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Impact of Ovine Whey Protein Concentrates and Clarification By-Products on the Yield and Quality of Whey Cheese

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Summary

The effects of the addition of whey protein concentrates and clarification by-products obtained from ovine cheese whey and deproteinized whey (Sorelho) on the yield and quality of the whey cheese (Requeijão) have been evaluated. Whey protein concentrates were obtained by ultrafiltration of skimmed whey and Sorelho. The clarification by-products were obtained after the treatment of the skimmed whey and Sorelho by thermocalcic precipitation and microfiltration with two membranes (0.20 and 0.65 μm pore size). Next, the liophilization of the corresponding retentates was carried out. Each powder was added in three different mass ratios: 0.5, 1.0 and 1.5 %. The addition of the powders caused higher yields of the whey cheese – mainly the one with the additional whey powder – but it did not affect the strength of the products. The retention of water and other components of whey and milk in the whey cheese was influenced by the protein composition of the powders. In relation to colour parameters, the whey cheese manufactured with ultrafiltration and microfiltration retentate powders showed lower values of ligthness than the control whey cheese – mainly the whey cheese with 1.5 % of added powders. The microstructure constituted of small aggregates in the whey cheese manufactured with ultrafiltration and 0.20-μm microfiltration retentate powders and also by large, smooth structures in the other whey cheeses, especially in batches with added Sorelho powders.

Key words: whey cheese, ovine cheese whey, deproteinized whey, whey protein concentrates, yield, quality parameters

Introduction

Whey cheese is a dairy product manufactured mainly from ovine whey in the Mediterranean countries. The production of whey cheese is important because the heating process in the manufacture of this dairy product allows the recuperation of whey proteins from whey. These cheeses have different names depending on the country where they are produced. For example, in Italy, Spain and Portugal, the whey cheeses obtained from ovine cheese whey are called Ricotta, Requesón and Requeijão, respectively (*1*). Requeijão is obtained by heating the whey at a temperature ranging from 90 to 100 °C for 15–30 min, with or without the addition of 10–20 % of ovine/caprine/bovine milk. The residual »deproteinized« whey called Sorelho, resulting from the manufacture of Requeijão, contains approximately 60 % of the original dry matter of the whey. Lactose and minerals largely contribute to its dry mass, but residual fat and non-thermally precipitated nitrogen components are still present.

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Hence, attempts to recover some of the solid components present before the final disposal may be of interest (*2*).

In previous works (*2–4*), we studied the composition and some functional properties of ultrafiltration retentate powders obtained from ovine cheese whey and deproteinized whey (Sorelho). These whey protein concentrates have different composition and functional properties depending on their origin (whey or Sorelho). Moreover, in these studies, both by-products of ovine cheese manufacture were clarified by thermocalcic precipitation and microfiltration using two pore size membranes (0.20 and 0.65 μm). Next, the filtrates were ultrafiltrated/diafiltrated and then, the corresponding retentates were freeze dried. The clarification improved posterior ultrafiltration rates and improved some functional properties of the whey and Sorelho protein concentrates and also generated microfiltration retentates for potential use in food manufacturing. These microfiltration retentates also showed interesting functional properties.

Whey protein concentrates (WPC) have been widely used in the food industry in a variety of products (*5*). One important use is in dairy products, such as yoghurts, cheeses, *etc*. The addition of denatured whey proteins to cheese milk leads to an increased yield attributed to an increased retention of serum in the cheese matrix and to the incorporation of whey proteins, but it may also result in a slightly poorer quality of cheese flavour and texture (*6*). All the investigations were done with bovine whey proteins and in dairy products manufactured with bovine milk. There are no studies about the effect of the addition of whey proteins on the properties of ovine whey cheeses. This addition could increase the yield of the whey cheeses as well as allow the recuperation of more whey proteins. However, some quality parameters of the whey cheese (colour, texture) might be affected.

The objective of this work was to study the influence of the addition of ultrafiltration retentate powders and clarification by-products (microfiltration retentate powders) obtained from ovine cheese whey and deproteinized whey (Sorelho) on some parameters (yield, dry matter content, texture, colour and microstructure) of a whey cheese (Requeijão).

