Val: valine.

Lactose, a disaccharide composed of α/β-D-glucose and β-D-galactose linked by a β (1→4) glycosidic bond, is the main carbon source in milk (Iskandar et al., 2019). As the primary fermentation substrate, it underwent gradual degradation during storage in all drinks, indicating ongoing bacterial metabolism. This behaviour was consistent with the observed microbial counts, the progressive increase in lactic acid concentration, and the associated decrease in pH (Table 2, Fig. 3, and Fig. 4A). In BY24h4C, a more pronounced decrease in lactose content was detected, possibly related to yeast metabolism at this temperature, which is consistent with the simultaneous increase in lactic acid, indicating a coherent metabolic activity during this period. Regarding glucose, it was undetectable in all CP. Glucose also remained at extremely low levels (0.05–0.17 g/mL) under all storage conditions and for all drinks, indicating that fermentative bacteria rapidly consumed it at the beginning of storage. In K-, FW-, and QU-drinks, glucose levels were also below the detection limit, except for QU24h20C, where a slight increase was observed at 24h20C, likely due to microbial activity. As to galactose, CP showed higher contents compared to the freshly prepared drinks, attributed to a dilution effect. A significant increase in galactose concentration was observed during storage, reaching the highest values at 24h20C in all drinks except for BY-drinks, which showed no significant differences. This behaviour can be attributed to microbial metabolism, as lactose is hydrolysed into glucose and galactose, the latter being less efficiently utilised by microorganisms since not all lactic acid bacteria can metabolise galactose from milk (Iskandar et al., 2019). In fact, although some strains of *Streptococcus thermophilus* possess an active Leloir pathway for galactose utilisation, it is generally accepted that most strains of this species only partially ferment lactose. Consequently, galactose tends to accumulate in the medium as a residual sugar excreted by *S. thermophilus* (Wu et al., 2015). Similarly, glycerol concentrations increased over time in all formulations, with significant rises observed in Y-, FW-, and QU-drinks after 24 h at 20 °C.

In summary, it can be concluded that the different microbial populations in each product determine the fermentation rate and sugar metabolism, leading to variations in lactose content and the release of glucose, galactose, and acids into the medium during storage.

### 3.6. Free amino acids

Fifteen major free amino acids (FAAs) identified in the protein-fortified drinks were categorised into essential (EAA) (Table 3) and non-essential (NEAA) (Table 4) groups. Complementary data regarding the FAA composition of the individual ingredients is provided in Table S4. Regarding milk, it exhibited a baseline FAA profile dominated by leucine, isoleucine and phenylalanine among the EAA, and asparagine, cysteine, and proline among the NEAAs. Notably, the low levels of free lysine observed in M, are likely due to the industrial thermal treatments. According to Li et al. (2021) and Rabbani et al. (2025), heat-induced Maillard reactions between the ε-amino group of lysine and lactose cause 'lysine blockage', reducing both protein bioavailability and digestibility. Total FAA concentrations in the milk were considerably lower than those found in the fermented CP. Baseline analysis of whey protein (WPC) revealed minimal FAA levels (5.93 mg/100 g for EAAs and 48.12 mg/100 g for NEAAs), confirming the non-hydrolyzed nature of the fortifier; thus, any subsequent FAAs increase in the formulated drinks is attributable to microbial proteolytic activity rather than the fortifier itself. In contrast, fermented CP already exhibited notable FAA levels prior to fortification, due to proteolysis occurred during processing. Among these, K0h exhibited the lowest initial FAA content, consistent with Irigoyen et al. (2012), who reported lower baseline FAA levels in kefir compared to other fermented milks. FW0h presented the highest initial concentration of NEAA, particularly proline

