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Author: S. Agboola, H. H. Chan, J. Zhao and A.-U. Rehman

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Author Address: sagboola@csu.edu.au;jzhao@csu.edu.au

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Can the use of Australian cardoon (*Cynara cardunculus* L.) coagulant overcome the quality problems associated with cheese made from ultrafiltered milk?

Samson O. Agboola^{1*}, Hwa H. Chan¹, Jian Zhao¹ and Ata Rehman²

¹*School of Wine and Food Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia*

²*NSW Department of Primary Industries, Pine Gully Road, Wagga Wagga, NSW, Australia*

* Corresponding Author

Address for correspondence: School of Agricultural and Wine Sciences, Charles Sturt University, Private Bag 588, Wagga Wagga, NSW 2650, Australia.

e-mail: sagboola@csu.edu.au

Abstract

Concentrated cow's milk, obtained by either limited ultrafiltration to arrive at a concentration factor of 1.4x (UF) or by mixing 4x UF milk with regular milk (MX) was used to manufacture cheeses coagulated with calf rennet or aqueous extract from *Cynara cardunculus* L. (cardoon). The manufactured cheeses were tested and compared with those made from regular milk for chemical and sensory properties, yield, textural and biochemical indices over a 60-day ripening period. There was no significant difference ($P > 0.05$) in the chemical properties with the type of coagulant but in general, a lower yield and greater bitterness was observed in the cheeses made using cardoon, while ultrafiltration led to reduced casein hydrolysis, less bitterness and harder, more crumbly cheeses irrespective of coagulant type. The MX process was successful in reducing the textural problems which occurred in cheese made with UF milk alone. The ultrafiltration process itself was apparently detrimental to the textural quality of cheeses, rather than the associated increase in concentration

Keywords: ultrafiltration; cheese-making; plant coagulant

1. Introduction

The use of extracts from the flowers of the Australian cardoon plant (*Cynara cardunculus* L) as a coagulant for cheese making has received a lot of attention in recent years (Agboola, 2002; Chen, Agboola and Zhao, 2003a; Agboola, Chen and Zhao, 2004; Zhao, Chen and Agboola, 2004). While it has been shown to be largely successful for making hard and semi-hard cheese products from ovine milk (Cordeiro, Jakob, Puhan, Pais and Brodelius, 1992; Macedo, Malcata and Oliveira, 1993; Sousa and Malcata, 1996), its application to cow's milk has resulted in poor quality cheeses, especially in terms of poor (soft) texture and bitter flavour. The indicators are, however, that, if cow's milk could be modified in such a way that its properties approximate those of sheep milk, then plant coagulants such as cardoon extract may be commercially exploited for cheese making.

Sheep milk is significantly different from cow's milk in a number of ways including fatty acid profile, size of fat globules, casein micelles, casein profile, protein level, minerals and total solids content (Kalantzopoulus, 1999). Of these differences, the most significant for milk clotting and cheese-making properties is the low protein content, since caseins are directly involved in coagulation. Furthermore, there is an established process for manipulating the protein level in cheese milk, i.e., ultrafiltration (UF). Consequently, the effects of plant coagulants such as ficin, papain and cardoon extract on UF milk samples whose protein level had been increased up to four times that in the regular milk were studied, comparing their clotting properties and products of enzymatic proteolysis to those obtained using commercial calf rennet (Low, Agboola, Zhao and Lim, 2006). It was shown that the undesirable proteolysis inherent in the application of plant coagulants to regular cow's milk can be

significantly reduced when concentrated UF milk was employed as the milk source. Furthermore, clotting properties of the different milk samples suggest that there were structural changes in the UF milk due to the process of ultrafiltration itself which could be detrimental to the quality of the final product (Low et al. 2006). These structural changes also increased with the extent of milk concentration.

Although clotting properties of milk can be good indices for cheese-making performance (Dalgleish, 1993), it is still important to study the actual cheese making process, due to the influence of other ingredients and the processing conditions on the final product. Significantly, it has also been shown that different coagulants may show similar clotting properties and extent of hydrolysis early in the process, but differ significantly after the maturation has progressed to a reasonable extent (Agboola, 2002). Therefore, in this study, the effect of the ultrafiltration process itself on suitability of UF milk for cheese-making with cardoon extract was investigated, by comparing, throughout a 60 d maturation period, biochemical, textural and sensory attributes of cheeses made using milk with similarly enhanced protein content but differing proportions of UF milk.

2. Materials and Methods

All chemicals and reagents used were of analytical grade and were purchased from Sigma-Aldrich Pty. Ltd., (Sydney, Australia), unless otherwise stated. Double-strength calf rennet [NaturenTM, 230 International Milk Clotting Units per mL, (IMCU/ mL)] and TCC-20, a thermophilic cheese starter culture containing a mixture of *Streptococcus thermophilus* and *Lactobacillus* were both supplied by Chr. Hansen

Pty. Ltd., (Bayswater, Victoria, Australia). An aqueous extract of cardoon [15 g/100 mL fresh flowers] was prepared as described by Chen et al. (2003a).

2.1 Ultrafiltration of bovine milk

Raw bovine milk was supplied by Fonterra Wagga Wagga, (NSW, Australia) at temperatures between 2°C and 4°C. Ultrafiltration was carried at a transmembrane pressure of 50 kPa out using a Masterflex[®] 7549-32 Pump Drive together with a Milipore Prep/Scale-TFF spiral-wound (0.23 m²) cartridge with 10,000 Da nominal molecular weight cut-off. Prior to ultrafiltration, the milk was analysed for fat and protein using a Milko-scan 133 (FOSS Pacific, Nunawading, Vic, Australia) and then heated to 50±2 °C. To monitor the ultrafiltration process, samples were collected from the retentate line for fat and protein analysis on the Milko-scan every 10 min. The ultrafiltration process was stopped when the desired protein concentration was obtained, which was at 1.4 (1.4x) or 4 times (4x) the initial protein content of the milk. The 1.4x milk was used as is, (designated UF) while the 4x milk was mixed with regular milk to make up to 1.4x initial protein concentration (designated MX) prior to cheese making.

