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Production of processed cheese using kasseri cheese and processed cheese analogues incorporating whey protein concentrate and soybean oil

S KAMINARIDES* and S STACHTIARIS
Laboratory of Dairy Technology, Agricultural University of Athens, Iera Odos 75, Votanikos, 11855 Athens, Greece

A control processed cheese (A) made mainly from kasseri cheese (60%) without whey protein concentrate or soybean oil, and three other cheese products B, C and D containing increasing amounts of whey protein concentrate (UF) and soybean oil were manufactured simultaneously. All the cheeses were produced to contain 50–51% moisture and 53–54% fat-in-dry-matter and were submitted to microbiological, physicochemical, rheological and organoleptic tests 1 day after production and after 90 days in cold storage under vacuum. The mesophilic and psychrotrophic microflora of all the cheeses was very low; coliforms were not found. All the cheeses differed significantly in their content of total protein, soluble protein, lactose, ash, acidity (ADV) and in the oxidation of unsaturated fatty acids (cheese D). In contrast, no significant differences in pH, moisture or fat were noted because of the standardization of the blends. Rheological tests of the products indicated that there were marked differences in hardness, adhesiveness, elasticity, gumminess and chewiness. The cheeses were subjected to sensory analysis and showed differences in flavour, texture and spreading ability on day 1 and, moreover, in appearance after 90 days.

INTRODUCTION

The production of processed cheeses started a decade before World War I and, because of their great advantages,[1,2] world production had reached 1350000 metric tonnes by 1981.[3] The numerous investigations recently carried out in many parts of the world reflect, without doubt, increasing interest in this important dairy product,[4–6] for processed cheese belongs to a group of dairy products where there are considerable possibilities for incorporating materials into the blends to create a variety of types.

Whey, the main by-product of cheesemaking, is responsible for environmental pollution and represents a loss of milk constituents of excellent nutritive value. Indeed, about half the original milk solids are left in whey during the manufacture of most cheeses, and it has an especially high biochemical oxygen demand (BOD), equal to 35–55 g of oxygen per litre of whey.[2] In Greece approximately 700000 tons of whey are produced from the manufacture of various cheeses, of which half is used for whey cheeses. Since there is an ever-increasing need for proteins and energy,[7] many researchers have proposed improved technological procedures to incorporate whey proteins into cheese[8–11] or other foods.[12–14]

So, it was decided to produce a control-processed cheese based on Greek kasseri cheese, along with other processed cheese analogues, where the kasseri cheese and butter are substituted by UF whey protein concentrate and soybean oil. In this context, the objectives of this work were to investigate: (a) the production of processed cheese based on Greek kasseri cheese without UF whey protein concentrate and soybean oil, (b) the preparation of processed cheese analogues incorporating increasing amounts of UF whey protein concentrate and soybean oil to replace cheese casein and milk fat, (c) the combined effects of incorporation of UF whey protein concentrate and soybean oil on the physicochemical, microbiological, textural and organoleptic properties of these products and (d) the keeping quality of these products.

*Author for correspondence.

MATERIALS AND METHODS

Selection of raw materials

Cheeses

Cheeses, based on chymosin curd, were obtained from the pilot plant of the Dairy Laboratory of the Agricultural University of Athens. To ensure the desired structure and flavour of the products, a large quantity of kasseri cheese, with a high portion of unhydrolysed casein, and a small amount of mature kopanisti cheese was used. Kasseri cheese is a semi-hard cheese with a mild flavour and its fat and total solids content were 25% and 55.5%, respectively. Kopanisti cheese is a soft cheese with a sharp flavour and its fat and total solids content were 21% and 51%, respectively.

Emulsifying salts

A combination of trisodium citrate, disodium monophosphate and sodium hexametaphosphate (Graham's salt) in a ratio of 1:3:6,

TABLE 1
Proportions of material in the processed cheese blends (%)

Cheese type	Percentage proportion of material						
	Greek cheeses						
	Kasseri cheese	Kopanisti cheese	Ultrafiltrated whey protein concentrate (UF retentate)	Emulsifying salts	Soybean oil	Butter	Water
A	60.50	4.32	0.00	2.59	0.00	7.89	24.70
B	55.42	4.62	9.24	2.31	2.31	6.00	20.10
C	49.58	4.96	19.83	1.98	4.96	3.87	14.82
D	39.25	4.91	39.25	1.48	9.81	0.00	5.30

respectively was chosen and used according to the various characteristics of emulsifying salts and other raw materials of the blend, as well as the properties desired in the end products. The amount of emulsifying salts added to each blend was related to the cheese and its casein content, as shown in Table 1.

