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Article

Sheep's and goat's frozen yoghurts produced with ultrafiltrated whey concentrates

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Featured Application: The present work envisages the valorization of cheese whey concentrated by ultrafiltration as the main ingredient of frozen yoghurts

Abstract: The objective of this work was the use of goat and sheep liquid whey concentrates (LWCs) produced by ultrafiltration (UF) for the manufacture of frozen yoghurts with or without different concentrations of inulin. In a first step, natural yoghurts using only goat and sheep LWCs as raw material were obtained. One day after production, these yoghurts were used to produce frozen yoghurts with different concentrations of added inulin. The physicochemical charateristics of ewe´s and goat´s yoghurts were significantly different regarding dry matter, protein, fat and minerals. Ewe´s yoghurts were solids while goat´s yoghurts behaved as a viscous liquid. Frozen yoghurts with different levels of inulin addition also presented significant differences concerning physicochemical and microbiological characteristics. Overrun was similar for all formulations except for the one produced with ewe's LWC containing 5.0% inulin, which presented a significantly higher value. Higher meltdown rates in goat's whey frozen yoghurts were observed. The survival rates of lactic acid bacteria were lower than data reported for similar products. Concerning sensory acceptance, both products showed encouraging results. It can be considered that the production of frozen yoghurts by using LWCs as main ingredient can be an interesting option to broaden the product's portfolio of small/medium scale dairy producers.

Keywords: Ovine; Caprine; Cheese whey; Ultrafiltration; Frozen yoghurt; Inulin

1. Introduction

The dairy industry originates the production of wastewaters and effluents that can have a significant environmental impact because of their pollutant characteristics [1-3]. Whey is the by-product of the cheese industry that received more attention, not only due to its environmental impact if disposed without prior treatment but also as a result of the progressive evidence of the nutritional importance of its constituents [4-6].

The high organic load of whey arises from the presence of residual milk compounds, being lactose its major constituent (*ca.* 5% w/v). It has a very high BOD and COD load (30-50 g/L and 60-80 g/L respectively). Its annual production surpasses 160 million tones and shows an estimated growth rate of 1–2% yearly, as a result of a similar trend in cheese production [7]. The quality of whey components depends on the type of the process used and the operation conditions occurring during its production [8,9]. Various technological approaches have been employed to valorize whey [10-13]. These technologies are mainly applied to whey resulting from cow's milk. The technological advancements have enhanced whey utilization and about 50% of the total produced whey is nowadays transformed into value-added products such as whey powder, whey protein concentrates, whey protein isolates, individual whey proteins, bioactive peptides, whey permeate, bioethanol, biopolymers and other valuable materials [14]. Among various value-added products, the transformation of whey into proteinaceous products is attractive and with increased demand. These proteinaceous products have applications as functional, nutritional, and therapeutic commodities.

In the case of the whey resulting from the manufacture of ewe's and goat's cheeses, this by-product is often used to produce whey cheeses, such as *Ricotta*, *Requesón* or *Requeijão*, in Italy, Spain and Portugal respectively. However, not all this whey is transformed in whey cheeses, being a large proportion used as animal feed or directly discarded. These results from the constraints associated to whey cheeses production, namely the high energy inputs required for the thermal precipitation of whey proteins which, associated to the short shelf-life of whey cheeses, discourage its application [15,16]. In previous works, we proposed solutions for the valorization of whey resulting from the manufacture of small ruminant's milk cheeses [17-23]. Most of the solutions proposed envisage the direct utilization of such by-product after a concentration step performed by ultrafiltration (UF). However, the adoption of such technologies by cheese producers is hindered by several factors, of which the small dimension of the cheese production units and the lack of technical skills are determinant. Besides, these producers are mainly focused on cheese production and are not familiar with the production and marketing strategies of other dairy products. However, the possibility of valorization of these by-products through the production of innovative dairy products targeting local consumers and/or niche markets, may stimulate novel approaches in line with circular economy imperatives.

Yoghurt is a fermented dairy product obtained by the activity of a mixed culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* on milk. Fermentation of lactose produces lactic acid, which acts on milk protein to give yoghurt its texture and characteristic taste. Frozen dairy products have properties of both yoghurt and ice cream and can be the carriers of lactic acid bacteria and/or probiotics. In recent years, the production of frozen yoghurts or frozen yoghurts with added prebiotics and probiotics created new opportunities for the development of products with health promoting properties beyond their nutritional value. The production of ewe's and goat's yoghurts and frozen yoghurts has also followed this trend. Alves and coworkers [24] produced goat's milk frozen yoghurt with 1% inulin, 0.25% starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and 0.25% probiotic culture (*Bifidobacterium animalis* and *Lactobacillus acidophilus*) to verify its probiotic potential after 7 and 120 days of storage. During storage probiotic bacteria were reduced, but the product showed probiotic potential due to both *B. animalis* and *L. acidophilus*, which reached the minimum desirable numbers until the end of storage. The product received good scores for global appearance, color, aroma and taste, while texture and residual taste had lower acceptance rates.

Other researchers investigated four experimental groups of caprine frozen yoghurt produced with the addition of fresh and spray dried jambolan fruit pulp and *Bifidobacterium animalis* subsp. *lactis*. The addition of *B. animalis* decreased the pH of frozen yoghurt samples. Low overrun values (14.2-22.6%) were observed for all samples. Higher phenolic content for samples with the probiotic strain was observed suggesting a possible biotransformation that would lead to enhanced phytochemical level. The frozen yoghurt samples with probiotic presented high cell counts (9 log CFU g^{-1}) and a high probiotic survival rate throughout the 90 days of frozen storage. The frozen yoghurt samples with probiotic bacteria received lower flavor scores and similar results were obtained for appearance, aroma and consistency among frozen yoghurt samples [25].

Frozen yoghurt formulations were developed by Abreu et al. [26] using powdered sheep milk. It was found that the concentration of powder preparation presented a significant positive effect on protein, lactose, and calcium and a significant negative effect on pH.

Goat milk and goat milk plus inulin were used as encapsulating agents of Bifidobacterium BB-12 and applied in frozen yoghurt to evaluate the antagonistic effect against *Escherichia coli.* The production of organic acids by bifidobacteria was directly related to the decrease in the *E. coli* counts. In plate counts, *E. coli* was not detected in the descending colon. However, when quantified by qPCR the sample presented amplification that corresponded to 3 log CFU g^{-1}. It was therefore possible to observe the phenomenon of the viable but not-culturable cells of *E. coli.* The authors recommended the production of microcapsules with goat milk and inulin for application in goat milk products, due to the better antagonist effect against *E. coli* [27].

