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Nedanovska, Emilija, Jakopović, Katarina Lisak, Daniloski, Davor, Vaskoska, Rozita, Vasiljevic, Todor and Barukčić, Irena (2022) Effect of storage time on the microbial, physicochemical and sensory characteristics of ovine whey-based fruit beverages. International Journal of Food Science and Technology, 57 (8). pp. 5388-5398. ISSN 0950-5423

International Journal of Food Science + Technology

Original article

Effect of storage time on the microbial, physicochemical and sensory characteristics of ovine whey-based fruit beverages

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(Received 14 February 2022; Accepted in revised form 23 May 2022)

Summary The aim of this study was to establish the physicochemical, sensory and microbiological quality of ovine whey-based fruit beverages produced from pasteurised ovine whey (mango flavoured–sugar sweetened [M-Su], pineapple flavoured–extra sugar sweetened [P-ESu], and tropical fruit flavoured–stevia sweetened [T-St]), and the changes in quality of these beverage types during the 15-day storage. The beverages were evaluated for their pH, colour, sediment and particle size distribution, microbiological count and sensory quality. No significant differences in the pH and the microbial counts among the beverages were noted during the storage period; however, the particle size distribution reflected the sediment formation that significantly decreased during the same period in all beverage types. The beverages were getting lighter during storage, which was evident by increasing L^* and decreasing a^* values for colour. All three beverage types had very high scores for taste and odour after 9 days, and colour and sediment after 5 days of storage. Beverage-type T-St was most preferred by consumers, with the highest scores for taste, odour and sensory appearance. These results can assist in the better determination of the deteriorative changes in a variety of ovine whey-based beverages, necessary changes in product composition that might result in improved product quality, and yet reducing losses incurred during ovine milk processing. In this sense, this research's findings contribute to the utilisation of whey beverages by the dairy industry to the development of functional products.

Keywords Beverage, ovine whey, quality, shelf life, storage.

Introduction

Just a few decades ago, whey was a serious environmental problem. Its polluting ability stems from its high biochemical oxygen demand, as 4000 L of whey causes equivalent environmental damage as the faecal waste of 1900 humans (Božanić *et al.*, 2014). On a global scale, ovine milk production accounts for only 1.4% (Gerosa & Skoet, 2013), making the ovine whey fairly a minor factor in the global environmental pollution, although it still can present environmental threat locally. Utilisation of ovine whey as a raw ingredient without further processing in the production of functional whey beverages should be considered because of the small scale production of ovine milk (Balthazar *et al.*, 2017). This is mainly related to the implementation of modern approaches, such as membrane processing, high-pressure processing, pressure-assisted thermal sterilisation or pulsed-electric field treatment, as they may be unfeasible techniques due to two primary reasons, namely the cost associated with these technologies (D'Incecco *et al.*, 2021) and the fact that ovine milk and whey are being perceived as nutritionally superior to bovine milk, and thus, further processing may diminish these benefits (Lordan *et al.*, 2019). The literature suggests that these benefits

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doi:10.1111/ijfs.15870

stem from the presence of compounds in ovine milk with immunomodulatory properties (Balan *et al.*, 2010), as well as anticancerogenic and antimicrobial properties (Kerasioti *et al.*, 2014; Kerasioti *et al.*, 2016; Kafantaris *et al.*, 2019; Kerasioti *et al.*, 2019). Compared with other sources of proteins, whey proteins in general have higher concentration of essential amino acids, as well as branched-chain amino acids (BCAAs), leucine, isoleucine and valine, which are important for protein and glucose homeostasis, lipid metabolism and regulation of body weight (Sharma & Chauhan, 2018; Kerasioti *et al.*, 2019). Additionally, greater circulating increase in BCAAs is noticed after ingestion of ovine milk (Milan *et al.*, 2020), thus suggesting that ovine whey may be a better source of BCAAs compared with bovine whey. Apart from that, unique ovine whey proteomes were found to be involved in numerous biological processes, including cellular development and immune responses (Ha *et al.*, 2015). Moreover, compared with bovine and caprine whey, ovine whey contains higher amounts of fat, proteins and ash, making it an excellent source of essential amino acids, in addition to a greater taste and mouthfeel (Kaminarides *et al.*, 2020).

One of the most effective ways to utilise raw whey is production of whey beverages. As a result of the increased awareness of people regarding environmental pollution, and the connection between diet and health, whey is now considered as a nutritional basis for the production of healthy beverages (da Mata Rigoto *et al.*, 2019). The process is cheap and simple, and the result is a highly nutritional beverage for human consumption, relatively well accepted by the consumers (Macwan *et al.*, 2016). These beverages can be non-fermented or fermented, alcoholic or non-alcoholic, and carbonated or not, and made with various sweeteners, and fruit or vegetable flavours (Mudgil & Barak, 2019). The challenges of producing ovine whey beverages include unacceptable salty sour taste of whey that has to be masked by another strong flavour, high water activity, which renders these beverages prone to microbial deterioration and sediment formation as a result of thermal treatment above 60 °C, which is undesirable in this type of beverage (Macwan *et al.*, 2016). This sediment is mainly influenced by heat-induced whey protein denaturation and their interaction with minerals present in the whey.

