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Ovine ice cream made with addition of whey protein concentrates of ovine-caprine origin

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A B S T R A C T

The use of whey protein concentrates (WPC) of ovine/caprine origin in the manufacture of ovine ice cream (5% fat) was evaluated. Three ice cream mixes were prepared using ovine skimmed milk and cream, stabilisers/emulsifiers, mix of sucrose:fructose (50:50) and bovine skimmed milk powder (SMP) (35% protein; ice cream A), WPC 65% protein (ice cream B) or WPC 80% protein (ice cream C). Ice cream A melted faster than ice creams B and C, and presented higher overrun (76%), compared with ice cream B (46%) and ice cream C (36%); for hardness the opposite was true. Ice cream A was significantly brighter, ice creams B and C were more yellow. The flavour scores of ice cream B was similar to those of ice cream A. The substitution of bovine SMP with ovine/caprine WPC (65% protein) in ovine ice cream to produce a product with no ingredient of bovine origin seems to be feasible.

1. Introduction

Ice cream is the most popular dairy dessert in the world. It consists of several different components that make it a complex food with multiple phases/structure. Structure of ice cream is formed by serum, ice crystals, air cells, fat globules and clusters, and protein/stabilisers (Goff & Hartel, 2013). Generally, ice cream should contain fat, milk-solids-non-fat (MSNF) that are mainly proteins, lactose and minerals, sweeteners, stabilisers, emulsifiers, water, and other substances, i.e., flavours and colours. When making the formulation of ice cream mix, addition of skimmed milk powder (SMP) or whey powder products is a practice to increase the MSNF and in the latter case to take the advantage of whey proteins functionality (Heino, Uusi-Rauva, Rantamaki, & Tossavainen, 2007; Hernández-Ledesma, Ramos, & Gómez-Ruiz, 2011). Thus, depending on their protein and lactose content, whey powders, whey protein concentrates (WPC) or whey protein isolates (WPI) are used in ice cream at such ratios that will not cause crystallisation defect due to lactose content.

The vast majority of ice cream production worldwide is made from bovine milk. However, ovine dairy products are unique with regard to their quality and in general, they are evaluated as more delicious and nutritious than their bovine counterparts (Hayaloglu & Karagul-Yuceer, 2011; Moschopoulou & Moatsou, 2017; Pandya & Ghodke, 2007).

Numerous research works conducted in recent years for bovine ice cream concern mainly the incorporation of prebiotics, probiotic microorganisms, dietary fibre, low glycaemic index sweeteners or natural antioxidants to increase the bio functionality (Ozturk, Demirci, & Akin, 2018; Tsevdou et al., 2019). Soukoulis, Fisk, and Bohn (2014) have overviewed the innovative functional ingredients and the techniques for incorporating them in ice cream. Furthermore, WPI, WPC and inulin have been reported as fat replacers to produce low- or reduced-fat bovine ice creams (Akalin, Karagözlü, & Ünal, 2008; Khillari, Zanjad, Rathod, & Raziuddin, 2007; Mahdian & Karazhian, 2013; Pinto, Prajapati, Patel, Patel, & Solanky, 2007). For the same reason, WPC modified by high pressure has been used (Lim, Swanson, Ross, & Clark, 2008).

On the other hand, literature for ovine ice cream is scarce. Previous studies concern formulation issues (Martinou-Voulasiki & Zerfiridis, 1990; O'Kane & Wilbey, 1990; Wilbey, Allen, Anstis, & Cameron, 1995), while recent research on ovine ice cream deals with incorporation of prebiotics and/or probiotic microorganisms (Balthazar et al., 2017a,b, 2018; Nadelman et al., 2017).

As mentioned, SMP and whey powders are used to increase the MSNF of ice cream. However, in ovine ice cream, dairy powders of ovine or/and caprine origin are particularly important to keep the identity of the product and to avoid allergens derived from bovine powders. The aim of the present research

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work was to produce a dietary (low-fat) ice cream with no ingredient of bovine origin using ovine or/and caprine WPC instead of bovine SMP and to investigate its effects on the main quality characteristics.

