



HAL
open science

**Kishk - a dried fermented milk / cereal mixture. 2
Assessment of a variety of protein analytical techniques
for determining adulteration and proteolysis**

Adnan Y. Tamime, Margaret N.I. Barclay, Andrew J.R. Law, Jeffrey Leaver,
Emmanuel M. Anifantakis, Thomas P. O'Connor

► **To cite this version:**

Adnan Y. Tamime, Margaret N.I. Barclay, Andrew J.R. Law, Jeffrey Leaver, Emmanuel M. Anifantakis, et al.. Kishk - a dried fermented milk / cereal mixture. 2 Assessment of a variety of protein analytical techniques for determining adulteration and proteolysis. *Le Lait*, 1999, 79 (3), pp.331-339. hal-00929655

HAL Id: hal-00929655

<https://hal.science/hal-00929655v1>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Kishk – a dried fermented milk/cereal mixture. 2. Assessment of a variety of protein analytical techniques for determining adulteration and proteolysis

Adnan Y. Tamime^{a*}, Margaret N.I. Barclay^a, Andrew J.R. Law^b,
Jeffrey Leaver^b, Emmanuel M. Anifantakis^c, Thomas P. O'Connor^d

^a Food Standards & Product Technology Department, SAC Auchincruive,
Ayr KA6 5HW, Scotland, United Kingdom

^b Hannah Research Institute, Ayr KA6 5HL, Scotland, United Kingdom

^c Department of Food Science & Technology, Agricultural University of Athens,
Laboratory of Dairy Technology, 75 Iera Odos, 11855 Athens, Greece

^d Department of Food Chemistry, University College Cork, Cork, Republic of Ireland

(Received 6 March 1998; accepted 24 November 1998)

Abstract — A variety of protein assays were applied to the analysis of 25 commercial samples of Lebanese Kishk to detect the adulteration of goat's milk with cow's milk. Polyacrylamide gel electrophoretic (PAGE) methods could detect the degree of adulteration ~ 25 %. Using sodium dodecyl sulphate (SDS-PAGE), reverse phase high performance liquid chromatography (RP-HPLC) and anion-exchange fast protein liquid chromatography (FPLC) failed to detect the degree of adulteration in Kishk due to a very extensive proteolysis that made the distinction of the original caseins very difficult. Moreover, the immunoassay technique (Cortecs method) had limited application for powder products and could not be used effectively to calculate the degree of adulteration of goat's milk Kishk with cow's milk. © Inra/Elsevier, Paris.

Kishk / PAGE / SDS-PAGE / RP-HPLC / immunoassay

Résumé — Le Kishk – un mélange lait/céréales fermenté et séché. 2. Test de différentes techniques d'analyse des protéines pour déterminer l'adulteration du lait et la protéolyse. Différentes méthodes de détermination des protéines ont été testées pour l'analyse de 25 échantillons de Kishk libanais du commerce pour détecter l'adulteration du lait de chèvre par du lait de vache. Les méthodes par électrophorèse en gel de polyacrylamide (PAGE) permettent la détection d'un degré d'adulteration d'environ 25 %. L'utilisation de l'électrophorèse en présence de sodium dodecyl sulphate (SDS-PAGE), de la chromatographie liquide haut performance en phase inverse (RP-HPLC) et de la

* Correspondence and reprints. a.tamime@au.sac.ac.uk

chromatographie liquide des protéines par échange d'anion (anion-exchange FPLC) ne permettait pas de déterminer le degré d'adultération dans le Kishk en raison d'une forte protéolyse qui rendait la distinction entre caséines très difficile. De plus, le test immunologique (méthode 'Cortecs') présentait une application limitée sur les produits en poudre et ne pouvait pas être utilisé avec efficacité pour calculer le degré d'adultération du lait de chèvre du Kishk par du lait de vache. © Inra/Elsevier, Paris.

Kishk / PAGE / SDS-PAGE / HPLC phase inverse / immuno-essai

1. INTRODUCTION

Kishk is a dried mixture of low-fat yoghurt and Burghol (parboiled cracked wheat), and usually made from goat's milk. Information on the compositional and nutritional properties of Kishk have been reviewed by Tamime and O'Connor [44], and the product is a good source of protein [45]. Limited labelling data are available on the type of milk used in Kishk-making in Lebanon [46], and this could be against the interest of the consumer.

Analytical techniques such as polyacrylamide gel electrophoresis (PAGE) and the ratios of certain fatty acids have been used widely in the past for detecting the adulteration of cow's milk in goat's and sheep's milk and dairy products [26–29]. The PAGE method tends to differentiate the milk from different mammalian species based on the mobility of α_{s1} -casein, and the adulteration sensitivity may be limited. Determination of the ratio of fatty acids $C_{12:0}/C_{10:0}$ was recommended as a means of detecting the presence of cow's milk in goat and sheep products, and this analytical technique is still used [31, 33, 38].

