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## Biochemical characteristics of reduced-fat cheese made from high-heat treated goat's milk supplemented with *Penicillium candidum*

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### Βιοχημικά χαρακτηριστικά τυριού με μειωμένη περιεκτικότητα σε λίπος που παρασκευάστηκε από κατσικίσιο γάλα υψηλής θερμικής επεξεργασίας με επιφανειακή ανάπτυξη του μύκητα *Penicillium candidum*

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**ABSTRACT.** Novel reduced-fat goat-cheese (R) was produced from high-pasteurized milk using *Penicillium candidum* as an adjunct. A full-fat goat-cheese (F) from pasteurized milk without mold addition was produced for comparison reasons. Physicochemical analyses of the two cheeses were performed through the 14-d period of ripening. The effect of *P.candidum* on proteolysis of goat-cheese caseins and the production of hydrophilic and hydrophobic peptides during cheese ripening were investigated. To our knowledge, similar results for reduced-fat, mold-ripened, goat-milk cheeses have not been previously reported before. R-cheese exhibited a higher organoleptic score and developed properties similar to Kopanisti, which is a Protected Designation of Origin Greek soft cheese with specific intense flavour manufactured from raw milk without the use of starters. Moreover, R-cheese had significantly higher moisture, protein in dry matter and water soluble nitrogen contents than F-cheese and was less adhesive. The high-pasteurization improved the texture and cheese yield, while the use of *P. candidum* as an adjunct improved the flavour, increased and accelerated proteolysis in R-cheese. According to the results, the technology for R-cheese employed in the present study can be easily adopted and could be used to produce a reduced-fat goat-cheese.

**Keywords:** Reduced-fat goat-cheese, physicochemical characteristics, proteolysis, texture, sensory attributes

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**ΠΕΡΙΛΗΨΗ.** Παρασκευάστηκε ένα νέο κατσικίσιο τυρί με μειωμένη λιποπεριεκτικότητα (R) από γάλα υψηλής θερμικής επεξεργασίας στο οποίο προστέθηκε ως συμπληρωματική καλλιέργεια ο μύκητας *Penicillium candidum*. Παράλληλα, για λόγους σύγκρισης, παρασκευάστηκε ένα κατσικίσιο τυρί από πλήρες παστεριωμένο γάλα (F) χωρίς την προσθήκη του μύκητα. Μελετήθηκαν οι φυσικοχημικές ιδιότητες των δύο τυριών κατά την διάρκεια της ωρίμανσής τους στους 20°C για 14 ημέρες. Επίσης, ερευνήθηκε η επίδραση του *P. candidum* στην πρωτεόλυση των καζεϊνών του κατσικίσιου τυριού και η παραγωγή υδρόφιλων και υδρόφοβων πεπτιδίων κατά την ωρίμανσή του. Στη βιβλιογραφία, δεν έχουν αναφερθεί μέχρι τώρα παρόμοια αποτελέσματα για κατσικίσιο τυρί με μειωμένη λιποπεριεκτικότητα που έχει παρασκευαστεί από γάλα υψηλής θερμικής επεξεργασίας και έχει ωριμάσει με επιταχυνόμενο ρυθμό από τον μύκητα *P. candidum*. Το τυρί R παρουσίασε υψηλότερη βαθμολογία στα οργανοληπτικά χαρακτηριστικά από το τυρί F και ανέπτυξε ιδιότητες παρόμοιες με το τυρί Κοπανιστή, το οποίο είναι ένα ελληνικό μαλακό τυρί Προστατευόμενης Ονομασίας Προέλευσης με ιδιαίτερη έντονη γεύση που παρασκευάζεται από νωπό γάλα χωρίς τη χρήση εκκινητών. Επιπλέον, το τυρί R είχε σημαντικά μεγαλύτερο ποσοστό υγρασίας, πρωτεΐνης υπολογισμένη σε ξηρή ουσία και υδατοδιαλυτού αζώτου από το τυρί F και λιγότερη συνάφεια. Η υψηλή παστερίωση βελτίωσε την απόδοση και την υφή του τυριού R, ενώ η χρήση του *P. candidum* ως πρόσθετη καλλιέργεια βελτίωσε τη γεύση, αύξησε και επιτάχυνε την πρωτεόλυσή του. Σύμφωνα με τα αποτελέσματα, η τεχνολογία που χρησιμοποιήθηκε για το τυρί R στην παρούσα μελέτη μπορεί να υιοθετηθεί εύκολα και να χρησιμοποιηθεί με επιτυχία στην παραγωγή κατσικίσιου τυριού με μειωμένη λιποπεριεκτικότητα.

**Λέξεις κλειδιά:** Κατσικίσιο τυρί με μειωμένη λιποπεριεκτικότητα, φυσικοχημικά χαρακτηριστικά, πρωτεόλυση, ρεολογικά χαρακτηριστικά, οργανοληπτικά χαρακτηριστικά.