2. Materials and methods

2.1. Materials

Raw milk was obtained from the sheep farm of Agricultural University of Athens. Partially skimmed milk (0.5% fat) and ovine cream (45% fat) were obtained after fat separation of the raw milk. Medium heat bovine SMP (Arla Foods, Visby, Sweden) contained on average 1.25% fat, 35% protein, 52% lactose and 8% ash. Two WPC products produced from Feta cheesemaking, i.e., from mixed ovine/caprine whey containing caprine whey up to 30%, were supplied by Hellenic Protein S.A. (Ioannina, Greece). WPC65 contained on average 2% fat, 65% protein, 26% lactose and 3% ash, while WPC80 contained 4% fat, 80% protein, 10% lactose and 2% ash. Moreover, the foam expansion (FE, %) as well as the foam stability (FS, %) of the three powders (4%, w/v, aqueous solution) were determined according to the method described by Diaz, Pereira, and Cobos (2004). FE was 100%, 65% and 85% for the SMP, WPC65 and WPC80, respectively, while FS was 0%, 15.2% and 17.5%, respectively. Stabilisers and emulsifiers were the commercial blend Cremodan SE 334 VEG that contained mono- and diglycerides of fatty acids, guar gum, cellulose gum and carrageenan (DuPont, supplied by Alteco S.A. Food Ingredients, Athens, Greece). Sucrose, fructose, and vanilla flavour were obtained from the retail market.

2.2. Ice cream mix formulation

Three ice cream mixes using SMP (ice cream mix A), or WPC-65% protein (ice cream mix B), or WPC-80% protein (ice cream mix C) and the above mentioned other materials were formulated as shown in Table 1. The goal was to prepare ice cream mixes with 5% fat and 12% MSNF contents. According to the current EC regulations, the minimum fat content of dairy ice creams is 5%, while in countries outside the EC, i.e., USA, New Zealand, Australia it is 8–10% (Goff & Hartel, 2013; Mullan, 2016). In general, the composition of the three mixes where within the limits for low-fat ice creams, i.e., min fat 5%, sweeteners 18–21%, emulsifiers/stabilisers 0.8%, MSNF 12–14% and total solids 28–32% (Goff & Hartel, 2013). Therefore, the ice cream of the present study will be referred to as low-fat ice cream.

2.3. Ice cream production

About 15 kg ovine raw milk was preheated to 40 °C and was skimmed on a cream separator (Milky, FJ12ERR, JANSCHITZ GMBH, Althofen, Austria). Ice cream mix was made by mixing skimmed milk, cream and all other ingredients, except vanilla flavour. After mixing, it was heated at 65 °C under continuous agitation and then was homogenised on a pilot scale homogeniser (HP 202 GEA, OMVE, Netherlands) at 250/50 bar. Then, the mix was batch pasteurised at 75 °C for 10min, followed by cooling to 25 °C. Vanilla flavour was added to the cooled pasteurised mix and the mix was aged at 4 °C for 24 h. Freezing of mix took place in a laboratory vertical batch freezer (Arktic Hendi B.V., Netherlands) for 30 min at −4 °C. One and half kg of ice cream was produced per batch, which was packed in 150 mL plastic containers and stored at −22 °C. The experiment was carried out in triplicate.

2.4. Analyses of the mix

Gross composition of ice cream mix was determined using Milkoscan FT-120 134 (Foss, Hilleroed, Denmark) after dilution with ultra-pure water at a ratio of 1:1. Determination of ash content was made by heating the ice cream mix at 525 °C ± 25 °C for 5.5 h. Calcium, magnesium, potassium and sodium contents were determined by the atomic absorption spectrometry method of International Dairy Federation (IDF, 2007) on a Shimadzu AA-6800 atomic absorption spectrophotometer equipped with the autosampler Shimadzu ASC-6100 and the software WizAArd v. 2.30 (Shimadzu Corporation, Kyoto, Japan).

pH was directly measured using a digital pHmeter (MultiLine IDS 3510, WTW) and acidity, expressed as (%) lactic acid, was determined by titration using NaOH N/9 solution and the indicator phenolphthalein.