Over the past decade new analytical techniques have been developed in order to detect the adulteration of goat's and sheep's milk products with cow's milk. These techniques include: (a) modified PAGE methods which are suitable for dairy products, especially where the milk is heated to 90 °C for 30 s [8, 14, 15]; (b) gel isoelectric focusing which is sensitive in detecting the adul-

teration levels ≥ 0.5 % of cow's γ_2 - and γ_3 -caseins [1, 25, 34, 35]; (c) reverse phase high performance liquid chromatography (RP-HPLC) separates and identifies cow's whey proteins (β -Lg A and α -La) in human, goat, sheep, buffalo and mare products [36, 47]; and (d) capillary zone electrophoresis measures the migration time of α_{s1} -casein fraction of milk of different species and is capable of detecting cow's milk (~ 8 %) in sheep's and goat's milk [17]. However, a wide range of immunological analytical techniques have been developed to detect specific cow's or goat's protein fractions in goat's and sheep's milk or in sheep's milk, respectively. Adulteration can be detected at low levels, and an example of such a technique is given in the European Union (EU) reference methods [20]. The immunoassay methods rely on using specific mono- or polyclonal specific antibodies reactive to: (a) cow's β -Lg [16, 21, 32, 41]; (b) cow's IgG [10, 11, 37]; (c) cow's κ -casein [12]; (d) α_{s1} -casein [43]; (e) goat's whey protein [22, 23]; (f) γ_2 - and γ_3 -caseins [2]; (g) goat casein [24, 39, 40, 42]; and (h) cow's β -casein [6, 7]. The sensitivity of such methods is also ≥ 0.5 %. Furthermore, comparison of different analytical techniques to determine the sensitivity of the presence of cow's milk in goat and sheep products have been reported by Amigo et al. [3, 4].

The objectives of this study were to investigate in detail the origin of the milk used, the nutritional properties and the microbiological qualities of 25 Lebanese Kishk samples obtained from different outlets. This paper details the application of

chromatographie liquide des protéines par échange d'anion (anion-exchange FPLC) ne permettait pas de déterminer le degré d'adultération dans le Kishk en raison d'une forte protéolyse qui rendait la distinction entre caséines très difficile. De plus, le test immunologique (méthode 'Cortecs') présentait une application limitée sur les produits en poudre et ne pouvait pas être utilisé avec efficacité pour calculer le degré d'adultération du lait de chèvre du Kishk par du lait de vache. © Inra/Elsevier, Paris.

Kishk / PAGE / SDS-PAGE / HPLC phase inverse / immuno-essai

1. INTRODUCTION

Kishk is a dried mixture of low-fat yoghurt and Burghol (parboiled cracked wheat), and usually made from goat's milk. Information on the compositional and nutritional properties of Kishk have been reviewed by Tamime and O'Connor [44], and the product is a good source of protein [45]. Limited labelling data are available on the type of milk used in Kishk-making in Lebanon [46], and this could be against the interest of the consumer.

Analytical techniques such as polyacrylamide gel electrophoresis (PAGE) and the ratios of certain fatty acids have been used widely in the past for detecting the adulteration of cow's milk in goat's and sheep's milk and dairy products [26–29]. The PAGE method tends to differentiate the milk from different mammalian species based on the mobility of α_{s1} -casein, and the adulteration sensitivity may be limited. Determination of the ratio of fatty acids $C_{12:0}/C_{10:0}$ was recommended as a means of detecting the presence of cow's milk in goat and sheep products, and this analytical technique is still used [31, 33, 38].

Over the past decade new analytical techniques have been developed in order to detect the adulteration of goat's and sheep's milk products with cow's milk. These techniques include: (a) modified PAGE methods which are suitable for dairy products, especially where the milk is heated to 90 °C for 30 s [8, 14, 15]; (b) gel isoelectric focusing which is sensitive in detecting the adul-

teration levels ≥ 0.5 % of cow's γ_2 - and γ_3 -caseins [1, 25, 34, 35]; (c) reverse phase high performance liquid chromatography (RP-HPLC) separates and identifies cow's whey proteins (β -Lg A and α -La) in human, goat, sheep, buffalo and mare products [36, 47]; and (d) capillary zone electrophoresis measures the migration time of α_{s1} -casein fraction of milk of different species and is capable of detecting cow's milk (~ 8 %) in sheep's and goat's milk [17]. However, a wide range of immunological analytical techniques have been developed to detect specific cow's or goat's protein fractions in goat's and sheep's milk or in sheep's milk, respectively. Adulteration can be detected at low levels, and an example of such a technique is given in the European Union (EU) reference methods [20]. The immunoassay methods rely on using specific mono- or polyclonal specific antibodies reactive to: (a) cow's β -Lg [16, 21, 32, 41]; (b) cow's IgG [10, 11, 37]; (c) cow's κ -casein [12]; (d) α_{s1} -casein [43]; (e) goat's whey protein [22, 23]; (f) γ_2 - and γ_3 -caseins [2]; (g) goat casein [24, 39, 40, 42]; and (h) cow's β -casein [6, 7]. The sensitivity of such methods is also ≥ 0.5 %. Furthermore, comparison of different analytical techniques to determine the sensitivity of the presence of cow's milk in goat and sheep products have been reported by Amigo et al. [3, 4].