In the last decades, a large number of whey beverages have been developed successfully and were found to have the necessary acceptability with the addition of fruit with different percentage of dry matter (5–20%) (Djurić *et al.*, 2004; Barukčić *et al.*, 2019). Most often, citrus and tropical fruit flavours, including mango, papaya and banana can be added, as they have been proven to be the most effective in concealing the unwanted aroma of heat-treated milk and sweet–sour taste of the fresh whey (Djurić *et al.*, 2004). Furthermore, stevia extract containing stevioside, used as a sweetener, expands the nutritional value of the beverages. As shown by Lima *et al.* (2019), whey protein supplements enriched with extract from the leaves of *Stevia rebaudiana* Bertoni, containing steviol glycosides, can be very beneficial for increasing the strength and muscular mass of athletes. Notably, before the current study was conducted, it was expected that the addition of stevia extract would initiate a decrease in the microbial count, as some studies suggested (Ortiz-Viedma *et al.*, 2017; Pina-Pérez *et al.*, 2018; Ahmad *et al.*, 2020). Further, the possibility of increased sedimentation with stevia extract addition was not excluded considering the results reported previously Rad, Delshadian (Rad *et al.*, 2012).

The current study investigated the physicochemical, microbiological and sensory properties among ovine whey-based fruit beverages. The main challenges of the study were placed on solving the problem with sour taste by adding different aroma and sweeteners, avoiding sediment formation by applying pasteurisation at 72 °C / 35 s to evade significant denaturation of proteins and ensuring relatively long shelf life.

Materials and methods

Production of whey and whey beverages

Three batches of fresh whole ovine milk from the island of Pag (Croatia) were used for the production of cheese, on three separate occasions. Prior to cheese production, milk was pasteurised at 90 °C for 10 min and skimmed with centrifugal separator (EP-80, Technica, Dulles, VA, USA) at 4 °C for 12 min at 3225 *g* (Cavalcanti *et al.*, 2019). The milk was fermented using a mesophilic culture (Choozit BT 01 LYO 50 DCU, DuPont, Sassenage, France). The process lasted 11–12 h with the final pH measuring 4.6, upon which the curd was cut into pieces and heated to max 60 °C to induce greater syneresis. At the end, the cheese and the whey were separated by 30–60 min of draining through a strainer. The remaining whey was collected overnight, by leaving the cheese to drain in a mould placed in a refrigerator. The entire volume of the collected whey was then used for beverage production.

Preliminary optimisation of beverage recipes

In a preliminary study, samples of whey subjected to three different pasteurisation regimes, including 72 °C / 35 s, 78 °C / 35 s and 85 °C / 40 s, were examined for sediment formation and pH, and according to the obtained results, the pasteurisation regime 72 °C / 35 s was chosen in the current study (Table S1). In addition, as a part of the preliminary study, conducted in order to optimise the current design, nine beverages were grouped as M-type, P-type and T-type, and contained mango, pineapple, and tropical fruit aroma, respectively. Each type was subdivided into three subtypes based on the sweetener added: Su for sugar (3.2 g in 100 mL whey beverage: Viro, Virovitica, Croatia), ESu for extra sugar (5 g in 100 mL whey beverage: Viro, Virovitica, Croatia), and St for stevia powder (Naturex, Avignon, France), containing 90% stevioside. The last sweetener was prepared as 2% solution from the Stevia powder and 0.9% of the solution being added in 100 mL of beverage, resulting in the final concentration of 0.018% stevia powder in 100 mL beverage. The whole design thus contained nine different beverages: mango–sugar (M-Su), mango–extra sugar (M-ESu), mango–stevia (M-St), pineapple–sugar (P-Su), pineapple–extra sugar (P-ESu), pineapple–stevia (P-St), tropical fruit–sugar (T-Su), tropical fruit–extra sugar (T-ESu), and tropical fruit–stevia (T-St). Based on the preliminary hedonic sensory analysis, three out of these nine beverages were chosen for further examination within this study including M-Su, P-ESu and T-St, the best rated in each type (Table S2).