The freezing point of the mix was determined using a cryoscope (CryoStar 1, Funke Gerber) after dilution with ultra-pure water at a ratio 1:2 to bring it into the proper operating range (Goff & Hartel, 2013).

All analyses of mix were performed in triplicate.

2.5. Analyses of ice cream

Except overrun, analyses of ice cream were carried out after 2 weeks of storage at −22 °C. The percentage of overrun was determined in all ice creams immediately after they exited the freezing machine.

Overrun (%) was calculated based on weights of a specific volume of ice cream mix and ice cream according to Goff and Hartel (2013) using the equation:

$$\%\ \text{Overrun} = \frac{\text{Wt. of mix} - \text{Wt. of same vol. of ice cream}}{\text{Wt. of same vol. of ice cream}} \times 100\%$$

Water activity (a_w) of ice cream was measured on the instrument AQUALAB Dew Point Water Activity Meter (Aqualab, METER Group, Inc. USA) at ~22 ± 3 °C (sample and room temperature).

The determination of melting behaviour was based on a combination of the methods described by Sofjan and Hartel (2004) and Roland, Phillips, and Boor (1999). Briefly, 50 g of ice cream (−15 °C), a cylinder with 5 cm diameter and 4 cm length, were placed on a 2-mm stainless-steel screen with a funnel and graduated cylinder on a balance beneath to collect and weight the melt. Timing of the melt-down measurement began when the first drop of melt touched the bottom of the cylinder. Volumes were recorded once every 10 min until entire melting. Analysis was carried out in duplicate and the melting behaviour was expressed as the weight of melt as % of initial weight.

Hardness (in N) of ice creams was measured by an Autograph Shimadzu testing instrument, model AGS-500 NG (Shimadzu Corporation, Kyoto, Japan) according to Lim et al. (2008). Before analysis, samples were stored at −15 °C for 24 h and during analysis the sample temperature was −10 °C. A cylindrical probe of diameter 6 mm was used, the penetration speed was set at 2.0 mm s^{-1}, and the penetration distance was 20 mm.

Colour measurements were performed using a portable colorimeter (Lovibond Colour Measurement LC 100, Tintometer GmbH, Germany). The parameters L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were measured for the ice creams. In addition, due to the visible difference in colour among SMP and WPCs, an aqueous solution 4% (w/v) of each of them in pure water was analysed for the colour parameters.

All analyses of ice cream were performed in duplicate.

Sensory evaluation was performed by a panel composed of eight experienced laboratory staff members (5 males and 3 females). Each assessor was provided with ice cream samples individually packed in 150 mL plastic pots that were presented randomly. The characteristic flavor, sweet taste, sandy texture, watery body, wheying-off and quick melting were scored on a scale from 0 to 5.

2.6. Statistical analysis

The obtained data were statistically evaluated by comparing the means using the Least Significant Difference test (LSD, $P < 0.05$) using the software Statgraphics (Centurion v, xv, Manugistics software, Inc., Rockville, Maryland, USA).

3. Results and discussion

3.1. Mix freezing point

The freezing point of ice cream mix depends on the concentration of soluble ingredients and varies depending on the composition. However, even if whey proteins are dissolved, they have little effect on the freezing point (Goff & Hartel, 2013). Therefore, the freezing point of the mix depends to a large extent on its sugar and MSNF contents, and from the latter particularly on the contents of lactose and minerals. The increased amount of lactose depresses the freezing point. Consequently, ice cream mix A showed significantly ($P < 0.05$) lower freezing point (Table 1) since SMP contained more lactose (52%) compared with mixes B and C. The obtained results are in line with those of El-Zeini Hoda, Moneir El-Abd, Mostafa, and Yasser El-Ghany (2016) who used WPC for up to 3% substitution of MSNF in the formulation of buffalo ice cream.