**Table 4**
Non-essential free amino acids content (mg/100 g) of protein-fortified fermented dairy drinks.

| Codification | Ala | Asn | Cys | Gln | Glu | Pro | Ser | Tyr | Total |
|---|---|---|---|---|---|---|---|---|---|
| Y0h | 40.88 ± 20.55 a | 82.25 ± 36.01 a | 37.43 ± 3.10 a | 32.08 ± 22.36 a | 42.78 ± 24.62 a | 96.24 ± 8.59 ab | 9.17 ± 3.45 a | 143.72 ± 30.20 a | 499.98 ± 127.82 a |
| Y8h20C | 56.64 ± 25.06 a | 68.77 ± 40.68 a | 36.57 ± 3.31 a | 26.32 ± 12.21 a | 42.69 ± 22.95 a | 86.41 ± 9.18 b | 10.62 ± 3.24 a | 131.28 ± 30.22 a | 475.00 ± 135.41 a |
| Y24h20C | 55.14 ± 32.39 a | 69.79 ± 17.60 a | 34.48 ± 1.73 a | 13.10 ± 6.40 a | 35.12 ± 23.94 a | 114.08 ± 21.08 a | 12.60 ± 4.56 a | 166.16 ± 16.16 a | 512.00 ± 78.41 a |
| Y24h4C | 54.08 ± 46.98 a | 86.78 ± 25.36 a | 37.27 ± 2.94 a | 16.82 ± 9.62 a | 38.53 ± 26.73 a | 121.37 ± 18.03 a | 8.94 ± 5.57 a | 144.08 ± 14.60 a | 524.07 ± 87.64 a |
| BY0h | 74.31 ± 37.81 b | 90.48 ± 28.57 a | 39.38 ± 1.28 a | 20.35 ± 8.15 a | 43.14 ± 27.81 a | 158.11 ± 27.23 a | 11.29 ± 3.72 b | 105.19 ± 9.11 a | 562.04 ± 79.65 ab |
| BY8h20C | 58.01 ± 32.25 b | 79.35 ± 20.99 a | 35.19 ± 1.60 b | 16.94 ± 8.74 a | 33.66 ± 22.60 a | 154.32 ± 31.61 a | 11.44 ± 4.95 b | 117.54 ± 30.43 a | 522.59 ± 72.69 b |
| BY24h20C | 138.91 ± 6.52 a | 99.29 ± 7.52 a | 36.84 ± 1.61 ab | 27.04 ± 1.31 a | 69.03 ± 6.31 a | 139.45 ± 4.01 a | 22.37 ± 1.88 a | 123.30 ± 8.81 a | 687.16 ± 19.34 a |
| BY24h4C | 92.03 ± 7.05 ab | 117.24 ± 4.61 a | 38.99 ± 1.22 a | 18.24 ± 1.91 a | 54.47 ± 4.09 a | 133.90 ± 7.26 a | 9.97 ± 0.60 b | 50.06 ± 4.50 b | 545.80 ± 24.60 ab |
| K0h | 29.70 ± 1.36 b | 95.74 ± 3.75 a | 36.38 ± 0.76 a | 4.95 ± 0.93 a | 43.81 ± 0.79 ab | 120.41± 3.21 b | 4.05 ± 0.26 b | 93.60 ± 2.40 c | 451.15 ± 8.52 b |