2.2 Cheese making

Two batches of semi-hard cheeses were made in the Charles Sturt University commercial cheese factory using a mini-cheese making system (in 15 L containers). In each batch, six cheeses were made using a combination of the two coagulants and three milks. The combinations were cheeses made from regular milk using calf (animal) rennet (RA), regular milk using cardoon extract (RC), 1.4x direct UF milk using animal rennet (UFA), 1.4x direct UF milk using cardoon extract (UFC), 1.4x

mixed concentrated milk using calf rennet (MXA) and 1.4x mixed concentrated milk using cardoon extract (MXC). Prior to cheesemaking, each milk sample was pasteurised with constant stirring; regular milk was pasteurized at 72°C for 15 seconds while concentrated milk was pasteurized at 80°C for 1 min due to its higher solids content. Both milk types were cooled to 37 °C for cheese-making.

The cheese-making process commenced with the addition of starter culture and coagulant was then added after 20 minutes of ripening when the pH of the milk was about 6.45 ± 0.03 . Calf rennet and cardoon extract were added to milk at rates previously described by Chen, Zhao and Agboola (2003b) where the dosage of the both coagulants was standardized to the same amount of milk clotting activity, which was experimentally determined with a Gelograph InstrumentTM (Gel Instrument, Thalwil, Switzerland).

After allowing the milk to coagulate for 35 min, the curd was cut to approximately 1.5 cm square cubes. The cut curd was then allowed to heal for 10 min before gentle stirring for 15 min was applied and half of the whey removed at pH 6.20 ± 0.03 . The whey sample was collected for further analysis for fat, total solids and protein content. About 90 g of salt was then added and the curd stirred for a further 15 min, after which the salted curd was scalded with hot water of about 80 °C, raising the overall temperature to about 43 °C. The heated curd was then stirred for another 15 min before all the whey was removed. The curd was then placed in Gouda-type round hoops and pressed at 301 kPa for 24 h, followed by brining in a saturated salt solution for 4 h at room temperature (25 °C). The cheese was then allowed to dry overnight before being vacuum-packed. The packed cheese was allowed to mature in the cool

room at 10 ± 1.5 °C for up to 60 d. Samples were taken after 7 d for chemical analysis and at 7, 30 and 60 d for chromatic, textural and biochemical analyses. Sensory analysis was carried out after 30 and 60 d of maturation.

2.3 Chemical Analysis

Fat was determined by the Mojonnier method, moisture by the oven method, and salt by the Volhard method, as outlined by Marshall (1992). The pH of milk was measured with a glass electrode and Beckman 3500 digital pH-meter (Beckman Coulter Inc., CA, USA). Similarly, the cheese pH was determined by directly inserting the glass electrode into the cheese. The colour of the cheeses was measured using a Chroma Meter CR-300 with a CR-300 measuring head and Data Processor DP-301 (Biolab Ltd., Clayton, Vic, Australia). The measurements used were the L^*a^*b chromatic system.

2.4 Biochemical Analysis

The acid degree value (ADV) of the cheeses was determined by the titration (with alcoholic potassium hydroxide) method described by Marshall (1992). Extracts of cheese samples were prepared following the procedure described by Kuchroo and Fox (1982) and the filtrates were used for the determination of water-soluble nitrogen (WSN), trichloroacetic acid soluble nitrogen (TCASN) and phosphotungstic acid soluble nitrogen (PTASN). The total nitrogen (TN) content of WSN, TCASN and PTASN extracts as well as that of cheese and whey samples was determined using a Leco CNS-2000 total elementary analysis system (AOAC, 1996).

2.5 Capillary electrophoresis (CE)

CE analysis was carried out according to the method of de Jong, Visser and Olieman (1993) with modifications. CE was performed on a Beckman P/ACE system 5510 capillary electrophoresis unit controlled by a P/ACE System 5000 Series Software.

The capillary used was uncoated with a 50 µm internal diameter and of length 50 cm. Electrophoresis was conducted at 45 °C and a voltage of 20 kV. The diode array detector was set at 214 nm, and samples were injected under pressure for 40 sec; the separation time was 40 min. The capillary was rinsed with 0.1 M sodium hydroxide for 4 min, 0.1 M HCl for 2 min, distilled water for 2 min and run buffer for 3 min prior to every electrophoretic run.

2.6 Sodium dodecyl sulfate tricine polyacrylamide gel electrophoresis (SDS-tricine PAGE)

Aliquots (8.5 µL and 4.5 µL) of water-soluble fractions of cheeses made using animal rennet (RA, UFA and MXA) and cardoon (RC, UFC and MXC), respectively, were diluted in 30 µL of sample loading buffer, made by mixing 4.5mL of 1M dithiothreitol (DDT), 1.5 mL of 1M Tris-HCl (pH 6.8), 6 mL of 10 g/100 mL SDS, 12 mL of glycerol, 12 µL of bromophenol blue and 4 mL of deionized water in a 50 mL centrifuge tube. An aliquot (10 µl) of polypeptide SDS-PAGE standards (Bio-Rad, Regents Park, NSW, Australia) was diluted in 190µL of sample loading buffer. Samples were separated and stained on a 16g/100 mL tris-tricine gel as described by Ahmed (2005) at 12 mA for 17 h with power supplied using a Bio-Rad Power Pac 300 (Bio-Rad, Regants Park, NSW).

2.7 Sensory evaluation

Cheeses aged 30 and 60 d were subjected to sensory evaluation by a consumer panel of 7 men and 7 women consisting of staff and students from the School of Wine and Food Sciences, Charles Sturt University. They were pre-selected as regular cheese consumers (daily or at least weekly). The cheese samples were cut into standard bite size pieces of about 1 cm³. The samples were served in plates together with a consumer sensory evaluation questionnaire on a blind-labelled basis. The consumers

were asked to evaluate sensory characteristics such as appearance (colour), hardness, bitterness and creaminess on a structured scale (Poste, Mackie, Butler and Larmond 1991) and overall acceptability on a hedonic scale of 1 (dislike extremely) to 9 (like extremely).

2.8 Textural Profile Analysis (TPA)

The hardness and adhesiveness of cheese samples were determined using the TA-TX2 Universal Texture Analyzer (Stable Microsystem Ltd, Godalming, UK), which was operated by the Texture Expert for Windows software. Prior to testing, cheese samples were warmed to 20 °C in an incubator. The cheeses were then cut into a 5 x 5 x 3 cm cubes and placed onto the sample platform of the Texture Analyzer. The texture profile of each sample was measured by double penetration with a 3mm diameter stainless steel cylindrical probe. The test conditions were: test speed, 0.5 mm sec⁻¹; pre-test speed, 5 mm sec⁻¹; post-test speed, 2 mm sec⁻¹; penetration distance, 30 mm; and time pause between first and second bite, 5 sec. At least three measurements were performed on each sample. The textural measurement, including TPA hardness and TPA adhesiveness, were automatically recorded and calculated by the Texture Expert software. Samples tested were at 7, 30 and 60 d of maturation.