3.2. Mix composition

The composition of the three ice cream mixes showed significant differences ($P < 0.05$) in terms of protein, total sugars and ash contents (Table 2). Fat and total solids did not differ significantly ($P > 0.05$). Specifically, ice cream C had higher protein and lower sugar and ash contents than those of the other samples. The differences in protein, carbohydrates and ash contents were expected as they reflected the differences in the composition of the SMP or WPC. O'Kane and Wilbey (1990) have reported that in ovine ice cream protein content over the range 4.4–5.9% correlates well with the preference scores in sensory evaluation but the products with less than 5% protein taste watery.

Acidity and pH are related to the composition of the mix, an increase in MSNF increases the acidity and decreases the pH. According to Goff and Hartel (2013), the normal titratable acidity, i.e., that which has not increased by bacterial fermentation of lactose, depends on the percentage of MSNF and it ranges from 0.12 to 0.22 (% lactic acid) when MSNF content ranges from 7% to 13% respectively. Therefore, if fresh milk ingredients are used, the mix can be expected to have a normal acidity. The pH of the three ice creams differed significantly from each other (Table 2). The acidities of the three mixtures were within the above suggested range, but differed significantly ($P < 0.05$) firstly because of the ovine milk which contains more protein than bovine milk and secondly because of the increased protein content of WPC used. El-Zeini Hoda et al. (2016) have also reported significant differences in acidity when used WPC at a ratio from 1% to 4%. On the other hand, it seems that the use of lower ratio of WPC i.e., 1–1.5% has no significant effect on pH and acidity (Pinto et al., 2007).

The minerals calcium, magnesium, potassium and sodium make up most of the ash. Therefore, since the powders that were used differed in ash content, the results presented in Table 2 followed this differentiation. Ice cream A contained significantly ($P < 0.05$) more calcium, magnesium, potassium and sodium than ice creams B and C.

3.3. Ice cream physical characteristics

Overrun is the percent increase in volume that occurs because of air addition during agitation of the mix in the freezer. It is an important characteristic since it affects the weight per unit volume of the ice cream. Ice creams B and C showed significantly ($P < 0.05$) lower overrun (Table 3) than ice cream A. Overrun is affected by the total solids in the mix and the type of freezer used (Goff & Hartel, 2013). Since ice cream mix A, B and C had the same total solids content and were processed under the same conditions, the difference in the overrun could be attributed to the kind of the proteins and especially to the ratio of whey to casein. Ice cream A contained mainly caseins and native whey proteins, whereas ice creams B and C contained mainly whey proteins as they were made with WPC from processed cheese whey. Native whey protein powders have better foaming properties than cheese whey powders (Heino et al., 2007). In addition, it is known that the functional properties of whey proteins are affected by heating, although it has been reported that mild heat treatment, i.e., pasteurisation enhances the foaming properties of WPC (Mangino, Liao, Harper, Morr, & Zadow, 1987). As mentioned in the materials section, the foaming ability, expressed as foam expansion, of the SMP was 100%, while those of WPC65 and WPC80 were 65% and 80% respectively. The type of SMP used was medium heat and the WPC powders were manufactured from whey obtained from pasteurised Feta cheese. The denaturation degree of major whey proteins in WPC65 was insignificant as found by Moatsou, Hatzinaki, Kandarakis, and Anifantakis (2003) in a previous study. Moreover, it has been showed that proteins show selectivity in their adsorption at the air-serum interface and that caseins more easily adsorb than whey proteins, hence improving foaming (Zhang & Goff, 2004). Thus, the addition of milk protein concentrate (80% protein) in a mixture with high protein content (6–7%) causes positive effect on the aeration (Tomer & Kumar, 2013). In contrast, Daw and Hartel (2015) who

Table 1
Formulation (%) and physical characteristics of low-fat ovine ice cream mixes.[a]

Ingredient	A	B	C
Ovine milk (0.5% fat)	78.4	78.4	78.4
Ovine cream (45% fat)	7.6	7.6	7.6
Bovine SMP-35% protein	4	–	–
Ovine/caprine WPC-65% protein	–	4	–
Ovine/caprine WPC-80% protein	–	–	4
Mixed sucrose-fructose (1:1)	9	9	9
Emulsifiers/stabilisers	1	1	1
Freezing point	-0.510 ± 0.015^a	-0.466 ± 0.003^b	-0.453 ± 0.008^b

[a] Ovine ice cream mix contained bovine SMP-35% protein (A), WPC-65% protein (B) or WPC-80% protein (C) at same ratio. Freezing point was measured after dilution with ultra-pure water; (1:2); means with different superscript letters in the same row differ significantly ($P < 0.05$).