| K8h20C | 37.84 ± 4.91 b | 78.31 ± 5.23 b | 32.99 ± 2.81 a | 3.45 ± 0.21 a | 43.11 ± 2.03 ab | 130.05 ± 6.77 b | 7.11 ± 0.58 a | 121.58 ± 5.31 b | 474.46 ± 23.97 b |
| K24h20C | 64.07 ± 1.88 a | 78.91 ± 2.19 ab | 38.70 ± 1.65 a | 3.86 ± 0.16 a | 49.48 ± 6.36 a | 177.02 ± 6.20 a | 8.07 ± 0.24 a | 160.77 ± 6.51 a | 610.55 ± 20.27 a |
| K24h4C | 28.41 ± 6.10 b | 81.51 ± 12.40 ab | 34.46 ± 3.66 a | 6.32 ± 3.70 a | 37.89 ± 4.40 b | 129.80 ± 5.44 b | 4.25 ± 0.45 b | 86.73 ± 1.77 c | 431.30 ± 31.72 b |
| FW0h | 33.02 ± 6.47 a | 88.01 ± 8.18 a | 35.82 ± 4.72 a | 3.35 ± 0.19 b | 56.15 ± 10.35 a | 202.13 ± 16.42 a | 6.54 ± 1.14 b | 211.82 ± 22.32 a | 663.16 ± 69.25 a |
| FW8h20C | 35.47 ± 4.84 a | 83.00 ± 5.81 a | 37.29 ± 2.53 a | 3.00 ± 0.72 b | 58.64 ± 7.91 a | 214.10 ± 11.56 a | 8.71 ± 1.77 ab | 210.60 ± 20.06 a | 675.15 ± 43.69 a |
| FW24h20C | 32.45 ± 0.44 a | 79.46 ± 1.24 a | 36.06 ± 0.07 a | 5.01 ± 0.07 a | 50.54 ± 0.22 a | 210.30 ± 0.22 a | 9.13 ± 0.05 a | 206.20 ± 3.22 a | 647.13 ± 1.45 a |
| FW24h4C | 26.85 ± 0.77 a | 78.21 ± 2.63 a | 33.99 ± 1.77 a | 3.32 ± 0.23 b | 43.05 ± 1.19 a | 209.14 ± 4.14 a | 6.35 ± 0.10 b | 180.64 ± 6.63 a | 604.96 ± 14.50 a |
| QU0h | 45.39 ± 2.42 a | 92.20 ± 5.11 a | 38.71 ± 2.48 a | 10.35 ± 2.58 b | 13.08 ± 1.22 a | 139.29 ± 12.14 b | 11.10 ± 0.92 ab | 156.86 ± 13.24 a | 525.34 ± 38.58 a |
| QU8h20C | 43.37 ± 4.21 ab | 86.62 ± 5.32 a | 34.23 ± 2.70 a | 21.27 ± 1.73 a | 9.46 ± 0.41 b | 140.09 ± 7.87 b | 11.36 ± 0.98 ab | 164.92 ± 11.07 a | 526.75 ± 32.91 a |
| QU24h20C | 41.25 ± 7.85 ab | 77.16 ± 11.02 a | 35.70 ± 4.47 a | 24.31 ± 3.62 a | 5.83 ± 1.01 c | 153.07 ± 11.49 ab | 13.89 ± 1.60 a | 186.41 ± 16.72 a | 552.53 ± 52.25 a |
| QU24h4C | 32.27 ± 3.52 b | 74.71 ± 8.43 a | 35.00 ± 3.95 a | 10.44 ± 1.43 b | 8.13 ± 1.29 bc | 179.41 ± 11.70 a | 10.41 ± 1.23 b | 175.08 ± 9.63 a | 543.13 ± 37.89 a |