Retentate of ultrafiltered whey
Whey was obtained from the dairy plant 'Parnassos', after feta cheesemaking using goat's and ewe's milk. The whey was concentrated 14 times by ultrafiltration in a pilot plant from Paterson Candy International (PCI Membranes, Whitchurch, Hants, UK), and the fat and total solids contents of the retentate were 4.7% and 24%, respectively.

Butter and soybean oil
Milk butter and soybean oil were obtained commercially. Butter was used to adjust the fat content of the blends so that all the cheeses contained 53–54% fat-in-dry-matter (FDM).

Water
Distilled water was used to adjust the moisture content of the blends, so that all the cheeses contained 50–51% moisture.

Cheesemaking technology
The manufacturing procedure for processed cheese was carried out in the following order:

- Selection of a stock of raw materials.
- Computation of the ingredients in the various blends.
- Preparation of cheese (removing the rind from kasseri cheese and coarse cutting of the cheese).
- Weighing and mixing of the raw materials in containers before processing.
- Premixing the emulsifying salts with the required amount of water.
- Thermal processing (heating with agitation at 80°C for 5 min in a water bath).
- Distribution of the hot mixture in cups of 250 ml volume and evacuation and sealing in plastic pouches.
- Cooling of the products at room temperature.
- Holding in cold storage at 5°C.

Experimental planning and sampling
Four different blends of processed cheese (Table 1) were manufactured on the same day with five replications. The four blends were a control processed cheese (A), made from mainly kasseri cheese (60%) and with no whey concentrate or soybean oil, and three other cheese products B, C and D, in which the kasseri cheese was replaced by increasing proportions of ultra-filtered whey and soybean oil. The processed cheeses were analysed when fresh and after 90 days' storage at 5°C.

Enumeration of micro-organisms
The cheeses were examined on days 1 and 90. Samples of cheese (50 g) were transferred under aseptic conditions to a petri dish and analysed on the day of sampling. A subsample (5 g) was suspended in 20 g l^{-1} tri-sodium citrate (45 ml) to give a 1:10 dilution. Further decimal dilutions were prepared in 25% strength Ringer's solution. The total and psychrotrophic floras were enumerated by the pour-plate method of the American Public Health Association (APHA)[15] using Plate Count Aga (Difco, Michigan, USA) and incubation at 32°C for 2 days for the total colony counts, and 7°C for 10 days for the psychrotrophic counts. Coliforms were enumerated according to IDF[16] using MacConkey broth (Unipath Ltd., Basingstoke, UK) with incubation at 37°C for 2 days.

Physicochemical analyses
Samples of the cheeses were analysed for total N,[17] total solids,[18] NaCl,[19] fat,[20] soluble N (SN) and pH,[21] lactose,[22] ash, as specified in AOAC,[23] acid degree value (ADV)[24] and thiobarbituric acid reaction according to King,[25] except that the 17.6 ml milk was replaced by 4 g cheese homogenized in 15 ml distilled water at 30°C.

All analyses were performed in triplicate and the results are given as the average of 16 analyses from four trials.

Rheological and organoleptic properties
A Shimadzu testing instrument, model AGS-500 NG (Shimadzu Corporation, Japan) equipped with a 5-kg load cell was used to perform the texture profile analysis (TPA) of the