The objectives of this study were to investigate in detail the origin of the milk used, the nutritional properties and the microbiological qualities of 25 Lebanese Kishk samples obtained from different outlets. This paper details the application of

various protein assays to samples of Kishk carried out in laboratories in Scotland, Ireland and Greece in an attempt to identify properly those products that have been made with cow's milk, goat's milk or mixtures of both.

2. MATERIALS AND METHODS

2.1. Kishk samples

Twenty-five samples of commercial Kishk (~1 kg each) were obtained from different retail outlets [46]. Each sample of Kishk (~100 g) was dispensed into sterile screw-capped containers and despatched by airmail to Ireland and Greece using a 2nd-day delivery.

2.2. Protein analytical methods

2.2.1. Urea-PAGE (method A)

Electrophoresis in polyacrylamide (12.5 % separation, 4.2 % stacking) gel was performed in a Protean II xi (Bio-Rad Laboratories Ltd., Watford, Hertfordshire, UK) vertical slab cell according to the method of Andrews [5], with modifications. The gels were stained by the method of Blakely and Boezi [13]. Four standards were injected (cow, goat and sheep α_2 -caseinate and wheat flour). The milk origin was determined by comparing α_2 - and β -casein bands in the controls and in the Kishk samples.

2.2.2. Discontinuous PAGE (method B)

A discontinuous PAGE method was used to detect the adulteration of goat's milk with cow's milk in the Kishk samples as described by Anifantakis and Massouras [8] with a few modifications regarding the electrophoresis conditions and sample preparation.

The electrophoretic apparatus (model Pharmacia GE-2/4 LS), a cooling thermostat (DESAGA Frigastat), and a power supply (LKB 2197) were used. The gels were made using a Pharmacia kit 18 cm \times 14 cm \times 0.7 mm slab. Electrophoresis was carried out at 8 °C, initially pre-running at 150 V for 1 h, followed by final running under constant current (60 mA) for approximately 2 h. The proteins were fixed with 15 % trichloroacetic acid (TCA) for 30 min, and

stained with Coomassie blue according to the method described by EU [20]. The stained gels were stored in 10 % (v/v) acetic acid.

The milk protein concentration of each Kishk sample prior to electrophoresis was adjusted to approximately 5 mg·mL⁻¹ sample buffer assuming that the yoghurt to Burghol ratio was 4:1 (w/w). Typically, 150–230 mg of Kishk was diluted with 5 mL sample buffer and centrifuged at 3 000 g for 5 min. Supernatant (10–15 μ L) was used for electrophoresis. Milk protein standards at a concentration of 10 mg·mL⁻¹ were also used. Standards consisted of isolated caseins from: (a) cheese made from cow's milk; (b) goat's milk; (c) cheese made with a mixture of goat's and sheep's milks; and (d) a wheat sample (*Triticum aestivum* var. Chinese spring).

2.2.3. SDS-PAGE

Acid precipitates from Kishk samples and standard goat and cow caseins were reduced with 2-mercaptoethanol and boiled for 5 min in the presence of excess sodium dodecyl sulphate (SDS), and separated by PhastSystem™ SDS-PAGE on 20 % homogeneous gels as described by the manufacturers (Pharmacia Biotech, Saint-Albans, Hertfordshire, UK).

2.2.4. RP-HPLC

Skimmed cow's and goat's milk was diluted in 4 V of a dissociating buffer consisting of 8 mol·L⁻¹ urea/40 mmol·L⁻¹ bis-tris-propane, pH 7.0, containing 0.1 % (v/v) 2-mercaptoethanol, and the samples were incubated with stirring for a minimum of 1 h at room temperature. The Kishk samples and ground Burghol were stirred with the dissociating buffer at a concentration of 20 mg·mL⁻¹, and all samples were filtered through 0.2 μ m filters.

The Kishk samples or Burghol (50 and 20 μ L, respectively) were loaded onto an Apex C18 WP reverse phase column (25 cm \times 4.6 mm internal diameter; Jones Chromatography Ltd., Hengoed, Mid Glamorgan, UK) and the component proteins were separated using a 30–50 % acetonitrile gradient in 0.1 % trifluoroacetic acid (TFA) at a flow rate of 1.5 mL·min⁻¹ and 46 °C, detection being at 205 nm.