Materials and Methods

Sample preparation

Ovine cheese whey and deproteinized whey (Sorelho) were obtained from the Association of Producers of Serra da Estrela Sheep (ANCOSE, Portugal). Sample preparation was similar for both products. Immediately after reception, the products were skimmed by means of a Westfalia™ separator type ADB. Approximately 100 L of each product (cheese whey or Sorelho) were used in each trial; 50 L were submitted to ultrafiltration and 50 L were used for thermocalcic precipitation and microfiltration. All experiments were done in triplicate. More details and a diagram of the process can be found in the work by Pereira *et al.* (*2*).

Two microfiltration pore size membranes (0.20 and 0.65 μm) were used in the clarification procedure. Membrane retentates were freeze-dried in a Lyph-Lock freeze dryer (Labconco Corporation, Kansas City, USA). Six different powders were obtained: three from ovine whey and three from Sorelho. The three powders from whey were: the ultrafiltration retentate powder (UFRP), the microfiltration retentate powder using a 0.65-μm pore size MF membrane (MFRP 0.65) and the microfiltration retentate powder using a 0.20-μm pore size MF membrane (MFRP 0.20). The other three powders were obtained from Sorelho using similar treatments: SUFRP (ultrafiltration retentate powder), SMFRP 0.65 and SMFRP 0.20 (microfiltration retentate powders).

Whey cheese preparation

Nineteen different batches of whey cheese were manufactured. The control batch was made with 900 g of cheese whey and 100 g of bovine milk standardized to 12.2 % dry matter, 3.1 % protein and 3.5 % lipids. The whey constituted of 11.35 % dry matter, 1.53 % protein, 1.11 % lipids and 2.9 % ash. The remaining eighteen whey cheese batches were manufactured with the addition of the three different powders from ovine whey and the three powders from Sorelho. Each powder was added in three different mass ratios: 0.5, 1.0 and 1.5 %. The chemical and protein composition of these powders is shown in Table 1 [adapted from (*2*,*3*)].

The mixture was heated in a stainless steel kettle under smooth stirring conditions. When it began to boil, 20 mL of cold water were added and the temperature was maintained at 95 °C for 10 min. Afterwards, the mixture was poured into cheesecloth bags so it could drain for 24 h at 2 °C.

Three cheeses from the control batch and one cheese from the other batches were manufactured and analysed in each experiment. This experiment was repeated four times.

Dry matter content and whey cheese yields

Whey cheeses were analysed for dry matter by oven drying at 105 °C for 12 h. All determinations were made in duplicate.

Whey cheese yield was determined by dividing the mass of the whey cheese by the mass of the original mixture (whey, milk and powders) and multiplying by 100.

Yield adjusted to 30 % dry matter content was calculated as follows:

$$\text{Adjusted yield} = (\text{dry matter in whey cheese in }\%/30)\cdot \cdot\ \text{whey cheese yield}$$

Dry matter recovery was calculated as follows:

$$\text{Dry matter recovery} = [(\text{mass of cheese in g} \cdot \text{dry matter in cheese in }\%)/(100 \cdot 12.2 + 900 \cdot 11.35 + \text{mass of powder in g} \cdot \text{dry matter in powder in }\%)] \cdot 100$$

where 100 is the mass of the added milk in g, 12.2 is the percentage of dry matter in the added milk, 900 is the mass of the added whey in g and 11.35 is the percentage of dry matter in added whey.

Table 1. Chemical and protein composition (g/100 g powder) of ovine cheese whey and Sorelho powders

Ovine cheese whey	UFRP	MFRP 0.65	MFRP 0.20
Moisture	11.23±0.32	11.01±1.05	9.71±2.68
Total nitrogen	5.65±0.58	2.98±0.13	5.05±0.72
Fat	0.36±0.03	0.47±0.10	0.42±0.11
Ash	26.70±0.01	28.06±1.12	25.41±3.73
Calcium	0.65±0.03	0.90±0.11	0.88±0.23
Chloride	9.28±0.26	12.57±0.64	11.78±1.52
True protein	32.58±4.74	16.39±0.36	29.64±1.60
α-Lactalbumin	5.83±0.55	2.35±0.24	4.63±0.38
β-Lactoglobulin	23.81±3.77	11.78±1.24	19.75±0.79
Bovine serum albumin	0.88±0.58	0.52±0.08	0.27±0.15
NPNC	2.18±0.28	1.49±0.37	1.86±0.23
Sorelho	**SUFRP**	**SMFRP 0.65**	**SMFRP 0.20**
Moisture	10.54±0.39	8.68±0.50	8.79±2.67
Total nitrogen	4.56±0.90	2.69±0.32	3.45±0.70
Fat	0.44±0.07	0.49±0.02	0.52±0.14
Ash	27.24±0.33	29.83±0.21	27.69±1.29
Calcium	0.71±0.21	0.86±0.03	0.75±0.35
Chloride	15.12±0.56	15.07±0.50	14.17±0.62
True protein	25.38±6.15	15.90±1.32	19.99±3.83
α-Lactalbumin	6.02±1.89	3.09±0.94	5.17±1.02
β-Lactoglobulin	7.60±1.26	3.91±1.83	4.11±0.54
Bovine serum albumin	0.58±0.36	0.82±0.18	0.63±0.21
NPNC	2.65±0.96	1.41±0.07	1.95±0.34