2.9 Statistical Analysis

All the experiments were performed at least in triplicate for each of the two batches of cheese, and mean results (with standard deviation) are reported. Unless otherwise stated, analysis of variance (ANOVA) was used to compare the means and Fisher's least significant difference (LSD) test was used to separate the means that were significantly different ($P < 0.05$), using SPSSTM Statistical software version 1.1 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Chemical properties and colour

Table 1 shows the chemical composition of the cheeses obtained from two batches produced using the two different types of coagulant and three different milk concentrations. There was no significant difference in moisture, salt content and pH of all cheeses when analysed within seven days of manufacture. However, cheeses made from milk samples of higher solids concentration (UF and MX) showed slightly higher salt-in moisture levels. It is noteworthy that cheeses made with concentrated UF milk (UFA and UFC) had the lowest fat content while irrespective of the milk type, cardoon coagulant tended to result in lower fat content and yield. Also, cheeses made with mixed concentrated milk (MX) generally had the highest fat and protein levels. The composition of whey also suggested significantly higher loss of fat into the UF milk whey, partially accounting for lower fat retention in cheeses (Table 2). Incidentally, the whey composition after cheese-making from milk samples containing limited (MX) or no UF milk (R) were very similar.

Cheeses made from concentrated milk were brighter than those from regular milk (Table 3). Also, the use of cardoon extract resulted in much darker and more yellow cheeses than those made with animal rennet, irrespective of the milk type. However, the greenest and most yellow cheeses were made with regular milk, especially in combination with cardoon extract, while cheese made from concentrated milk (UF and MX) showed lower but similar cheese greenness irrespective of coagulant type.

3.2. Biochemical properties

Figure 1 shows the acid degree value (ADV) of the cheeses over a maturation period of 60 days. An ADV of 0.7 – 1.1 means lipids have been slightly hydrolysed, 1.2 –

1.4 moderately hydrolysed, while values more than 1.4 means the milk fat has been extremely hydrolysed (Marshall, 1992). In general, cheeses made using cardoon were found to be more hydrolysed than those made using animal rennet. Similarly, those made with regular milk were more hydrolysed than those made using concentrated milk and the effect of mixing UF and regular milk resulted in ADV values between the two extremes. Over the 60 d maturation, the ADV of all cheeses increased almost at the same rate and those made using animal rennet all recorded relatively low and similar ADV at 7, 30 and 60 days. Cheeses made from regular and direct UF milk using cardoon extract were extremely hydrolysed at 60 d, with ADV of 1.42 and 1.39 respectively.

Cheeses made with cardoon were found to have higher levels of water-soluble nitrogen compared to cheeses made with animal rennet (Figure 2A). In fact, there were two distinct groups of results based on the choice of coagulant. However, within the cardoon extract group, very similar results were obtained for the concentrated milk samples, especially at 60 days of maturation. In the animal rennet group, the cheese made from mixed concentrated milk recorded higher level of WSN/TN than in the other samples, which became more distinct during maturation at 30 and 60 days.

Changes in the TCASN/TN show a similar trend to the WSN/TN ratio where cheeses made using cardoon had a higher level of TCASN/TN than those made using animal rennet at 7 d of ripening (Fig. 3B). The exception in this case was, however, cheese made from direct UF with cardoon, which showed a slight drop in the TCASN/TN ratio between 30 and 60 d of maturation. Over the 60 d of maturation, a large increase in TCASN/TN for cheese made from regular milk using cardoon was observed,

increasing to more than double the level recorded at 7 d, from 1.68 g/100 mL to 3.56 g/100 mL. The effect of cardoon extract on the higher concentration milk types showed the lowest increase (about 0.4 g/100 mL) after 60 d. Each of the three cheeses made using animal rennet underwent an almost similar pattern in TCASN/TN during maturation, whereby at the end of the 60 day-maturation, the ratio doubled compared to values at 7 d, in these three samples.

Cheese made from regular milk using cardoon was found to have the highest level of phosphotungstic acid-soluble-nitrogen by the end of the 60 d maturation, with 1.2 g/100 mL of PTASN/TN (Fig. 2C). Cheeses made from regular milk and mixed concentrated milk coagulated using animal rennet showed almost no significant changes in PTASN/TN over the 60 d ripening period. In fact, they both had a similar level of phosphotungstic acid soluble nitrogen throughout maturation with 0.54 g/100 mL (RA at 7 d) and 0.84 g/100 mL (at 60 d). The ratio of PTASN/TN increased most significantly in cheeses made from regular milk with cardoon and direct UF milk with animal rennet. Compared to 7 d values, both have more than double the value of PTASN/TN at the end of the 60 d. Cheese made from direct UF milk using cardoon has the lowest level of phosphotungstic acid- soluble-nitrogen throughout the ripening period with only 0.71 g/100 mL of PTASN/TN at the end of 60 d.

Figure 3 shows the CE profiles of the water-soluble fractions of cheeses made from regular milk, 1.4x direct UF milk and mixed concentrated milk using animal rennet and cardoon extract over a 30-day maturation period; the peaks represent the peptides resulting from the degradation of milk proteins while the combined area of the peaks is a measure of the total peptide material. After 7 d of maturation, cheeses made using

cardoon have far more total peptide material than those made using animal rennet. Furthermore, there were more peptides from cheeses manufactured with cardoon extract compared to those made with animal rennet. The area of total peptide material (not shown) for cheeses made using cardoon were almost five times the area for cheeses made using animal rennet in the same milk group. Significantly, cheeses made from regular milk underwent more proteolysis compared to those made from higher concentration milk types with the same coagulant on comparing the number of peaks and intensity of peptides. However, cheese made from direct UF milk using cardoon had the greatest number of peaks, albeit at much lower intensity, as evidenced by the significantly lower total peak area. Over the maturation period of 30 and 60 days (not shown), the number of peaks for all cheeses was found to be reduced, although the area under each peak appeared to have increased generally.