Production of test beverages

Approximately 1200 mL of the whey–fruit juice mixture was prepared from 960 mL of pasteurised whey mixed with 240 mL of the mixture of pear and passion fruit syrup (Bio&Bio, Zagreb, Croatia) on three separate occasions. Postmix pasteurisation of the prepared beverages was not performed in order to avoid further denaturation of the whey proteins. The mixture was divided into three groups of 400 mL, each containing different aroma and sweetener: mango flavoured–sugar sweetened (M-Su) (mango flavour and 3 g of sucrose on 100 mL of beverage), pineapple flavoured–extra sugar sweetened (P-ESu) (pineapple flavour and 5 g of sucrose on 100 mL of beverage) and tropical flavoured–stevia sweetened (T-St) (tropical fruit flavour and stevia extract as a sweetener). The flavour of the beverages was achieved by adding 3–4 drops of each extract (mango, pineapple and tropical) per 100 mL of beverage. The 400 mL of each beverage was further divided into four 100 mL samples, and stored at 4 °C for further testing. Analytical tests were performed at day 1 (day of preparation), 5, 9 and 15, whereby the following parameters were determined: physicochemical (acidity [pH], colour, particle size distribution, sediment formation) and microbiological properties and sensory characteristics.

Determination of physicochemical properties

Determination of pH

The acidity of whey was determined as active acidity. The pH measurements were performed using a potentiometer (WTW pH 3110, Wissenschaftlich Technische Werkstätten, Weilheim, Germany). The measurement was performed at room temperature, using 100 mL of whey beverage (Barukčić *et al.,* 2015).

Determination of colour

Colour was determined by a spectrophotometer (CM-3500d, Konica Minolta, Nieuwegein, Netherlands) as described by Baccouche *et al.* (2013) with some modifications. During the spectrophotometric colour determination, the whey samples were placed in the cuvette, which was moved into a dark chamber and recorded in a colorimeter (wavelength range: 400 nm to 700 nm; wavelength pitch: 20 mm). The obtained results were expressed as L^* (lightness), a^* (green/red colour) and b^* (blue/yellow colour) values.

Determination of particle size distribution

The particle size distribution and mean particle diameter of the beverages were determined by the laser diffraction method with a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) (Costa *et al.,* 2018). The measurements were performed at 25 °C. Distilled water was used as a dispersant with its refractive index of 1.33, while the refractive index for the whey beverages was 1.52 (Jenness, 1962).

Determination of sediment formation

Determination of the sediment was carried out by centrifugation. The samples were placed in plastic centrifugal tubes with conical bottom, which had been previously weighted on an analytical scale. The determination was performed in a centrifuge (Rotofix 32A, Hettich, Tuttlingen, Germany) for 10 minutes at 2068 *g* at room temperature. After removing the supernatant, the tube was weighed with the sediment and the mass of the sediment was determined by subtracting the tube's weight (Jelen *et al.,* 1987).

Microbiological analysis

Total viable cell counts of bacteria, yeasts and moulds and *Enterobacteriaceae* were determined by the traditional pour plate method, based on the detection of microbial propagation following a previously described method of counting (Jeličić *et al.,* 2012) and according to International Organization for Standardization (ISO, 2013). For enumeration of the total number of bacteria, *Enterobacteriaceae*, yeasts and moulds, enriching agars such as Tryptic Glucose Violet, Red Bile and Sabouraud Dextrose were used, respectively (all from Biolife, Italy). Samples for enumeration of total viable cell count were incubated at 30 °C for 72 h, *Enterobacteriaceae* samples were incubated at 37 °C for 48 h, and yeasts and moulds samples were incubated at ambient temperature for 72 h. The results were expressed as log CFU mL^{-1}.

Preliminary sensory analysis

Sensory evaluation of the beverages was conducted using the weighted points and hedonic scale analysis (ISO, 2009). The sensory study was approved by the Ethics Committee of the University of Zagreb. Five trained panellists were included in the first part of the sensory evaluation. The beverages were equilibrated at room temperature, poured into identical laboratory beakers and encoded. The beverages were given points from 1 to 5 for the attributes including taste, odour, colour, sediment and appearance. Each attribute of the beverage had its own weighting factor; thus, the taste and the smell had the highest weighting factors (1.5 and 1, respectively), while colour, sediment and appearance had lower weighting factors (0.9, 0.4 and 0.2, respectively). The average grade of each beverage was multiplied by the weighting factor representing each attribute, and using this method, the maximum point one beverage could reach was 20 (Molnar & Örsi, 1982). In addition, at the end of the study 30 evaluators, all at once, performed a 9-point hedonic scale analysis to evaluate the acceptance of the beverages on a scale from 1 indicating 'extremely dislike' to 9 meaning 'extremely like' (Stone *et al.*, 2012).

Data analysis

Statistical analysis was performed in Minitab using Analysis of Variance (version 19; Minitab, Pennsylvania, USA) and Origin (Origin Pro 2021, v. 95E, OriginLab Corporation, Northampton, MA, USA). The data for the physicochemical and microbiological characteristics were analysed with beverage type and storage time as fixed factors and replicate as random factor with $P < 0.05$ considered as the significance level. The sensory data were analysed with beverage type and storage time as fixed factor and panellist as random factor. The replication for each treatment was three ($n = 3$), corresponding to the three separate production batches of whey beverages.

