Scientific Posters Presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors

18-20 April 2007, Alghero, Italy
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The Challenge to Sheep and Goats Milk Sectors
Posters of an International Symposium, April 18-20, 2007, Alghero - Sardinia, Italy

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The Challenge to Sheep and Goats Milk Sectors

Foreword

This issue of the Bulletin contains the Scientific Posters presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors, which took place from 18 to 20 April 2007, in Alghero, Italy.

About 300 delegates, including a significant number of postgraduate students from 30 countries, attended the event. 24 main lectures (which are being published in the peer reviewed journal “Small Ruminant Research”) were presented, and 163 posters exhibited.

The Symposium, held under the auspices of the IDF, was the fifth in a series, presenting latest findings in the research area related to the sheep and goats dairy sector. The overall objective of the Symposium was to provide comprehensive insight into the most recent knowledge including latest research findings on husbandry and milk production, technology, chemistry, physics, microbiology, nutrition etc, without losing sight of the significance of markets and appropriate policies. The core themes were related to the sheep and goat milk, processing and product, characteristics of the product and market and perspectives.

Several aspects were presented and discussed over a three-day programme. In the field of raw milk, genetic, analytical and quality aspects were approached, particularly to enhance the nutritional and beneficial effects for human health. New process treatments for this sector were presented while particular interest was given to functional products. Nutritional and health components together with the analytical and sensorial aspects were presented in the session dedicated to the characteristics of the products. Finally market aspects focused on the valorisation of the traditional and “artisanal” cheeses.

The high number of delegates that attended the Symposium demonstrate that the sheep and goat sector is one of the most dynamic and increasingly stimulates interest in the world.

The IDF is most grateful to the Istituto Zootecnico e Caseario per la Sardegna and the Italian National Committee of the IDF for organizing and hosting the event, and more particularly to Dr A. Pirisi and Dr G. Piredda, as well as to the members of the Programme and Organizing Committees and to all authors for their valuable contribution to the work of IDF.

Christian Robert
June 2008

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Posters Session II. Processing and Product

II-P098: Effects of an Innovative Manufacture System on Chemical Composition, Lipolisys and Volatile Fraction in the PDO Fiore Sardo Cheese

M. Addis¹, F. Tavera¹, R. Comunian¹, R. Di Salvo¹, M.F. Scintu¹,G. Piredda¹

Summary

This work aimed at evaluating the effects of an innovative production system upon chemical composition, lipolisys and volatile fraction in PDO Fiore Sardo cheese.

According to a previous survey, a farm supplied with facilities for both traditional and innovative cheese making was selected. Five cheese making were carried out monthly during dairy season from February to May.

Chemical analysis were carried out on cheese samples during ripening (3.5 and 5 months).

Both employed technologies (traditional and innovative) did not influence either chemical composition, fatty acids composition, lipolysis or volatile fraction in produced cheese. Fatty acids profile evolved during the season following animal feeding changes. In both traditional and innovative cheese lipolisys increased during ripening.

Ketones were very high in both the unripened cheeses. In ripened cheeses alcohols became the main volatile components, immediately followed by ketones and esters.

In conclusion, we can assert that it is possible to introduce subtle innovations in Fiore Sardo cheese making technology in order to shorten cheese makers’ working loads and times without modifying the traditional features of cheese.

1. Introduction

Fiore Sardo PDO is a traditional Italian uncooked sheep milk cheese, produced exclusively in Sardinia island from ewe's raw milk. Since 1985 it has had the Protected Designation of Origin status, which has been recently acknowledged by the European Community (C.D. 1996). The requirement of the cheese making protocol of Fiore Sardo cheese permit the use of both traditional lamb or kid paste rennets and autochthonous natural starters. Fiore Sardo cheese was mainly produced in small dairy farms, using a traditional manufacturing process. Recently, some technological innovations and facilities has been introduced in the production system in order to assist the cheese maker work and to increase the cheese production. This work aimed at evaluating the effects of an innovative production system upon chemical composition, lipolisys and volatile fraction in the Fiore Sardo PDO cheese.

2. Material and methods

According to a previous survey, a farm supplied with facilities for both traditional (handwork and traditional tools) and innovative cheese making (use of an autochthonous natural starter and automation of some phases in the production line) was selected. Five cheese making were carried out monthly during dairy season from February to May.

Chemical analysis including chemical composition (Addis et al., 2005), fatty acids composition (Jiang et al., 1996 ; Chin et al., 1992), lipolysis (De Jong et al., 1990) and volatile fraction (Addis et al., 2006), were carried out on the cheese samples during ripening (3.5 and 5 months).

3. Results and discussion

Both employed technologies (traditional and innovative) did not influence either chemical composition (Table 1), fatty acids composition (Table 2), lipolysis (Table 3) or volatile fraction

¹ Istituto Zootecnico e Caseario per la Sardegna 07040 Olmedo Sassari.
(Figure 1) in produced cheese. Anyway a high variability was observed among the various cheese makings under both used technologies. That variability is caused by many influencing factors, among which the most relevant are environment temperature, animal feeding, lactation stage and number of milking animals, which affect in similar ways both cheese making technologies.

Fatty acids profile evolved following animal feeding changes during the season. In both traditional and innovative cheese, lipolysis rose during ripening owing to the use of rennet paste for milk coagulation.

Ketones coming to the citrate metabolism were very high in both the unripened cheese (traditional and innovative). The cheese volatile fraction changed during ripening, and alcohols became the main chemical components, immediately followed by ketones and esters.