## INTRODUCTION

Greece is among the world's top ten producers of goat's milk and second within the European Union. According to FAO (2014) the goat population of Greece amounted to 4.255.000 for the year 2014. Many varieties of goat milk cheeses are produced around the world and especially in France. In Greece, goat's milk is mainly used to make cheese, but almost always mixed with sheep's milk, and only a small variety of home-made goat's cheese is produced. This is mainly due to a lack of data on the production methods and biochemical characteristics of goat cheeses, which precludes their production on an industrial scale. Goat's milk possesses unique nutritional and health properties (Jenness, 1980; Haenlein, 2004; Park *et al.*, 2007), such as high digestibility due to its fatty acid composition, the small size of the fat globules and its low levels of  $\alpha_{s1}$  casein, as well as high hypoallergenicity due to structural differences in  $\alpha$ -Lactalbumin ( $\alpha$ -La) and  $\beta$ -Lactoglobulin ( $\beta$ -Lg), when compared with bovine milk. Goat's milk is richer than cow's milk in various elements, such as calcium, magnesium, phosphorus, potassium, and vitamins: vitamin A, niacin, thiamine and riboflavin (Kondyli *et al.*, 2007). Due to their desirable properties, goat's milk products have been increasingly developed over the past 15 years. Goat cheeses ripened with molds represent only a small proportion of the world cheese production. However, these cheeses are

increasingly popular thanks to their distinct flavour and their soft texture, which is derived from the metabolism of lactose, fat, protein, citrate, and organic acids during ripening (Fox *et al.*, 1993).

Kopanisti cheese, according to Greek Codex Alimentarius (2014) is a soft traditional Greek cheese with Protected Designation of Origin (PDO) that is produced in the Cyclades islands from whole cow's, sheep's or goat's milk alone or mixed. The acidity of this cheese is produced by the flora of the raw milk without the use of lactic starter. It has a spreadable texture and a rich flavour similar to that of mold-ripened cheeses. Yeasts and molds are an important part of the essential microbial flora of Kopanisti cheese, with the species varying from production to production (Kaminarides and Anifantakis, 1989).

The last 30 years there is an increasing trend in favour of reduced-fat and reduced-energy products (Ritvanen *et al.*, 2005) to combat obesity, which contributes to serious health problems, such as arteriosclerosis, diabetes, cardiovascular damage, some forms cancer in the colon and breast (Hu *et al.*, 1997). Nevertheless, reduced-fat cheeses may be less acceptable to some consumers than full-fat cheeses due to defects in texture and flavour. Therefore, several attempts have been made to improve the flavour and texture of reduced-fat cheeses, for example by mod-

ification of conventional manufacturing process, the use of enzymes and additives (e.g., fat replacers), the introduction of adjunct cultures, or combinations of all these (Fenelon *et al.*, 2000; Mistry, 2001; Rodriguez, 1998).

In the present study, two soft goat-cheeses differing in fat content were produced, taking into consideration both the special dietary characteristics of goat's milk and consumers' preferences: a full-fat goat's cheese made from pasteurized milk using a mesophilic lactic starter and which is addressed to a wide consumer range. And a reduced-fat goat's cheese, made from high-pasteurized milk with the addition of *P. candidum*, which is aimed at customers who are on low-fat diets.

The first objective of this study was to encourage goat milk production in larger quantities for the production of high-quality, reduced-fat cheese. The second objective of this study was to determine whether *P. candidum* combined with high-pasteurization of milk could be used to improve appearance, flavour, yield, texture and to accelerate proteolysis and shorten the ripening of reduced-fat cheese.

## MATERIALS AND METHODS

### Milk, cultures and rennet

Raw goat's milk was obtained from the herd of the Agricultural University of Athens. The main constituents of 30 Kg whole raw milk, for the production of full-fat cheese (F), as determined by the Milkoscan apparatus (model 255 A/B, type 25700, Fosselectric, Denmark), were as follows (mean values  $\pm$  the standard errors of the means):  $3.01 \pm 0.01\%$  fat,  $2.76 \pm 0.03\%$  total protein,  $4.84 \pm 0.03\%$  lactose,  $0.76 \pm 0.01\%$  ash and  $11.10 \pm 0.03\%$  dry matter. A further quantity of milk from the same source was subjected to separation for up to  $\sim 50\%$  fat reduction. The mean values ( $\pm$ ) the standard errors of the means of the standardized milk used for reduced-fat cheese (R) were:  $1.51 \pm 0.01\%$  fat,  $2.86 \pm 0.03\%$  total protein,  $4.91 \pm 0.03\%$  lactose,  $0.79 \pm 0.01\%$  ash and  $9.68 \pm 0.03\%$  dry matter.

A 0.044% (w/w) freeze-dried lactic acid bacterial culture (DVS MO-10-Chr. Hansen Holding A/S, Hoershom, Denmark) of mixed mesophilic homofermentative species (*Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris*) was used.

A spore suspension of the mold *P. candidum* (Chr.

Hansen Swing FD PC A1) was made in sterile distilled water to an optical density of 0.4 at 525 nm ( $5.5 \times 10^9$  spores/ml). From this suspension was poured 0.2% to the surface of R-cheese drained curd.

Calf rennet powder HALA (0.3 gr per 100 l of milk; Chr. Hansen) was used in the cheese milk.

### Cheese making and sampling

Two soft goat cheeses were produced at the Laboratory of Dairy Science and Technology on the basis of traditional Kopanisti cheese technology (Greek Codex Alimentarius, 2014), modified as shown in Figure 1. Full-fat cheese (F) was made as a control from full-fat milk pasteurized at 68°C for 10 min and using mesophilic lactic starter. Reduced-fat cheese (R) was made from 50% reduced-fat milk, pasteurized at 80°C for 10 min and using mesophilic lactic starter and spores of *P. candidum* as adjunct. Four replicates of each type of soft cheese were produced on four successive weeks. The cheeses from each treatment were weighed, sampled and analyzed. For the assessment of the physicochemical characteristics, cheeses were examined during ripening. Textural and sensory properties were examined in the ripened cheeses (14 days after manufacture).