The addition of jujube pulp weakened greatly the goaty flavor, improving the sensory acceptance, and increased the antioxidant activities of goat milk yoghurt. No significant differences were found regarding the viable counts, pH values and titratable acidities of all formulations over 28 days refrigerated storage. All formulations showed the viable counts above log 6 $CFUmL^{-1}$ over the assessed storage period [28].

Regarding the physicochemical, microbiological, and sensory characteristics of frozen yoghurts, several research works have been performed. Inoue et al. [29] reported that viable lactic acid bacteria decreased in number with increasing storage period. Besides, different sensory rates were obtained for assessors who were familiar with yogurt and for assessor who were not. The product having a pH value of 5.5 was the most preferred frozen yoghurt type in the products examined. Other researchers reported that experimental storage conditions resulted in the survival of LAB in frozen yoghurts even after a 60 to 67 weeks period. The total LAB in three batches was higher log 7 $CFUg^{-1}$, but one of them was comprised exclusively of streptococci [30]. Results obtained by Abdelazez et al. [31] revealed that there was no significant difference between frozen yoghurt fermented by *Bifidobacterium* spp. and yoghurt culture and that fermented by yoghurt culture only.

The physical and sensory properties of frozen yoghurt produced from yoghurts with varying levels of sugar (18, 20 or 22%) and fruit concentration (15, 20 or 25%) were evaluated by Güven & Karaca [32]. An increase in the amount of sugar in vanilla frozen yoghurt

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softened the structure, whereas in strawberry frozen yoghurt the structure hardened in parallel with the increase in fruit concentration. Frozen yoghurts with high sugar and fruit concentrations were the most preferred by tasters.

The high levels of fat and sugar in frozen yoghurt and related products such as frozen yoghurt can lead to undesirable health effects. In fact, Ruopeng & Jiang [33] reported that frozen yoghurt and ice cream were found to be less healthy than yoghurt, and adding toppings further reduced their nutrition value due to a decreased intake of most vitamins/minerals, associated to an increased intake of sugar, total/saturated fat, cholesterol, fiber, and iron and daily energy intake.

Therefore, several authors evaluated the characteristics and sensory acceptability of low-fat and/or low-sugar frozen yoghurts. Furthermore, the incorporation of prebiotics and probiotics is often associated with such approach envisaging the production of safe, attractive, and healthy products. Isik et al. [34] produced frozen low fat and no added sugar yoghurt using polydextrose, aspartame and acesulfame-K mixture, and different levels of inulin and isomalt. The addition of inulin and isomalt increased viscosity by 19 to 52% compared with that of control. The average calorie values of samples substituted with sweeteners were about 43% lower than that of control sample. Low-calorie frozen yogurt samples melted about 33 to 48% slower than the reduced-fat control sample at 45 min. LAB counts between 8.12 and 8.49 log CFU g^{-1} were found 3 months after production. Shehzad and coworkers [35] tested the effect of inulin and glycerol supplementation on physicochemical properties of probiotic frozen yoghurt. The results showed that 4% and 6% inulin supplementation increased the overrun by 3% and 5% and decreased the hardness by 7% and 11%, respectively. Inulin supplementation did not have a significant effect on ice crystal size. Glycerol supplementation increased the stickiness from 2.4% to 18.7%, decreased the hardness from 8.0% to 14.5%, and did not have a significant effect on overrun or melting rate. Yang et al. [36] developed a symbiotic oat-based frozen yogurt containing fermented oat, probiotics, and inulin. The new product had 4.10% protein, 8.50% fat, and 2.10% fiber, respectively. Bifidobacterium remained log 6 $CFUmL^{-1}$ up to 8 weeks. Results indicated that consumption of oat- based frozen yogurt could decrease serum lipids levels in rats.

The aim of this work was the manufacture of frozen yoghurts with or without different concentrations of inulin using goat and sheep liquid whey concentrates produced by ultrafiltration, and the study of their physico-chemical, microbiogical and sensory properties in order to evaluate the feasability of the use of these by-products by the dairy industry. As follows from the literature reports, there are not studies about the production of frozen yogurst with goat and sheep liquid whey protein concentrates.

2. Materials and Methods

2.1. Production of liquid whey concentrates

Sheep and goat cheese wheys, supplied by external dairy companies, were taken to the pilot plant of the Escola Superior Agrária de Coimbra (Portugal), where it was processed. 500L of each type of whey (ewe's or goat's) were subjected to ultrafiltration (UF) in

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a Proquiga Biotech SA pilot plant (A Coruña, Spain), equipped with a UF organic membrane (3838 PVDF/polysulfone) with an effective filtration area of 7 m^2 and 10 kDa cutoff, supplied by FipoBiotech, Spain. The process was carried out at 40-45 ºC, at a transmembrane pressure of 3.5 bar aiming at a volumetric concentration factor (VCF=Vol. Feed/Vol. retentate) of 20, obtaining 25 L of concentrate. The concentrate was pasteurized (65 ºC, 30 min) and then homogenized at 10 MPa using a homogenizer APV Rannie™ model Blue Top (Copenhagen, Denmark). Liquid whey concentrates (LWCs) were frozen at – 25 ºC until the moment they were used for the production of yoghurts and frozen yoghurts.

2.2. Manufacture of yoghurt and frozen yoghurt

Twelve liters of sheep´s or goat's LWCs were thawed under refrigeration for 24 h. Subsequently, LWCs were heated at 65 °C and homogenized using APV Rannie™ model Blue Top (Copenhagen, Denmark) at 10 MPa. Subsequently, the samples were heated to 85 ± 5 ºC and homogenized again in order to achieve a particle diameter of whey protein aggregates less than 10 μm to avoid granularity and increase the smoothness of the concentrate. The concentrates were then cooled to 44 °C and inoculated with Yoflex™ (YF-L903) CHR (Hansen, Denmark) thermophilic yoghurt starter culture at a concentration of 0.005% (w/v).

The inoculated goat and sheep LWCs were placed in an incubation chamber (Jenogand Y 1000) at 43 °C and the pH and titratable acidity were monitored until the products reached a pH value of 4.6. Fermentation was stopped by rapid cooling to 20 °C in less than 30 minutes. Afterwards, the yoghurts were placed in the refrigeration chamber at 2 ± 2ºC for 12 hours.

For each type of LWC, three frozen yoghurts with increasing inulin concentrations were produced. The remaining ingredients used in frozen yoghurt formulations were added in the proportions presented in Table 1.

Table 1. Formulations used for the production of frozen yoghurts (% w/w).