Figure 4 shows the number and size of the hydrolysed milk proteins in cheeses as determined by SDS-Tricine gel electrophoresis, comparing results from 7, 30 and 60 d of maturation. A comparison of the number and intensity of the bands indicates increased proteolysis in cheeses manufactured with cardoon than in those made using animal rennet particularly considering that about half the concentration of cardoon extract samples compared to the animal rennet samples was loaded on the gel. Furthermore, greater proteolysis can be observed in the cheeses made from regular milk than the high concentration milk types, with direct UF milk showing the least proteolysis. Cheeses made with cardoon also had a higher number of smaller peptides (less than 1.4 kDa) than those made with animal rennet. Finally, as indicated in Figures 4B and 4C, the concentration of breakdown peptides increased with maturation time.

3.3. Sensory and textural properties

Table 4 shows mean scores of sensory attributes of cheese samples during maturation for up to 60 d. Cheeses made using only UF milk were significantly whiter than those made with either regular or mixed concentrated milk while cardoon-coagulation led to significantly softer, yellower and creamier cheeses. Most panellists also rated the cheeses as not being bitter (bitterness score < 2, except for the RC cheese) although those made from cardoon coagulation were significantly more bitter than their rennet-coagulated counterparts. It also appeared that cheeses became more acceptable with age, with MXC cheese having the best acceptability after 60 d of maturation.

Figure 5 shows the textural changes in TPA hardness and adhesiveness of all cheese samples during maturation for 7, 30 and 60 d. Cheeses made using animal rennet were harder and more adhesive than those made using cardoon for the same milk type. Overall, the cheese made from UF milk using animal rennet (UFA) was hardest and most adhesive followed by the cheeses made from mixed concentrated milk using animal rennet (MXA) and UF milk coagulated with cardoon (UFC) while the RC cheese was the softest and the least adhesive. In general, there does not appear to be any significant differences in the hardness and adhesiveness of cheeses due to their maturation.

4. Discussion

Lower yield was obtained for cardoon-coagulated cheeses made from regular cow's milk, confirming previous results (Garg and Johri, 1994; Sousa and Malcata, 2002), and correlating with the results of whey compositional analysis. Notably, however, UF did not lead to an improvement in the yield value of cardoon-coagulated cheese,

which is in contrast to the results obtained by Agboola (2002), although 2x UF milk was used in the latter study. It is also possible that the process of ultrafiltration itself could be detrimental to the gross properties of the cheese samples such as yield and fat retention. Possible changes to casein micellar structure as a result of the UF process could affect the cheese curd microstructure during clotting, which could, in turn, allow for reduced fat retention within the cheese matrix. Low et al., (2006) reported significant differences in UF milk clotting properties even when adjusted to a similar concentration to that of regular milk. Meanwhile, reducing the amount of the milk which passed through the UF membrane by mixing in a significant portion of regular milk resulted in cheese with compositional and biochemical attributes that approximate those of regular milk coagulated with calf rennet.

The influence of coagulant was greater on biochemical parameters than the influence of milk type or concentration. For example, use of cardoon extract led to enhanced lipolysis in the cheeses, which increased with age, suggesting slight lipase activity in the extract which may be due to inherent lipolytic activity of the proteolytic enzymes or may be microbial in nature, due to the handling of the isolation and purification processes. However, lipolytic activity in other proteases such as those used in the commercial microbial coagulant has been reported (Somkuti and Babel, 1968).

According to Kuchroo and Fox (1982), the release of water-soluble nitrogen (WSN) in cheese is primarily a result of casein solubilisation caused by action of proteolytic enzymes and is traditionally referred to as a measure of “ripening index”. WSN values have been mostly linked to products of rennet activity in ovine cheese made using different coagulants (Agboola et al., 2003a). Compared to WSN, however, the

trichloroacetic acid-soluble nitrogen (TCASN) normally contains much smaller peptides (ranging between two and 20 amino acid residues) and is regarded as an index of “ripening depth”. TCASN value has been known to be mainly products of proteolytic activities of starter cultures and indigenous microbial flora. The phosphotungstic acid-soluble nitrogen (PTASN) is known as the “free amino acid index”, being composed of mainly low molecular weight peptides less than 600 Da (Sousa and Malcata, 1996). The peptidases of micro-organisms are believed to be the main sources of their production using peptides obtained from coagulant and microbial degradation of caseins.

Protein hydrolysis in the cardoon-coagulated cheeses was more extensive, leading to the formation of a separate group of samples with high “ripening index” as denoted by their WSN/TN levels (Kuchroo and Fox, 1982). A similar trend was generally shown in the TCASN/TN and PTASN/TN levels based on the dependence of the latter indices on substrates generated by the coagulants. It was also shown that the extent of hydrolysis throughout maturation was reduced with increased milk concentration as reported by Agboola (2002). Most significantly, however, as indicated by both SDS-Tricine and CE results, milk concentrated only by passing through the UF membrane was less hydrolysed by either calf rennet or cardoon extract compared to milk of the same concentration but containing mostly non-UF milk. This difference in the extent and pattern of hydrolysis supports earlier results on the changes in the coagulation properties of regular milk compared with UF milk diluted with milk ultrafiltrate to the same protein concentration (Low et al., 2006). The probable changes in casein structure as a result of the UF process requires further investigation.

Cheeses made using cardoon were darker and more yellow than cheeses made from animal rennet as confirmed by both objective and subjective (sensory) measurements. This may be due to the fact that the volume of dark coloured cardoon extract (400 mL per 100 L of milk) added to the cheese milk was much higher than that of the calf rennet (20 mL per 100 L of milk). Cheeses made from higher concentration milk were brighter and less yellow, with direct UF milk cheeses being the whitest and least creamy amongst the cheeses. It is thus apparent that the UF process itself resulted in much whiter milk and cheese.

It has been reported that cheeses produced from plant coagulants show inferior qualities to those made from calf rennet, resulting in bitter taste and weak (soft) body (Egito et al., 2007). On the other hand, cheese made using UF milk and calf rennet were found to be hard and crumbly, with less developed flavours (Renner and Abd el Salam, 1991). In this study, cheese made from regular milk using cardoon extract was the creamiest coloured but was found from both sensory evaluation and instrumental analyses to be bitter, very soft and weak bodied. In contrast, cheese made from milk concentrated by direct UF was the least creamy, exhibited no bitterness and was hard and crumbly even when the cardoon extract was used as a coagulant. The application of cardoon to mixed concentrated milk resulted in a cheese with better textural and sensory attributes compared to all other cheeses including the control cheese made using calf rennet and regular milk.