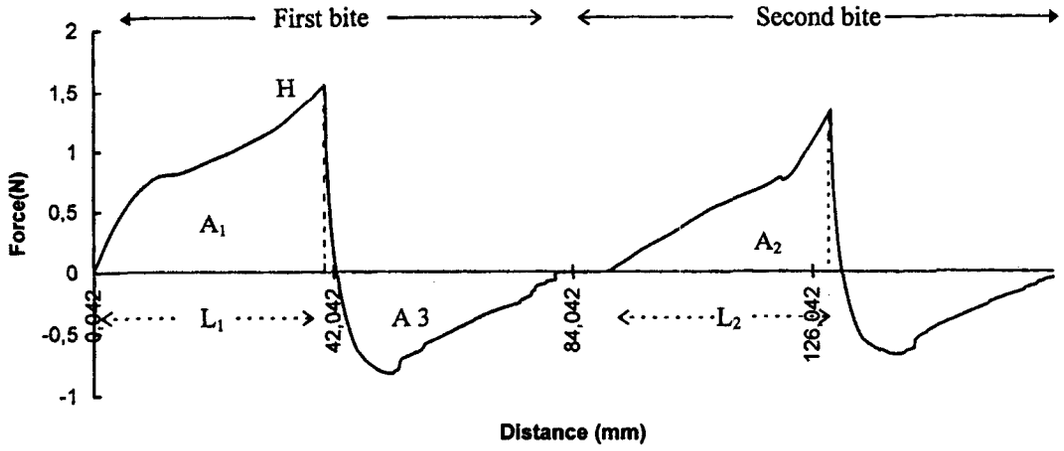

Fig. 1. Typical texture profile curve obtained by the double bite test on the surface of processed cheese. H, hardness; A_1, area of first down stroke; A_2, area of second down stroke; A_3, adhesiveness.

cheeses. A plunger with a diameter of 6 mm was attached to the moving crosshead. The speed of the crosshead was set at 2.5 cm min^{-1} in both upward and downward directions. The cheese sample was placed on a flat holding plate at 20°C and the plunger inserted 20 mm below the cheese surface. Two consecutive bites were taken. The following six textural characteristics were calculated from the resulting curve (Fig. 1):

- Hardness (N), defined as the peak force (H) during the first compression cycle (first bite), is the force necessary to attain a given deformation.
- Cohesiveness (N mm), defined as the ratio of the positive area under the curve during the second compression to that during the first compression (A_2/A_1).
- Adhesiveness (N mm), defined as the negative force area for the first bite (A_3), is the work necessary to overcome the attractive forces between the surfaces of the cheese and the plunger with which the cheese comes into contact.
- Elasticity (mm), defined as the ratio of the base line of the positive curve during the second compression to that during the first compression (L_2/L_1), is the height that the cheese recovers during the time that elapses between the end of the first and the start of the second bite.
- Gumminess (N), which is the product of hardness × cohesiveness ($H \times A_2/A_1$), is the energy required to disintegrate a cheese to a state ready for swallowing.
- Chewiness (N), which is the product of gumminess × elasticity ($H \times A_2/A_1 \times L_2/L_1$), is the energy required to masticate a cheese to a state ready for swallowing.

The textural characteristics of cheeses from the four treatments were evaluated simultaneously. Three replicate measurements were made on each cheese and the average values and standard error for the four cheesemaking trials are reported.

On days 1 and 90, the cheeses from each trial were evaluated by a panel of five judges familiar with judging dairy products. Scoring was based on the hedonic scale (0 = dislike extremely, 8 = like extremely). Also, the spreading ability of the processed cheeses was assessed by this panel on a scale of 1 to 5 (1 = not spreadable, 5 = spreadable). The results are expressed as a mean score for the whole panel for each cheese.

Statistical analysis

The measurements for the four treatments were subjected to analysis of variance (ANOVA) using the statgraphics program (Statistical Graphics Corp, Rockville, MD, USA, 1995). A randomized complete block design was used and paired comparisons of means were made using the least significant difference (LSD) test ($p \leq 0.05$).

Results and discussion

Microflora of processed cheese

The mean total colony counts of mesophilic bacteria for the four types of cheese, which ranged from 10^2–10^4, were very low due to the thermal processing of blends. Cheese D, containing about 40% UF whey retentate, showed the highest counts. This could be attributed to the greater proportion of lactose in this type of cheese (Table 2), and the UF whey retentate probably contains a greater number of microorganisms than the other materials used in the production of these types of cheese. The psychrotrophic count ranged from 10^2–10^3 cfu/g of cheese and followed the same trend as that for the total mesophilic bacteria. There were no differences between the bacterial floras of cheeses on day 1 after production and after 90 days' cold storage under vacuum; no coliforms were found.

Physicochemical characteristics of the cheeses

The results of the physicochemical analysis of all the experimental cheeses 1 day after

TABLE 2

Physicochemical characteristics of cheeses A, B, C and D 1 day after production and after 90 days' cold storage under vacuum (means ± standard error of mean)[1]