Table 1: Evolution of chemical composition in traditional and innovative Fiore Sardo cheeses during ripening (mean ± sd)

<table>
<thead>
<tr>
<th>Ripening</th>
<th>Technology</th>
<th>DM (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>NS/NT(%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 months</td>
<td>T</td>
<td>68.20 ± 2.72</td>
<td>31.65 ± 0.60</td>
<td>28.18 ± 1.68</td>
<td>26.66 ± 2.69</td>
<td>5.08 ± 0.11</td>
</tr>
<tr>
<td>3.5 months</td>
<td>I</td>
<td>68.05 ± 2.65</td>
<td>31.85 ± 2.25</td>
<td>27.63 ± 1.56</td>
<td>27.42 ± 1.86</td>
<td>5.06 ± 0.17</td>
</tr>
<tr>
<td>5 months</td>
<td>T</td>
<td>69.73 ± 0.08</td>
<td>32.13 ± 0.53</td>
<td>28.56 ± 0.11</td>
<td>29.14 ± 0.50</td>
<td>5.17 ± 0.01</td>
</tr>
<tr>
<td>5 months</td>
<td>I</td>
<td>69.61 ± 0.62</td>
<td>32.38 ± 0.53</td>
<td>28.16 ± 2.33</td>
<td>29.17 ± 1.75</td>
<td>5.11 ± 0.13</td>
</tr>
</tbody>
</table>

T, traditional cheese making; I, innovative cheese making.

Table 2: Fatty acid composition (mg/g of fat, mean ± sd) in traditional and innovative Fiore Sardo cheeses at different stages of ripening

<table>
<thead>
<tr>
<th>Ripening</th>
<th>Technology</th>
<th>SFA</th>
<th>UFA</th>
<th>PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 months</td>
<td>T</td>
<td>552.59 ± 17.82</td>
<td>262.61 ± 25.01</td>
<td>61.19 ± 8.90</td>
</tr>
<tr>
<td>3.5 months</td>
<td>I</td>
<td>551.82 ± 12.94</td>
<td>270.28 ± 15.64</td>
<td>63.66 ± 7.94</td>
</tr>
<tr>
<td>5 months</td>
<td>T</td>
<td>549.83 ± 5.61</td>
<td>263.96 ± 6.51</td>
<td>55.98 ± 1.41</td>
</tr>
<tr>
<td>5 months</td>
<td>I</td>
<td>557.40 ± 9.47</td>
<td>257.07 ± 3.75</td>
<td>65.01 ± 4.19</td>
</tr>
</tbody>
</table>

SFA = Saturated Fatty Acids; UFA = Unsaturated Fatty acids; PUFA = Polyunsaturated Fatty Acids.

Table 3: Evolution of free fatty acids (mmol/kg of cheese, mean ± sd) in traditional and innovative Fiore Sardo cheeses during ripening

<table>
<thead>
<tr>
<th>Ripening</th>
<th>Technology</th>
<th>C4:0-C10:0</th>
<th>C12:0-C16:1</th>
<th>C17:0-C18:3</th>
<th>TFFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 months</td>
<td>T</td>
<td>75.62 ± 18.84</td>
<td>37.30 ± 13.20</td>
<td>14.92 ± 4.70</td>
<td>127.84 ± 35.13</td>
</tr>
<tr>
<td>3.5 months</td>
<td>I</td>
<td>84.65 ± 36.62</td>
<td>40.87 ± 16.84</td>
<td>17.10 ± 5.66</td>
<td>142.63 ± 57.32</td>
</tr>
<tr>
<td>5 months</td>
<td>T</td>
<td>87.72 ± 8.69</td>
<td>48.08 ± 8.06</td>
<td>18.51 ± 1.66</td>
<td>154.32 ± 1.03</td>
</tr>
<tr>
<td>5 months</td>
<td>I</td>
<td>117.86 ± 33.07</td>
<td>57.10 ± 18.97</td>
<td>20.30 ± 3.33</td>
<td>195.26 ± 55.38</td>
</tr>
</tbody>
</table>

TFFA = Total Free Fatty Acids
In conclusion, we can assert that it is possible to introduce subtle innovations in Fiore Sardo cheese making technology in order to shorten cheese makers’ working loads and times without modifying the traditional features of cheese.

References
II-P099: Changes in Chemical, Textural and Sensory Characteristics of Crottin de Chavignol Cheese Manufactured from Frozen Curd and Packaged Under Modified Atmosphere

O. Esmer¹, P. Balkir², A.K. Seckin³

Summary
In this research, the effect of vacuum and modified atmosphere packaging techniques on the Crottin de Chavignol cheese, manufactured from frozen and thawed curd, was investigated. The cheeses were packaged with PA/PE film in vacuum packaging and PP/EVOH/LDPE for top part and PET/EVOH/LDPE for bottom part in modified atmosphere of %20 CO₂ + %80 N₂. The control cheese samples were packaged under ambient atmosphere.

Cheeses were sampled for physicochemical, textural and sensory analyses on the 0th day, 3rd week, 6th week and 9th week. The cheese group packaged in ambient atmosphere could not be evaluated organoleptically after the 6th week because of mould and yeast growth.

Keywords: Packaging, Crottin de Chavignol, curd freezing.

1. Introduction
In this research, the effect of modified atmosphere packaging and vacuum packaging techniques on the quality characteristics of Crottin de Chavignol cheese produced from frozen and thawed curd was investigated.

2. Materials and methods
Raw goat milk was provided from a local breeding farm. Mesophilic starter culture containing Lactococcus lactis subsp. lactis + Lactococcus lactis subsp. cremoris (LC Mix F02-01) was obtained from Wisby Starter Cultures and Media.

2.2.1. Cheese manufacturing
Crottin de Chavignol cheese was manufactured according to Devoyod [1]. The cheeses were packaged with PA/PE film in vacuum packaging (VP) and PP/EVOH/LDPE for top part and PET/EVOH/LDPE for bottom part in modified atmosphere of %20 CO₂ + %80 N₂ (MAP).

2.2.2. Analytical methods
In the cheese samples, the following analyses were made; dry matter by gravimetric method [2], total nitrogen by Kjeldahl method [3], milk fat by Van-Gulik method [4], salt by Mohr method [5] and ash content by gravimetric method [6], pH and titratable acidity, color, texture profile analysis, sensory analyses and statistical analysis. Milk fat and total nitrogen contents were expressed on dry matter basis.

