EVALUATION OF FOOD SAFETY IN A WHEY-BASED FERMENTED PRODUCT

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Abstract
Although long considered a by-product of cheesemaking, whey has been proven by this study to be a resource with high nutritional and functional value. Its utilization in the form of a fermented beverage, plain or enriched with elderberry extract, has been confirmed as an efficient strategy both technologically and sensory-wise. The study confirmed the potential of acid whey as a substrate for functional fermented beverages, with physicochemical values showing a low pH (4.45 ± 0.00), high titratable acidity and a nutritional composition characterized by 1.02% protein, 3.74% lactose and 0.72% ash. Microbiological analyses showed a low microbial load (NTG ~2 × 10^4 UFC/mL), below the limit set by EC Regulation no. 1441/2007, and the absence of E. coli and coliform bacteria colonies confirmed the safety of the sample. From a sensory point of view, the variants with the addition of elderberry extract were the best appreciated, due to their balanced taste, natural aroma and pleasant appearance. The results obtained indicate that fermented whey beverages can be classified as safe, nutritious and attractive products, with potential for use in the functional food industry and within the circular economy.

Keywords: whey, fermented product, food safety

Functional foods and beverages constitute a highly inventive and dynamic sector within the food industry, attracting increasing consumer attention due to their health benefits that extend beyond basic nutrition (Corbo *et al.,* 2014).

As consumers become increasingly aware of the special characteristics and, above all, the health benefits of fermented milks, the dairy industry is challenged to manage the large-scale production of these products in a sustainable way, without compromising the technological and functional qualities that define the traditional products from which they are derived. Whey and its constituents are increasingly used as functional ingredients in foods and nutritional goods, while bioactive proteins are facing increased demand in the pharmaceutical and food sectors (Jelicic, 2008). To date, research has focused on the formulation of sweet-sour whey beverages, whether native or powdered, delactosized or deproteinized (Skryplonek *et al.,* 2019).

As the dairy industry continues to develop and produce large volumes of whey, its intelligent valorization becomes increasingly important. Beyond the challenges related to its management, whey offers considerable potential in the development of functional food products, including fermented whey-based beverages. These have gained increased interest due to their nutritional properties, their ability to support the development of a probiotic microbiota and their technological versatility.

Whey is the liquid component, known as cheese whey, which is derived from the separation of the curd during milk coagulation by enzymes or proteolytic acids. (Buchanan *et al.,* 2023). Approximately 80–90% of milk processed in cheese factories is converted into whey, resulting in an estimated global production of 180–190 million tonnes of waste, of which 100 million tonnes is whey annually (Chandrapala *et al.* 2015; Flinois *et al.,* 2019). Currently, the reuse rate of whey in Europe exceeds 75%, but in other regions of the world it is only 50%, highlighting the waste of a product with high nutritional value.

Whey can be used both as a functional ingredient in food and as animal feed (Macwan *et al.,* 2016). Fermented foods, obtained by the controlled growth of microorganisms and enzymatic conversion of food components, represent one of the main sources of food globally, contributing to approximately one third of the human diet (Terpou *et al.,* 2025).

The fermentation of whey with lactic acid bacteria modifies its structural and biochemical profile, leading to the generation of bioactive peptides with functional roles in immunity, oxidative balance, gut microbiota composition, and

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the regulation of hypertension and inflammation (Marco *et al.,* 2017). Fermented whey beverages represent complex microbial ecosystems shaped by the lactic substrate, processing conditions, and the inoculated microorganisms. Unprocessed whey retains a diverse microbiota originating from both the raw milk and the processing environment, including lactic acid bacteria, wild yeasts, micrococci, and occasionally coliforms or bacterial spores (Pescuma *et al.,* 2010). Modern fermentation technologies address this by combining pasteurisation with selective inoculation, resulting in a controlled microbial community dominated by beneficial bacteria.

The research aims to validate the functional potential of the beverage, in line with current trends in the food industry, focusing specifically on microbiological stability and the ability to provide a safe, viable food adapted to the needs of responsible and modern consumption.

MATERIAL AND METHOD

In this study, both the raw material whey and the fermented beverage obtained as follows were analyzed: a control sample of whey without the addition of elderberry syrup and two samples of fermented whey, obtained by adding elderberry syrup in quantities of 5 mL and, respectively, 10 mL. The investigations included both physicochemical and microbiological analyses.