### Physicochemical analyses

The pH of the two cheeses was determined using a pH-meter (632 Metrohm, Herisau, Switzerland). Total solids were determined according to IDF (1982). Fat was analyzed according to the volumetric method of Gerber- Van Gulic (Schneider, 1954). Ash was determined by drying at 550°C to constant weight (IDF, 1964). Total nitrogen (TN), was determined by the Kjeldahl method according to IDF (1993). Water-soluble nitrogen (WSN) was obtained by homogenizing 25g cheese with 100 ml H<sub>2</sub>O according to the method for WSN extraction, cited in Butikofer and Ruegg (1993) and determined by the Kjeldahl method (IDF, 1993). Chloride content was analyzed according to IDF (2006). Ca, Mg, K and Na contents were determined by Atomic Absorption Spectrometry (IDF, 2007). All analyses were performed in quadruplicate.

### Electrophoretic analysis of cheese proteins (SDS-PAGE)

The electrophoresis of cheese proteins was performed using a Hoefer SE 600 Vertical Electrophoresis System according to the method of Kaminarides *et al.* (1995).

### Analysis of waters extracts by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)

Analysis of the water-soluble extracts of F- and R-cheese was carried out using the HPLC system of WATERS (WATERS, 34 Marple Street, Milford, MA 01757, USA) consisting of a pump capable of mixing four solvents (WATERS 600E), a photodiode array detector (WATERS 996), a helium degasser and an autosampler (WATERS 717). A Nucleosil 300-5-C18 column was used; the flow rate was 0.75 ml/min at room temperature. Solvent A was 0.1% trifluoroacetic acid (TFA, Serva Electrophoresis, Heidelberg, Germany) in ultra pure water (v/v) and solvent B was 0.1% TFA in 60% acetonitrile, (Lichrosolv grade, Merck, Darmstadt, Germany) (v/v).

The elution was as follows: 10 min, 100% A; 80 min 80% B, 101min, 100% A and 112 min, 100% B. Quantification was based on absorbance at 220 nm. 0.6 mL of WSN of each cheese sample (F- and R-cheese) was diluted with 0.6 mL solvent A. Diluted samples were kept for 10-15 min at room temperature. All samples were centrifuged at 8000 rcf for 5 min and filtered through 0.45 $\mu$ m membrane filters (Whatman, Rockwood, Germany) before injection; 100  $\mu$ L of each sample were analyzed.

### Textural evaluation

The textural properties of the cheese were measured with a Shimadzu testing instrument, (model AGS-500NG T, Shimadzu Corporation, Kyoto, Japan), as described by Kaminarides and Stachtiaris (2000).

### Sensory evaluation

The cheese sensory characteristics were graded by a fourteen-member taste-panel of the Dairy Science and Technology Laboratory of the Agricultural University of Athens. Panel members, who were familiar with this type of cheese, evaluated each sample 14 days after its manufacture for appearance, texture and flavour on a 10-point scale, from one signifying the worst to ten signifying the best quality. More importance was given to flavour and to texture than appearance of the cheese as advised by the IDF Standard 99c (1997) by multiplying their scores by 5 and 4 respectively. The total score was obtained by adding the scores based on the three attributes. An excellent cheese would receive a total score of 100. The panel was asked to note any flavour defects.

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the statistical program Statgraphics Plus for Windows (v. 5.2, Manugistics, Inc., Rockville, MO, USA) to test the effect of different goat's cheese type on cheese parameters.