Oxygen transmission rates of packaging materials were determined with MOCON OX-TRAN 2/21 equipment according to the ASTM D – 3985 method at 23°C and % 0 RH. Water vapor transmission rates of packaging materials were determined with MOCON PERMATRAN-W 3/33 equipment according to the ASTM F – 1249 method at 38°C and % 90 RH.

3. Results
The effect of packaging technique on color (a* and b* values), on some textural parameters (hardness, gumminess and chewiness) and flavor scores was found significant (p<0.05). However, no significant difference was found between MAP and vacuum packaged cheese groups except for some textural characteristics as hardness, gumminess and chewiness.

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² Ege Technical and Business College, Ege University, 35100, Bornova, Izmir, Turkey.
³ Department of Food Engineering, Celal Bayar University, Muradiye, Manisa, Turkey.
Table 1: Oxygen and water vapor transmission rates of the packaging materials

<table>
<thead>
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<th>Packaging material</th>
<th>OTRa (cc/m²-day)</th>
<th>WVTRb (g/m²-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA/PE</td>
<td>97.6</td>
<td>3.875</td>
</tr>
<tr>
<td>PP/EVOH/LDPE</td>
<td>3.73</td>
<td>2.09</td>
</tr>
<tr>
<td>PET/EVOH/LDPE</td>
<td>8.54</td>
<td>2.45</td>
</tr>
</tbody>
</table>

a: Oxygen transmission rate; b: Water vapor transmission rate

Table 2: Chemical composition of cheese samples

<table>
<thead>
<tr>
<th>Cheese group</th>
<th>Dry matter (%)</th>
<th>Fat in dry matter (%)</th>
<th>Total protein (%)</th>
<th>Salt in dry matter (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>36.71</td>
<td>43.58</td>
<td>16.00</td>
<td>16.60</td>
<td>1.011</td>
</tr>
<tr>
<td>MAP</td>
<td>37.26</td>
<td>44.28</td>
<td>15.65</td>
<td>12.45</td>
<td>1.384</td>
</tr>
<tr>
<td>VP</td>
<td>36.94</td>
<td>43.31</td>
<td>15.02</td>
<td>8.12</td>
<td>1.181</td>
</tr>
</tbody>
</table>

4. Conclusion

Modified atmosphere and vacuum packaging provided a good preservation up to 9 weeks for Crottin de Chavignol cheese manufactured from frozen and thawed curd, which had naturally some quality defects when compared to the fresh one. The ambient atmosphere packaged group could not be evaluated organoleptically because of visible yeast and mould growth. Bonferroni multiple comparison analysis showed that there was no significant difference was found between MAP and vacuum packaged cheese groups except for some textural characteristics as hardness, gumminess and chewiness. So, these two packaging techniques can be used successfully for lactic goat cheeses manufactured from frozen curd. However, further research for the shelf life evaluation for the packaging techniques is recommended.

References

II-P100: Variation of Whey Protein Content in Goat Milk and Impact on Cheese Yield

P. Barrucand¹, K. Raynal-Ljutovac¹

Summary

In order to better know whey protein: total protein ratio (WPR) variations and their impact on cheese yield, 15 French goat herd milks and 8 bulk milks (about 10 to 15 herd milks) were selected and divided each into two classes according to their total protein (TP) content. Whey protein content was determined after analysis of nitrogen fractions. The results showed that WPR of herd milks may vary from 18 to 26 %. Goat milks containing high TP content presented the highest WPR. Technological tests were carried on with standardised raw and pasteurised milks having 3 TP levels and 3 WPR. WPR had a significant impact on whey draining and total protein and whey protein recovering.

1. Introduction

Protein content, as well as fat, has a great effect on cheese yield and is one of the main criteria used for milk payment. Nevertheless, whey proteins, included in total protein content (TP), may impair cheese making. Variation of goat milk TP according to lactation stage is well known but few data concern variation of Whey Protein: total protein Ratio (WPR) in goat milk. The objectives of this work were to study WPR variations and their impacts on cheese making.

2. Material and methods

15 French goat herd milks and 8 bulk milks (about 10 to 15 herd milks) were selected. Herd milks were divided into two classes according to their total protein content. Milks from six consecutive milkings were sampled monthly during a whole lactation period. Total nitrogen, non casein nitrogen and non protein nitrogen of raw milks were analysed according to Kjeldhal method in order to determine whey proteins.

In a second step, technological tests were then carried out with raw and pasteurised (65°C/30 min in a water bath) milks having 3 TP levels (28, 32 and 36 g/l corresponding respectively to 31, 35.5 and 40 g fat/l) and 3 WPR (18, 21 and 24%). 2 replicates were realised. Fat was standardised in order to obtain the same Fat/TP ratio for all TP levels. Acidification rate, whey drainage of rennet (by centrifugation) and lactic curds (vaccum system) and total proteins, whey proteins and fat recovering after drainage were followed.

3. Results and discussion

Mean WPR varied from 20 to 24 % with low values during summer (extreme individual values being 18 and 26%). Moreover, goat milks containing high TP content presented the highest WPR (Figure 1), especially at the beginning of lactation (in March): comparing the composition of the 15 herd milks, 1 more gram of total protein brought 0.37 g whey protein (figure 2). These variations observed for goat milks are more accentuated than for cow milks (Coulon et al, 1998). For the 8 bulk milks, the increase in whey protein at the end of lactation was higher than this of casein. Mean WPR varied from 19 to 26%.

Concerning acidification rate, TP level was the only influent parameter (buffering effect of caseins). WPR had no effect. Fat recovering did not depend on TP and WPR in this studied range. WPR had a significant (P<0.05) impact on protein recovering for raw milks (Table 1). Increase in WPR (= decrease in casein content), impaired total protein recovering, especially in rennet curd (- 5.5% when WPR value increase from 18% to 24%). WPR had a marked effect on whey protein recovering, especially for lactic curd, in which they are highly entrapped. This impact was enhanced for heat treated milks, WP recovering being 19-33% for raw milks and 22-55%.