Mean values±standard deviation (*N*=3), adapted from (*2,3*)
NPNC: non-protein nitrogen components
UFRP, SUFRP: ultrafiltration retentate powders; MFRP, SMFRP: microfiltration retentate powders, using the 0.65 or 0.20-μm microfiltration membrane

Strength of whey cheeses

The strength of the whey cheeses was measured using a Stevens LFRA Texture Analyser (Stevens & Sons Ltd., UK) fitted with the cylindrical probe TA 24 (diameter of 4 mm). The whey cheeses were penetrated to a depth of 20 mm at a rate of 1 mm/s. The maximum force (*g*) required to penetrate the gel was taken as a measure of the relative gel strength. All determinations were made in duplicate.

Colour coordinates

The colour of the samples was measured using a Minolta CR 200 colorimeter (Minolta Camera Co., Osaka, Japan). All measurements were made in the Hunter Lab colour space using the D65 standard illuminant and 10° standard observer. The instrument was standardized with a white (L=97.8, a=–0.6, b=2.1) tile before sample measurements. The colour values were expressed as L (lightness), a (redness/greenness) and b (yellowness/blueness). All determinations were made in duplicate.

Microstructure of whey cheeses

Scanning electron microscopy (SEM) was used to study the microstructure of the whey cheeses. The samples consisted of 5-mm cubes, which were left in a 100 mL/L trichloromethane-water solution until analysis.

Whey cheese samples were sequentially dehydrated with successive treatments in aqueous ethanol solutions, with ethanol concentration ranging from 30 to 100 %. Afterwards, ethanol was substituted by 100 % acetone and the samples were dried with the critical point method under CO_2 using a Bal-Tec CPD 020 critical point dryer (Bal-Tec AG, Balzers, Liechtenstein). Dried specimens were fractured with a razor blade, mounted on stubbs and sputter coated with gold ions (300 Å at 1200 V and 10 mA) in a JEOL model JFC-1100 ion sputtering device (JEOL Ltd., Tokyo, Japan), and examined by SEM in a JEOL JSM-T220A scanning electron microscope, operated at 15 or 20 kV.

Statistical analysis

One-way ANOVA with Dunnett´s multiple comparison test ($p<0.05$) was carried out to identify the differences between the control batch and the other batches (SPSS 12.0S version 12.0.1 for Windows, 2003).

Results and Discussion

Whey cheese yields and dry matter recovery

Table 2 shows the effects of the addition of ovine cheese whey and Sorelho powders on yield, adjusted yield and dry matter recovery of the experimental whey cheeses. The control whey cheeses showed a yield of 5.3 %, which was lower than the typical yield of whey cheeses (6 %) reported by Pintado *et al.* (*1*). The lower heating temperature used in our work and the fact that no ovine milk was added could have influenced the differences observed.

In general, the addition of UF retentate and MF retentate powders increased whey cheese yields, as they were significantly higher than the control batch at the three levels of incorporation with MFRP 0.20, and only significantly higher when 1 or 1.5 % of the rest of the whey powders or 1.5 % of Sorelho powders were used. The yields increased proportionally with the levels of incorporation of the powders.