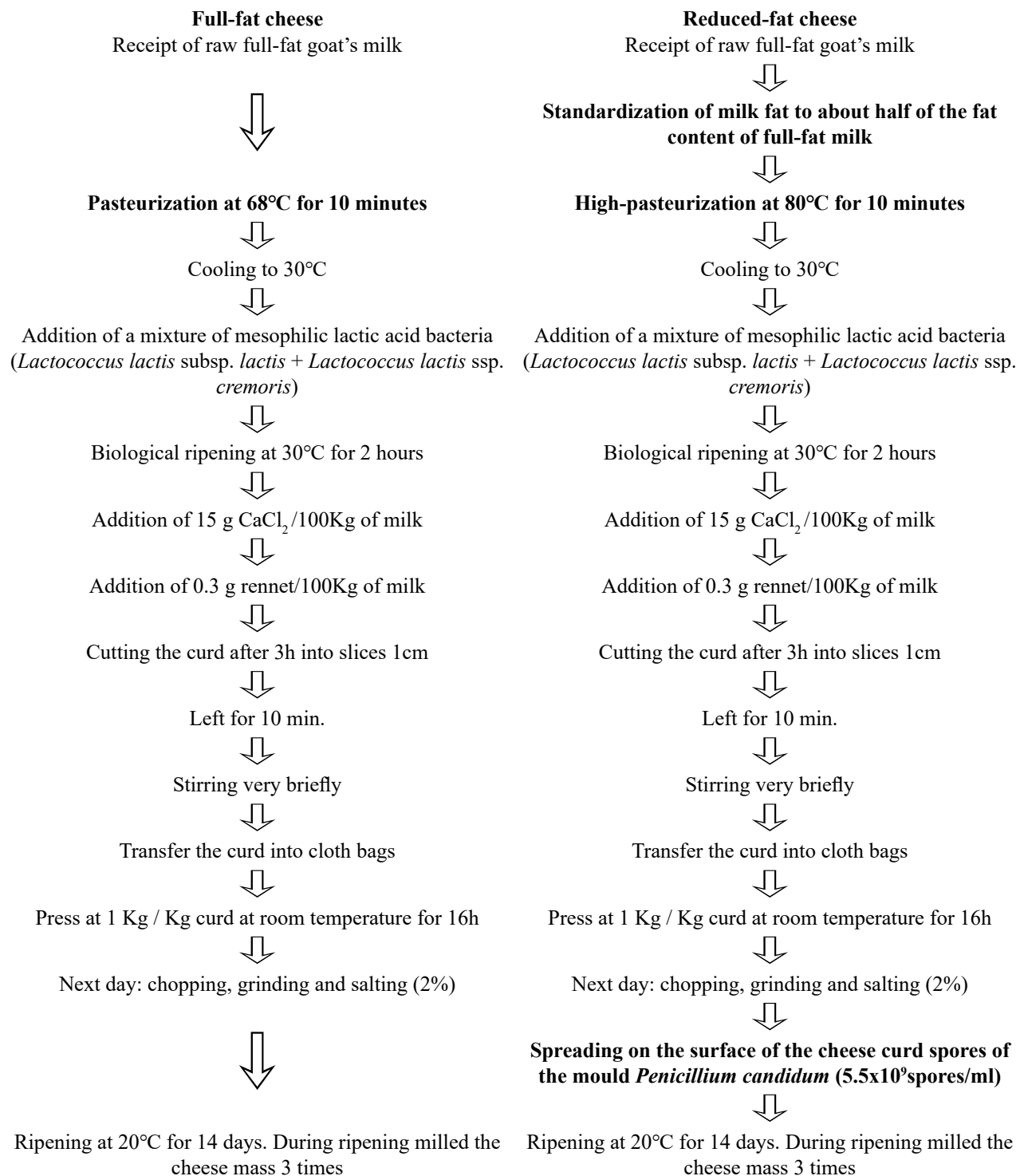
## RESULTS AND DISCUSSION

### Yield and physicochemical characteristics

Cheese yield, depends on the composition and quality of the milk used, the technology applied for cheese production and the conditions of cheese storage. Some reduction of cheese yield is inevitable during the production of cheese from milk with a low fat content, because the fat in the milk is one of the main components determining cheese yield (Romeih *et al.*, 2002). From the results (Table 1), it appears that the average yield of the full-fat cheese, one day after preparation, was 17.48%, compared with 17.91% for the reduced-fat cheese. Although the fat content of the R-cheese was half that of the F-cheese, the yield of the two cheeses did not differ significantly due mainly to the higher moisture content of the reduced-fat cheese (71.21%, day 1) compared to that of the full-fat cheese (65.01%, day 1) and also to the fact that in the case of R-cheese technology more proteins are transferred to the cheese (proteins in dry matter 49.06%) compared to that by F-cheese technology (proteins in dry matter 39.10%). The latter can be attributed to the incorporation of denatured serum proteins (mainly  $\beta$ -lactoglobulin) with  $\kappa$ - and  $\alpha_{s2}$ -caseins in casein micelles via sulphur bridges (Walstra and Jenness, 1984) during high temperature pasteurization of the milk (80  $^{\circ}$ C/10 min) in R-cheese technology (Figure 1).

There were no significant differences ( $P > 0.05$ ) in pH values between the two cheeses, although the pH values of both increased significantly ( $P < 0.05$ ) from day 1 to day 14 during ripening (Table 1). This increase in pH may be attributed to the use of lactic acid by yeasts and molds, the numbers of which increased during Kopanisti cheese ripening according to Kaminarides and Anifantakis (1989).

The moisture content of the R-cheese was significantly higher ( $P < 0.05$ ) than that of the F-cheese both on day 1 and day 14. The higher moisture content of ripe R-cheese (66.34%), compared with that of ripe F-cheese (55.99%), resulted from the use of high-pasteurization which is known to denature serum proteins (mainly  $\alpha$ -La and  $\beta$ -Lg), resulting in enhanced water-retaining ability.



**Figure 1.** Flow chart for the production of soft goat-cheeses from full-fat, pasteurized milk and from reduced-fat, high-pasteurized milk supplemented with *Penicillium candidum*. Bold letters indicate the modifications between the two technologies

Moisture, expressed as % in non-fat ingredients (MNFS), did not differ significantly between the experimental cheeses on day 1 (Table 1), which proves that it was not affected by fat reduction. However, a statistical difference occurred after maturation (day 14) due to differences in moisture loss during ripen-

ing. R-cheese is characterized by fat ~10 %, moisture ~66% and MNFS~75%. According to the Greek Codex Alimentarius (2014), this cheese is classified as a soft cheese. The total solids of each type of cheese followed a trend that was the reverse of that relating to moisture.