¹ Institute for Research in Goat milk products (ITPLC), BP 49, 17700 Surgères, France.
for heat treated milks (data not shown). WPR reduced whey drainage of lactic curds, especially for low TP raw milks (Figure 3). For a TP content of 28 g/l, an increase from 4.8 to 6.6 g/l of WP induced the same whey draining reduction than the heat treatment. Heat treatment of milks induced a 10 to 15% decrease in whey draining, especially for high TP, either with 18 or 21% WP (e.g. 6.4-7.5 g WP/l). The impact of heat denatured whey proteins and their negative impact on whey draining has previously been described on rennet curds for small ruminant milks (Raynal and Remeuf, 1998 ; Masle et al, 2002) but was less known (quantified) for lactic type cheeses.

**Figure 1.** Changes in total protein (TP g/l) and whey protein ratio (WPR %) of goat herd milks according to TP classes and lactation stage

**Figure 2.** Whey protein content brought per gram of total protein in goat herd milks according to lactation stage
Conclusion

As WPR induced marked effects on technological tests, some negative consequences in cheese- makings could be observed. Trials are about to be realised in order to validate these findings and to quantify the cheese making impairment.

References

II-P101: Influence of β-lactams on Manchego Cheese Manufacture

M.I. Berruga¹, G. Battacone², M.P. Molina³, M. Román⁴, A. Molina¹

Summary
The presence of antibiotics in milk may inhibit acid production and affect cheesemaking processes. In order to know how concentrations near to the Maximal Residue Limits (MRLs) could affect this process, artisanal Manchego cheese was elaborated with milk spiked with 5 different β-lactams (penicillin G, amoxicillin, ampicillin, ceftiofur and cephalexin) at 3 different concentrations and the pH decrease was evaluated during the cheesemaking process. With all assayed antibiotics delays were observed in the decrease of pH, but they were only significant when ceftiofur was present.

1. Introduction
Spanish ewe milk is destined for cheese manufacture, and approximately one third of this production comes from the Castilla-La Mancha region, where the main Spanish cheese recognized by an Appellation of Origin (44%) is produced: Manchego cheese (MAPYA, 2006).

β-lactams are widely used in ovine therapy, and if their residues were to reach milk destined to be cheese, they could harm people's health. This situation can be prevented by using routine controls and by observing the Maximal Residue Limits (MRLs) as per European Union norms. It is assumed that if MRLs are safe for consumers, then they will also be safe for the correct development of dairy fermentation processes. However, for the cheese industry the presence of antibiotics could perhaps present a problem for lactic acid bacteria by stopping or slowing down their growth and their acid production (Packham et al., 2001).

The aim of this experiment was to determine how five β-lactams, at concentrations close or inferior to the MRL, could affect the pH evolution in artisanal Manchego cheese manufacture.

2. Material and methods
Three penicillins (penicillin G, ampicillin and amoxicillin) and two cephalosporins (cephalexin and ceftiofur) were separately spiked in raw ewe milk at three different concentrations (0.5*MRL, 1*MRL and 1.5*MRL). Two 30 L vats of from each antibiotic at each concentration were made by using the Manchego cheese manufacturing protocol (CRDOQM, 2006) and the subsequent decrease in pH was studied. Delvotest SP test was used to control antibiotics in the milk (Berruga et al., 2005).

The effect of the concentration of antibiotic was calculated by a GLM according to the equation: $y_{ijk} = \mu + T_i + C_j + [T \times C]_{ij} + \varepsilon_{ijkl}$, where $y_{ijk}$ is ln pH, $\mu$ is the intercept, $T_i$ is the effect of time, $C_j$ is the effect of antibiotic concentration ($j = 0, 2, 4$ or $6 \mu$g/kg for penicillins, and $0, 50, 100$ or $150 \mu$g/kg for cephalosporins), $[T \times C]_{ij}$ is the interaction effect, and $\varepsilon_{ijkl}$ is the residual error. Data used for the GLM was taken in the time elapsed from the cut of the curd to the moment when the pH of the cheese lowered to between 5.4–5.3 (point of cheese introduction to brine).

3. Results and discussion
As the level of each antibiotic increased, the time required to complete the manufacturing process also increased (Figure 1), and it took longer to reach the desired pH. However, significant delays were only observed in the rate of pH decrease when ceftiofur was present (Figure 2 &

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Table 1). Delays on the evolution of acidification parameters when β-lactams are present have been previously observed by others (Cogan, 1972; Packham et al., 2001), but at concentrations superior to those used in this experiment. At concentrations corresponding to the MRL these antibiotics provoked delays which ranged from 5 (cephalexin) to 295 min (ceftiofur). These results suggest the possibility that technical problems might be found in cheese manufacture at concentrations authorized by the EU and that they may be undetectable by screening tests.

**Figure 1.** Time (mean ± sd) required to reach the pH established for the introduction of cheeses to the brine (values within 5.4-5.3) when elaborated with antibiotic spiked raw ewe milk (Point for brine in control cheeses was 255 min).

**Figure 2.** Effects of time and antibiotic concentrations on the ln pH evolution during cheese elaboration when ewe milk was spiked with β-lactams.
**Table 1:** GLM model coefficients for the β-lactams studied during Manchego cheese elaboration

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Function</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>ln pH=1.932 – 0.0009*[T]</td>
<td>&lt;0.0001</td>
<td>77.46</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>ln pH=1.926 – 0.0009*[T]</td>
<td>&lt;0.0001</td>
<td>84.53</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>ln pH=1.921 – 0.001*[T]</td>
<td>&lt;0.0001</td>
<td>94.28</td>
</tr>
<tr>
<td>Ceftiofur®</td>
<td>ln pH=2.0995 – 0.00226*[T] -0.2014*[C] + 0.0014*[T*C]</td>
<td>&lt;0.0001</td>
<td>93.23</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>ln pH=1.927 – 0.001*[T]</td>
<td>&lt;0.0001</td>
<td>88.77</td>
</tr>
</tbody>
</table>

[T]: time; [C]: antibiotic concentration; [T x C]: interaction within time x concentration.