The adjusted yield results allowed us to eliminate the effect of the variation in water content of the whey cheeses. The values were also higher in whey cheeses with added powders than in the control batch, but there were no significant differences when Sorelho powders were incorporated. A similar pattern was observed in the results of dry matter recovery. It is clear that, if a dry ingredient is added to a product, the yield or the adjusted yield should increase. However, when whey powders were incorporated, the increases in dry matter recovery were higher than those expected even with a full retention of the dry ingredients added, which did not happen in the same proportion when Sorelho powders were used. The higher increase in yields and dry matter recovery in batches with the added whey powder com-

Table 2. Effect of the addition of ovine cheese whey and Sorelho powders on yields, dry matter recovery, dry matter content and strength of whey cheeses

Whey cheeses	Yield/%	Adjusted yield/%	Dry matter recovery/%	Dry matter/%	Strength/g
Control	5.30±0.78	5.42±0.89	14.22±2.33	30.62±1.59	111.7±32.10
UFRP/%					
0.5	6.64±0.72	6.08±0.26	16.03±0.68	27.70±2.70	114.7±19.62
1.0	(8.15±1.31)*	(7.47±1.22)*	(19.78±3.23)*	27.56±2.14	108.0±32.50
1.5	(8.79±1.08)*	(7.74±0.97)*	(20.58±2.59)*	(26.51±2.45)*	81.25±24.59
MFRP 0.65/%					
0.5	5.67±0.27	5.58±0.15	14.71±0.38	29.56±0.66	133.5±13.52
1.0	(6.95±1.22)*	7.03±1.31	(18.61±3.46)*	30.38±2.36	115.0±23.24
1.5	(7.84±1.23)*	(7.42±1.10)*	(19.74±2.91)*	28.44±1.00	88.50±21.30
MFRP 0.20/%					
0.5	(7.15±0.93)*	(7.12±1.17)*	(18.76±3.08)*	29.78±1.92	127.0±40.46
1.0	(8.69±0.59)*	(7.58±0.76)*	(20.06±2.02)*	(26.23±3.04)*	114.0±34.13
1.5	(9.62±0.36)*	(9.15±0.65)*	(24.35±1.72)*	28.55±1.69	122.2±13.40
SUFRP/%					
0.5	6.62±1.43	6.65±1.23	17.52±3.25	30.31±1.63	106.5±47.91
1.0	6.54±1.04	6.49±1.45	17.17±3.84	29.53±2.28	104.5±11.36
1.5	(7.08±0.78)*	6.74±1.02	17.93±2.72	28.47±1.94	97.25±20.20
SMFRP 0.65/%					
0.5	6.08±0.62	6.45±1.01	16.99±2.66	31.70±2.07	96.25±20.06
1.0	6.44±0.54	6.23±0.87	16.49±2.31	29.03±3.19	111.7±34.01
1.5	(7.04±0.14)*	6.65±0.52	17.69±1.38	28.34±2.31	120.5±22.17
SMFRP 0.20/%					
0.5	5.05±0.51	5.14±0.72	13.55±1.90	30.68±4.17	106.5±52.13
1.0	6.38±0.17	5.84±0.35	15.48±0.92	27.49±0.94	114.5±63.16
1.5	(7.26±0.39)*	6.85±0.72	18.23±1.91	28.30±2.38	73.25±49.49

Mean values±standard deviation (*N*=4), except control (*N*=12)
*Means differ significantly ($p<0.05$) from control
UFRP, SUFRP: ultrafiltration retentate powders; MFRP, SMFRP: microfiltration retentate powders, using the 0.65 or 0.20-μm microfiltration membrane

pared to the Sorelho ones could be due to the higher proportion of β-lactoglobulin in the former, but also to the higher ratio β-lactoglobulin/α-lactalbumin [5/1 in whey powders and 1/1 in Sorelho powders, approximately (*3*)]. β-lactoglobulin is the main protein in the composition of whey cheeses (*7*) and it forms aggregates in higher proportion than α-lactalbumin in concentrated whey even at lower temperatures than those used in whey cheese production (*8*). The added protein probably interacted with the proteins of the whey and the milk added; it is also possible that the additional amount of β-lactoglobulin was associated with the residual fat globule membranes, an effect described by other authors in whole milk during heating (*9,10*). These interactions may explain the increased yields in batches with added whey powder in relation to the control batch, mainly when 1.0 and 1.5 % of whey powders were used.

The higher yields of the MFRP 0.20 batches in relation to the UFRP ones cannot be attributed to the true protein and β-lactoglobulin contents of the powders (Table 1). This may be due to the fact that the MFRP 0.20 is the by-product obtained from thermocalcic aggregation during the clarification procedure of the whey, so it is expected that phospholipoproteins are concentrated in this product [the objective of the pretreatment; (*11*)] together with a certain amount of the main whey proteins. It might be possible for these proteins to interact in a different way with other components of the whey/milk mixture during whey cheese manufacture than UFRP proteins. This would allow for their retention in higher amounts, as can be observed in dry matter recoveries. MFRP 0.65 batches showed yields similar to those for UFRP, although this product had a lower protein concentration. It is also possible that the different nature of the proteins after thermocalcic aggregation affected the yields more than the protein content.