PRODUCT	0% INULIN	2.5% INULIN	5.0 INULIN
LWC yoghurt	89.4	86.9	84.4
Inulin	0.0	2.5	5.0
Guar gum	0.1	0.1	0.1
Xanthan gum	0.1	0.1	0.1
Honey	5.0	5.0	5.0
Sugar	5.0	5.0	5.0
Citric acid	0.4	0.4	0.4

The ingredients were added to yoghurts and stirred until a homogeneous distribution was obtained. The mix was allowed to maturate for 12 hours at 0 ± 2 ºC chamber. For each formulation, two 1L batches of frozen yoghurts were produced in a laboratory scale frozen yoghurt freezer (Klarstein, Electronic Star) for 40 minutes. After this process, the frozen yoghurts were stored under refrigeration in a Zanussi freezer chest at -21 ± 1 ºC for 21 days. Formulations 1, 2 and 3 correspond to ewe's frozen yoghurts with 0, 2.5 and 5.0 %

added inulin, while formulations 4, 5 and 6 correspond to goat's frozen yoghurts with 0, 2.5 and 5.0 % added inulin.

2.3. Physico-chemical analysis

2.3.1. Compositional analysis

Total solids were determined by drying the samples in a Schutzart DIN 40050-IP20 Memmert™ oven, according to NP 703: 1982 for yoghurt [37] and AOAC (1997) for frozen yoghurt [38]. The ash content was determined by incineration of dry samples in a HD-23 Hobersal™ electric muffle furnace. The fat content was determined by the Gerber method (SuperVario-N Funke Gerber™ centrifuge) according to NP 469: 2002 [39] and by the Soxhlet method using 2050 Soxtec Auto Extraction Unit Foss Tecator™. The total N content was determined by the Kjeldahl method in the Digestion System 6 1007 Digester Tecator™ following the AOAC (1997) standard and the conversion factor of 6.38 was used to calculate the percentage of protein [38]. All analyzes were performed in triplicate.

2.3.2. pH and titratable acidity

The pH was determined with a HI 9025 HANNA Instruments pH meter, in order to monitor the evolution of the pH over yoghurt fermentation, immediately after production of yoghurts and on the 7^{th}, 14^{th} and 21^{st} days of storage of frozen yoghurts. The titratable acidity, expressed in g of lactic acid per liter, was determined by titration using a 0.1 N NaOH solution according to the technique described in NP 701: 1982 for yoghurts [40] and AOAC (1997) for frozen yoghurts [38].

2.3.3. Color analysis

The color of the yoghurt and frozen yoghurt was determined with a Minolta Chroma Meter, model CR-200B colorimeter calibrated with a white standard (CR-A47) using the CIELAB L*a*b* system. Five measurements were taken for each sample.

2.3.4. Viscosity of yoghurts

The evaluation of the yoghurt viscosity was carried out in triplicate for 10 min (30 s intervals), in a rotational Brookfield Viscometer, model DV2T, with a concentric cylinder RV (spindle 3) at a constant angular speed (100 rpm for goat's yoghurt and 1 rpm for sheep's yoghurt). The viscosity of the samples was the mean value of the viscosity measurements recorded during the 10 min of analysis.

2.3.5. Rheological analysis of yoghurts

The rheological properties of the yoghurts were evaluated in a rheometer (Rheostress 1, ThermoHaake™) in oscillatory mode. The measurement system consisted of a cone and plate geometry, C60/Ti-0.052mm (35mm diameter and 1° angle). Stress sweep tests were performed at 1 Hz to investigate the rheological linear viscoelastic behavior of the yoghurts. The elastic modulus (G'), the viscous modulus (G'') and the complex viscosity (η*) of the products were evaluated in the range of 0.3 to 6.5 rad/s at 3 Pa.

2.3.6. Texture of yoghurts

A Stable Micro Systems texture analyzer, model TA.XT Express Enhanced, was used to perform the texture analysis of the yoghurts one day after production (n=5) and the results were calculated using the Specific Expression PC software. A TPA-type test was

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run with a penetration distance of 20 mm at 2 mm/s using an acrylic cylindrical probe with a diameter of 25.4 mm and a height of 38.1 mm.

2.3.7. Overrun

Overrun is the increase in the volume of the yoghurt when making frozen yoghurt due to the incorporation of air. The method described by Skryplonek *et al.* [41] was followed, measuring the weight of the yoghurt and that of the frozen yoghurt with the same volume.

Overrun [%] = (weight of yoghurt - weight of frozen yoghurt/weight of frozen yoghurt) x 100

2.3.8. Meltdown rate

The meltdown rate was determined by the method described in Skryplonek *et al.* [42] by placing 30 g of frozen yoghurt on a stainless-steel screen with 1×1 mm opening, located on top of a beaker. After 45 min at 20 ± 1 °C, the weight of the sample collected in the beaker was measured. The meltdown rate was expressed as the percentage of the melted frozen yoghurt weight divided by the initial frozen yoghurt weight].

Meltdown [%] = (weight of melted frozen yoghurt/initial weight of frozen yoghurt) x 100

2.4. Microbiological analysis

The microbial counts of lactic acid bacteria (LAB) of the genera *Streptococcus* and *Lactobacillus* were analyzed after production and during storage at -21 °C of frozen yoghurts. Streptococci and lactobacilli were enumerated on plates at 37 °C for 48h on M17 agar (in aerobiosis) and on MRS agar (in anaerobiosis) (Biokar Diagnostics, France), respectively, according to ISO 7889, IDF 117 (2003) [43]. 1 mL of dilutions -5 to -8 were inoculated in triplicate along with two controls for each medium.

2.5. Sensory analysis

Consumer preference tests were conducted with an untrained panel within 6 days of storage. A hedonic test was performed for the yoghurts indicating the preference and evaluating the aroma, texture, flavor and global appreciation on a scale from 1 to 5 (1 = I don't like it at all to 5 = I like it very much) using a non-trained panel with 34 members. For the frozen yoghurts, a preference test was also performed; the control frozen yoghurt with 0% inulin was compared to the frozen yoghurts with 2.5% and 5% inulin. Panelists were asked to evaluate the characteristics of consistency, appearance, aroma, flavor, and global evaluation using a hedonic scale from 1 to 5. Thirty consumers participated in the panel.

3. Results

Ewe's and goat's UF concentrated whey yoghurts produced with UF concentrated whey were significantly different regarding their composition and textural properties. As it can be observed in Fig. 1, ewe's yoghurts presented lower solids content than goat's yoghurts. The main differences resulted from the significantly lower fat content of ewe's yoghurts when compared to goat's yoghurts. However, ewe's yoghurts presented significantly higher amounts of protein and minerals. Ewe´s yoghurts presented a solid nature and were much harder than goat's yoghurts, which behaved as a viscous liquid (hardness

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values of 13.5 and 0.5 N respectively for ewe's and goat's yoghurts). Large differences were also observed in the viscosity of both types of yoghurt (Table 2). These differences result mainly from the protein content differences, which in the case of goat's LWCs was probably below the minimum required to form a structured gel.