4. Conclusions

Compared to controls, during the cheese manufacture the presence of ewe milk spiked with β-lactams at the corresponding MRL concentration provoked delays in pH decrease that ranged from 5 to 295 min, which were only significant in the presence of ceftiofur.

References

3. CRDOQM. 2006. Resolución 02-10-2006, de la consejería de Agricultura, por la que se adopta la decisión favorable de modificación del pliego de condiciones de la Denominación de Origen Protegida Queso Manchego. DOCM, 209, 20695-20702.
II-P102: High Hydrostatic-pressure Technology Applied in Fresh Sheep Milk Cheese

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Summary

The aim of this work was to extend shelf-life of ovine fresh cheese by means of high hydrostatic pressure technology. Cheese were obtained from Sardinian sheep milk and treated at high pressure. The behavior of cheese throughout ripening was studied, both in high-pressured cheese sample and untreated cheese sample, that was used as control. Microbiological, chemical, textural and sensorial analysis were carried out at different storage times on both samples.

An increase of shelf life has been obtained for high-pressured cheese, from 15 days of storage for the untreated cheese up to 45 days of storage for the treated cheese, due to the reduction of microbial cell count.

Sensorial analyses, performed by a trained sensory panel, revealed significant differences between samples, that increased throughout storage.

1. Introduction

High hydrostatic pressure (HHP) is an innovative technology for food treatment and preservation. Recently HHP application on milk and cheese has been increasing, and its effects on milk coagulation, texture, proteolysis and lipolysis of cheese have been assessed (Trujillo et al., 2002). Many authors have published on microrganisms inactivation, both in milk and in cheese, where pressures between 300 and 600 MPa can inactivate microrganisms, including infectious food-borne pathogens (López-Pedemonte et al., 2007).

Objective of the present work is to study the behaviour of ovine fresh cheese after HHP treatment; in particular the extension of shelf life was evaluated, in comparison with the untreated cheese.

2. Materials and methods

An HPP410100/QFP-6 research press machine (Flow Autoclave Systems, Inc., Columbus, Ohio) has been used to process cheese at 400 MPa, for 10 minutes at 20°C. Treated (T) and untreated (U) cheese was stored at +4°C. Microbiological (Total, Lactic acid bacteria, Coliform and Staphilococci counts), chemical (DM, Fat, NT, NS, NNP, NaCl, pH), textural (TPA and cutting-shear test) and sensorial (Triangle test, Acceptance test) analyses were carried out on treated and untreated cheese at 1, 15, 30, 45 and 60 days of ripening.

3. Results and discussion

High pressure treatment did not influence the chemical composition of cheeses (pH, fat, protein and NaCl content). Proteolysis increased in both cheeses during ripening, although U cheese showed a NS content higher than T product (Table 1).

Cheese hardness was the same, both in T and U samples, immediately after HHP treatment (Figure 1). The untreated cheese softened during the ripening, due to the increase of microbial count and proteolysis (NS content). The treated cheese showed a gradually increasing of hardness up to 45 days of storage. Moreover, the cutting-shear test revealed a smoother structure on the cutting surface in the treated cheese (Figure 2).

Cheeses evaluated by a trained sensory panel using a Triangle test, were judged different between them. Differences increased with storage time. At 1 day, both T and U cheeses were different.

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judged *like fairly well* by Acceptance test using a 9-point scale (from *dislike extremely* to *like extremely*), although the mean value of consumer’s acceptance for the untreated cheese was slightly higher than for the treated counterpart (6.36 and 5.90, respectively). The average difference between T and U products was significantly different from zero (Paired t-test, p < 0.05).

An overall reduction of microflora has been obtained immediately after HHP treatment (Figure 3), lactic acid bacteria and coliform were most affected. A reduction of the fresh cheese deleterious bacteria led to an extension of the product shelf life up to 45 days. Shelf life of untreated cheese ends within 15 days of storage, due to the high coliform cell number.

**Table 1:** Evolution of chemical parameters during ripening in treated and untreated cheeses

<table>
<thead>
<tr>
<th>Ripening</th>
<th>Technology</th>
<th>SS</th>
<th>Fat</th>
<th>NT</th>
<th>NS</th>
<th>NNP</th>
<th>NaCl</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>T</td>
<td>34.45</td>
<td>16.25</td>
<td>2.05</td>
<td>0.196</td>
<td>0.15</td>
<td>1.80</td>
<td>6.41</td>
</tr>
<tr>
<td>1 day</td>
<td>U</td>
<td>33.60</td>
<td>16.00</td>
<td>2.05</td>
<td>0.217</td>
<td>0.13</td>
<td>1.58</td>
<td>6.48</td>
</tr>
<tr>
<td>15 days</td>
<td>T</td>
<td>36.89</td>
<td>17.50</td>
<td>2.11</td>
<td>2.1</td>
<td>0.11</td>
<td>1.66</td>
<td>6.36</td>
</tr>
<tr>
<td>15 days</td>
<td>U</td>
<td>37.18</td>
<td>17.75</td>
<td>2.17</td>
<td>0.26</td>
<td>0.09</td>
<td>1.66</td>
<td>6.27</td>
</tr>
<tr>
<td>30 days</td>
<td>T</td>
<td>36.67</td>
<td>18.50</td>
<td>2.29</td>
<td>0.255</td>
<td>0.11</td>
<td>1.64</td>
<td>6.1</td>
</tr>
<tr>
<td>30 days</td>
<td>U</td>
<td>37.54</td>
<td>18.25</td>
<td>2.21</td>
<td>0.353</td>
<td>0.09</td>
<td>1.73</td>
<td>6.1</td>
</tr>
<tr>
<td>45 days</td>
<td>T</td>
<td>37.40</td>
<td>19.25</td>
<td>2.17</td>
<td>0.287</td>
<td>0.14</td>
<td>1.40</td>
<td>5.65</td>
</tr>
<tr>
<td>45 days</td>
<td>U</td>
<td>39.23</td>
<td>20.75</td>
<td>2.36</td>
<td>0.395</td>
<td>0.13</td>
<td>1.37</td>
<td>5.66</td>
</tr>
<tr>
<td>60 days</td>
<td>T</td>
<td>40.10</td>
<td>21.25</td>
<td>2.39</td>
<td>0.329</td>
<td>0.12</td>
<td>1.65</td>
<td>5.53</td>
</tr>
<tr>
<td>60 days</td>
<td>U</td>
<td>37.83</td>
<td>20.25</td>
<td>2.09</td>
<td>0.437</td>
<td>0.13</td>
<td>1.56</td>
<td>5.27</td>
</tr>
</tbody>
</table>