**Table 1.** Yield and physicochemical characteristics of the two types of soft goat cheeses during their ripening. (Means of 4 trials  $\pm$  standard error of mean)

Yield and physico-chemical characteristics	F		R	
	Day 1	Day 14	Day 1	Day 14
Cheese yield (%)	17.48 <sup>a</sup> $\pm$ 0.49	-	17.91 <sup>a</sup> $\pm$ 0.49	-
pH	4.09 <sup>a</sup> $\pm$ 0.11	4.73 <sup>b</sup> $\pm$ 0.13	4.15 <sup>a</sup> $\pm$ 0.11	4.63 <sup>b</sup> $\pm$ 0.13
Total solids (%)	34.99 <sup>b</sup> $\pm$ 1.21	44.01 <sup>c</sup> $\pm$ 1.33	28.43 <sup>a</sup> $\pm$ 1.21	33.67 <sup>b</sup> $\pm$ 1.33
Moisture (%)	65.01 <sup>b</sup> $\pm$ 1.20	55.99 <sup>a</sup> $\pm$ 1.31	71.21 <sup>c</sup> $\pm$ 1.20	66.34 <sup>b</sup> $\pm$ 1.31
MNFS (%)	77.48 <sup>b</sup> $\pm$ 1.96	71.59 <sup>a</sup> $\pm$ 1.96	78.66 <sup>b</sup> $\pm$ 1.96	74.75 <sup>a</sup> $\pm$ 1.96
Ash (%)	2.74 <sup>a</sup> $\pm$ 0.12	3.76 <sup>b</sup> $\pm$ 0.14	2.89 <sup>a</sup> $\pm$ 0.12	3.71 <sup>b</sup> $\pm$ 0.14
Fat (%)	16.66 <sup>c</sup> $\pm$ 0.49	19.47 <sup>d</sup> $\pm$ 0.57	8.81 <sup>a</sup> $\pm$ 0.49	10.02 <sup>b</sup> $\pm$ 0.57
Fat in dry matter (%)	46.75 <sup>b</sup> $\pm$ 0.62	46.65 <sup>b</sup> $\pm$ 0.72	31.03 <sup>a</sup> $\pm$ 0.62	30.96 <sup>a</sup> $\pm$ 0.72
Protein (%)	13.68 <sup>a</sup> $\pm$ 0.58	16.75 <sup>b</sup> $\pm$ 0.65	13.95 <sup>a</sup> $\pm$ 0.61	16.94 <sup>b</sup> $\pm$ 0.71
Protein in dry matter (%)	39.10 <sup>a</sup> $\pm$ 0.75	38.06 <sup>a</sup> $\pm$ 0.88	49.07 <sup>b</sup> $\pm$ 0.80	50.31 <sup>b</sup> $\pm$ 0.90
Water soluble nitrogen –WSN-(%)	0.105 <sup>a</sup> $\pm$ 0.012	0.196 <sup>b</sup> $\pm$ 0.015	0.105 <sup>a</sup> $\pm$ 0.014	0.289 <sup>c</sup> $\pm$ 0.013
Maturation index (WSN/TN%)	-	8.261 <sup>a</sup> $\pm$ 0.96	-	10.44 <sup>b</sup> $\pm$ 0.86
Salt (%)	2.14 <sup>a</sup> $\pm$ 0.16	2.89 <sup>b</sup> $\pm$ 0.15	2.10 <sup>a</sup> $\pm$ 0.16	2.69 <sup>b</sup> $\pm$ 0.15
Ca (mg/100g cheese)	-	97.76 <sup>a</sup> $\pm$ 2.72	-	98.77 <sup>a</sup> $\pm$ 2.72
Mg (mg/100g cheese)	-	11.49 <sup>a</sup> $\pm$ 0.50	-	12.75 <sup>a</sup> $\pm$ 0.50
Na (mg/100g cheese)	-	40.34 <sup>a</sup> $\pm$ 7.96	-	55.26 <sup>a</sup> $\pm$ 7.96
K (mg/100g cheese)	-	179.06 <sup>a</sup> $\pm$ 7.45	-	179.94 <sup>a</sup> $\pm$ 7.45

a,b,c,d: Means in the same row bearing a common superscript did not differ significantly ( $p > 0.05$ ).

F& R: as in Material and methods

The ripe R-cheese (14 days) had a fat content of 10.02%, compared with 19.47% in ripe F-cheese (14 days). Clearly, this statistically significant difference in fat between the two types of cheese was due to the 50% reduction of fat in the milk used for R-cheese production. According to the Commission Regulation (1924/2006) of the European Parliament and Council, R-cheese can be described as “reduced-fat cheese” as its fat content has been reduced by ~50% in relation to F-cheese.

A small, but statistically insignificant ( $P > 0.05$ ) increase in protein content was found in R-cheese, which when expressed on a dry matter basis, was significantly higher in ripe R-cheese (50.31%) than in ripe F-cheese (38.06%) as shown in Table 1. This difference could be attributed to the denaturation of  $\beta$ -lactoglobulin during the high temperature pasteurization of milk for R-cheese production and its association with  $\kappa$ - and  $\alpha_{s2}$ -casein in the milk through disulfide bond formation (Walstra and Jenness, 1984), thus coagulating with casein during cheese making.

There were no significant differences ( $P > 0.05$ ) in ash and NaCl contents between the two types of cheese. Ash and NaCl contents were significantly higher at 14 days than at day 1 owing to moisture loss during ripening.

No significant difference ( $P > 0.05$ ) was observed

in the water soluble nitrogen (WSN) content of F- and R-cheese on day 1, but the levels of WSN increased during aging and differed significantly ( $P < 0.01$ ) on day 14 (Table 1).