2.2.5. Anion-exchange FPLC

Aqueous suspensions of different types of Kishk (20 g·L⁻¹) were prepared, adjusted to pH

7.0, and warmed to 40 °C. The samples were kept at 40 °C for 20 min and then cooled to 20 °C, and subsequently passed through Whatman No. 54 filter paper. The filtrates were adjusted to pH 4.6 to precipitate the caseins by the addition of equal volumes of acetate buffer (0.1 mol·L⁻¹ sodium acetate, 0.42 mol·L⁻¹ acetic acid), and centrifuged at 1 000 g for 10 min. The pellets were washed once with water at pH 4.6, and the supernatants discarded.

The pellets prepared from Kishk, and control samples of Burghol, goat and cow caseins, were dissolved in bis-tris-propane buffer (0.005 mol·L⁻¹ bis-tris-propane, 8.0 mol·L⁻¹ urea; pH 7.0) and adjusted to pH 7.0. The proteins were reduced by adding 2-mercaptoethanol (about 1 µL·10 mg⁻¹ protein) and stirring for 1 h. Samples were filtered (0.2 µm) and then analysed by anion-exchange fast protein liquid chromatography (FPLC) as described previously [19].

2.2.6. Cortecs diagnostics casein assay

Kishk (10 g) was added to 100 mL of saline solution, and mixed thoroughly to ensure that the powder was fully dissolved. After standing for a few minutes to allow insoluble material to settle out, the extract was diluted 1:40 (v/v) by adding 50 µL to 2 mL working assay diluent solution and mixing well. The casein enzyme immunoassay was carried out as described previously [9], and the absorbance measured at 405–420 nm.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of Kishk samples

The average chemical composition of 25 samples of Kishk has been reported by Tamime et al. [46], and the protein content ranged between 14.7 and 21.4 g·100 g⁻¹ on dry matter basis (DMB).

3.2. Protein assay

Kishk is a dried fermented milk/Burghol mixture that contains milk proteins, mainly caseins and wheat proteins (e.g. glutenin and gliadins). Since the wheat proteins are of high molecular weight, positively charged,

insoluble in water and only soluble in acidic solutions [18], they require specific electrophoretic conditions in order to be separated [30]. Thus, the electrophoretic methods used pose no problem for analysis of the milk proteins present in Kishk.

3.2.1. PAGE methods

Adulteration of goat's milk by cow's milk could be detected using PAGE methods A and B. These methods separate protein on the basis of their net charge based on the electrophoretic mobility of α_{s1} -casein in cow's milk. The electrophoretic assays of some of the controls and Kishk samples are shown in *figure 1A* and *B* (PAGE methods A and B, respectively).

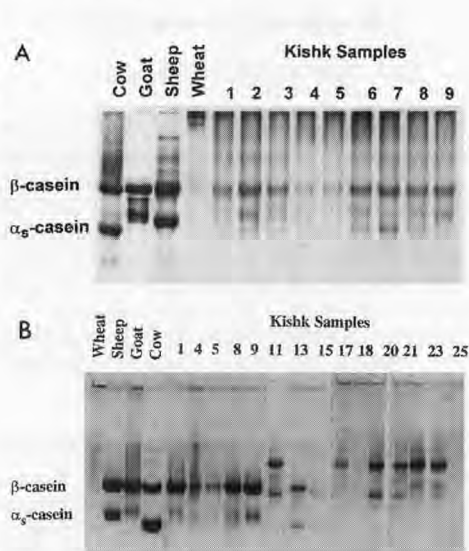


Figure 1. Polyacrylamide gel electrophoresis (PAGE; methods A and B) assay of some Kishk samples. (For sample identification refer to Tamime et al. [46].) **A:** urea-PAGE (method A); **B:** discontinuous PAGE (method B). (After Tamime et al. [45].)

Figure 1. Électrophorèse en gel de polyacrylamide (PAGE; méthodes A et B) de quelques échantillons de Kishk (voir Tamime et al. [46]) pour l'identification des échantillons. **A:** PAGE-urée (méthode A); **B:** PAGE discontinue (méthode B). (D'après Tamime et al. [45].)

The results obtained using PAGE method A suggest that milk from more than one species was used to prepare the yoghurt, and only the predominant species could be identified. Kishk samples 1→6, 8→10, 22 and 23 appear to have been made with predominantly goat's milk mixed with cow's milk, while samples 7, 11→14 and 17 are made predominantly from cow's milk (only some samples are shown in *figure 1A*). However, samples 15, 16, 18→21 and 25 appear to have been made with cow's milk only, and only Kishk sample 24 was made from sheep's milk [45].

PAGE method B (*figure 1B*) could estimate the adulteration of Kishk with cow's casein at a level either ~25 %. The results indicate that: (a) Kishk samples 1→5, 9 and 17→19 appear to have been made from a mixture of goat's ($\geq 75\%$) and cow's milk ($\leq 25\%$); (b) samples 6→8, 11, 12, 14, 22 and 23 were made with added cow's milk at a level of $> 25\%$; and (c) only Kishk samples 10 and 24 were made solely from goat's milk, and sample 25 appears to contain no milk proteins at all [45].