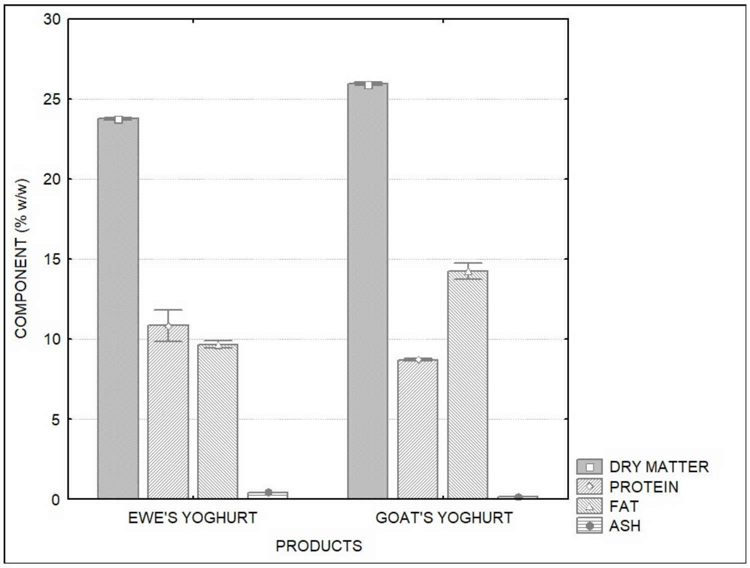

Fig. 1. Gross composition of ewe's and goat's yoghurts produced with UF concentrated cheese whey.

Table 2. Texture parameters of ewe's and goat's yoghurts one day after production (mean values and standard deviation).

PARAMETER	EWE'S YOGHURT		GOAT'S YOGHURT	
	Mean	±	Mean	±
Hardness (N)	13.55[a]	0.33	0.50[b]	0.08
Adhesiveness (N.s)	0.53[a]	0.03	-0.51[b]	0.10
Gumminess (N)	13.52[a]	0.34	0.47[b]	0.08
Cohesiveness	1.00[a]	0.01	0.93[b]	0.02
Resilience	0.9 [a]	0.02	0.04[b]	0.01
Viscosity (Cps)	16 946.67[a]	46.19	143.33[b]	12.66

Different superscript letters indicate significant ($p<0.05$) differences between values in rows.

Small strain oscillatory tests also confirmed the differences in texture of both products. Goat's yoghurts behaved as liquids with the viscous modulus (G'') higher than the elastic modulus (G') while ewe's yoghurts presented higher values for G' (Fig.2). Clear difference can also be seen concerning the complex viscosity of both products, with higher values for ewe's yoghurt.

Regarding color parameters (Fig.3), it could be observed that ewe's yoghurts were significantly darker (lower L* values) when compared to goat's yoghurts. Goat's yoghurts presented slightly lower values for parameter a* (green-red axis) and slightly higher values for parameter b* (blue-yellow axis).

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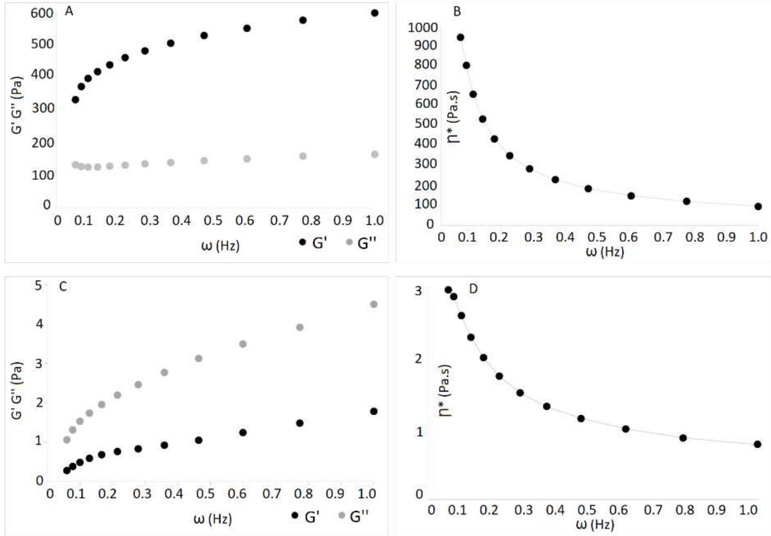

Fig. 2. Elastic (G') and viscous (G'') modulii and complex viscosity of ewe's (A, B) and goat's (C, D) yoghurts produced with UF concentrated cheese whey.

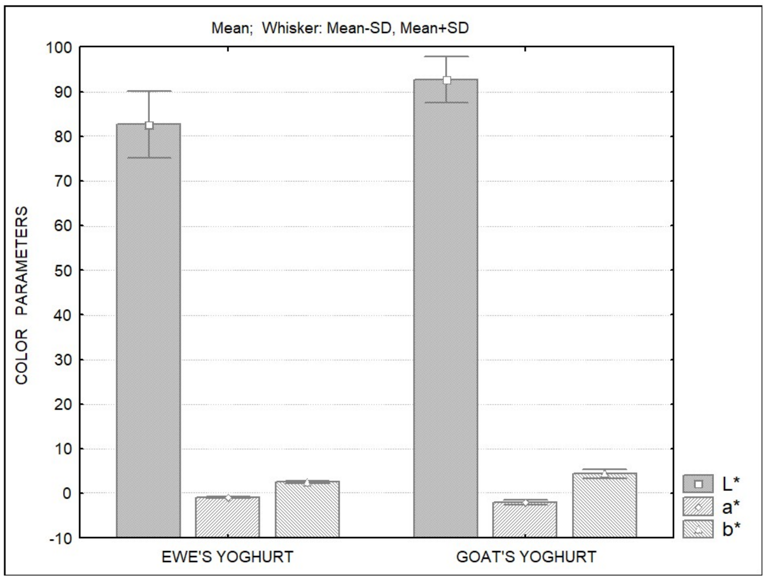

Fig. 3. CIEL*a*b* color parameters of ewe's and goat's yoghurts produced with UF concentrated cheese whey.

As expected, the different composition of yoghurts was reflected in the composition of frozen yoghurts with different levels of added inulin (Fig.4). Ewe's frozen yoghurts presented higher levels of protein and lower levels of fat. The fat content of formulation 4 (goat's frozen yoghurt without inulin addition) is significantly higher than all other formulations. This difference might have resulted from an uneven distribution of fat, possibly resulting from fat separation during the freezing process which took 40 minutes.