**Figure 1.** Cheese hardness evaluated via TPA test, using a TA-XT2 Texture Analyser (Stable Micro System, UK).
**Figure 2.** Texture of cheese after HHP treatment analysed via cutting-shear test, using a TA-XT2 Texture Analyser (Stable Micro System, UK).

**Figure 3.** Microbial counts in cheese immediately after HHP treatment.
4. Conclusion

A reduction of bacterial number due to the high pressure treatment allowed to extend shelf life of cheese. Differences in texture and chemical profile, observed throughout cheese ripening, could be ascribed to a difference in the microflora development.

The sensorial analysis showed that although HHP treatment does modify the product features, the changes are not so extensive to affect the common consumer's choice.

References

II-P103: Characteristics of a Probiotic “Caprino” Fresh Cheese with Lactobacillus Acidophilus

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Summary

Aim of the research is to compare the aroma compounds and the lactic flora in a fresh goat cheeses, produced using: 1) \textit{Lactococcus lactis subsp. lactis} and \textit{subsp. cremoris}, as starter culture, for “conventional” cheese; 2) the same starter associated to \textit{Lactobacillus acidophilus} as “probiotic”; both cheeses were analysed during the refrigeration at 4 °C, from the end of cheesemaking to 28 days. Aroma compounds were determined by SPME/GC-MS; the microbiological and physicochemical characteristics were reported, too. The data elaboration using ANOVA demonstrated that probiotic cheeses had a higher content of free fatty acid and lower of alcohols, ketones and hydrocarbons than that conventional. During the refrigeration equivalent quantitative and qualitative changes in aroma compounds have been observed both for conventional and probiotic samples. Starter and probiotic culture count decreased during refrigeration in both cheeses.

1. Introduction

The potential health-promoting effect of dairy products that incorporate \textit{Lactobacillus} species and other probiotic organisms has stimulated considerable research in recent years. Due to its manufacturing process, fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria [Buriti et al., 2005]. The objective of the present study was to examine two kind of “caprino” fresh cheese: conventional (\textit{C}), inoculated with a 1% freeze-dried culture of \textit{Lc. lactis subsp. lactis} (\textit{Lll}) strain [R-704 (Chr. Hansen, Corsico, Milan, Italy)] and probiotic (\textit{P}), that was obtained with the probiotic \textit{Lactobacillus acidophilus} (\textit{La}) (1%) (FD-DVS LA-5\textsuperscript{®} – Probio-Tec \textsuperscript{™}, Chr Hansen) added to the starter culture.

2. Material and methods

Whole milk obtained from a flock of “Maltese” goats breed was used for cheese making trials that were carried out in a small dairy of West Sicily, following the indications reported by S. del Prato (1998) and referred to fresh caprino cheese with slow renneting. The vat was inoculated with a 1% freeze-dried culture of \textit{Lll} and \textit{Llc} strain that was suspended in liquid milk; the amount of liquid rennet was 0.5 ml·10 l\textsuperscript{-1} milk. Probiotic cheese was obtained with starter associated to a probiotic culture using the same cheese making procedure.

Cheese samples, cylindrical shaped, were individually wrapped in parchment paper and then placed inside some little metal containers. Samples were received in our laboratory under refrigerated conditions (4°C) and analysed immediately upon receipt and after 15 and 28 days of storage.

\textbf{SPME/GC-MS}

Volatile fraction was determined as in previous researches (Verzera et al.2004; Condurso et al., 2005). Data elaboration were carried out by ANOVA.

\textbf{Microbiological analysis}

For each cheese type, 10 g cheese was aseptically homogenized with 90 ml on buffered peptone water; serial dilutions were prepared by adding 1 ml to 9 ml sterile peptone water. Samples were tested for counts of \textit{La} on acidified (pH 5.4) MRS agar (Oxoid) and incubated anaerobically (Gas Generating System, Oxoid) at 37°C for 3 days. Starter lactococci were enumerated on M17 agar (Oxoid) and incubated aerobically for 48 h at 37°C.

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3. Results and discussion

Thirty-six volatile components were identified in each sample analyzed. Table 1 reports the composition as classes of substances for the samples analyzed during the refrigeration at 4°C; for both C and P sample, at the production time and during the refrigeration, main components were free fatty acids; hexanoic, octanoic (cheesy, rancid, sweet-like), and decanoic acid (cheesy, rancid) essential to the intense aroma of goat cheese; free fatty acids constituted the 70%-79% about of the total volatile components after manufacturing and, the 74-84% after 28 days of refrigeration, respectively for C and P samples.

The latter showed, after manufacturing and during the refrigeration, a higher amount of free fatty acids and lower of 2-ketones, alcohols and hydrocarbons; similar amount resulted for esters, aldehydes and terpenes. Statistically significant increase resulted for each aliphatic free fatty acid between 15 - 28 days; for 2-methyl-ketones and aromatic hydrocarbons between 0 - 15 and 15 - 28 days; a decrease resulted for esters and aldehydes between 0-15 and for alcohols, terpenes and aliphatic hydrocarbons between 15-28 days. Altogether considered, the differences observed during the period considered were particularly evident after 15 days, when each aroma compounds showed values significantly dissimilar. Terpenes and most of hydrocarbons, probably due to the goat’s feeding, could give significant information on the freshness of the cheese.