Physicochemical characteristics	*Cheese type on day 1*			
	A	*B*	*C*	*D*
pH	5.97 ± 0.01	5.93 ± 0.03	5.85 ± 0.07	5.76 ± 008
Moisture, %	50.37 ± 0.40	50.40 ± 0.15	50.08 ± 0.40	49.74 ± 0.78
Fat, %	26.90 ± 0.10	27.00 ± 0.20	26.80 ± 0.2	26.90 ± 0.10
Fat on dry matter, %	54.11 ± 0.33	54.49 ± 0.37	53.33 ± 0.57	53.51 ± 0.76
Total protein, % (Total N × 6.38)	17.29^{a} ± 0.19	17.53^{a} ± 0.18	17.75^{a} ± 0.11	18.73^{b} ± 0.37
Protein on dry matter, %	34.84^{a} ± 0.44	35.35^{a} ± 0.28	35.38^{a} ± 0.15	37.27^{b} ± 0.62
Soluble protein, % (Soluble N × 6.38)	1.72^{a} ± 0.06	2.30^{b} ± 0.09	2.74^{b} ± 0.19	4.08^{c} ± 0.39
Lactose, %	0.45^{a} ± 0.04	$0.74^{a,b}$ ± 0.02	1.12^{b} ± 0.05	1.99^{c} ± 0.30
Ash, %	4.82^{c} ± 0.05	4.42^{b} ± 0.06	4.08^{b} ± 0.13	3.46^{a} ± 0.15
NaCl, %	0.95 ± 0.07	0.87 ± 0.06	0.88 ± 0.05	0.83 ± 0.04
ADV (mEq. KOH/100 g fat)	0.31^{a} ± 0.01	0.35^{b} ± 0.01	0.37^{c} ± 0.01	0.46^{d} ± 0.01
	Cheese type on day 90			
pH	5.91 ± 0.03	5.87 ± 0.03	5.81 ± 0.05	5.71 ± 0.07
Moisture, %	50.96 ± 0.27	51.04 ± 0.03	50.95 ± 0.52	50.99 ± 0.47
Total protein, % (Total N × 6.38)	17.36^{a} ± 0.16	17.56^{a} ± 0.13	17.91^{a} ± 0.16	18.62^{b} ± 0.32
Protein on dry matter, %	35.40^{a} ± 0.37	35.87^{a} ± 0.26	36.52^{a} ± 0.27	37.98^{b} ± 0.36
Soluble protein, % (Soluble N × 6.38)	2.23^{a} ± 0.14	2.49^{a} ± 0.15	3.25^{b} ± 0.21	4.12^{c} ± 0.17
Lactose, %	0.34^{a} ± 0.02	0.66^{b} ± 0.02	0.99^{c} ± 0.04	1.66^{d} ± 0.08
ADV (mEq. KOH/100 g fat)	0.33^{a} ± 0.02	0.42^{b} ± 0.01	0.47^{c} ± 0.02	0.55^{d} ± 0.02

[1]Cheese A, B, C and D are defined in Table 1.
[a,b]Means in the same row without a superscript or bearing a common superscript did not differ significantly.

production and after 90 days are shown in Table 2. There were no significant differences ($p > 0.05$) in the contents of moisture, fat and FDM of the various cheeses (Table 2), and the pH values were the same after 90 days as they were on the day after production. The mean pH values ranged from 5.71 to 5.97, the lowest value being observed for cheese D after 90 days' cold storage.

As can be seen from Table 2, there were statistically significant differences ($p < 0.05$) between cheese D and the other types of cheese with respect to total protein and protein in dry matter. Cheese D had the highest protein content (followed in decreasing order by C, B and A) since it contained the highest amounts of UF retentate, which contained about 66% of its dry matter in the form of protein.

Significant differences were also found in the soluble protein contents of the different cheeses (Table 2). These were attributed to compositional differences with respect to casein and UF whey protein concentrate in the cheese blends, and to the denaturation of the whey proteins during heating of the blends. The soluble protein contents increased from cheese A to cheese D. This trend was anticipated, since one of the main objectives of this work was to partially replace the casein of the control cheese A by the addition of proportionally increasing amounts of UF whey protein concentrate (Table 1). Also, an increase in the soluble protein content of all the experimental cheeses occurred during cold storage as a result, perhaps, of the proteolytic activity of the psychrotrophic bacteria.

The observed differences in ash, which were statistically significant ($p < 0.05$), may be attributed to differences in the emulsifying salt content of the blends (Table 1). Cheese A had the highest content of ash, since more emulsifying salts were added to this blend than to others, and this was followed, in decreasing order, by B, C and D cheeses. Therefore, as the percentage incorporation of emulsifying salts in the processed cheese blends decreased (Table 1), the ash contents of the cheeses also decreased.

No differences in percentage NaCl were found between the cheeses ($p > 0.05$). The lower level of NaCl in cheese D may be due to the lower proportion of cheese used in this blend.