Results and discussion

Physicochemical analyses

pH assessment

The pH of the beverages ranged between 4.11 and 4.22 (Table 1), while pH of the fresh whey, measured on the first day of examination, was 4.63. The pH of whey beverages with fruit juice is expected to be lower than that of fresh whey, which is either a result of the involved heat treatment as shown in whey-based prickly pear beverages (Baccouche *et al.*, 2013) or the concentration of fruit juice in the beverage observed in whey-based banana juice beverage (Dhamsaniya & Varshney, 2013). The beverage type and the storage period did not affect the pH of the beverages significantly ($P > 0.05$; Table S3). Reduction in pH has a beneficial impact on the storage of beverages, as acidification decreases the activity of undesired microorganisms and their ability for propagation (Thakkar *et al.*, 2018).

Colour

The colour of the beverages was mostly governed by highly concentrated, dark purple, pear and passion fruit syrup. The positive b^* values during storage indicate the prominence of the yellow notes. The a^* values are indicative of the red colour, while the L^* values

Table 1 Physicochemical properties of ovine whey beverage samples stored at 1, 5, 9 and 15 days of storage at 4 ± 1 °C

		Colour					PSD	
Day	Sample	L^*	a^*	b^*	Sediment	pH	d (0.5)	d (0.9)
Day 1	M-Su	33.01	15.91	49.09	0.37	4.21	56.06	1297.288
	P-ESu	35.87	15.41	51.19	0.34	4.21	50.68	806.35
	T-St	34.29	15.85	50.17	0.43	4.22	49.38	1494.43
Day 5	M-Su	31.11	16.10	46.86	0.31	4.18	54.09	122.61
	P-ESu	33.88	15.77	49.39	0.32	4.13	47.58	103.25
	T-St	33.65	15.27	49.05	0.34	4.15	40.56	101.13
Day 9	M-Su	33.43.	14.99	47.64	0.30	4.19	60.60	200.80
	P-ESu	35.22	14.61	48.86	0.28	4.17	65.47	542.94
	T-St	33.91	14.68	48.19	0.31	4.18	52.51	151.08
Day 15	M-Su	38.78	14.18	50.56	0.28	4.11	55.40	212.39
	P-ESu	40.63	13.68	50.78	0.29	4.11	54.88	179.62
	T-St	40.23	14.02	49.77	0.23	4.11	46.21	158.18
SEM		2.78	0.64	1.25	0.03	0.03	4.46	1.17

P-values of main factors can be found in Table S3 (beverage type x storage time $P > 0.05$).
Mango flavoured–sugar sweetened (M-Su); pineapple flavoured–extra sugar sweetened (P-ESu); and tropical fruit flavoured–stevia sweetened (T-St).

showed that the black tone was more prominent (Table 1). Storage time significantly changed a^* and L^* ($P < 0.05$), but not the b^* values ($P > 0.05$: Table S3). The significantly descending trend of a^* suggests that the beverages lost some red colour intensity during storage, which could result from the increasing content of regenerated riboflavin, which initially degraded during heat treatment but reformed in the presence of mineral nutrients during storage (Baba *et al.,* 2016). The L^* values decreased during first 5 days of storage, followed by an increase afterwards, with the beverages at day 15 having a greater L^* value than at day 1. According to Giroux *et al.* (2008), pasteurisation of beverages did not modify the colour when compared to unheated samples. They suggested that pasteurisation only involved the initial phase of the Maillard reaction, but without colour changes. On the contrary, protein aggregation can alter the lightness of the beverages (Giroux *et al.,* 2008); thus, the increased L^* coincided with the particle size distribution, which depicted larger particles during first days of storage but a substantial decline later. Tranjan *et al.* (2009) did not detect the presence of Maillard reaction products (MRPs) in caprine cheese whey-flavoured beverages after 14 days of storage as well.

Sediment

Over time, there was significantly less sediment in the products ($P < 0.01$) with a greatest reduction between day 1 and day 5, followed by a smaller decline afterwards (Table 1). The interaction between beverage type and storage time had no significant effect on the sediment formation ($P > 0.05$) (Table S3). Usually, sediment formation in whey beverages is caused by whey protein aggregation, which is highly dependent on the heat treatment and pH (LaClair & Etzel, 2010; Ahmadi *et al.,* 2018). Upon denaturation, whey proteins can aggregate with themselves or other proteins by creating covalent bonds and weak attractions (Lucey *et al.,* 2022). Weak interactions prevail at pH below isoelectric point of whey proteins (~ 4.8–5.2) since the presence of hydrogen ions prevents to a certain extent the formation of sulfhydryl bridges (Dissanayake *et al.,* 2013). This understanding has been drown from numerous studies carried out on thermally induced denaturation of bovine whey proteins and subsequently their sediment formation (Donovan & Mulvihill, 1987; Bramaud *et al.,* 1997; Wijayanti *et al.,* 2019); on the other hand, a limited research has been done on the relation between the ovine milk and sedimentation (Tribst *et al.,* 2020). However, major whey proteins, β-Lg and α-La, are found in similar concentrations in bovine and ovine milk, namely β-Lg: 8.07 and 8.11%, respectively, and α-La: 2.70 and 3.70%, respectively (Panopoulos *et al.,* 2020). In addition, these major whey proteins share great sequence similarities and the structural features between ovine and bovine whey (El-Zahar *et al.,* 2004; Sawyer, 2013); thus, the findings in the present study may be compared with studies carried out using bovine whey proteins. Our results align with the study of Baccouche *et al.* (2013) who examined whey pear beverages and reported gradual disappearance of the sediment during initial stages of the storage period. Such an occurrence can be related to, first, low initial pH, which prevented the formation of strongly linked whey protein aggregates and, second, gradual pH decline during storage (Table 1), which likely affected electrostatic attractions by increasing positive charge among the particles (Dissanayake *et al.,* 2013). Hydrophobic attractions, one of the main drivers of the aggregation at a pH below pI of whey proteins, are at minimum during cold storage; hence, the aggregated proteins are attracted by other weak interactions (Liyanaarachchi & Vasiljevic, 2018).