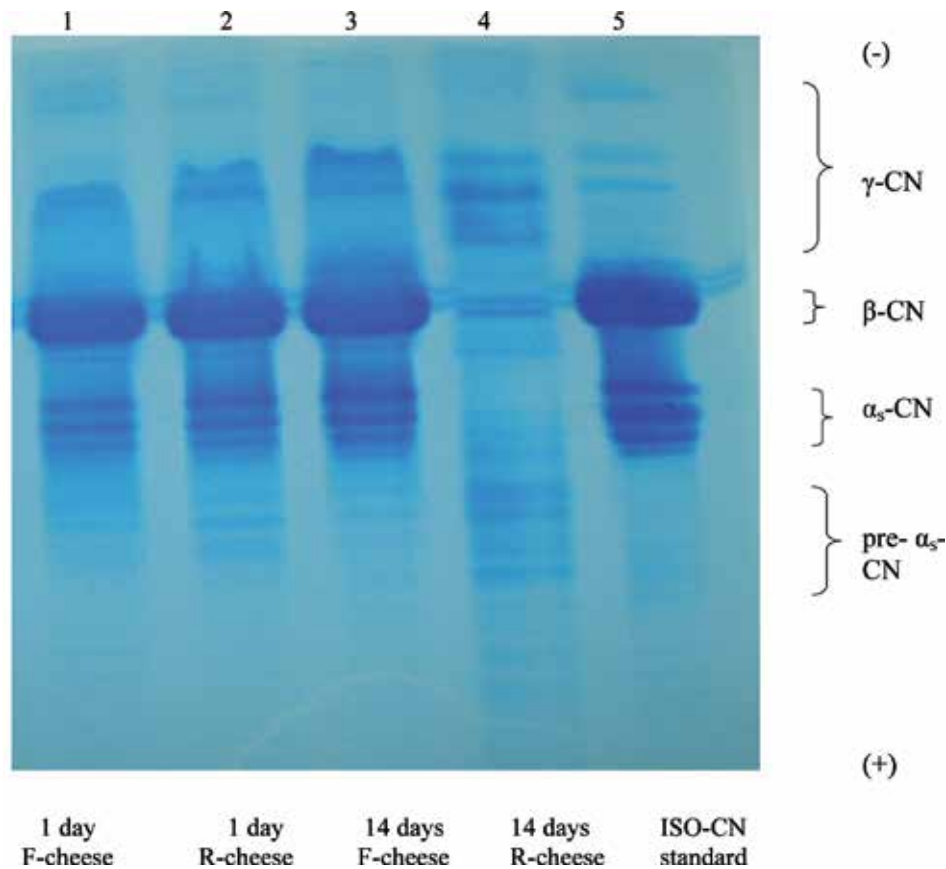
Water soluble nitrogen expressed as percentage of total nitrogen (WSN, % of TN), is used as a ripening index, and differed significantly ( $P > 0.05$ ) between the two cheeses. Thus, R-cheese had a ripening index of 10.4%, which was significantly higher than that of F-cheese (8.3%), and was attributable to more intense proteolytic activity in R-cheese due to the presence of *P. candidum*. The ripening index is an indication of the extent of proteolysis and varies in cheeses from very limited (e.g. Mozzarella) to very extensive (Blue cheese, cheeses that mature with fungi) (Upadhyay *et al.*, 2004).

### Electrophoretic profile analysis

Figure 2 demonstrates the electrophoretic patterns of proteins of the two types of cheese during ripening, together with that of pure isoelectric casein (ISO-CN) of caprine milk which was used as a standard (lane 5). The first strong bands correspond to  $\beta$ -caseins, and the other strong bands (following the  $\beta$ -caseins) correspond to  $\alpha_s$ -caseins (Franco *et al.*, 2003). In goat cheeses,  $\beta$ -caseins are predominant. On day 1, the first strong band of R-cheese (lane 2), appears to be diffusion (a tail), which probably corresponds to serum

protein denaturation by heat during high-pasteurization of milk and complexing of  $\beta$ -lactoglobulin with caseins (Walstra and Jenness, 1984; Considine *et al.*, 2007). The hydrolysis rate of  $\alpha_s$ - and  $\beta$ -caseins differed significantly between the two cheeses. R-cheese

(inoculated with *P. candidum*) had the higher hydrolysis rate of  $\alpha_s$ - and  $\beta$ -caseins than those in F-cheese. The bands below  $\alpha_s$ -caseins and above  $\beta$ -caseins correspond to peptides derived from their degradation by enzymes during cheese ripening (Franco *et al.*, 2003).



**Figure 2.** Polyacrylamide gel electrophoretic patterns of proteins of two goat cheeses during ripening. Lanes: 1 ; 2; 3; 4 and 5