There were statistically significant differences ($p < 0.05$) in the lactose contents of the four cheese products on day 1 and after 90 days' cold storage (Table 2). The highest lactose content was found in cheese D (1.66%), followed, in declining order, by cheeses C, B and A, due to the decreasing participation of the UF whey retentate (which contained 2.5% lactose). The lowest level of lactose occurred in cheese A, which contained no UF whey retentate. The values of lactose in the products were not high, so there should be no adverse effect for persons suffering from lactose malabsorption. The greatest decrease in lactose content during cold storage was observed in the case of cheese D, and may be due to the larger psychrotrophic flora of this cheese.

Lipolysis in the experimental cheeses was followed by determination of the acid degree

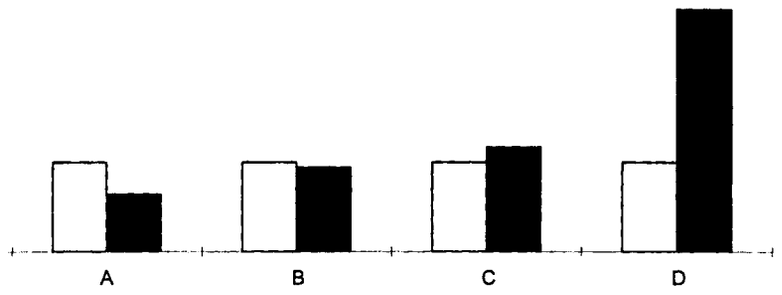

Fig. 2. Relative change of cheese oxidation (thiobarbituric acid test) of various type of cheeses A–D after 90 days' cold storage under vacuum (■) in comparison with the first day after production (□). Cheese A, B, C and D are defined in Table 1.

value (ADV). There were significant differences ($p < 0.05$) in the ADV of the four cheese products on day 1 and after 90 days' cold storage (Table 2). ADV was highest in cheese D followed, in declining order, by C, B and A. This pattern could be attributed to the presence of more free fatty acids in soybean oil, which was added to blends B, C and D in increasing amounts, than in butter, which was highest in A. Although the level of lipolysis was low, due to the fact that the lipoprotein lipase was inactivated by the thermal kneading of the blends, an increase in lipolytic activity occurred in all cheeses during cold storage.

TABLE 3

Rheological properties of cheeses A, B, C and D 1 day after production and after 90 days' cold storage under vacuum (means ± standard error of mean)[1]

Rheological characteristics	*Cheese type* A	B	C	D
Hardness [N]	$1.56^{c} \pm 0.48$	$0.37^{b} \pm 0.05$	$0.54^{b} \pm 0.10$	$0.36^{a} \pm 0.01$
Cohesiveness [N mm]	0.57 ± 0.03	0.60 ± 0.02	0.62 ± 0.03	0.66 ± 0.03
Adhesiveness [N mm]	$8.17^{c} \pm 2.63$	$2.47^{b} \pm 0.38$	$3.79^{b} \pm 0.71$	$1.12^{a} \pm 0.07$
Elasticity [mm]	$0.85^{c} \pm 0.04$	$0.66^{b} \pm 0.05$	$0.78^{b} \pm 0.05$	$0.35^{a} \pm 0.09$
Gumminess [N mm]	$0.90^{c} \pm 0.27$	$0.22^{b} \pm 0.03$	$0.32^{b} \pm 0.06$	$0.10^{a} \pm 0.01$
Chewiness [N mm]	$0.81^{c} \pm 0.28$	$0.15^{b} \pm 0.03$	$0.26^{b} \pm 0.06$	$0.04^{a} \pm 0.01$

[1]Cheese A, B, C and D are defined in Table 1.
[a,b]Means in the same row without a superscript or bearing a common superscript did not differ significantly.

TABLE 4

Organoleptic evaluation of cheeses A, B, C and D 1 day after production and after 90 days' cold storage under vacuum (means ± standard error of mean)[1]