It is evident from the data shown in *figure 1B* that not all the Kishk samples contain

the same amounts of yoghurt and Burghol (i.e. ratio of 4:1), especially samples 4, 5, 15→18 and 25 gave poor resolution of the β - and α_s -caseins. Quantitative determination of cow's milk adulteration in these Kishk samples was possible when the theoretical milk protein concentration used for the preparation of the Kishk samples was increased from 5 to 10 mg·mL⁻¹ and 15 μ L was analysed for electrophoresis.

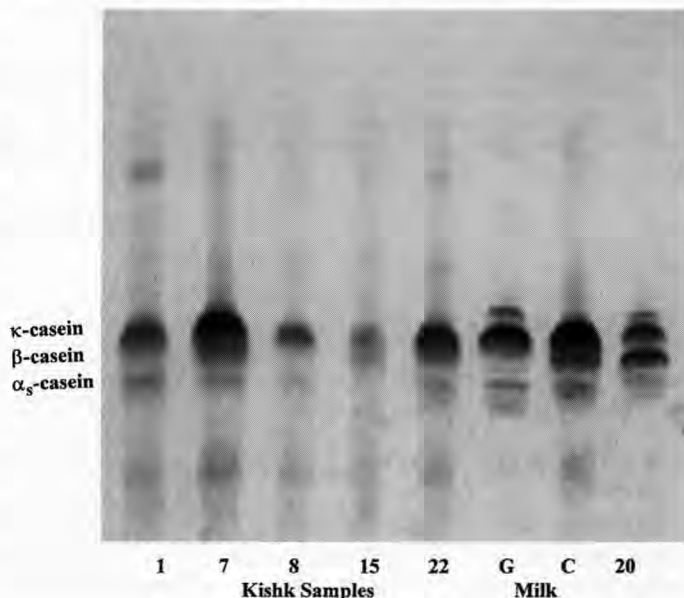
The results obtained by PAGE revealed disagreements with the milk type claimed by the manufacturers [46]. In particular, Kishk samples 13 and 21, which were claimed to be made entirely from goat's milk, were found to be made exclusively from cow's milk. In addition, sample 15, which was supposedly made from sheep's milk, was found to contain only cow's milk.

3.2.2. SDS-PAGE

This technique separates proteins on the basis of their molecular weight. Good separation of κ -, β - and α_s -casein components in goat's and cow's milk was achieved using SDS-PAGE (*figure 2*). However, all the Kishk samples exhibited extensive proteol-

Figure 2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) assay of some Kishk samples. (For sample identification refer to Tamime et al. [46].) Slots 1, 7, 8, 15, 22 and 20 are Kishk samples; G: goat's milk; C: cow's milk.

Figure 2. Électrophorèse en gel de polyacrylamide-sodium dodecyl sulphate (SDS-PAGE) de quelques échantillons de Kishk (voir Tamime et al. [46] pour l'identification des échantillons). Les puits 1, 7, 8, 15, 22 et 20 sont du Kishk. G : lait de chèvre ; C : lait de vache.



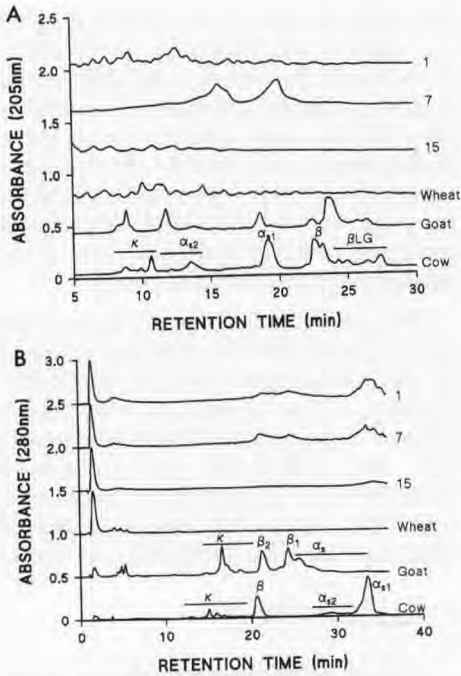


Figure 3. Elution profiles of reverse phase high performance liquid chromatography (A) and anion-exchange fast protein liquid chromatography (B) of some Kishk samples. (For sample identification refer to Tamime et al. [46].) Samples 1, 7 and 15 are Kishk samples.