Table 2
Composition, mineral content, pH and titratable acidity of low-fat ovine ice cream mixes.[a]

Parameter	A	B	C
Fat (%)	4.90 ± 0.11[a]	5.34 ± 0.25[a]	4.97 ± 0.49[a]
Protein (%)	6.92 ± 0.05[a]	8.15 ± 0.11[a,b]	8.30 ± 0.72[b]
Carbohydrates (%)	13.71 ± 0.50[a]	12.69 ± 0.15[b]	11.73 ± 0.45[b]
Ash (%)	1.12 ± 0.05[a]	0.89 ± 0.06[b]	0.88 ± 0.02[b]
Total solids (%)	26.19 ± 0.22[a]	26.88 ± 0.52[a]	25.83 ± 1.77[a]
Milk solids non-fat (%)	12.16 ± 0.02[a]	12.09 ± 0.04[a]	12.15 ± 0.07[a]
Ca (mg 100 g^{-1})	134.45 ± 3.73[a]	107.91 ± 5.38[b]	112.49 ± 5.25[b]
Mg (mg 100 g^{-1})	25.74 ± 0.20[a]	24.41 ± 0.13[b]	23.18 ± 0.62[c]
K (mg 100 g^{-1})	193.09 ± 18.09[a]	170.21 ± 12.54[a]	160.23 ± 13.69[a]
Na (mg 100 g^{-1})	127.74 ± 42.29[a]	109.12 ± 3.16[a]	116.56 ± 4.78[a]
pH	6.74 ± 0.02[a]	6.69 ± 0[b]	6.67 ± 0[c]
Titratable acidity (% lactic acid)	0.18 ± 0.01[a]	0.19 ± 0[b]	0.21 ± 0[c]

[a] Ovine ice cream mix contained bovine SMP-35% protein (A), WPC-65% protein (B) or WPC-80% protein (C) at same ratio. Means in the same row with different superscript letters differ significantly ($P < 0.05$).

Table 3
Physical characteristics of low-fat ovine ice creams.[a]

Characteristic	A	B	C
Overrun (%)	76.47 ± 0.48[a]	46.15 ± 19.69[b]	35.64 ± 6.71[b]
Hardness (N)	5.48 ± 1.87[a]	14.06 ± 4.87[a]	29.52 ± 6.22[b]
a_w	0.970 ± 0[a]	0.957 ± 0[b]	0.958 ± 0[b]
Colour			
L*	82.97 ± 1.06[a]	80.30 ± 0.79[b]	80.80 ± 1.04[b]
a*	−2.60 ± 0.10[a]	−2.50 ± 0.10[a]	−2.30 ± 0.00[b]
b*	6.83 ± 0.29[a]	8.30 ± 0.52[b]	8.83 ± 0.49[b]

[a] Ovine ice cream contained bovine SMP-35% protein (A), WPC-65% protein (B) or WPC-80% protein (C) at same ratio. Means in the same row with different superscript letters differ significantly ($P < 0.05$).

used SMP and/or WPI in ice cream with protein contents of 6−10% have found that overrun was not significantly affected by different combinations of SMP/WPI or by the protein source. On the other hand, El-Zeini Hoda et al. (2016) have reported increase of the overrun of buffalo ice cream using WPC for up to 3% substitution of MSNF in the formulation. However, when the ratio of whey protein in the mix is higher than that of casein, the emulsion stability, structure and texture have been reported to be negatively affected (Goff, Kinsella, & Jordan, 1989). Taking into consideration the formulations of the mixes showed in Table 1 as well as the composition of the milk, cream and each powder, the calculated ratios of whey protein to casein were about 1:4, 1:1 and 1:0.88 for ice cream A, B and C respectively. Therefore, the significant difference in the overrun of our ice cream samples, could be attributed to a greater extend to the whey protein to casein ratio, rather than to the other factors discussed before.