Values followed by a different letter within the same column and sample are significantly different ($p < 0.05$) for the Tukey test. 0h (freshly prepared sample), 8h20C (8 h at 20 °C), 24h20C (24 h at 20 °C) and 24h4C (24 h at 4 °C). Y: Yoghurt; BY: Bifidus yoghurt; K: Kefir; FW: Fresh whipped cheese; QU: Quark. Ala: alanine; Asn: asparagine; Cys: cysteine; Gln: glutamine; Glu: glutamic acid; Pro: proline; Ser: serine; Tyr: tyrosine.

and tyrosine, while QU0h and BY0h were the main sources of EAA, such as leucine and phenylalanine. Across all formulated drinks, the FAA profile was dominated by isoleucine, leucine, and phenylalanine for the EAA, and asparagine, proline, and tyrosine for NEAA. This distribution aligns with the amino acid composition of dairy caseins and whey proteins (Fox et al., 2015) and is consistent with Gu et al. (2021), who identified proline as a predominant FAA due to its key role in the stress response of lactic acid bacteria and its limited catabolism during fermentation. The high prevalence of branched-chain amino acids (BCAAs: isoleucine, leucine, and valine), especially leucine, is of significant clinical interest for the elderly. Leucine acts as a key signaling molecule for muscle protein synthesis to counter sarcopenia (Deutz et al., 2014). However, no health claims for BCAAs are currently authorised by the European Commission due to insufficient evidence (European Commission, 2012). Conversely, lysine and serine were the minority EAA and NEAA fractions. Low lysine levels stem from thermal treatments, while minimal serine reflects low baseline levels. FAAs remained generally stable during refrigerated storage (24h4C), except for BY-drinks, which showed a significant decrease in EAAs after 24 h. This decline is potentially attributed to the metabolic activity of the present yeasts, which consume free amino acids as a nitrogen source during growth (Fox et al., 2015; Zhumakadyrova & Mazhitova, 2024). Following storage at 24h20C, a general increase in FAA levels was observed, indicating stimulated proteolytic activity by LAB, consistent with results by Gu et al. (2021). However, K-drinks were the only matrix to exhibit a significant increase in both EAA and NEAA fractions during storage at 24h20C, notably doubling their EAA content. This pronounced proteolytic activity is consistent with the previously described data. Specifically, the observed FAAs accumulation is consistent with the observed increase in microbial counts, a pronounced pH drop, and high lactic acid production, alongside substrate depletion (Fig. 3, Fig. 4A, and Table 2). These findings suggest that the mesophilic consortia in kefir reach peak enzymatic efficiency at 24h20C. This acceleration in FAA formation may also be attributed to the autolysis of LAB, which releases intracellular peptidases into the matrix, further stimulating proteolysis (Gu et al., 2021). In contrast, FW-drinks showed a significant reduction in EAAs at 24h20C. This suggests that microbial consumption outweighed proteolytic release, a competition described by Gu et al. (2021) and Nielsen et al. (2022) where FAA levels decrease if metabolic requirements exceed their rate of release. Conversely, Y, BY, and QU-drinks remained the most stable matrices throughout the study. Beyond their biochemical significance, these FAA profiles also support the sensory differences observed in the PCA (Fig. 2). As noted by Zhumakadyrova & Mazhitova (2024), specific amino acids act as 'taste-active' molecules, where their balance and concentration directly influence the sweetness, sourness, and aftertaste persistence identified in our drinks. Therefore, future research should further explore the evolution of the FAA profile during storage and its specific contribution to the development of taste and off-flavors in these fermented drinks. Ultimately, these findings underscore that the final FAA profile—and consequently the nutritional quality—is a result of a dynamic metabolic interplay where the specific proteolytic efficiency of each microbial consortium dictates the final product characteristics.

## 4. Conclusions

All dairy bases produced protein-fortified drinks without off-flavors, showing sensory profiles adaptable to different preferences. LAB populations were maintained in all dairy blends assayed, as well as under the different storage conditions. The results indicated that QU and K promote greater microbial viability, and K promotes more intense acidification during storage at 24h20C, which may benefit certain functional properties such as maintaining the balance of the intestinal microbiota, but may also compromise sensory stability if not properly controlled. Changes in pH, color, and metabolites (lactic acid, glucose, galactose) indicate that fermentation and storage modify the matrix, which could affect its quality and sensory perception. However, these moderate transformations allow a 24-h domestic shelf life. Furthermore, the enhanced proteolytic activity observed during storage, especially in kefir-drinks, doubled the EAAs. Further studies are needed to optimise home-made formulations and evaluate their stability during refrigerated and room temperature storage, paying particular attention to safety and quality, as well as consumer studies to test their acceptability among people over 65 years of age.

## 5. Limitations

This study was conducted at a laboratory scale, which may limit the extrapolation of the results to real domestic conditions.

Sensory evaluation was based on descriptive analysis rather than consumer testing, so actual acceptance among the target population remains to be confirmed.

Only a limited number of dairy bases were tested, which restricts the generalization of the findings to other formulations or raw materials, such as fresh fruits or cereal addition.

## Ethical Statement

**Statement for studies in humans:** The sensory evaluation presented in this study was performed by an internal panel of researchers for product characterization purposes. All ingredients used in the formulations were food-grade and safe for human consumption. The evaluation was conducted under strict hygienic conditions in a controlled laboratory environment. No external human participants or consumers were recruited for this study; therefore, formal institutional ethical approval was not required.

**Statement for studies in animals:** The authors declare that this study did not involve any experiments with intermediate or higher animal species; therefore, no animal ethical approval was required.

## CRediT authorship contribution statement

**Marta Rodríguez-Soriano:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Ana M. Solivella-Poveda:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Ana B. Ropero:** Writing – review & editing, Conceptualization. **Marta Beltrá:** Writing – review & editing, Conceptualization. **Marina Cano-Lamadrid:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Conceptualization. **Esther Sendra:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.102202.

## Data availability

Data will be made available on request.

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