Microbiological and physicochemical determinations were performed on the whey used as raw material. The physicochemical determinations focused on parameters such as density, pH, titratable acidity, volatile acidity, alcoholic strength, sugar content and dry matter content.

The microbiological analyses aimed to quantify the total number of bacteria, as well as to identify and enumerate coliform bacteria and the species *Escherichia coli.*

The total bacterial count in the control whey and in the fermented beverages was determined by the serial dilution method, based on the identification of viable mesophilic aerobic bacteria capable of developing on specific culture media at temperatures of 30–37 °C. Additionally, simple Gram staining was performed to confirm cellular morphology.

The determination of coliform bacteria and E. coli was carried out using the chromogenic medium RAPID'E. coli 2 Agar (Bio-Rad). The medium was prepared by suspending 37 g of powder/L in 1000 mL of distilled water, followed by sterilisation at 121 °C for 15 minutes in an autoclave. After cooling, the medium was poured into sterile Petri dishes and used for the enumeration of total coliforms and *E. coli* in the analysed samples.

Figure 1. **Sampling and performing successive dilutions**

Figure 2. **Appearance during inoculation by the Spread Plate Technique**

The physico-chemical analyses were performed using standard analytical procedures, in compliance with the relevant regulations and methodological guidelines established by the Association of Official Analytical Chemists (AOAC, 2010) and (Gavril *et al.* 2024).

The technological process carried out within the dairy microproduction workshop began with the collection of fresh whey, followed by its defatting through the separation of fat using a centrifugal separator. The raw material was subsequently subjected to pasteurisation and an additional centrifugation step to ensure the complete removal of lipids, in accordance with the objective of obtaining a fat-free final product.

Figure 3
pH determination

Figure 4
Creamed whey

Next, the whey was heated to approximately 30 °c to provide optimal conditions for the inoculation of selected yeasts. Following this thermal treatment, the whey was gradually cooled to room temperature and left to rest overnight, an essential step for the complete sedimentation of protein fractions, which were subsequently removed by filtration through sterile cheesecloth.

To adjust the flavour profile and improve fermentability, an invert sugar syrup (50%) was added. The composition of this syrup included 20% water, while sugar and citric acid accounted for 2% of the total whey–sugar mass, with the proportions measured precisely.

Primary fermentation was initiated by inoculating the mixture with 6.3 g of active culture of *Saccharomyces cerevisiae bayanus* per 9 l of whey. The yeast was activated in 100 ml of syrup at 26 °c to prevent thermal inactivation, thereby obtaining an active starter culture distributed uniformly throughout the whey. The inoculum was prepared by adding 1% sugar to a volume of

filtered whey, followed by incubation at 25 °c for 48 hours.

The inoculated mixture was fermented at 22 °c for 14 days, during which the yeast partially metabolised the available sugars, generating carbon dioxide and aromatic compounds. At the end of primary fermentation, the beverage was bottled, leaving a headspace of approximately 5% for gas accumulation, and sealed with crown cork caps.

Figure 5 **The primary fermentation process**

Secondary fermentation, champagne-type (in bottle), was performed by adding two different concentrations of elderberry syrup, adjusted according to the previously determined sugar content, so that the final product did not exceed 17 g/l of sugars. Specifically, 5 ml (z5) and 10 ml (z6) of diluted elderberry syrup were added, while the control sample (z4) contained no syrup. The yeast was reactivated (0.66 g for the remaining whey volume) under the same rehydration conditions.

In total, nine bottles of fermented beverage were obtained: three with 5 ml elderberry syrup, three with 10 ml, and three control bottles (yeast only). These samples were subsequently subjected to physico-chemical, microbiological, and sensory analyses in order to achieve a comprehensive characterisation of the final product.Carbonation of the beverage was achieved naturally, through the fermentation of residual sugars by yeast. The samples were coded as follows: *Z1* – raw whey, *Z2* – inoculated whey, *Z3* – post-primary fermentation whey, *Z4* – control sample (without elderberry syrup), *Z5* – whey with 5 mL elderberry syrup, *Z6* – whey with 10 mL elderberry syrup

Figure 6 **The fermented beverage samples**

The physico-chemical examinations of the fermented beverage were carried out in the Oenology Laboratory to establish key quality indicators. Analyses were conducted in accordance with OIV standards and national regulations.