Particle size

The particle size distribution over the storage time and the values of mass median diameter (MMD) $d_{(0.5)}$ and $d_{(0.9)}$ are presented in Fig. 1. On day 1, the beverages contained a pronounced amount (~ 4–6%) of particles with a size equal or greater than 1000 μm. Due to their decomposition, the $d_{(0.9)}$ values significantly decreased over the storage time ($P \leq 0.001$) (Table S3). Loss of attractive hydrophobic interactions during cold storage and pH change affecting electrostatic interactions could encounter for some of the aggregate disintegration into smaller particles (Liyanaarachchi & Vasiljevic, 2018), although structural rearrangement of polysaccharides and their repositioning on the surface of denatured proteins limiting aggregation and/or driving aggregate breakdown may also be involved (Wagoner & Foegeding, 2017). Similar to our results, Koffi *et al.* (2005) measured the greatest diameter of the particle surface in a UHT-processed whey–banana beverage in the initial phase of the study.

These observations align well with the sediment determination (Fig. 1 and Table 1), since the larger particles gave greater sediment. In addition, the particle size affected the visual appearance of the beverage as they affected its colour. The beverages M-Su and P-ESu had greater $d_{(0.5)}$ values than those of T-St ($P \leq 0.05$), and the interaction between the beverage type and the storage time influenced the $d_{(0.9)}$ values significantly, suggesting that sucrose played some role. According to Kulmyrzaev *et al.* (2000), although sucrose increases the proteins' denaturation temperature, once the proteins unfold, sucrose straightens the protein–protein interaction due to increased exposure of hydrophobic groups.