Figure 3. Profil d'élu­tion en chromatographie liquide haute performance en phase inverse (A) et chromatographie liquide des protéines par échange d'anion (B) de quelques échantillons de Kishk (voir Tamime et al. [46] pour l'identification des échantillons). Les échantillons 1, 7 et 15 sont du Kishk.

ysis, and it was difficult to differentiate the bands due to the original caseins. Thus, SDS-PAGE was not suitable for detecting adulteration of goat's with cow's milk in Kishk-making, but is useful as a means of determining the extent of proteolysis.

3.2.3. RP-HPLC

Although good separations of the various proteins in cow's and goat's milk were achieved by RP-HPLC, all the Kishk sam-

ples showed evidence of very extensive proteolysis with little or none of the original caseins being detected. An illustration of the results using selective samples is shown in figure 3A. RP-HPLC was, therefore, not a suitable method for distinguishing between Kishk samples made solely from goat's milk and those adulterated with cow's milk. The high degree of proteolysis would also make such a distinction very difficult by any other chromatographic method.

3.2.4. Anion-exchange FPLC

Adjusting the pH of the Kishk filtrates at pH 4.6 resulted in precipitation in 16 samples, but not in the remaining nine. The elution profiles obtained by anion-exchange FPLC of the precipitates showed that there were only small amounts of protein corresponding to the major components in goat and cow casein controls, and an illustration is shown in figure 3B. There was also an increase in the amount of less tightly bound proteins in all the Kishk samples. The results indicate, therefore, that there was extensive proteolysis, and possibly dephosphorylation, of the caseins in the Kishk. This extensive modification of the caseins, particularly of the cow's α_{11} -casein, which can normally be detected in mixtures of cow's and goat's milk by anion-exchange FPLC, made detection of adulteration of Kishk with cow's milk difficult.

3.2.5. Immunoassay method

Results of the immunoassay techniques (Cortecs method) of 25 samples of Kishk were reported by Tamime et al. [45] and only samples 10, 22 and 23 were made from pure goat's milk; the rest of the samples were adulterated with cow's milk. Comparing the results with those obtained with PAGE methods A and B appeared to confirm that Kishk sample 10 was made solely from goat's milk, and samples 22→25 may also have contained only goat's milk.

Although the immunoassay methods can detect adulteration of goat's milk products at very low concentration, the Cortecs test kit could not effectively be used to calculate the degree of adulteration. This may be because: (a) this assay method may have limited application for powder products such as Kishk; (b) the presence of Burghol in the product made it difficult to clarify the test sample prior to analysis; and (c) the test kit supply did not provide a formula to calculate the degree of adulteration of the Kishk's milk expressed as percentage.

4. CONCLUSION

Urea-PAGE and discontinuous PAGE gave relatively similar results and indicated that the majority of the Lebanese Kishk samples were adulterated with cow's milk. This was confirmed by immunoassay. SDS-PAGE, RP-HPLC and anion-exchange FPLC methods were not suitable for detecting Kishk samples solely from goat's or cow's milk or a mixture of both. The extent of proteolysis in all the Kishk samples was high and this suggests that antibodies reactive to the specific peptide fragments and/or whey protein fractions would be an alternative method(s) suitable for distinguishing Kishks made from milk from different species of mammals.

ACKNOWLEDGEMENTS

The SAC and the Hannah Research Institute receive financial support from the Scottish Office of Agriculture, Environment and Fisheries Department (SOAEFD).