Determination of alcohol content- Alcohol strength, expressed as % v/v at 20 °C, quantifies the ethanol concentration. The method involves neutralisation with calcium hydroxide, distillation using the Dujardin-Salleron apparatus, and measurement of the distillate with an alcoholmeter.

Determination of density-Density was assessed with a hydrostatic balance based on Archimedes' principle, representing the ratio of mass to volume of the beverage at 20 °C, expressed in g/cm³.

Determination of titratable acidity- Acidity was determined by titration with standardised NaOH, using bromothymol blue as indicator after CO_2 removal. The endpoint corresponds to a stable blue-green hue, in line with STAS 6128/1-79.

Determination of volatile acidity- Volatile acidity, mainly from acetic acid and its salts, was quantified by distillation of the sample acidified with tartaric acid, followed by titration of the distillate with 0.1 N NaOH using phenolphthalein as indicator (STAS 6182/2-86).

Determination of reducing sugars- Reducing sugars (glucose, fructose, lactose, maltose, invert sugar) were evaluated refractometrically, by measuring the refractive index of the solution.

The colour analysis of the fermented beverage samples was carried out using a Konica Minolta Chroma Meter CR-410, applying a D50 illuminant, a 2° standard observer, and the CIE L*, a*, b* colour space parameters were analysed following the procedure described by Dag *et al.* (2017).

Determination of sensory quality- Sensory evaluation was conducted after 24 h of cold stabilisation by a trained panel of 15 assessors, in accordance with ISO 22935-1 and ISO 8586-1 standards.

RESULTS AND DISCUSSIONS

In order to evaluate the quality of whey, a series of physico-chemical parameters were analysed. The pH was 4.45 ± 0.00, indicating the pronounced acidity of acid whey, while density was 1.025 ± 0.02 g/cm³, consistent with fresh whey and suggesting a moderate level of lactose and salts with good digestibility.

Table 1

Physico-chemical parameters of whey

Parameters	Value
pH	4.45±0.00
Density g/cm^3	1.025±0.02
Water, %	93.51±0.00
Non-fat dry matter, %	6.47±0.06
Dry matter %	6.48±0.05
Fat, %	0.01±0.01
Protein, %	1.02±0.02
Ash, %	0.72±0.00
Lactose	3.74±0.00
Titrable acidity (g/L acid lactic)	10.60±0.05

Water content reached 93.51 ± 0.00%, underlining the aqueous nature of whey and the need for preservation methods such as pasteurisation or fermentation. Total dry matter was 6.48 ± 0.05%, very close to the fat-free dry matter of 6.47 ± 0.06%, showing negligible fat content. Indeed, fat accounted for only 0.01 ±

0.01%, confirming its classification as skimmed whey, suitable for dietary applications.

Proteins were present at 1.02 ± 0.02%, mainly α-lactalbumin and β-lactoglobulin, known for their high biological value and digestibility. Ash content was 0.72 ± 0.00%, reflecting an adequate supply of essential minerals, while lactose, at 3.74 ± 0.00%, dominated the dry matter, contributing both to energy value and fermentation potential.

Titratable acidity averaged 10.6 ± 0.05 g lactic acid/L, reinforcing the acidic character and offering insight into freshness and fermentation status. Together, these findings define the specific profile of acid whey and highlight its potential as a valuable raw material for functional and fermented food production.

As shown in *table 2,* NTG values for the control whey (Z1) and yeast-inoculated sample (Z2) ranged between 16.63 × 10^3 and 21.68 × 10^3 CFU/mL, remaining well below the maximum permitted limit of 100,000 CFU/mL set by Regulation (EC) No. 1441/2007. After the onset of fermentation (Z3), NTG values stabilised at ~19 × 10^3 CFU/mL. With further fermentation (Z4–Z6), a sharp reduction was recorded, reaching minimum values of 3.5 × 10^2 CFU/mL. These results indicate effective process control and confirm the improved microbiological stability of the final fermented whey products.