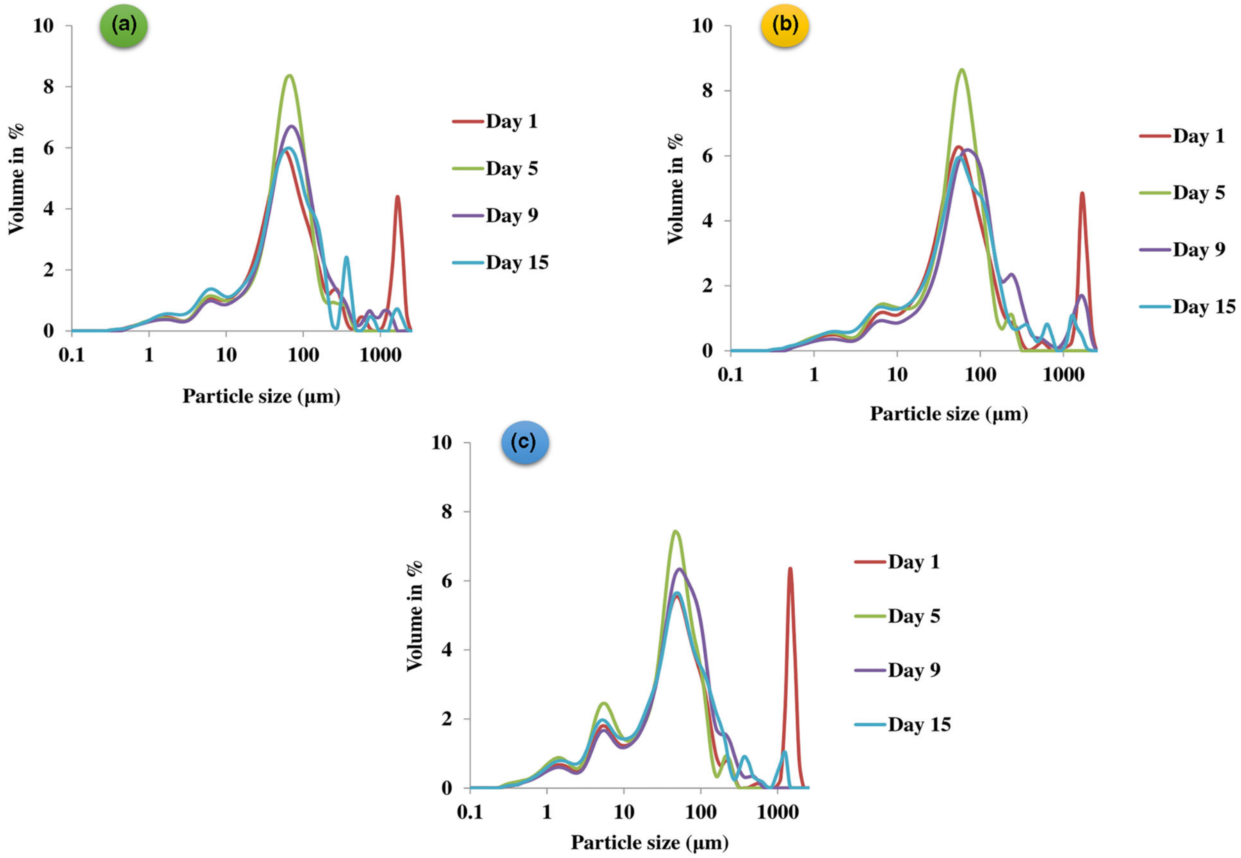

Figure 1 Particle size distribution for mango flavoured–sugar sweetened (a: M-Su), pineapple flavoured–extra sugar sweetened (b: P-ESu) and tropical fruit flavoured–stevia sweetened (c: T-St) whey beverages kept at the temperature of 4 ± 1 °C over the examined period of time.

Microbiological analysis

The number of bacteria (Fig. 2a), yeast and moulds (Fig. 2b) in the beverages increased over time independent of the beverage type (Table S4). On the first day, that is immediately after the heat treatment of the whey and the preparation of the beverages, the bacterial count was accordingly low (1.0 log CFU mL^{-1}). The microbial growth was linear during the storage time. Considering the low pH, increase in bacterial counts was not expected, and despite the fact that the beverages were produced in a sterile environment and stored at 4 °C, the possibility of contamination cannot be discounted. This was likely a reason for greatest bacterial counts obtained for the T-St beverage, which contradicted to a number of studies, which have suggested the potential antimicrobial effects of stevia (Ortiz-Viedma *et al.*, 2017; Pina-Pérez *et al.*, 2018; Ahmad *et al.*, 2020).

Increase in yeast and mould counts in our study aligns with the study of Sakhale *et al.* (2012), but differed from those reported by Ismail *et al.* (2011), where yeast and mould count decreased over time. The microbial counts at day 1 and the increase with storage time were not surprising as the beverages were produced by mixing ingredients and without downstream heating (Fig. 2). This short shelf life points to the opportunity of reintroducing microbial contamination after the milk pasteurisation step. It is noteworthy that in our study, the presence of *Enterobacteriaceae* that would indicate faecal contamination of the beverages was not noticed. Due to the linear growth of microorganisms, to achieve better preservation for the beverages, pasteurisation of the whey beverage by conventional or novel methods is demanded.

Preliminary sensory analysis

Considering the weighted score sensory analysis, the storage period significantly affected the sensory characteristics of the beverages, including taste, odour, colour and sediment parameters ($P < 0.05$) (Table S5). Overall, the beverages were most acceptable on the 5th day of examination (Table 2). According to the