It was clear that, despite having the same total solid concentration as mixed concentrated milk, the quality of the cheese made from direct UF milk in the experiment was inferior. Factors contributing to such difference could be the

undesirable changes occurring in the milk during the ultrafiltration process. Although there is no evidence yet in published literature, it is possible that some casein micelles may be physically damaged when being filtered through the membrane. This may in turn affect the coagulation process when making cheese, resulting in a hard and crumbly product. In addition, the UF process in this study was usually operated at 50°C. Such relatively high temperature, combined with the stress applied on milk might alter the structure of casein micelles and also result in possible denaturation of whey proteins, especially α -lactalbumin (Brew & Gobler, 1992). Finally, although the cheese milk contained added CaCl_2 as a coagulation aid, UF milk generally contains more colloidal calcium than regular milk (Renner & El-Salam, 1991; Low et al., 2006), which may adversely influence the texture of coagulated milk.

Conclusions

This study indicated a possible effect of the UF process itself on the downstream processes of milk coagulation and cheese proteolysis and maturation. Proteolytic, textural and sensory studies showed that while UF may have reduced the extent of milk and cheese proteolysis in the presence of cardoon extract, it did not result in improved sensory qualities unless a significant amount of regular milk was present. The study also showed that the superior properties of cheeses from naturally concentrated milks, e.g., ovine, in comparison to less concentrated cow's milk, when coagulated with cardoon, may not be merely concentration related. They are also probably due to the intrinsic properties of its casein micelles, e.g., size and proportion of individual casein molecules, which were left mostly undisturbed. Further studies on the microstructure and dynamic rheological properties of the milk and cheese samples should help in elucidating the molecular changes that underpin these gross structural observations reported in this study. The application of the cardoon coagulant to

concentrated milk for the manufacture of commodity-type cheeses such as Cheddar and Mozzarella are currently being investigated.

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1 Table 1 Composition (g/100g cheese) at 7 days and yield (g/100 mL milk) of cheeses made with different coagulants and milk concentrations¹

Chemical properties	Cheese type ²					
	RA	RC	UFA	UFC	MXA	MXC
Protein	25.45 ^a ± 1.11	25.67 ^a ± 2.29	25.89 ^a ± 1.03	25.72 ^a ± 1.21	26.73 ^b ± 0.46	26.77 ^b ± 2.45
Fat	27.75 ^a ± 1.65	27.48 ^a ± 0.65	23.22 ^b ± 1.33	22.30 ^b ± 0.45	28.15 ^c ± 0.61	27.20 ^a ± 1.50
Moisture	44.32 ± 2.47	44.53 ± 1.23	43.45 ± 0.93	43.22 ± 1.64	42.77 ± 1.07	43.14 ± 0.68
Salt	1.95 ± 0.25	2.01 ± 0.05	2.06 ± 0.35	2.04 ± 0.34	2.08 ± 0.25	1.97 ± 0.15
Fat in dry matter	49.84 ^a ± 1.63	49.54 ^a ± 0.67	41.06 ^b ± 1.35	39.27 ^b ± 0.40	49.19 ^c ± 0.62	47.84 ^a ± 1.52
Salt in moisture	4.33 ^a ± 0.21	4.41 ^a ± 0.04	4.74 ^b ± 0.39	4.72 ^b ± 0.37	4.86 ^b ± 0.25	4.57 ^b ± 0.11
pH	4.81 ± 0.33	4.82 ± 0.24	4.73 ± 0.65	4.77 ± 0.35	4.84 ± 0.33	4.84 ± 0.02
Yield ³	11.56 ^a ± 0.98	10.74 ^b ± 1.33	12.44 ^c ± 0.66	10.86 ^b ± 1.36	12.35 ^c ± 0.64	12.14 ^c ± 0.57

2 ¹ Results are means ± standard deviation of at least three replicates for each property excluding the yield which was based on two batches

3 ² Cheese designated by milk and coagulant types. Milk type was regular milk (R) or 1.4x concentrated milk made from direct UF of regular milk
 4 (UF) or by mixing 4x UF milk with regular milk (MX). Coagulant designated as either A for animal rennet or C for cardoon.

5 ³ All yield values were adjusted to 1x milk concentration equivalent.

6 ^{a-c} Data in the same row with the same superscript (or with no superscript) are not significantly different (P > 0.05).

7 Table 2 Composition (g/100 g whey)) of whey from cheeses made with different coagulants and milk concentrations¹

Composition	Cheese type ²					
	RA	RC	UFA	UFC	MXA	MXC
Protein	0.78 ^a ± 0.07	0.80 ^a ± 0.13	0.98 ^b ± 0.11	1.11 ^c ± 0.37	0.83 ^a ± 0.13	0.86 ^a ± 0.15
Fat	0.20 ^a ± 0.03	0.20 ^a ± 0.07	0.40 ^b ± 0.06	0.36 ^b ± 0.03	0.26 ^c ± 0.06	0.29 ^c ± 0.03
Total solid	5.57 ^a ± 0.41	5.59 ^a ± 0.32	5.98 ^b ± 0.94	5.97 ^b ± 1.01	5.43 ^a ± 0.41	5.59 ^a ± 0.93

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9 ¹ Results are means ± standard deviation of at least three replicates

10 ²See Table 1 for explanation of each cheese type.

11 ^{a-c} Data in the same row with the same superscript are not significantly different (P > 0.05).

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16 Table 3 Colour measurements for cheeses

Measurements ¹	Cheese type ²					
	RA	RC	UFA	UFC	MXA	MXC
Brightness	86.38 ^a ± 1.11	83.12 ^b ± 2.52	91.05 ^c ± 0.31	88.18 ^a ± 1.74	92.53 ^c ± 0.77	87.65 ^a ± 1.42
Red/greenness ³	-3.93 ^a ± 1.10	-5.08 ^b ± 1.27	-3.17 ^c ± 0.36	-3.21 ^c ± 0.64	-3.40 ^c ± 0.34	-3.33 ^c ± 0.65
Yellow/blueness ⁴	+24.90 ^a ± 2.22	+27.91 ^b ± 1.72	+17.88 ^c ± 3.01	+20.67 ^d ± 1.22	+18.26 ^c ± 0.6	+21.73 ^d ± 1.13

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18 ¹Based on the L*a*b chromatic system (see materials and methods for details). Results are means ± standard deviation of at least three replicates
 19 for each measurement

20 ²See Table 1 for explanation of each cheese type.