Table 2

Results regarding the total number of germs in the analyzed samples

Sample	Sample	Dilution	NTG		Conclusion Reg. 1441/2007 of the Commission in 05/12/2007
			PCA	GPCA	
Z1	Whey control	10^{-1}	-	$21.68x10^3$	Satisfactorily
		10^{-2}	-	$19.99x10^3$	
Z2	Whey inoculated with yeast	10^{-1}	-	$20.59x10^3$	Satisfactorily
		10^{-2}	-	$16.63x10^3$	
Z3	Whey after first fermentation	10^{-1}	$19.05x10^3$	-	Satisfactorily
		10^{-2}	$18.95x10^3$	-	
Z4	Fermented whey	10^{-1}	$8.55x10^3$	-	Satisfactorily
		10^{-2}	$8.15x10^2$	-	
Z5	Fermented whey+5 ml syrup	10^{-1}	$8.25x10^2$	-	Satisfactorily
		10^{-2}	$8.10x10^2$	-	
Z6	Fermented whey+10 ml syrup	10^{-1}	$4.15x10^2$	-	Satisfactorily
		10^{-2}	$3.50x10^2$	-	

From a microbiological perspective, the use of a chromogenic medium ensured both sensitivity and specificity in detecting potential contaminants, while its application alongside standard decimal dilutions allowed for precise quantification. The results (Figure 5) showed a complete absence of *Escherichia coli* and coliforms across all samples (Z1–Z6), regardless of the technological stage. The control whey (Z1) exhibited no faecal contamination, confirming the quality of the raw material, and inoculation with yeast cultures (Z2) did not alter this profile. Similarly, the fermentation stages (Z3–Z6), including syrup addition, revealed no microbial risk.

These findings highlight the effectiveness of hygienic control throughout processing and confirm compliance with Regulation (EC) No. 2073/2005. The constant absence of coliforms and *E. coli* indicates that the fermented whey beverages meet safety standards and can be considered suitable functional products with minimal microbiological risk, supporting their integration into the category of health-promoting foods.

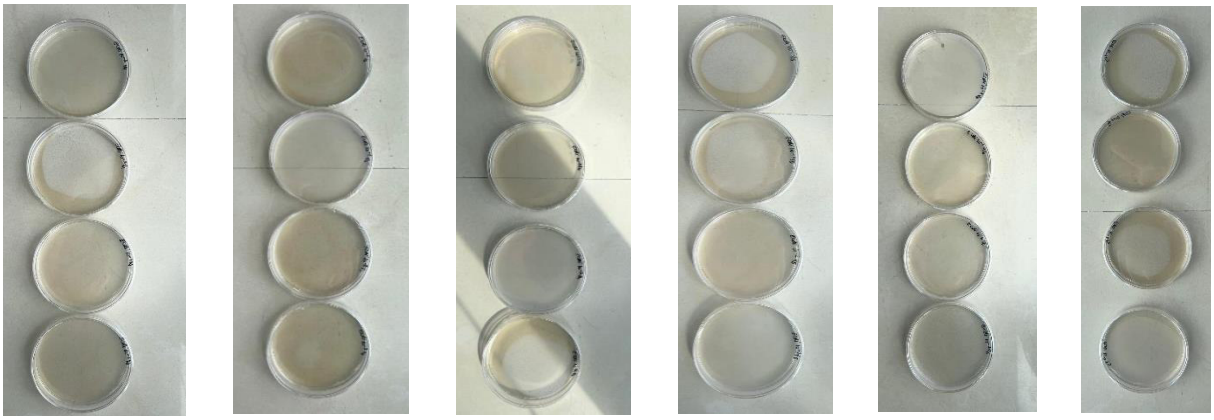

Figure 5 **Rapid *E-coli* medium inoculated with control whey (Z1), whey inoculated with yeast (Z2) and whey after the two fermentations (Z3,Z4,Z5,Z6) to highlight the possible presence of the *E. Coli* species. The results were negative (original photo)**

Table 3 presents the results obtained for the physico-chemical parameters of the fermented beverage with the addition of elderflower syrup. The density shows only minor variations, increasing gradually from 0.983 g/cm³ in Z4 to 0.991 g/cm³ in Z6, a change attributable to the incorporation of syrup, which contributes to higher dissolved solids and residual fermentation metabolites.