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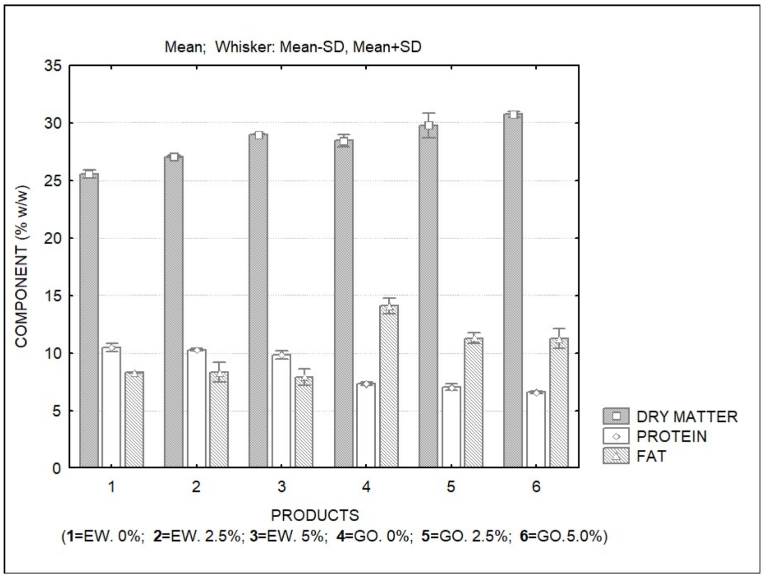

Fig. 4. Gross chemical composition of ewe's and goat's frozen yoghurts produced with UF concentrated cheese whey over 21 days of refrigerated storage (EW. 0%=Ewe's yoghurt with 0% inulin addition; EW. 2.5%=Ewe's yoghurt with 2.5% inulin addition; EW. 5.0%=Ewe's yoghurt with 0% inulin addition; GO. 0%=Goat's yoghurt with 0% inulin addition; GO. 2.5%=Goat's yoghurt with 2.5% inulin addition; GO. 5%=Goat's yoghurt with 5% inulin addition).

The pH and titratable acidity of ewe's and goat's frozen yoghurts are displayed in Fig. 5. These parameters were evaluated weekly over 3 weeks of frozen storage. For each formulation, a slight increase in pH and a decrease in titratable acidity values was observed during storage. Formulations 5 and 6 (goat's yoghurts with 2.5 and 5.0% added inulin) presented significantly lower pH and higher acidity values when compared to all other formulations. The goat´s whey presented an initial acidity value higher than the ewe´s whey. The acidity increased slightly during the UF concentration process and the obtained yoghurts presented pH values around 4.0. Most probably, the reduction in pH much below the target value defined for yoghurt production (4.5) resulted from the slow refrigeration of the 2 L flasks in which they were produced. The presence of inulin can not be considered a possible explanation for the drastic pH reduction of those two formulations since the same pattern was not observed with ewe's frozen yoghurts.

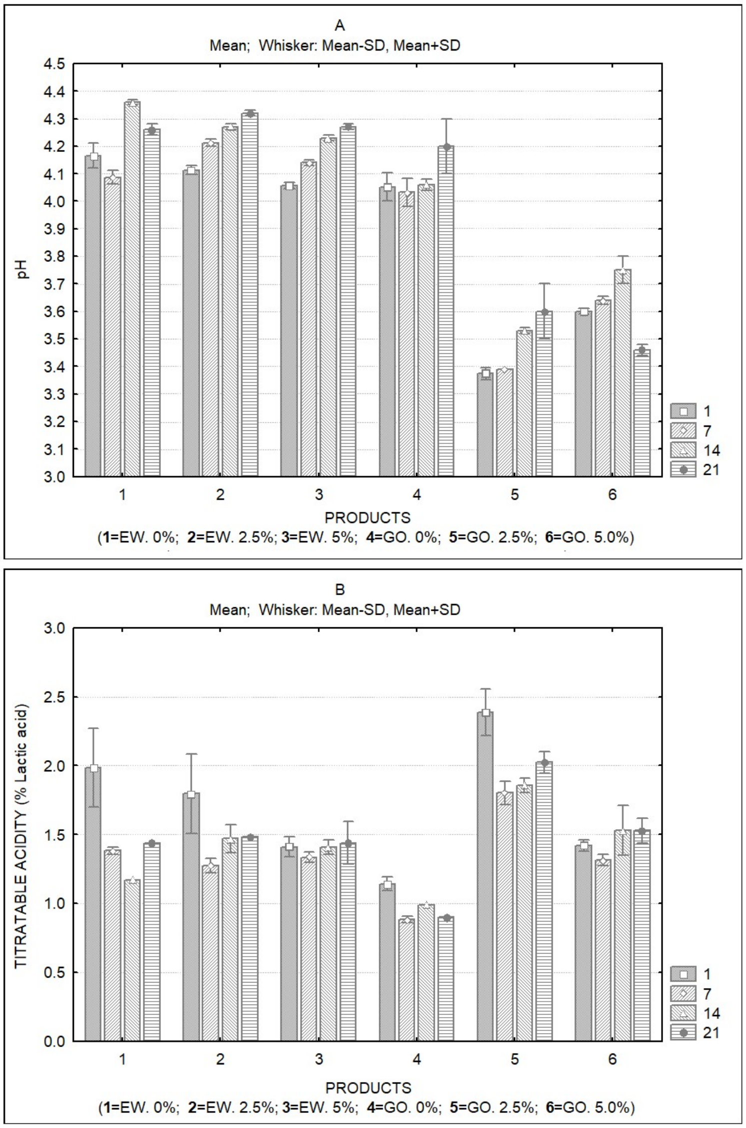

Fig. 5. (A) pH and (B) titratable acidity of ewe's and goat's frozen yoghurts produced with UF concentrated cheese whey over 21 days of refrigerated storage (EW. 0%=Ewe's yoghurt with 0% inulin addition; EW. 2.5%=Ewe's yoghurt with 2.5% inulin addition; EW. 5.0%=Ewe's yoghurt with 0% inulin addition; GO. 0%=Goat's yoghurt with 0% inulin addition; GO. 2.5%=Goat's yoghurt with 2.5% inulin addition; GO. 5%=Goat's yoghurt with 5% inulin addition).

The color parameters of frozen yoghurts (Fig. 6) were slightly different from those of the original yoghurts, being the differences more marked in ewe's frozen yoghurts. The whiteness (L*) increased in ewe´ frozen yoghurts as compared to the original products, and similar trend was observed regarding the b* parameter (increased yellowness). These changes are probably the result of air incorporation during the freezing process.

With regard to overrun (Table 3), with the exception of formulation 3 (ewe's frozen yoghurt with 5% inulin addition) that presented the highest value (40.29%), no significant differences were observed between ewe's and goat's frozen yoghurt formulations. However, with regard to the meltdown rate (Table 3), ewe's frozen yoghurts presented values below 10, while goat´s frozen yoghurts had values above that value. In these yoghurts, it appears that the addition of inulin improved this property.