### RP-HPLC profiles of Water Soluble Extracts (WSN)

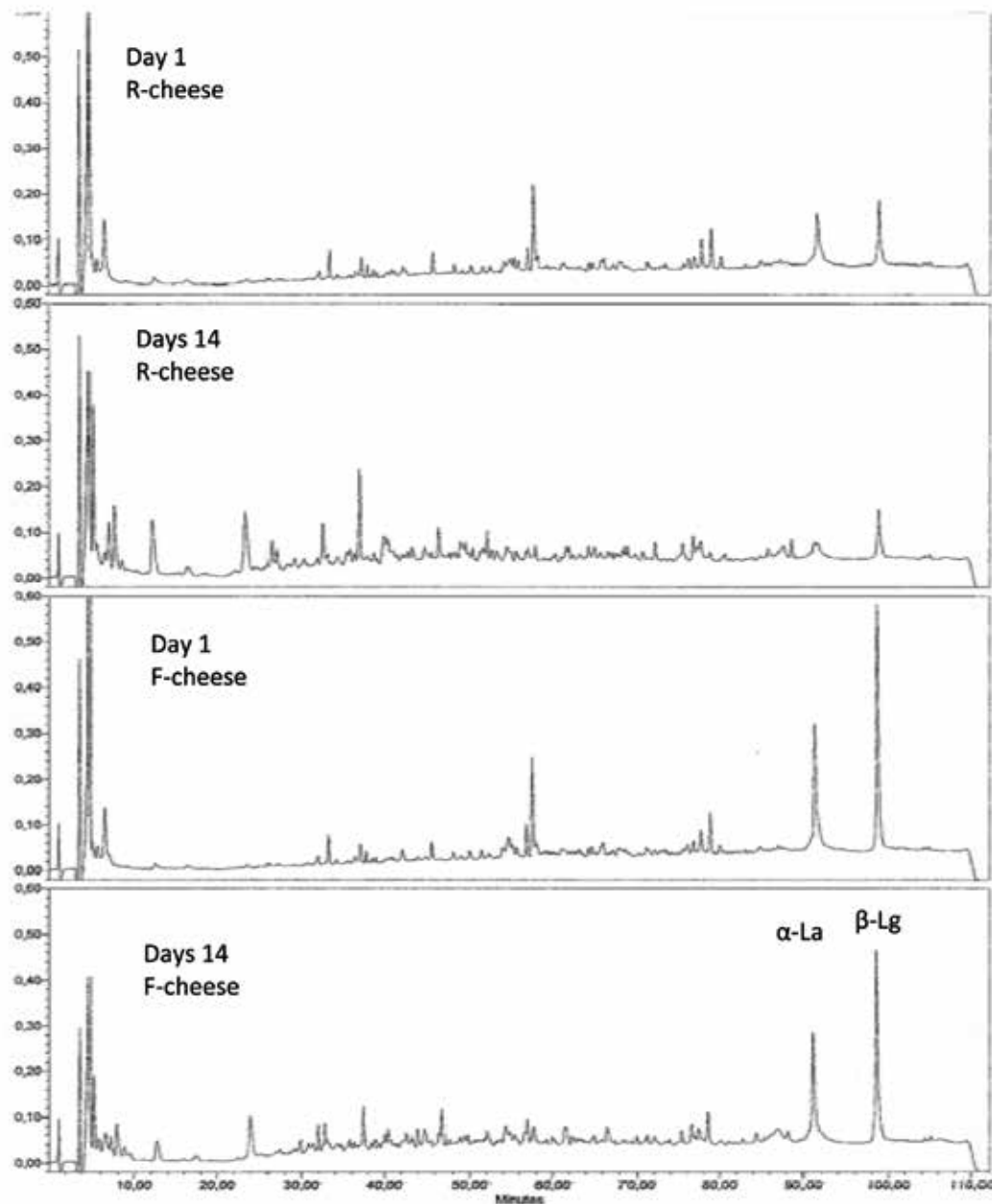
Figure 3 shows the peptide profiles of the water soluble fraction of experimental F-cheese and R-cheese on day 1 and after ripening for 14 days, obtained by means of RP-HPLC. Visual inspection of these chromatograms reveals that after 14 days of ripening new peaks appeared in both cheeses chromatograms, while the height of other peaks that existed on day 1 had changed. Michaelidou *et al.* (1998) reported that the first peaks eluted between 0 and 10 min in similar chromatographic analysis were free amino acids such as tyrosine and phenylalanine, the peaks eluted in the middle of the chromatogram corresponded to small and medium peptides mainly derived from the degradation of  $\alpha_{s1}$ - and  $\beta$ -caseins, while the peaks eluted at the end of the chromatogram corresponded to

$\alpha$ -La and  $\beta$ -Lg. Also, the peaks in the front region of RP-HPLC profiles (from 10-55 min) include hydrophilic peptides -HL-, while the peaks eluted between 55 and 80 min correspond mainly to the hydrophobic peptides -HB- (Nega and Moatsou, 2012). Differences were observed between the elution profiles of F- and R-cheese (Figure 3). It was evident that the cheese-making conditions strongly affected the major whey proteins  $\beta$ -Lg and  $\alpha$ -La. On day 1, R-cheese contained less non-denatured (natural) whey proteins, apparently due to the high-heat treatment,  $\beta$ -Lg being more intensely affected (Sakkas *et al.*, 2014). Moreover, from day 1 to day 14 the area of  $\alpha$ -La in R-cheese greatly decreased, indicating strong proteolytic activity of the mold. For quantitative analysis the chromatograms were divided into six parts according to the elution times given above, and the total area of the



peaks in each part was used for the assessment (Figure 4). The area of the part from 70-100 min was lower in R-cheese than in F-cheese due to denaturation. The area of peaks eluted between 0-55 min, which contains the hydrophilic peptides, was higher in R-cheese (with mold) than in F-cheese (no mold), indicating more intense proteolysis in the former. In contrast, the area of peaks eluted within 55-100 min, which contains the hydrophobic peptides and whey protein, was lower in the mold-ripened R-cheese than in F-cheese. For the assessment of proteolysis, the HB/HL peptide

ratio of WSN profiles was calculated. After 14 days of ripening, HL peptides had increased in both cheeses, whereas the HB peptides decreased (Figures 3, 4). In particular, the ratio HB / HL of R-cheese decreased from 1.93 to 1.00 within 14 days. The respective values for F- cheese were 2.08 and 1.51. The decrease in the ratio HB/HL observed during ripening could be attributed to proteolytic activity, as reported for other cheese varieties (e.g. Gonzalez *et al.*, 1995; Katsiari *et al.*, 2000), again indicating higher proteolytic activity in R-cheese due to *P. candidum*.