REFERENCES

- [1] Addeo F., Moio L., Chianese L., Nota G., Evaluation of bovine and water buffalo milk in mixtures of liquid milk and Mozzarella cheese by gel isoelectric focusing, *Ital. J. Food Sci.* 1 (1989) 71–80.
- [2] Addeo F., Nicolai M.A., Chianese L., Moio L., Musso S.S., Bocca A., del Giovine L., Spagna-Musso S., del Giovine L., A control method to detect bovine milk in ewe and water buffalo cheese using immunoblotting, *Milchwissenschaft* 50 (1995) 83–85.
- [3] Amigo L., Ibanez I., Fernandez C., Santa-Maria G., Ramos M., Comparison of an electrophoretic and an immunological method for the determination of goat and cow milk cheese, *Milchwissenschaft* 44 (1989) 215–218.
- [4] Amigo L., Ramos M., Calhau L., Barbosa M., Comparison of electrophoresis, isoelectric focusing and immunodiffusion in determinations of cow's and goat's milk in Serra de Estrela cheese, *Lait* 72 (1992) 95–101.
- [5] Andrews A.T., Proteinases in normal bovine milk and their actions on caseins, *J. Dairy Res.* 50 (1983) 45–55.
- [6] Anguita G., Martin R., Garcia T., Morales P., Haza A.J., Gonzales I., Sanz B., Hernandez P.E., Immunostick ELISA for detection of cow's milk in ewe's milk and cheese using a monoclonal antibody against bovine β -casein, *J. Food Protect.* 59 (1996) 436–437.
- [7] Anguita G., Martin R., Garcia T., Morales P., Haza A.J., Gonzales I., Sanz B., Hernandez P.E., Detection of bovine casein in ovine cheese using digoxigenated monoclonal antibodies and a sandwich ELISA, *Milchwissenschaft* 52 (1997) 511–513.
- [8] Anifantakis E., Massouras Th., Detection of cow's milk in yoghurt made from mixtures of cow's and goat's milk, in: *Proceedings of the 2nd National Congress on Food Science and Technology*, Athens, Greece, 1989, pp. 511–512.
- [9] Anonymous, Biokits Casein Assay Kit, Cortecs Diagnostics, Clwyd, Wales, 1992.
- [10] Aranda P., Oria R., Calvo M., Detection of cow's milk in ewes' milk and cheese by an immunoblotting method, *J. Dairy Res.* 55 (1988) 121–124.
- [11] Aranda P., Sanchez L., Perez M.D., Ena J.M., Puyol P., Oria R., Calvo M., Rapid immunoenzymatic method for detecting adulteration in ewe's milk, *Food Control* 4 (1993) 101–104.
- [12] Bitri L., Rolland M.P., Besancon P., Immunological detection of bovine caseinomacropptide in ovine and caprine dairy products, *Milchwissenschaft* 48 (1993) 367–371.
- [13] Blakely R.W., Boezi J.A., A new staining technique for polyacrylamide gels using Coomassie Brilliant Blue G250, *Anal. Biochem.* 82 (1977) 580–582.
- [14] Brehmer H., Klinger A., Tierärztliche untersuchungen von frischkäse mit hilfe der polyacrylamidegelelektrophorese (PAGE) – nachweis von kuhmilch in schaf- und ziegenkäse, *Arch. Lebensmittelhyg.* 40 (1989) 34–36.