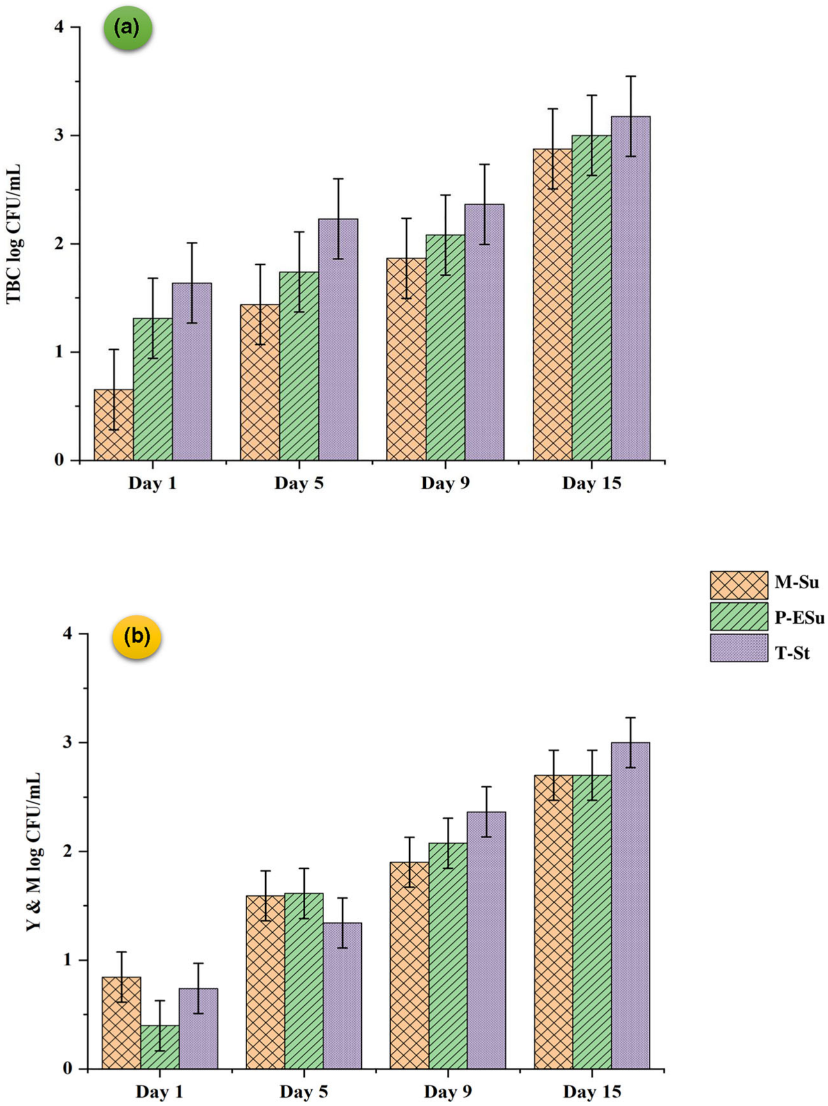

Figure 2 Total bacterial count (**a:** TBC log CFU/mL, SEM - TBC = 0.38) and total yeast and mould count (**b:** Y&M log CFU/mL, SEM - Y&M = 0.23) in mango flavoured–sugar sweetened (M-Su), pineapple flavoured–extra sugar sweetened (P-ESu) and tropical fruit flavoured–stevia sweetened (T-St) whey beverages kept at the temperature of 4 ± 1 °C over the examined period of time. *P*-values of main factors can be found in Table S4 (beverage type × storage time, *P* > 0.05).

sensory evaluation, the acceptance slightly declined on day 9 but significantly decreased on the last, 15th day of storage. The most important factors for poor acceptability could be the proliferation of microorganisms and the decrease in pH. The colour as a sensory characteristic became less desirable after day 9. This can be linked to various physicochemical changes leading to a shift in particle size distribution, decreased sediment and enhanced brightness of the beverages, all of which lead to less appealing colour.

Sugar containing beverages were graded significantly different from the T-St beverage considering taste and odour ($P < 0.05$). The T-St beverage obtained the best grades on all days of examination. Evaluating the colour, sediment and appearance, the beverages did not show any substantial differences. Considering the total grades calculated by the weighted points system, the highest grade was assigned to the T-St beverage on all days of examination, while the beverage with the lowest score was P-ESu on days 1, 5 and 9 and M-Su on the last day of examination. Using the hedonic scale, the best-evaluated beverage was again T-St, achieving 7.8 points out of 9, while beverages M-Su and P-ESu acquired 6.3 and 6.9, respectively (Table 2). Considering the benefits of using stevia as a sweetener, and the good evaluations of T-St beverage, commercialisation of this beverage type may be considered. Punnagaiarasi *et al.* (2017) similarly found high sensory scores (9/10) for whey beverages; in their case, the whey was mixed with watermelon juice. Sakhale *et al.* (2012) analysed the acceptability of three whey–mango beverages containing different amounts of mango pulp and

Table 2 Mean sensory scores of ovine whey beverage samples stored at 1, 5, 9 and 15 days of storage at 4 ± 1 °C

	Day 1			Day 5			Day 9			Day 15			
	M-Su	P-ESu	T-St	M-Su	P-ESu	T-St	M-Su	P-ESu	T-St	M-Su	P-ESu	T-St	SEM
Colour	3.93	3.95	3.93	4.47	4.50	4.61	3.91	4.22	4.16	3.74	3.83	3.58	0.11
Odour	4.21	4.00	4.66	4.39	4.12	4.83	4.53	4.10	4.86	3.83	3.58	4.83	0.08
Taste	4.30	4.08	4.40	4.40	4.25	4.50	4.53	4.31	4.87	3.41	3.99	4.24	0.09
Appearance	3.58	3.83	3.89	4.17	4.15	4.41	3.97	3.98	4.31	3.66	3.66	3.41	0.10
Sediment	3.83	3.81	3.83	4.41	4.41	4.35	3.87	3.87	3.87	3.58	3.49	3.58	0.11
Total*	16.40	15.90	17.10	17.60	17.10	18.20	17.20	16.50	18.20	14.40	15.00	16.50	

*Every grade is multiplied by its weighting factor, and the corresponding numbers are summed to calculate the total grade). *P*-values of main factors can be found in Table S5 (beverage type x storage time, $P > 0.05$). Mango flavoured-sugar sweetened (M-Su); pineapple flavoured–extra sugar sweetened (P-ESu); and tropical fruit flavoured–stevia sweetened (T-St).

whey and found their most preferred beverage (consisting of 30% mango pulp, 100 g sugar, 1% stabiliser, and 599 mL whey) scored 8 out of 9 for overall acceptability on the initial day of study.