Table 3

Physico-chemical results of fermented whey beverage with added elderberry

Sample	pH	Density at 20 °C	Titratable acidity (g/L tartaric acid)	Volatile acidity (g/L acetic acid)	Alcohol concentration, %	Non-reducing sugars
Z4	4.00±0.01	0.98±0.03	8.50±0.01	0.62±0.01	2.30±0.04	5.50±0.5
Z5	3.79±0.1	0.98±0.2	8.80±0.12	0.57±0.02	3.30±0.06	4.19±0.01
Z6	3.8±0.12	0.99±0.1	9.10±0.3	0.50±0.04	3.80±0.01	3.50±0.07

Titratable acidity, expressed as g tartaric acid/L, increased slightly from 8.50 g/L in Z4 to 9.10 g/L in Z6, indicating intensified fermentative activity and the presence of organic acids derived from both fermentation and the acidic compounds of the syrup. Conversely, volatile acidity decreased with the increasing syrup concentration (0.62 g/L in Z4 to 0.50 g/L in Z6), suggesting a moderating effect of bioactive compounds from elderflower syrup on volatile acid production, particularly acetic acid. Alcohol concentration displayed a distinct upward trend, rising from 2.3% vol. in Z4 to 3.8% vol. in Z6, fully consistent with the additional fermentable carbohydrates supplied by the syrup. Non-reducing sugars decreased significantly from 5.5 g/L in Z4 to 3.5 g/L in Z6, confirming their consumption during alcoholic fermentation by Saccharomyces cerevisiae.

The pH decreased marginally from 4.0 (Z4) to 3.8 (Z6), indicating gradual acidification due to organic acid generation, while remaining within the acceptable range for fermented beverages and enhancing microbiological stability.

Overall, the incorporation of elderflower syrup positively influenced the fermentation process by increasing alcohol content and reducing residual sugars, while maintaining acid–base balance and ensuring the stability of the final product.

Table 4 presents the colour parameters (CIE L*, a*, b*) of fermented whey samples supplemented with elderflower syrup.

Table 4

Color parameter results

Sample	L*	a*(D65)	b*(D65)
Z4	66.21	-5.54	12.19
Z5	65.27	-5.33	11.20
Z6	66.38	-5.49	11.74

The lightness values (L*) remained relatively constant, ranging between 65.27 (Z5) and 66.38 (Z6), indicating minimal variation in brightness across samples. The a* values were consistently negative (from –5.54 to –5.33), reflecting the greenish hue characteristic of whey, with only slight shifts following syrup addition. Similarly, the b* coordinate showed positive values (11.20–12.19), confirming the presence of yellow tones, with minor reductions observed in the syrup-enriched variants compared to the control. Overall, the incorporation of elderflower syrup produced subtle changes in chromatic attributes, without significantly altering the visual profile of the fermented whey.

The quality of fermented whey beverages is closely linked to their sensory profile. In the interpretation of the results obtained from the sensory evaluation of the control sample (Z4) and those supplemented with elderflower syrup (Z5 and Z6), significant differences can be observed.

Figure 6 **Comparative sensory profile of fermented whey beverages**

Figure 6 shows that the control sample recorded lower and more uniform scores, with a simpler profile in terms of colour, aroma, and taste. In contrast, highlights that the beverage enriched with 10% elderflower syrup (Z6) obtained the highest ratings for colour, clarity, aroma, and

overall acceptability, reflecting the positive contribution of the syrup.

The intermediate sample (Z5) achieved scores between the two extremes, indicating a proportional improvement in visual and flavour attributes. The control sample was appreciated for its stability, but it lacked aromatic richness and balance. Overall, Z6 scored highest, reflecting enhanced sensory qualities and confirming the innovation brought by the addition of elderflower syrup.

CONCLUSIONS

In conclusion, the present research highlights and validates the high potential of whey, from both a technological and nutritional perspective, as a valuable substrate for the development of functional fermented beverages. This approach supports the sustainability of the food industry, contributes to the advancement of the circular economy, and promotes the intelligent conversion of a by-product into a value-added food, aligned with contemporary trends in nutrition and public health.

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