21 ³ +ve value shows the redness while -ve value shows the greenness.

22 ⁴ +ve value shows the yellowness while -ve value shows the blueness.

23 ^{a-d} Data in the same row with the same superscript are not significantly different (P > 0.05).

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25 Table 4 Means scores for sensory attributes of cheeses over 60 days of maturation

Sensory attribute	Cheeses aged 30 days					
	RA ¹	RC	UFA	UFC	MXA	MXC
Color ²	2.29 ^a ± 0.52	4.56 ^b ± 0.76	2.09 ^a ± 0.74	2.39 ^a ± 0.66	3.22 ^c ± 0.51	3.56 ^c ± 0.62
Hardness ³	2.83 ^a ± 0.65	1.11 ^b ± 0.57	2.06 ^c ± 0.75	2.78 ^a ± 0.61	2.83 ^a ± 0.72	2.33 ^c ± 0.54
Bitterness ⁴	1.89 ^a ± 0.51	2.56 ^b ± 0.65	1.50 ^b ± 0.67	1.94 ^a ± 0.73	1.61 ^b ± 0.61	2.22 ^c ± 0.77
Creaminess ⁵	1.56 ^a ± 0.67	3.73 ^b ± 0.75	2.44 ^c ± 0.73	2.28 ^c ± 0.72	2.39 ^c ± 0.74	2.89 ^c ± 0.82
Acceptability ⁶	4.06 ^a ± 0.91	3.44 ^b ± 1.18	3.33 ^b ± 1.01	3.56 ^b ± 1.05	3.00 ^c ± 0.98	3.61 ^b ± 1.11
	Cheeses aged 60 days					
Color ²	3.93 ^a ± 0.61	4.29 ^b ± 0.72	1.86 ^c ± 0.50	2.57 ^d ± 0.67	2.79 ^d ± 0.54	3.36 ^e ± 0.40
Hardness ³	2.71 ^a ± 0.63	1.14 ^b ± 0.32	3.43 ^c ± 0.69	2.71 ^a ± 0.84	2.57 ^a ± 0.73	2.14 ^d ± 0.61
Bitterness ⁴	1.57 ^a ± 0.67	2.86 ^b ± 0.69	1.71 ^a ± 0.78	1.93 ^a ± 0.82	1.81 ^a ± 0.76	1.86 ^a ± 0.76
Creaminess ⁵	1.86 ^a ± 0.68	3.93 ^b ± 0.61	1.57 ^a ± 0.71	2.21 ^c ± 0.63	1.86 ^a ± 0.65	3.00 ^d ± 0.63
Acceptability ⁶	3.57 ^a ± 0.91	3.36 ^a ± 0.84	2.86 ^b ± 1.07	3.64 ^a ± 1.17	3.36 ^a ± 0.81	2.36 ^c ± 0.75

26 ¹See Table 1 for explanation of each cheese type. Results are means ± standard deviation of at least three replicates for each batch of cheese

27 ² Color scores, 1 = very white to 5 = very dark; ³ Hardness scores, 1 = not hard to 5 = very hard; ⁴ Bitterness scores, 1 = not bitter to 5 = very bitter

28 ⁵Creaminess scores, 1 = not creamy to 5 = very creamy; ⁶ Acceptability scores, 1 = like extremely to 9 = dislike extremely.

29 ^{a-e} Data in the same row showing the same superscript are not significantly different (P > 0.05).

Figure captions

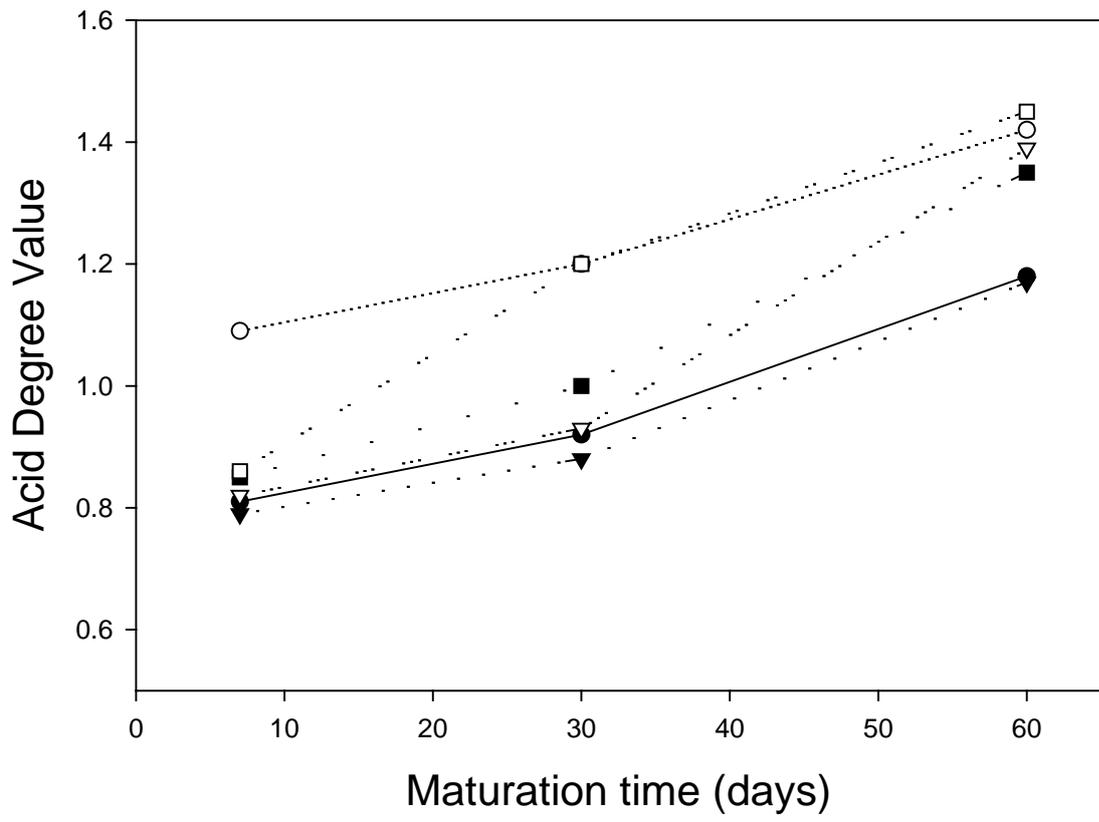
Figure 1. Changes in acid degree value of cheeses during maturation; ● RA; ■ MXA; ▼UFA; ○ RC; □ MXC and ▽ UFC. The standard deviation for the data points in this Figure ranged between 3 to 10 % of the mean (plotted) values. See Table 1 for explanation of cheese types.