- [15] Calvo M.M., Amigo L., Olano A., Martin P.J., Ramos M., Effect of thermal treatments on the determination of bovine milk added to ovine or caprine milk, *Food Chem.* 32 (1989) 99–108.
- [16] Castro C., Martin R., Garcia T., Rodriguez E., Gonzalez I., Sanz B., Hernandez P.E., Indirect enzyme linked immunosorbent assay for detection of cow's milk in goat's milk, *J. Food Agric. Immunol.* 4 (1992) 1118.
- [17] Cattaneo T.M.P., Nigro F., Greppi G.F., Analysis of cow, goat and ewe milk mixtures by capillary zone electrophoresis (CZE): preliminary approach, *Milchwissenschaft* 51 (1996) 616–619.
- [18] Coultrate T.P., *Food: The Chemistry of its Components*, 2nd edn., The Royal Society of Chemistry, Cambridge, UK, 1989, pp. 117–118.
- [19] Davies D.T., Law A.J.R., Quantitative fractionation of casein mixtures by fast protein liquid chromatography, *J. Dairy Res.* 54 (1987) 369–376.
- [20] EU, Establishing a reference method for the detection of cow's milk in cheese made from ewes' milk, Commission Regulation (EEC) No. 69092, Off. J. Eur. Commun. No. L 74, 1992, pp. 23–32.
- [21] Garcia T., Martin R., Rodriguez E., Hernandez P.E., Sanz B., Development of cow's milk identification test (COMII) for field use, *J. Dairy Res.* 56 (1989) 691–698.
- [22] Garcia T., Martin R., Morales P., Gonzales I., Sanz B., Hernandez P., Sandwich ELISA for detection of caprine milk in ovine milk, *Milchwissenschaft* 48 (1993) 563–566.
- [23] Garcia T., Martin R., Rodriguez E., Morales P., Gonzalez I., Sanz H., Hernandez P.E., Detection of goat's milk in ewes' milk by an indirect ELISA, *J. Food Agric. Immunol.* 6 (1994) 113–118.
- [24] Haza A.I., Morales P., Martin R., Garcia T., Anguita G., Gonzales I., Sanz B., Hernandez P.E., Use of a monoclonal antibody and two enzyme-linked immunosorbent assay formats for detection and quantification of the substitution of caprine milk for ovine milk, *J. Food Protect.* 60 (1997) 973–977.
- [25] Horn D., Verfälschung von schafkase mit kuhmilch – machweismöglichkeiten und Ergebnisse, *Deut. Milchwirtschaft.* 40 (1989) 116–118.
- [26] IDF, The Composition of Ewe's and Goat's Milk, Bulletin No. 140, International Dairy Federation, Brussels, Belgium, 1981.
- [27] IDF, Production and Utilization of Goat's and Ewe's Milk, Bulletin No. 158, International Dairy Federation, Brussels, Belgium, 1983.
- [28] IDF, Update on Existing Analytical Methods for Detecting Mixtures of Cow's, Ewe's and Goat's Milk, Bulletin No. 181, International Dairy Federation, Brussels, Belgium, 1984.
- [29] IDF, Production and Utilization of Ewe's and Goat's Milk, Bulletin No. 202, International Dairy Federation, Brussels, Belgium, 1986.
- [30] Inglett G., *Wheat – Production and Utilization*, AVI Publishing, Westport, CT, 1974, pp. 119–130.
- [31] Iverson J.L., Sheppard A.J., Detection of adulteration of cow, goat and sheep cheeses utilizing gas-liquid chromatographic fatty acid data, *J. Dairy Sci.* 72 (1989) 1707–1712.
- [32] Levieux D., Venien A., Rapid, sensitive two-site ELISA for detection of cow's milk in goat's or ewes' using monoclonal antibodies, *J. Dairy Res.* 61 (1994) 91–99.
- [33] Luf W., Stock A., Brandl E., Zum nachweis von fermdfett in milchfett über die triglyceridanalyse. 2. Verfälschungsnachweis durch auswertung von triglycerid-kombinationen, *Österr. Milchwirtschaft.* 42 (1987) 5, 29–35.
- [34] Mayer H., Rockenbauer C., Foissy H., Elektrophoretische differenzierung von kuh-, schaf- und ziegenmilch auf der basis von δ - und para- κ -caseinen, *DMZ Lebensm. Milchwirtschaft.* 116 (1995) 324–331.
- [35] Moio L., Sasso M.L., Chianese L., Addeo F., Rapid detection of bovine milk in ovine, caprine and water buffalo milk or cheese by gel electrophoresis on PhastSystem™, *Ital. J. Food Sci.* 2 (1990) 185–190.
- [36] Pellegrino L., de Noni I., Tirelli A., Resmini P., de Noni I., Determinazione del latte di vacca nei formaggi di specie minori mediante HPLC delle sieroproteine: Nota I. Applicazione alla Mozzarella di bufala, *Sci. Tec. Latt. Casearia* 42 (1991) 87–101.
- [37] Perez M.D., Sanchez L., Aranda P., Ena J.M., Calvo M., Use of an immunoassay method to detect adulteration of ewe's milk, in: Morgan M.R.A., Smith C.J., Williams P.A. (Eds.), *Food Safety and Quality Assurance – Applications of Immunoassay Systems*, Elsevier Applied Science, London, 1992, pp. 41–48.
- [38] Prager M.J., Differential characteristics of fatty acids in cheese for milk of various animal species by capillary gas chromatography, *J. Assoc. Off. Anal. Chem.* 72 (1989) 418–421.
- [39] Rodriguez H., Martin R., Garcia T., Hernandez P.E., Sanz B., Detection of cow's milk in ewes' milk and cheese by an indirect enzyme-linked immunosorbent assay (ELISA), *J. Dairy Res.* 57 (1990) 197–205.
- [40] Rodriguez E., Martin R., Garcia T., Azcona J.I., Sanz B., Hernandez P.E., Indirect ELISA for detection of goat's milk in ewes' milk and cheese, *Int. J. Food Sci. Technol.* 26 (1991) 457–465.
- [41] Rodriguez E., Martin R., Garcia T., Gonzales I., Morales P., Sanz B., Hernandez P.E., Detection of cow's milk in ewe's milk and cheese by sandwich enzyme-linked immunosorbent assay (ELISA), *J. Sci. Food Agric.* 61 (1993) 175–180.

- [42] Rodriguez H., Martin R., Garcia P., Morales I., Gonzales I., Sanz B., Hernandez P.E., Sandwich ELISA for detection of goat's milk in ewes' milk and cheese, *J. Food Agric. Immunol.* 6 (1994) 105–111.
- [43] Rolland M.P., Bitri L., Besancon P., Monospecificity of the antibodies to bovine α_{s1} -casein fragment 140–149: application to the detection of bovine milk in caprine dairy products, *J. Dairy Res.* 62 (1995) 83–88.
- [44] Tamime A.Y., O'Connor T.P., Kishk – a dried fermented milk/cereal mixture, *Int. Dairy J.* 5 (1995) 109–128.
- [45] Tamime A.Y., Barclay M.N.I., O'Connor T.P., Anifantakis E.M., Comparison of different methods to detect the adulteration of caprine milk Kishk with bovine milk, in: *Production and Utilization of Ewe and Goat Milk, Special Issue No. 9603, International Dairy Federation, Brussels, Belgium, 1996*, pp. 214–217.
- [46] Tamime A.Y., Barclay M.N.I., Amarowicz A., McNulty D., Kishk – a dried fermented milk/cereal mixture. 1. Gross components, carbohydrates, organic acids and fatty acids composition, *Lait* 79 (1999) 317–330.
- [47] Urbanke W., Luf W., Brandl E., Einsatz der HPLC bei der verfälschungskontrolle von milch und milchprodukten verschiedener species, *Z. Lebensm. Untersuch. Forsch.* 195 (1992) 137–142.