Ice creams B and C had lower a_w values than ice cream A (Table 3) and this fact could deliver them longer shelf life. Similar a_w values have been reported for commercial ice creams (Gougouli, Angelidis, & Koutsoumanis, 2008).

The hardness of ice creams is shown in Table 3. It is obvious that ice cream A containing SMP was significantly ($P < 0.05$) softer than ice creams B and C containing WPC. This difference was attributed not only to the higher overrun and the lower freezing point of ice cream A but also to the increased protein content of ice creams B and C. Hardness is principally affected by initial freezing point and the lower the freezing point the softer ice cream. Moreover, it is affected by total solids, overrun and amount and type of stabiliser (Goff & Hartel, 2013). In addition, Akalin et al. (2008) have reported that WPI (>95% protein) increased hardness as well as melting resistance and Sofjan and Hartel (2004) have shown that hardness of bovine ice cream with overrun 80% was higher than those with 120% overrun.

Colour is an important attribute of foods since it may influence consumers' preference. Colour analysis of the SMP, WPC (65% protein) and WPC (80% protein) aqueous solutions 4% (w/v), (the same ratio of addition in ice cream mix) revealed the following results: SMP, L* value 69.5, a* −3.0, b* −5.3; WPC65, L* 50.6, a* −2.5, b* 0; WPC80, L* 48.2, a* −2.2 and b* 2.3. a* varies from green (−) to red (+) and b* varies from blue (−) to yellow (+). The differences in colour parameters of the powders used affected similarly the colour of ice creams. It is obvious that the more yellow colour of WPC decreased the lightness of ice creams B and C significantly ($P < 0.05$) (Table 3) and increased the yellow colour of them (b* value) as it shown in Fig. 1. In contrast, when no powder is used in the formulation of ovine ice cream with 10% fat, inulin and *Lactobacillus casei* the L* value can be as high as 93 to 94.84 (Balthazar et al., 2018).

3.4. Ice cream meltdown

Ice cream meltdown is a significant characteristic which affects the consumer preference and in general may cause practical problems. Ice creams with high overrun tend to melt more slowly because air bubbles act as insulators in the product (Sofjan & Hartel, 2004). In contrast, in the present work, the ice cream A having the higher overrun started to melt earlier and the total melting time was the shortest, followed by ice cream B and C (Fig. 2). In fact, it was observed that 50% of the weight of ice cream A, B and C melted after about 40, 50 and 58 min, respectively. The melting behaviour of ice creams B and C may be

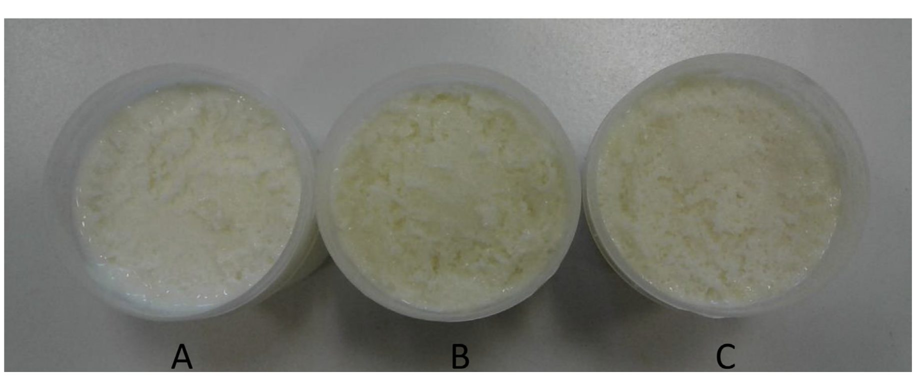

Fig. 1. Low-fat ovine ice cream made with addition of SMP (A), WPC-65% protein (B) or WPC-80% protein (C).