Dry matter content

Table 2 also shows the dry matter content of the whey cheeses. In general, the whey cheeses manufactured with the addition of whey or Sorelho powders showed lower dry matter content than the control batch, mainly when high concentrations were added. Although several components were retained in the whey cheeses (according to the dry matter recovery results), water was probably the main compound that the proteins of the powder retained.

Strength

The strength of the whey cheeses is shown in Table 2. Changes were not observed in the strength of the products with the addition of the powders. It is important to remark that the higher retention of water caused by the addition of retentate powders did not influence the texture of the whey cheeses.

Colour

The *L*, *a* and *b* values of whey cheeses are shown in Table 3. The whey cheeses manufactured with ultrafiltration and microfiltration retentate powders showed lower values of *L* than the control whey cheeses – mainly in those with 1.5 % of added powders. This observation may be explained by the content of dry matter in the whey cheeses, with the lowest values in control whey cheeses and the highest values in the whey cheeses with higher levels of added powders. In these cheeses, the decrease in *L* values could have a detrimental effect on the consumers´ acceptance. In general, the whey cheeses manufactured with ultrafiltration and microfiltration retentate powders showed higher *a* values than control whey cheeses. No effect of the retentate powders was observed in *b* values.

Table 3. Effect of the addition of ovine cheese whey and Sorelho powders on colour coordinates of whey cheeses

Whey cheeses	*L*	*a*	*b*
Control	94.85±0.78	–3.03±0.34	10.53±0.46
UFRP/%			
0.5	94.09±0.92	–2.70±0.38	10.46±0.58
1.0	93.74±0.55	–2.50±0.41	10.81±0.57
1.5	(93.06±0.38)*	–2.59±0.45	11.25±0.80
MFRP 0.65/%			
0.5	93.90±0.21	–2.71±0.60	10.36±1.06
1.0	(92.55±0.90)*	–2.40±0.57	10.20±1.16
1.5	(92.85±1.38)*	–2.46±0.53	10.85±0.81
MFRP 0.20/%			
0.5	94.75±0.38	–2.60±0.49	10.89±1.01
1.0	93.56±0.64	–2.62±0.45	9.98±0.68
1.5	93.92±0.44	–2.71±0.42	10.30±0.94
SUFRP/%			
0.5	94.15±1.08	–2.86±0.33	10.64±0.72
1.0	94.07±0.50	–2.55±0.51	9.99±0.84
1.5	93.71±0.62	–2.51±0.50	10.54±0.59
SMFRP 0.65/%			
0.5	94.20±0.94	–2.79±0.73	10.20±1.06
1.0	94.47±0.39	–2.87±0.59	10.38±1.01
1.5	(93.30±0.97)*	–2.85±0.71	11.00±1.26
SMFRP 0.20/%			
0.5	94.97±0.26	–3.02±0.45	11.01±0.64
1.0	94.34±0.54	–2.76±0.36	10.64±0.25
1.5	93.87±0.78	–3.12±0.37	11.29±0.42

Mean values±standard deviation (N=4), except control (N=12)
*Means differ significantly ($p<0.05$) from control
UFRP, SUFRP: ultrafiltration retentate powders; MFRP, SMFRP: microfiltration retentate powders using the 0.65 or 0.20-μm microfiltration membrane

Microstructure

SEM micrographs (1500 magnifications, Fig. 1) show different structures for each sample of whey cheese, although in most of them (except SMFRP 0.20 added batches), similar aggregates to those observed in whey protein gels of ovine (*4*) or bovine origin (*12,13*) have been found. However, the size of these aggregates and the presence of pores or of other structures show differences among the experimental whey cheeses. Control batch (Fig. 1, first micrograph) and MFRP 0.65 added batches have a microstructure formed by a network of small aggregates of different sizes (more compact in control than in MFRP 0.65 whey cheeses) and large, smooth structures of 20 μm or more incrusted in this network.

The microstructure of UFRP (Fig. 1, second micrograph) and MFRP 0.20 (Fig. 1, third micrograph) batches are only formed by aggregates having a similar appearance to thermal whey protein gels. These aggregates are more homogeneous in size in MFRP 0.20 batches in comparison with UFRP ones. Some pores can also be observed in both samples.