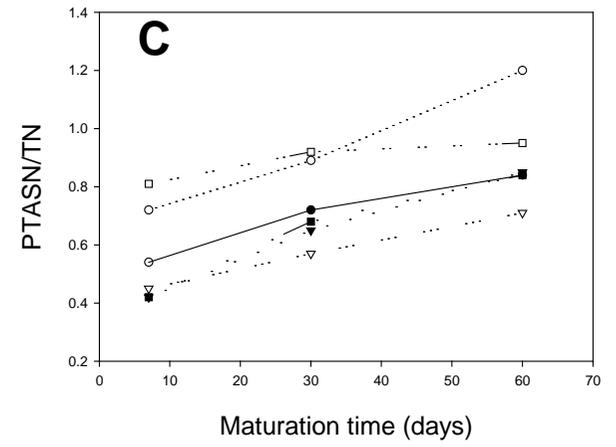
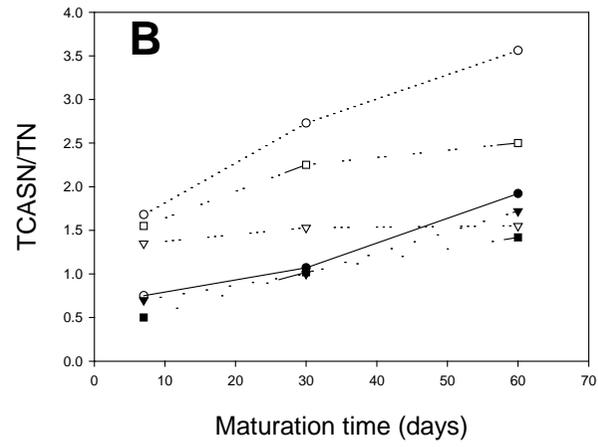
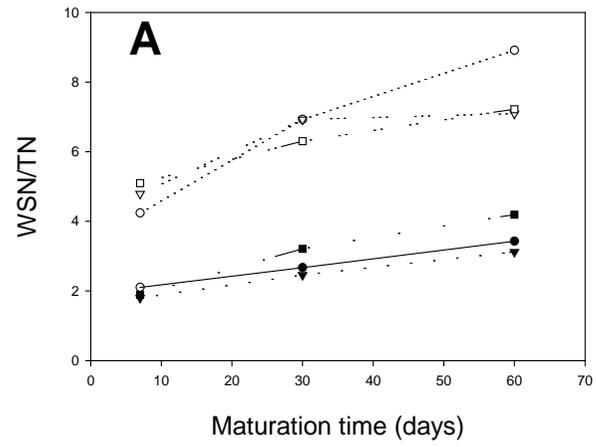
Figure 2. Changes in the ratio of (A) water soluble nitrogen (WSN); (B) trichloroacetic acid soluble nitrogen (TCASN) and (C) phosphotungstic acid-soluble-nitrogen (PTASN) to total nitrogen (TN) of cheeses during maturation. ● RA; ■ MXA; ▼UFA; ○ RC; □ MXC and Δ UFC. Values on y-axis are in g/100 TN. The standard deviation for the data points in this Figure ranged between 4 to 12 % of the mean (plotted) values. See Table 1 for explanation of cheese types.