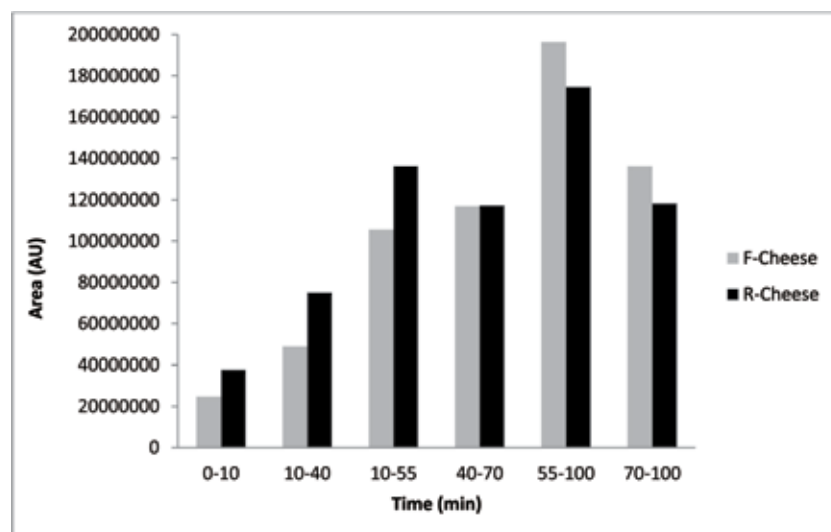
**Figure 3.** Reversed phase HPLC profiles of water-soluble extracts (WSN) of goat-milk cheeses during their ripening. F-cheese made from full-fat pasteurized milk without mold. R-cheese made from reduced-fat, high-pasteurized milk with the addition of *Penicillium candidum*.

**Table 2.** Textural and sensory properties of two types of soft goat cheeses after 14 days' ripening. (Means of 4 trials  $\pm$  standard error of mean)

Properties	Types of soft cheese from goat milk	
	F- cheese	R- cheese
Textural properties		
Hardness (N)	2.58 <sup>a</sup> $\pm$ 0.95	2.38 <sup>a</sup> $\pm$ 1.24
Cohesiveness (N.mm)	0.37 <sup>a</sup> $\pm$ 0.07	0.34 <sup>a</sup> $\pm$ 0.07
Adhesiveness (N.mm)	7.89 <sup>b</sup> $\pm$ 2.01	5.20 <sup>a</sup> $\pm$ 1.47
Sensory evaluation		
Taste and Flavour (0-50)	39.9 <sup>a</sup> $\pm$ 1.0	42.5 <sup>b</sup> $\pm$ 0.7
Texture (0-40)	32.5 <sup>a</sup> $\pm$ 0.6	33.3 <sup>a</sup> $\pm$ 0.8
Appearance (0-10)	8.1 <sup>a</sup> $\pm$ 0.3	8.9 <sup>b</sup> $\pm$ 0.1
Total (0-100)	80.5 <sup>a</sup> $\pm$ 1.2	84.7 <sup>a</sup> $\pm$ 1.9

a,b: Means in the same row bearing a common superscript did not differ significantly ( $p > 0.05$ ).

F& R: as in Material and methods



**Figure 4.** Area of isolated parts of the WSN profiles of goat-milk cheeses. F-cheese made from full-fat pasteurized milk without mold after 14 days of ripening. R-cheese made from reduced-fat, high-pasteurized milk with *Penicillium candidum* after 14 days of ripening.

### Textural characteristics

No significant difference ( $P > 0.05$ ) was observed in hardness and cohesiveness between F- and R-cheese. Although the reduction of fat content in R-cheese was half that of F-cheese, this did not increase its hardness, due to the high moisture content of the former (Table 1) and the incorporation of serum proteins into the curd, which loosened the casein matrix. Adhesiveness was found to differ significantly ( $P < 0.05$ ) between F-cheese and R-cheese (Table 2). Similarly, a decrease in the adhesiveness of Cheddar cheese has been reported to result from a reduction in its fat content (Bryan *et al.*, 1995).

### Sensory characteristics

Sensory evaluation results are given in Table 2. The mature cheeses obtained from both technologies were judged to be of good quality and characterized

by soft texture, good spread ability and a rich flavor. No significant difference in texture was detected. These results were in accordance with the results of rheological tests, which showed that both cheeses had similar hardness and cohesiveness. R-cheese was whiter in color and received significantly ( $P < 0.05$ ) higher scores for appearance, flavour and total sensory characteristics compared with F-cheese (Table 2) thanks to the mold, *P. candidum*, which improved the appearance and the flavour of R-cheese. No bitter taste was noticed by any member of the panel. In addition, R-cheese has less 'goaty' flavour due its lower level of milk fat.

### CONCLUSION

The two new cheeses manufactured in this study possessed the special dietary nutritional properties of goat's milk. So, the use of goat's milk in the pro-

duction of these cheeses will help for an increased utilization of goat's milk. The technology applied to R-cheese resulted in a fast ripening period of two weeks and the cheese produced was of good quality, soft texture, rich flavour and good spread ability. So, R-cheese, which was produced from goat's milk with 10.02% fat content, had a whiter color and higher scores for appearance flavour and total sensory characteristics compared to F-cheese, which resulted from the inclusion of *P. candidum* in its manufacture. This

mold in the R-cheese increased proteolytic activity during its ripening.

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## CONFLICT OF INTEREST STATEMENT

We have no conflict of interest to declare.

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