After 0, 15 and 28 days of refrigeration the following microbiological results were respectively registered: starter count in C was of 2.4x10^18, 8.7x10^7 and 5x10^5 cfu/g. In P samples starter count was 1x10^16, 5x10^7 and 6x10^3 cfu/g; La count was 1x10^15, 7.6x10^5 and 6x10^3 cfu/g. The value of 3.8x10^7 cfu/g in P was reached at 10th day; this value is required to produce health benefits claimed for probiotic cheese.

4. Conclusion

Proteolysis and lipolysis were the most important pathway for flavour development in our cheese. The extent of these processes was more extensive in P for a discrete synergistic effect between Lll, Llc and La (Buriti et al., 2005).

Decrease of both starter and probiotic cultures were due to growth ability under acidic condition. Likely shelf life of P cheese could be not detrimental for pleasant volatile compounds. It could be of 10 days, a period during which the health benefit could be assured.
References

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5. Salvadori del Prato O. Trattato di Tecnologia Casearia. Edagricole (Editors), Bologna, Italy, 1998
II-P106: Influences of Haccp System Application on “Fiore Sardo” Cheese Processing Contaminating Micro-organisms

S. Fresi¹, P. Mele¹, G. Porqueddu¹, A. Fadda¹

Summary
Fiore Sardo cheese is made with traditional technologies till today. However, the non-optimal processing hygienic conditions can cause economic losses due to the increase of rejected cheese for problems like precocious swelling.

To improve hygienic characteristics a self-control manual based on HACCP system was applied in a dairy sheep farm of 800 dairy Sarda ewes where milk is processed into Fiore Sardo. During two years the presence data of contaminating micro-organisms (coliforms, *Escherichia coli*, moulds, yeasts and Total Bacterial Count in bulk milk like hygienic parameter) were collected. On the first year data on dairy processing system were recorded; during this period a HACCP manual was studied observing management and hygiene lacks during processing.

On the second year manual procedures were applied, verifying the trend of hygienic and contamination parameters considered.

Comparison between processing of first and second year data demonstrated on one hand the clear improvement of processing hygienic conditions, on the other the cheese rejects decrease.

1. Introduction
The manufacturing process of Fiore Sardo cheese follows this scheme: milk coming from the afternoon’s milking (refrigerated at 4°C) and from the next morning’s milking is poured into aluminium tanks and then into a vat. During this last operation milk is filtered by clean cotton cloths, that withhold impurities.

Milk is heated from 4°C to 34°C in copper vats, its temperature is controlled with a thermometer.

Lamb rennet produced in the dairy farm business is added to the heated milk, stirred into the mass of milk homogenously with a special tool called “churn”. Coagulation occurs on an average in the space of 10-14 minutes.

After hardening, curd cutting follows, which is done energetically and vigorously, until the curd is reduced into grains of the dimension of two, three millimetres. Also curd cutting is done with the tool called “muriga”.

Once the operation of curd-cutting is over, the curd matter is allowed to settle on the bottom of the vat for several minutes. Once settled, the procedure’s next step is the pressing of the curd by hand.

Cheese moulding occurs through the operations of turning over and giving the final touch to the wholes on a draining table to complete the running of whey. Ripening occurs in two phases. In the first phase the product stays in a cellar for three months, at the temperature of 10°C. The cheese wholes are stacked into piles of two or three, directly in contact with the cement pavement.

The second phase of ripening begins with the transfer of the Fiore Sardo cheeses in a special fresh basements, but with a higher temperature (12 – 16°C), where the cheese wholes are periodically turned over and brushed with oil and vinegar.

HACCP consents a preventive assessment of the single hazards for the consumer, due to consumption of that product, and also a correct management of such risks, until they are possibly zero ed or at least reduced to an acceptable level. Such a system, nowadays largely used in the food industry, is the HACCP system. This system can be used not only in big

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industries but, with all the necessary correctives and adjustments, it can be adapted also to small structures with hand made manufacturing.

2. Material and methods

During the first year, the transformation of the milk, in all its phases, was carried out according to the usual procedure followed by the dairy and in force until that moment.

During the second year the dairy adopted methodologies and procedures that had been suggested by us.

The taking of samples for analysis was carried out as follows:
- FIL-IDF 50C:1995 (taking samples of milk and milk products)
- APHA,1992 (Taking environmental samples)

The search for *Escherichia coli* β-glucoronidasi positiva was performed by means of a method derived from the application of the following norms:
- ISO 16649-2 "Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucoronidase-positive *Escherichia coli* – Part. 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide”.

The search for Coliforms was performed by means of a method derived from the application of the following norms:

3. Results and discussion

The firm’s implementation of the HACCP system laid out by the manual made for the improvement of the hygienic-sanitary characteristics of the products. Moreover, it is proved by the reduction in firm ‘wastes’. During the first year Fiore Sardo had a percentage value of 5.66%, this went down to 1.92% during the second year. The majority of waste of this product is due to a processing defect known as "premature swelling". This is caused by the metabolic activity of contaminating microorganisms which are mainly coliforms and in particular *Escherichia coli*.

| Table 1: Biennial cheese production 2005/2006 |
|---|---|---|---|
| Cheese | Production in Kg | Waste in Kg | Waste in % |
| 2005 | 17.300 | 980 | 5.66 |
| 2006 | 18.200 | 350 | 1.92 |

4. Conclusion

The management procedures, cleaning and sanitation techniques prepared, regularly applied, led to general improvement of the hygiene characteristics of the products. This was noted in the second year.