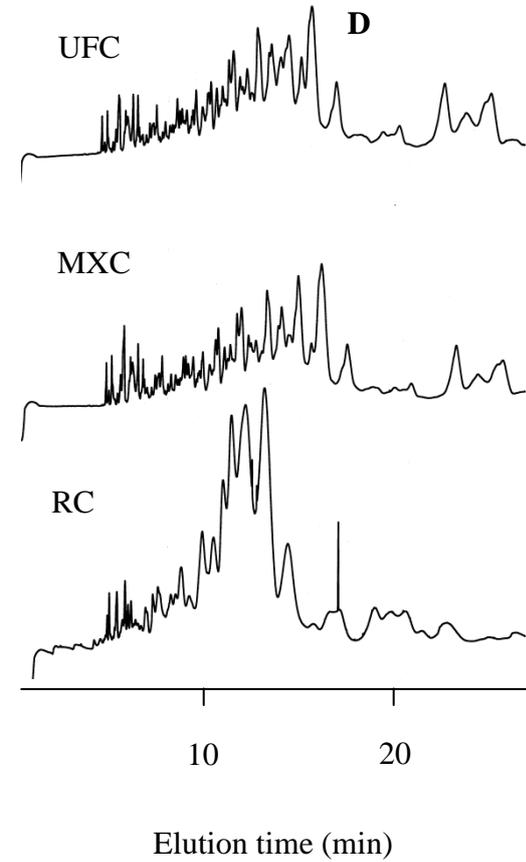
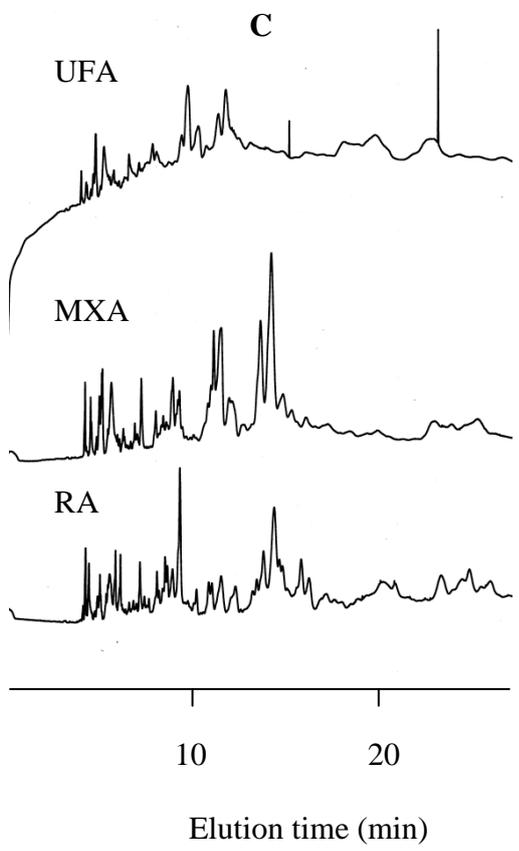
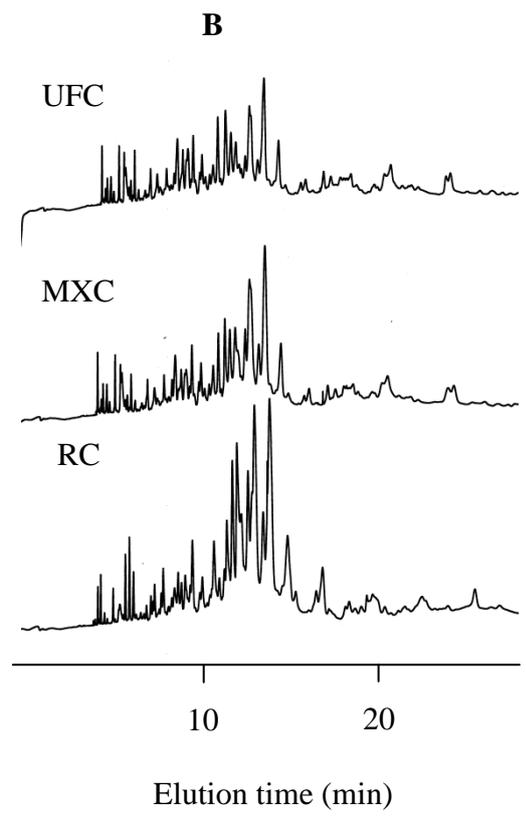
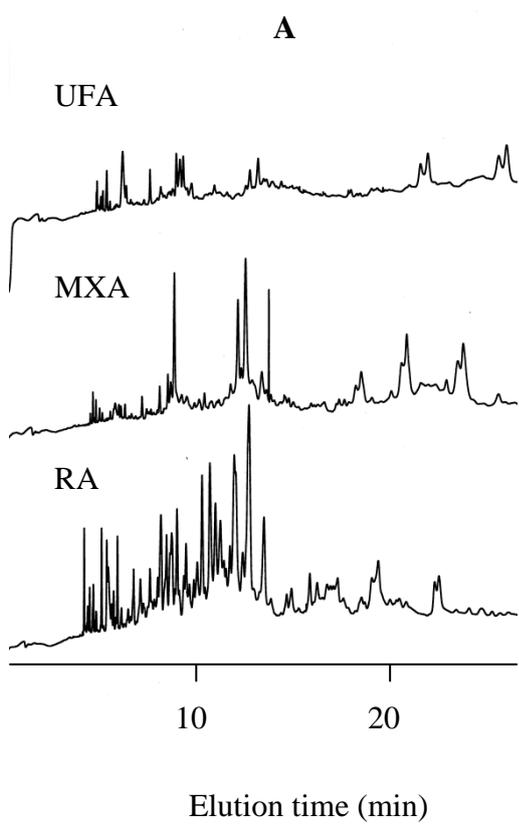
Figure 3. Typical capillary electrophoretograms of water soluble fraction of cheeses made using regular (R), direct UF or mixed concentrated milk (MX) and coagulated with animal rennet or cardoon extract. Cheeses were ripened for 7 days (A, B) or 30 days (C, D). The y-axes are in arbitrary units. See Table 1 for explanation of cheese types.

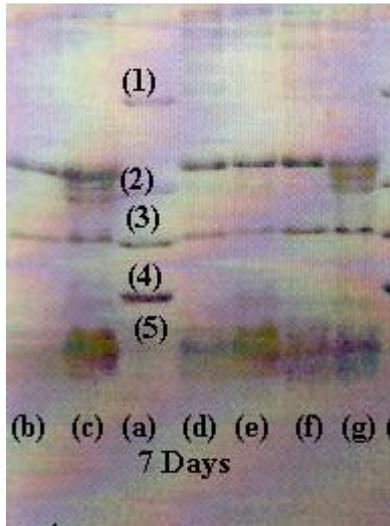
Figure 4. SDS-Tricine gels obtained from water-soluble fractions of cheeses during maturation for 7 days (A), 30 days (B) and 60 days (C). Lane a: molecular weight markers (1) myoglobin (16.9 kDa), (2) a-lactalbumin (14.4 kDa), (3) aprotinin (6.5 kDa), (4) insulin B chain (3.5 kDa), (5) bacitracin (1.4 kDa); lane b: MXA; lane c: MXC; lane d: UFA; lane e: UFC; lane f: RA; lane g: RC. See Table 1 for explanation of cheese types.

Figure 5. Changes in TPA (A) hardness and (B) adhesiveness of cheese samples during maturation for 7, 30 and 60 days respectively (from left to right for each cheese type). See Table 1 for explanation of cheese types.

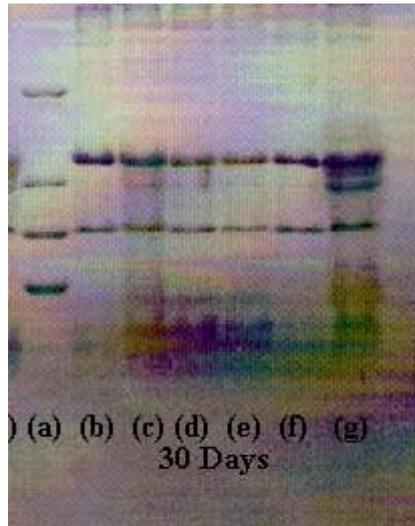




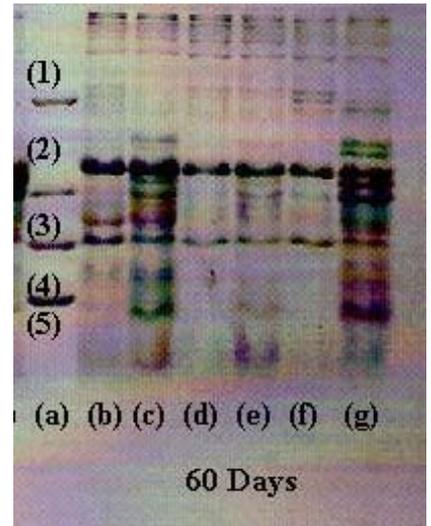




A



B



C