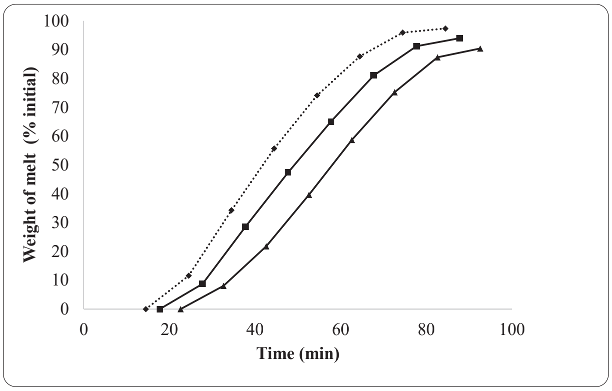

Fig. 2. Melting curve of low-fat ovine ice cream made with addition of SMP (...◆...), WPC-65% protein (—■—) or WPC-80% protein (—▲—).

related with the foam stability of the powders used (mentioned in 2.1 section) and/or with the water holding capacity of the whey proteins, which they contained. Pandiyan, Annal Villi, Kumaresan, Rajarajan, and Elango (2012), who replaced up to 40% of the SMP with WPC in 10% fat bovine ice cream, reported that the melting time decreased as the percentage of WPC increased. Similarly, Udabage, Augustin, Cheng, and Williams (2005) using a high heated blend of WPC35 (35% protein) and low heat SMP at a ratio 1:1 manufactured ice cream with better melting resistance among the samples containing only low heat SMP or only WPC35.

3.5. Sensory evaluation

In terms of organoleptic evaluation, all three ice creams scored similarly for the sweet taste as well as for the textural properties (gummy body and wheying-off) (Fig. 3). Ice cream A scored 3.75 out of 5 for the flavour whereas ice creams B and C scored 3.34 and 2.84 respectively, although no cheesy flavour was detected in ice creams B and C, which contained WPC produced from Feta cheese whey. On the other hand, ice cream A was evaluated as more watery and easily melted, compared with ice creams B and C. These scores agree with the results from the corresponding instrumental analyses. Moreover, ice creams B and C appeared to be slightly sandy. This could be related to the lactose crystallisation in WPC65 and WPC80 in combination with the ratio of them in the ice cream formulation.

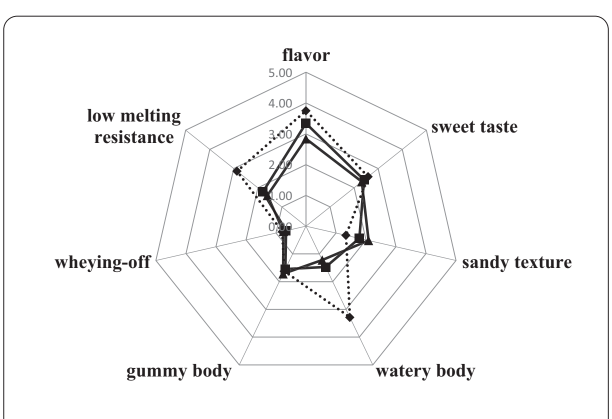

Fig. 3. Sensory evaluation of low-fat ovine ice cream made with addition of SMP (...◆...), WPC-65% protein (—■—) or WPC-80% protein (—▲—).

4. Conclusions

The source of the MSNF influenced the characteristics of the ovine ice cream mix and the properties of the final ovine ice cream. The substitution of bovine SMP with ovine/caprine WPC in ovine ice cream apart from the definition 'contains no ingredient of bovine origin', hence safe for allergenicity, resulted in a more nutritious ice cream, since it contained more protein and less carbohydrates. In addition, taking into consideration other characteristics such as the melting behaviour, the use of ovine/caprine WPC, especially those with 65% protein, seems to be feasible but further study is needed to optimise the production to improve overrun, hardness as well as the sensory characteristics.

Author contributions

EM, conceptualization, supervision, validation, writing - review & editing; DD, formal analysis, investigation; EZ, investigation, methodology development, writing – original draft.

Declaration of competing interest

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

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