Table 3. Overrun and meltdown rate of ewe's and goat's frozen yoghurts immediately after production (mean values and standard deviation).

	EW. 0%	±	EW. 2.5%	±	EW. 5.0%	±	GO. 0%	±	GO. 2.5%	±	GO. 5.0%	±
Overrun	31.04[a]	1.89	31.09[a]	2.04	40.29[b]	2.20	29.34[a]	1.45	31.13[a]	1.56	29.34[a]	2.23
Meltdown Rate	3.47[a]	0.03	6.33[b]	0.03	4.41[c]	0.10	27.99[d]	0.01	13.07[e]	0.50	15.00[f]	0.55

Different superscript letters indicate significant ($p<0.05$) differences between values in rows.

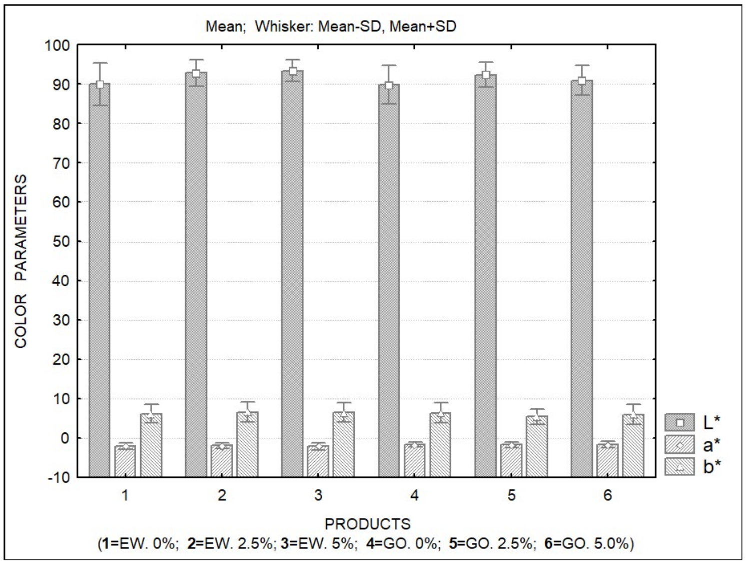

Fig. 6. CIEL*a*b* color parameters of ewe's and goat's frozen yoghurts produced with UF concentrated cheese whey over 21 days of refrigerated storage (EW. 0%=Ewe's yoghurt with 0% inulin addition; EW. 2.5%=Ewe's yoghurt with 2.5% inulin addition; EW. 5.0%=Ewe's yoghurt with 0% inulin addition; GO. 0%=Goat's yoghurt with 0% inulin addition; GO. 2.5%=Goat's yoghurt with 2.5% inulin addition; GO. 5%=Goat's yoghurt with 5% inulin addition).

With regard to the microbial characteristics of frozen yoghurts, Fig. 7 presents the counts of lactobacilli and streptococci at the day of production of the frozen yoghurt samples. With the exception of formulation 6 (goat's frozen yoghurt with 5.0% inulin incorporation), all products presented adequate levels (> log 6 UFC g^{-1}) of LAB. However, lower counts of streptococci in all goat's frozen yoghurts were observed. Table 4 presents the LAB counts of both frozen yoghurt types over a 21 days storage period. In the case of ewe's yoghurts without added inulin it can be observed that adequate levels of LAB were maintained for 2 weeks. However, at the 21st of storage, lactobacilli were present at low levels (*ca.* 5 log CFU g^{-1}) while streptococci were absent. Regarding goat's frozen yoghurts without inulin addition, adequate levels of LAB were observed for seven days, but streptococci were already presented low levels by the 7th day of storage. At the 14th and 21st days of storage, lactobacilli were absent while streptococci surpassed 6 log UFC g^{-1}. The formulation of goat's frozen yoghurt containing 2.5% inulin presented low levels of streptococci (5.42 log UFC g^{-1}) at the first day. Lactobacilli were absent at the 7th, 14th and 21st days of storage. Streptococci also presented low counts over frozen storage. Curiously, the formulation containing 5.0% inulin presented inadequate levels of LAB at the first day, while at the 7th day LAB counts surpassed 6 log CFU g^{-1}. At the 14th and 21st day of storage the pattern was similar to the one observed with formulation 5 (2.5% inulin). Generally, it can be indicated that ewe's concentrated whey was more effective in promoting LAB growth. However, further studies must be undertaken to confirm these results. In fact, several research works regarding frozen yoghurts indicate high survival rates for both LAB and probiotic bacteria. The results obtained in the present study for goat's frozen yoghurts can not be considered satisfactory. We could not ascertain whether this behavior resulted from the composition of goat's whey, as opposed to ewe's whey, or if it resulted from the characteristics of this goat's whey batch in particular.

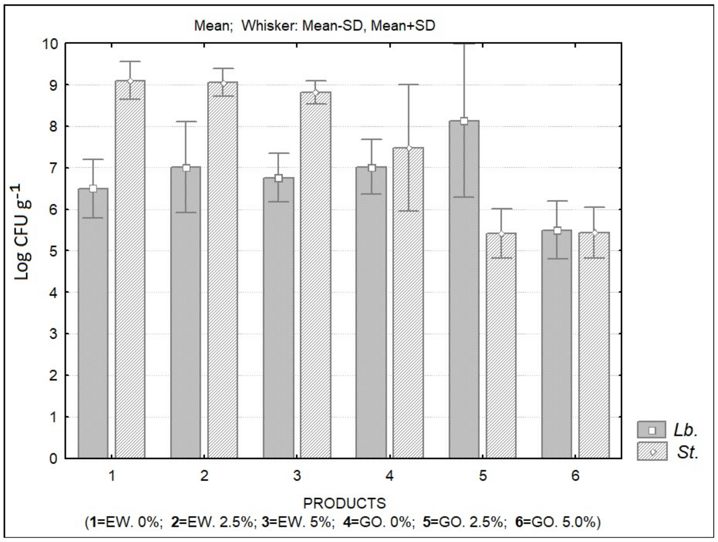

Fig. 7. *Lactobacilli* sp. (Lb.) and *Streptococci* sp. (St.) counts (log CFU g^{-1}) of ewe's and goat's frozen yoghurts produced with UF concentrated cheese whey at the day of production (EW. 0%=Ewe's yoghurt with 0% inulin addition; EW. 2.5%=Ewe's yoghurt with 2.5% inulin addition; EW. 5.0%=Ewe's yoghurt with 0% inulin addition; GO. 0%=Goat's yoghurt with 0% inulin addition; GO. 2.5%=Goat's yoghurt with 2.5% inulin addition; GO. 5%=Goat's yoghurt with 5% inulin addition).