Limitation of the study

The present study, using the set of three different ovine whey-based fruit beverages, found that by utilising various sweeteners, it would likely be possible to improve the quality of the product, by reducing losses incurred during ovine milk processing. Nevertheless, the choice for a specific research design and approach, with its corresponding selection of specific research methods, implies some limitations on this study. A first limitation pertains to the nature of data collection, that is the selection procedure for the preliminary sensory evaluation, which was performed with a limited number of panellists and evaluators. Therefore, more research with a larger number of panellists is needed to confirm the ability of sensory characteristics for the ovine whey-based fruit beverage discrimination. Additionally, larger scale and extensive data studies with more samples that would explain the content and estimate the levels of the individual ovine whey proteins, including the ratio of β-Lg and α-La and their possible interaction with caseins (Ali *et al.*, 2022; Pan *et al.*, 2022), are needed to further elaborate on the functionality of ovine milk and consequently the functionality of ovine whey-based fruit beverages (El-Zahar *et al.*, 2005). This would also include the impact of their polymorphic variants as well, which were not assessed in the current study, all of which can affect various interactions, mineral levels in ovine milk, technological and quality properties, such as heat stability, production traits, milk composition, and acid- and rennet-induced gels (Amigo *et al.*, 2000; El-Zahar *et al.*, 2005).

Conclusion

One of our main objectives was to prevent sediment formation in the whey beverages, which was attained since the sediment content decreased over time. The decreased pH values during storage time likely increased the surface charge of denatured whey proteins causing disintegration of initially formed sediment. At the same time, the particle size distribution showed a tendency of shifting towards smaller particles since it is closely correlated to the sediment content. The beverages M-Su and P-ESu showed greater PSD values compared to T-St, since sucrose likely strengthened the protein–protein interaction once the protein molecules unfolded. Since the lightness increased owing to a decreased particle size, the occurrence of products of the Maillard reaction was ruled out. The gradually increasing number of bacteria during storage time was likely due to contamination. To assure longer shelf life, conventional or novel pasteurisation methods appear warranted.

The challenge to mask the sour taste of the beverages appeared to be resolved considering the high acceptability demonstrated by the sensory evaluation. The beverage-type T-St consisting of whey, syrup from pear and passion fruit, aroma of tropical fruit and stevia as a sweetener showed the most desired sensory properties for the consumers, particularly in its odour, taste and sensory appearance. After the 9th day of observation, the proliferation of bacteria and decrease in pH declined the acceptability of the beverages. Findings of this study can help predict the deteriorative changes in a range of ovine whey-based beverages, and necessary modifications in the product composition can be made to result in improved quality of the products, therefore decreasing the losses incurred during ovine milk processing and possibility for functional products' development.

Acknowledgments

This work was supported by the Erasmus+ Programme scholarship (MK Bitola01, academic 2017/2018 year), University of Zagreb (Faculty of Food Technology and Biotechnology), Zagreb, Croatia; and St. Clement of Ohrid, University of Bitola (Faculty of Technology and Technical Sciences), Bitola, Republic of North Macedonia. The graphical abstract was prepared using BioRender.com. Open access publishing facilitated by Victoria University, as part of the Wiley - Victoria University agreement via the Council of Australian University Librarians. [Correction added on 02 July 2022, after first online publication: CAUL funding statement has been added.]

Author Contribution

Emilija Nedanovska: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Katarina Lisak Jakopović:** Data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting). **Davor Daniloski:** Conceptualization (lead); data curation (supporting); formal analysis (supporting); investigation (supporting); resources (supporting); software (lead); validation (supporting); visualization (supporting); writing – original draft (lead); writing – review and editing (lead). **Rozita Vaskoska:** Conceptualization (supporting); data curation (supporting); validation (supporting); writing – review and editing (supporting). **Todor Vasiljevic:** Conceptualization (supporting); formal analysis (supporting); validation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Irena Barukčić:** Investigation (supporting); methodology (supporting); supervision (lead); visualization (supporting); writing – review and editing (supporting).

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval

Ethics approval was obtained from the Ethics Committee of the University of Zagreb.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15870.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Measured sediment formation and pH for three different pasteurisation regimes.

Table S2. Sensory analysis of nine beverages differing by the aroma and sweetener added.

Table S3. Levels of significance (*P* value) for the physicochemical parameters in the whey beverages.

Table S4. Levels of significance (*P* value) for the microbiological parameters in the whey beverages.

Table S5. Levels of significance (*P* value) for the sensory parameters in the whey beverages.