Organoleptic characteristics	*Cheese type on day 1* A	B	C	D
Flavour (0–8)	$6.4^{b} \pm 0.3$	$6.3^{b} \pm 0.3$	$6.4^{b} \pm 0.2$	$5.6^{a} \pm 0.1$
Texture (0–8)	$6.7^{b} \pm 0.2$	$6.7^{b} \pm 0.2$	$6.6^{b} \pm 0.2$	$5.8^{a} \pm 0.2$
Appearance (0–8)	6.9 ± 0.4	7.1 ± 0.1	6.9 ± 0.2	6.2 ± 0.2
Spreading ability (1–5)	$3.1^{a} \pm 0.1$	$3.8^{b} \pm 0.1$	$4.0^{b} \pm 0.2$	$4.9^{c} \pm 0.1$
	Cheese type on day 90			
Flavour (0–8)	$6.1^{b} \pm 0.3$	$5.7^{b} \pm 0.2$	$4.9^{a,b} \pm 0.8$	$3.4^{a} \pm 0.7$
Texture (0–8)	$5.9^{b} \pm 0.4$	$6.4^{b} \pm 0.1$	$6.5^{b} \pm 0.1$	$4.8^{a} \pm 0.5$
Appearance (0–8)	$6.7^{b} \pm 0.1$	$6.6^{b} \pm 0.2$	$6.4^{b} \pm 0.2$	$4.8^{a} \pm 0.4$
Spreading ability (1–5)	$2.5^{a} \pm 0.1$	$3.6^{b} \pm 0.1$	$3.7^{b} \pm 0.2$	$4.8^{c} \pm 0.1$

[1]Cheese A, B, C and D are defined in Table 1.
[a,b]Means in the same row without a superscript or bearing a common superscript did not differ significantly.

This change could, perhaps, be attributed to lipases from the psychrotrophic flora.

The relative change in lipid oxidation after 90 days' cold storage, as determined by the thiobarbituric acid (TBA) reaction, is shown in Fig. 2. A significant difference ($p < 0.05$) was observed only between cheese D and the other cheeses. As the extent of oxidation is directly related to the unsaturated fatty acid levels in the cheese, the higher degree of oxidation observed in cheese D was a result of the higher amount of soybean oil.

Textural assessment

The rheological characteristics of the cheeses after 90 days' cold storage under vacuum are shown in Table 3. The textural differences observed between the cheeses are attributed to their compositional differences. It is evident from the results that cheese A was significantly harder ($p < 0.05$) than the other cheeses due to its higher casein content, since casein shows a strong correlation with cheese hardness,[26] as do higher salt and ash contents (Table 2). The softest cheeses were those made with the highest proportion of UF retentate. No significant difference ($p > 0.05$) was observed in hardness between cheeses B and C. The adhesiveness, gumminess, chewiness and elasticity followed the same trend and had the same differences of means between the cheese types. These differences were also attributed to the different amounts of cheese casein and whey proteins in the products. From the above, it is clear that casein contributes more than whey protein to elasticity and this confirms that the rheological role of casein in cheese is to provide a continuous elastic framework for the individual cheese granules.[27] The processed cheese A, made with the highest proportion of cheese in the blend, had the highest elasticity, while the processed cheese D, with the highest proportion of UF retentate, had the lowest elasticity. No significant difference ($p > 0.05$) in cohesiveness was observed between the cheeses after 90 days' cold storage.

Organoleptic evaluation

The results of the taste panel assessment of the quality of the four different cheeses are reported in Table 4. All the cheeses proved acceptable to the panelists, but the scores were higher for cheeses on day 1 than after 90 days. Statistical analysis of the mean scores for flavour and texture showed a significant difference ($p < 0.05$) only between the cheese D, with about 40% UF retentate and the lowest score, and the other cheeses. This significant decrease in flavour of cheese D was mainly due to the oxidation of unsaturated fatty acids (Fig. 2) as a result of the high proportion of soybean oil in its lipid.

By contrast, as the level of UF retentate in the cheese blend increased, the mean scores

for spreading ability at both sampling dates increased. The differences were significant ($p < 0.05$) and the highest spreading ability was reported for cheese D, which also had the lowest value for adhesiveness (Table 3). It should be noted that the spreading ability for all cheeses decreased slightly after 90 days' cold storage. No significant differences ($p > 0.05$) in appearance were observed between the cheeses 1 day after production but, after 90 days, a significant difference ($p < 0.05$) in appearance between cheese D and the other blends was noted.

CONCLUSIONS

Processed cheese analogues were successfully manufactured by incorporation of UF whey protein concentrate and soybean oil to replace a proportion of the cheese casein and milk fat of kasseri cheese. Cold storage of cheese D (highest level of UF whey protein concentrate and soybean oil) caused a significant deterioration in quality. The control processed cheese (A) was harder and had a higher content of ash, whereas the other cheese products were more spreadable, had higher rates of lipolysis and a higher content of soluble protein and lactose. The lactose contents were low in all cases.

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