Table 4. Microbial counts (log CFU g^{-1}) of ewe's and goat's frozen yoghurt over storage. *Lb.*=lactobacilli; *St.* streptococci (mean values and standard deviation).

DAYS		0% INULIN		2.5% INULIN		5.0% INULIN	
EWE´S		Mean	±	Mean	±	Mean	±
1	*Lb.*	6.50^a	0.71	7.02^a	1.09	6.76^a	0.59
	St.	9.11^a	0.45	9.05^a	0.33	8.82^a	0.28
7	*Lb.*	7.86^a	0.41	7.92^a	0.39	7.88^a	0.32
	St.	9.28^a	0.94	9.07^a	0.54	8.56^a	0.28
14	*Lb.*	6.00^a	1.41	6.05^a	0.02	5.80^a	0.30
	St.	8.22a	0.37	8.01^a	0.49	8.09^a	0.62
21	*Lb.*	5.33^a	0.47	5.86^a	1.26	5.00^a	0.10
	St.	0.00^a	0.00	0.00^a	0.00	0.00^a	0.00

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GOAT´S		Mean	±	Mean	±	Mean	±
1	*Lb.*	7.02[a]	0.66	8.14[a]	1.84	5.50[b]	0.70
	St.	7.48a	1.53	5.42a	0.60	5.44[b]	0.61
7	*Lb.*	6.99[a]	0.97	0.00[b]	0.00	6.14[a]	0.63
	St.	5.65[a]	0.49	6.68[a]	1.23	7.85[a]	1.58
14	*Lb.*	0.00[a]	0.00	0.00[a]	0.00	0.00[a]	0.00
	St.	6.94[a]	0.11	5.43[b]	0.51	5.74[b]	0.37
21	*Lb.*	0.00[a]	0.00	0.00[a]	0.00	0.00[a]	0.00
	St.	6.15[a]	1.63	5.33[a]	0.58	6.92[a]	0.99

Different superscript letters indicate significant ($p<0.05$) differences between values in rows.

Fig 8. shows the results of the sensory analysis performed to both yoghurts and frozen yoghurts. It is clear the consumers preference with regard to ewe's whey yoghurts. The sensory attributes that more penalized goat´s yoghurts evaluation were texture and taste. The liquid nature and the higher acidity of this yoghurt were pointed as the main negative factors. With regard to frozen yoghurts, it is clear that, for both types of yoghurts, the formulations containing the highest level of inulin were preferred (formulations 3 and 6). The improvement of goat's whey yoghurt's sensory attributes after freezing is evident. Consistency and appearance of frozen goat's yoghurts received similar scores to ewe´s yoghurts. Despite aroma and taste of goat's frozen yoghurts were improved as compared to the original goat's yoghurts, these attributes were less valuated when compared to the same attributes in frozen ewe's yoghurts. It should be referred that no flavoring agents were added to the formulations. The flavor of the products was directly linked to the specific composition of the LWCs and to the addition of sucrose and honey. Addition of fruit purees and flavoring agents could improve the sensory attributes of both products, further increasing their acceptability.

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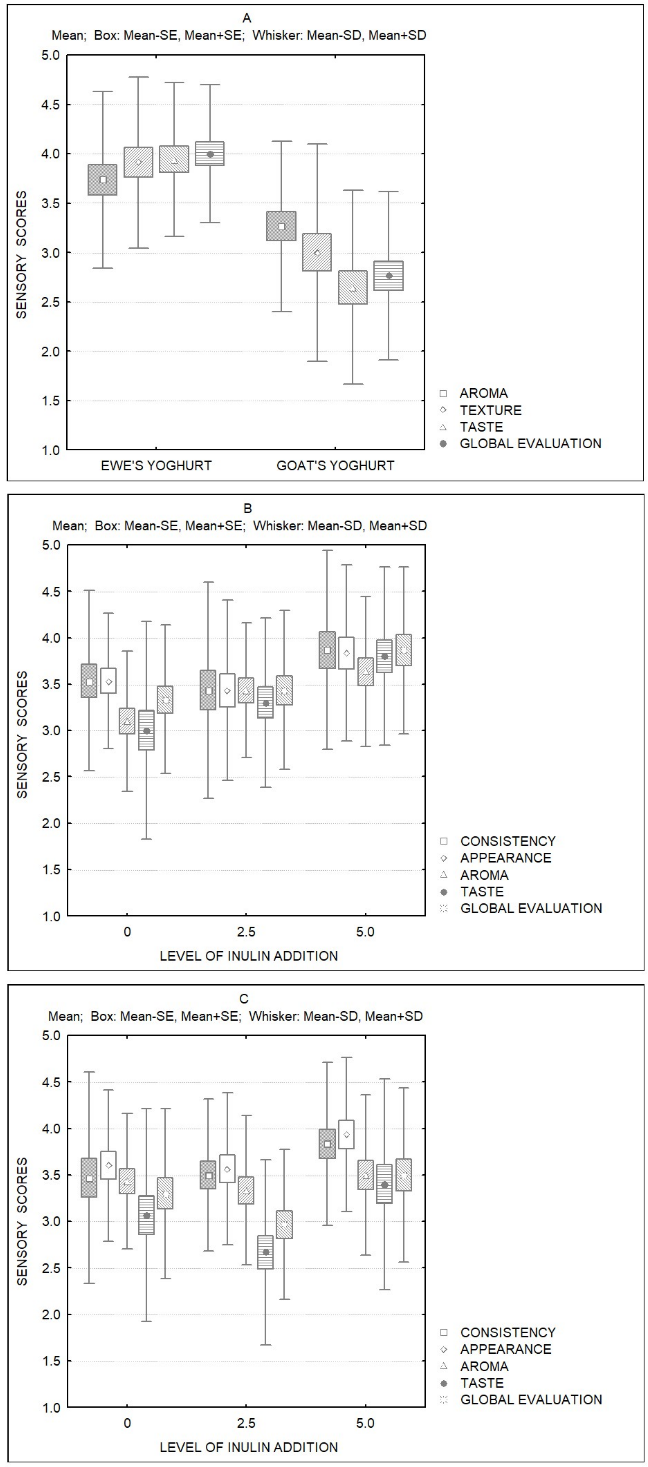

Fig. 8. Sensory scores of ewe's and goat's yoghurts and correspondent frozen yoghurts produced with UF concentrated cheese whey at the day of production (A=ewe´s and goat´s yoghurts; B=ewe´s frozen yoghurts, C=goat´s frozen yoghurts).