Whey cheeses with SUFRP additions have a microstructure similar to the control batch, with heterogeneous aggregates and smooth structures, with no pores. SMFRP 0.65 (Fig. 1, fourth micrograph) also shows this appearance, although aggregates are smaller and more homogeneous and compact than in the other whey cheeses. However, the structure of aggregates has almost disappeared in SMFRP 0.20 batches and the large, smooth structures predominate in these whey cheeses.

The small aggregates that constitute the main structure in most of the whey cheeses are probably formed by whey proteins, although some caseins from the added milk can be included in the structures, interacting with whey protein, as has been suggested for yogurts fortified with WPC (*14*). The smooth structures are not due to the addition of the experimental powders, and they may be formed by whey proteins interacting in a more disordered way than the proteins which generate the small aggregates. This effect might be related to the amount of soluble protein and its denaturation state: these structures are specially abundant in batches with added Sorelho powder, products that contain a low proportion of soluble protein (Table 1). Nevertheless, they do not appear in UFRP and MFRP 0.20 whey cheeses; these powders contained a high proportion of soluble β-lactoglobulin and a low quantity of denatured proteins.

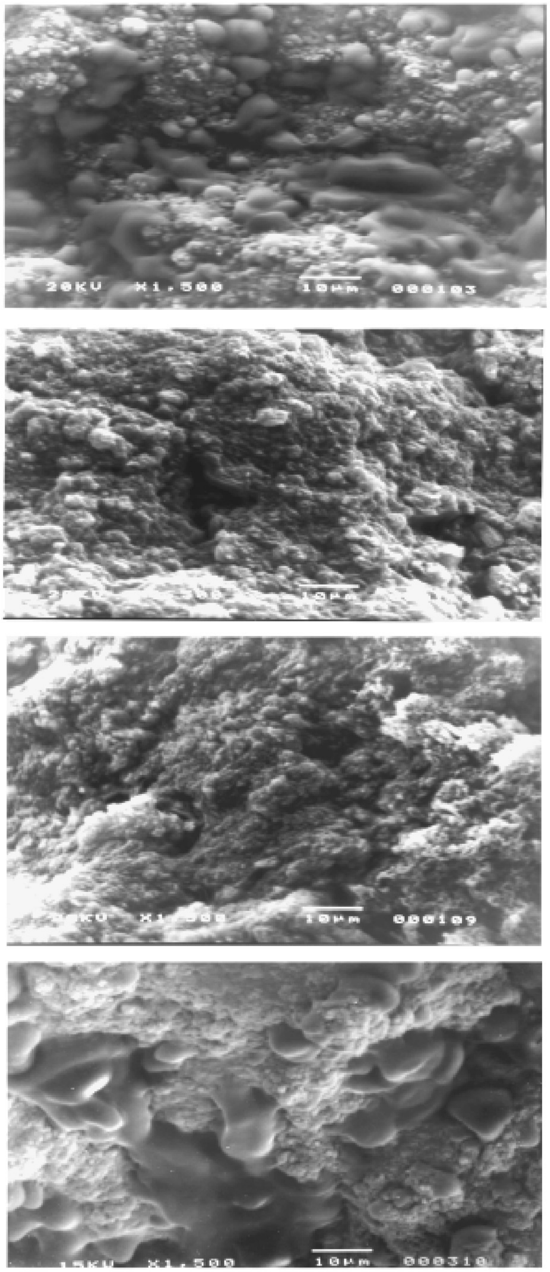

Fig. 1. Scanning electron micrographs of control whey cheese and those containing added ovine whey powders. From top to bottom: control; UFRP added whey cheese; MFRP 0.20 added whey cheese, and SMFRP 0.65 added whey cheese

Conclusions

The influence of the addition of ovine whey protein concentrates and clarification by-products on the manufacture of whey cheeses is very interesting since it allows an increase in yield without affecting the strength of the products. The increase in the yields was not due only to the retention of water but also of other milk components. The yields were influenced by the contents of protein, β-lactoglobulin and phospholipoproteins, and the ratio β-lactoglobulin/α-lactalbumin of the powders. Hence, the addition of powders obtained from ovine cheese whey could be more useful than powders obtained from deproteinized whey (Sorelho). Finally, some colour parameters (*L* and *a* values) and the microstructure of the whey cheeses can be influenced by the addition of the powders. The decrease in *L* values in whey cheeses with high levels of added powders could have a detrimental effect on the consumers' acceptance.

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