4. Discussion

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In previous work we highlighted the potential of bovine LWC's as primary raw material for acid dairy gels. Yoghurt type gels and gels produced by acidification with glucono-δ-lactone were characterized, and the ability of such concentrates to produce yoghurts or dairy desserts was reported [44].

Regarding the physicochemical characteristics of frozen yoghurts other research works report the manufacture of products with proximal compositions similar to the ones obtained in our study. However, all the studies are about frozen yoghurts manufactured with cow milk, which implies many differences in protein composition and interactions. Pereira et al. [45] evaluated the influence of pH on the characteristics of frozen yoghurts. The formulation consisted of 6 % of milk fat, 10 % of milk solids-not-fat, 11 % sucrose, 3 % corn syrup, 0.3 % emulsifiers and 0.5 % stabilizers. Strawberry fruit was added to the fermented frozen yoghurt mix at a level of 4 % (w/w). The treatments with final pH of 5.0 and 5.5 presented higher acceptability and acidity close to the ideal. It was also found that the physicochemical characteristics, acidity content and sugar concentration had effects on the acceptance of the product [45]. Soukoulis & Tzia [46] tested different acidification procedures and stabilizers as well as two protein-fortifying agents (skim milk powder and whey powder) regarding their influence on the quality characteristics of frozen yoghurts. Indirect acidification, by blending of plain acidified milk with frozen yoghurt mix, was found to favor texture while direct acidification (fermentation of frozen yoghurt mix with starter culture) improved viscosity but did not enhance the sensory acceptance of frozen yoghurts. The addition of 0.2% xanthan gum and the partial substitution of skim milk powder by whey powder increased overall acceptance and creaminess [46]. These frozen yoghurts presented values of overrun similar to the ones obtained by us. Milani & Koocheki [47] tested the effects of date syrup and guar gum on physical, rheological and sensory properties of low-fat frozen yoghurt. Guar gum softened the frozen yoghurt whereas date syrup increased the hardness of the product. Meltdown stability was improved with the increase in gum and date syrup concentration. Low-fat frozen yoghurts prepared showed good sensory properties [47]. Other authors refer that guar and arabic gum addition significantly improved physicochemical, sensory and flow behavior properties of frozen yoghurt. Gums significantly affected the viscosity, overrun and melting rate of frozen yoghurt [48]. Water-soluble polysaccharides (WSP) extracted from Chinese truffle were also tested in frozen yoghurts. WSP consisted of glucose, mannose, galactose, and lyxopyranose. The presence of WSP reduced significantly syneresis, improved the viscosity and titratable acidity and contributed to a better flavor in the tested yoghurt when compared to control frozen yoghurts [49]. Skryplonek et al. [41] produced lactose-free frozen yoghurts which presented significantly lower hardness and stickiness and higher viscosity than control frozen yoghurt. Moreover, lactose hydrolysis promoted a smooth and creamy consistency, whereas in the case of conventional products a coarse structure, due to the presence of large ice crystals, was identified. Hydrolysis of lactose also improved the sweetness and brightness of frozen yogurt [41]. Lactose free frozen yoghurts with added k carrageenan or corn starch were also tested [42]. The nutritional supplementation of frozen yoghurts with carrot pulp has also been reported [50].

The addition of inulin at 2 % level, improved overrun, viscosity and melting properties of frozen yoghurt with probiotics. Acceptability tests revealed that frozen yogurt with 2 % inulin had the most appealing sensory characteristics. The flow behavior of all samples showed pseudo-plastic nature. In terms of probiotic survival, the sample with 2 % inulin significantly improved the viability of *Lactobacillus acidophilus* and *Bifidobacterium lactis* [51]. Similarly, in the present study, the highest overall acceptability was obtained by the formulations containing 5.0% inulin (formulations 3 and 6).

With regard to survival of LAB and probiotic bacteria on frozen yoghurts, the microencapsulation of was also tested. Yoghurts were added with microcapsules containing Bifidobacterium BB-12 produced with different contents of carrier agents (reconstituted skim milk and inulin). The counts of bifidobacteria were maintained practically constant in the samples added with the microcapsules, while the frozen yogurt added with free bacteria showed a decrease of about 34% after 90 days [52]. Even with this significant reduction, these values are higher than those obtained in our study. Besides, Soodbakhsh and coworkers [53] evaluated the viability of encapsulated *L. casei* and *Bifidobacterium lactis* in synbiotic frozen yoghurt. The viable cell number of *L. casei* and *B. lactis* in frozen yoghurt mixture was $4.7*10^8$ and $5*10^8$ CFU g^{-1} at the day of production and, after 150 days of storage, these numbers were decreased to $4.5*10^6$ and $3.5*10^6$ CFU g^{-1}, respectively. These data for encapsulated bacteria were $7.3*10^8$ and $9*10^8$ CFU g^{-1} at the day of production and at the end of survey it decreased to $8.6*10^7$ and $9.6*10^7$ CFU g^{-1}. The addition of encapsulated probiotics and inulin had no significant effect on the sensory properties of frozen yoghurt, but inulin improves viscosity and melting properties [53]. These results are also higher than those obtained by us. In another work, a commercial yoghurt culture was used as starter culture while free and immobilized probiotic cells were added as adjuncts for frozen yoghurt production. The viability of the immobilized probiotic cells was maintained in high levels during 90 storage days at -18 °C, while the viability of free probiotic cells decreased ca. 10%. *L. bulgaricus* counts reduced by approximately 3 log cycles and no counts of *S. thermophilus* were detected by the end of freezing storage. Gastrointestinal simulation showed that cell immobilization offer protection to probiotic cells against the harsh environmental conditions of the gastro-intestinal tract and help in maintaining the minimum viable cell counts required to offer health benefits to the consumers (> log 7 CFU g^{-1}) [54]. According to these results, we conclude that further work with the aim of evaluating the survival of starter or probiotic cultures in LWCs should be further investigated in order to evaluate possible causes for the low LAB survival rates obtained in the present work.

5. Conclusions

Considering the results so far obtained, it can be concluded that the concentration of ewe's or goat's whey by means of ultrafiltration can give rise to the production of liquid whey concentrates that can be used in the production of novel dairy products such as yoghurts and frozen yoghurts. In both cases, levels of more than 10% protein in the LWC's are recommended. Besides, considering the information already available regarding the incorporation and survival of probiotic on such products, we consider that it will be possible to produce novel synbiotic yoghurts based on ewe's or goat's whey. This will represent an attractive way to valorize such by-products as a complement to the production of whey cheeses, which is already common in dairies manufacturing small ruminant's milk cheeses. The processes of production of yoghurts and frozen yoghurts do not require the acquisition of expensive equipment and the technical skills can easily be transferred to the existing personal. These will ease the introduction of such technologies in small and medium scale dairies.

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Conflicts of Interest: Authors declare no conflict of interest.

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