However, the handbook is not an arrival but a work foundation and a starting point from which obtaining a product which better responds to the demands of a market, which, besides an organoleptic quality, also demands an ever-increasing guarantee of the healthy characteristics of products.
References


4. FIL-IDF 50C: 1995 (taking samples of milk and milk products).

5. APHA, 1992 (Taking environmental samples).

6. ISO 16649-2 "Microbiology of food and animal feeding stuffs.

7. ISO 5541/1: 1986 "Milk and milk products –

8. ISO 7218: 1996 "Microbiology of food and animal feeding stuffs."
II-P107: Changes in Texture and Flavour During the Ripening of Murcian Wine Cheese Made with Lamb Rennet Paste

E. Ferrandini¹, M.B. López¹, M. Castillo², M. De Renobales³, M. Virto³, J. Laencina¹

Summary
The objective of this work is to determine the effect on texture and flavour of lamb rennet in Murcian wine cheese. Regarding to the texture the cheeses made with lamb rennet paste had less deformation and are harder than those elaborated with liquid rennet. The sensory analysis permit to conclude that lamb rennet paste produces a little bitterness and piquant taste that offers an interesting alternative for the regional cheese making sector.

1. Introduction
Mediterranean area is famous for its typical goat cheese most of them made using lamb or goat rennet pastes. For instance, the Italian cheeses Provolone, Pecorino Sardo and Pecorino Romano, from Greece Kefalotyri and Feta, from Spain Idiazabal and Majorero, from Portugal Serra D'Estrela, etc. are made using lamb rennet paste (1). The scope of this study is to determine the influence of lamb rennet paste in the texture and flavour of Murcian wine cheese compared to liquid rennet (2).

2. Material and methods
Cheese A and B were manufactured using liquid bovine rennet (80% chymosin, 180 IMCU) with a difference of two days between manufactures and cheese C and D were made employing lamb rennet paste (71,10% chymosin, 176,92 IMCU, lipase activity 4,57 U g⁻¹). Cheese samples were taken at 45 and 60 days of ripening and then analysed by means of a TA-TX2 (State Micro System, Survey, UK) texture meter for both uniaxial compression and stress relaxation tests, while the sensory analysis were done by a ten experts panel from de Consejo Regulador de los Quesos de Murcia y Queso de Murcia al Vino de la Región de Murcia.

3. Results and discussion
At 45 days cheese B (22,35 kPa) is significantly the more breakable one and at 60 days C and D had the highest values so they were firmer and reached a grade of maturity before than the others (Table 1). Fracture strain describes the deformability of cheese (3) and it is considered as a rheological parameter related to the behaviour of the casein gel net structure obtained by different type of milk coagulants (4). Along the time of maturity studied the cheeses with rennet paste (C, D) were less deformed -less elastic- then they showed stronger internal cohesion forces than A and B ones. In similar conditions Buffa et al. (2001) found lower values at 60 days (0,23=23%). At the end of maturity cheeses D and C have shown the highest values of fracture work (1,14-0,78 kJ m⁻³ respectively) this means that they needed more energy to be fractured then they were harder than A and B. At 45 days all the cheeses had the same elastic behaviour while at 60 days we only found significantly differences between A and B being cheese B the most elastic one of them (Table 2). No differences were observed for e parameter.

Panellists only determined significantly differences for taste and residual taste between cheeses made with rennet paste (C, D) and without it (A, B) reaching de lowest values the first ones because they strictly punished those attributes that were strange in relation to the normal ones that appear in cheese made with liquid bovine rennet -principally for its little piquant and

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bitterness-. Although C and D cheeses did not obtain higher punctuations they were considered by the experts' panel as an interesting good alternative for making this other kind of Murcian wine cheese.

**Table 1:** Mean and standard deviation for uniaxial compression tests of cheeses

<table>
<thead>
<tr>
<th></th>
<th>Fracture stress (kPa)</th>
<th>Fracture strain (dimensionless)</th>
<th>Fracture work (kJ m(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 days</td>
<td>60 days</td>
<td>60 days</td>
</tr>
<tr>
<td>A</td>
<td>34.80 ± 8.21</td>
<td>28.45 ± 2.77</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>22.35 ± 3.28</td>
<td>22.20 ± 3.10</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>40.99 ± 7.71</td>
<td>36.75 ± 5.31</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>38.58 ± 4.22</td>
<td>50.08 ± 5.66</td>
<td></td>
</tr>
</tbody>
</table>

Means with the same superscripts in the same column are not significantly different (P< 0.05).

**Table 2:** Mean and standard deviation for stress relaxation tests of cheeses

<table>
<thead>
<tr>
<th></th>
<th>r (s(^{-1}))</th>
<th>e (dimensionless)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 days</td>
<td>60 days</td>
</tr>
<tr>
<td>A</td>
<td>0.184 ± 0.004</td>
<td>0.193 ± 0.004</td>
</tr>
<tr>
<td>B</td>
<td>0.180 ± 0.014</td>
<td>0.157 ± 0.016</td>
</tr>
<tr>
<td>C</td>
<td>0.175 ± 0.007</td>
<td>0.188 ± 0.024</td>
</tr>
<tr>
<td>D</td>
<td>0.186 ± 0.011</td>
<td>0.176 ± 0.008</td>
</tr>
</tbody>
</table>

Means with the same superscripts in the same column are not significantly different (P< 0.05).

4. Conclusion

Lamb rennet paste influences the rheological parameter fracture strain during the ripening of Murcian wine cheese being those made with rennet paste less deformed so they are less elastic than ones made with liquid bovine rennet. Only at 60 days of ripening fracture stress is affected for the type of rennet used reaching the highest values those made with lamb rennet paste. What it means is that the cheeses made with the paste are harder than the others.

Finally, according to the expert panel’s results this Murcian wine cheese made with lamb rennet paste could be manufactured being a good alternative for the regional cheese making sector.
References


II-P108: The Quality of Galotyri Cheese Made with Different Starter Cultures

M.C. Katsiari¹, E. Kondyli², L.P. Voutsinas¹

Summary

The compositional and sensory characteristics of Galotyri cheese, a traditional Greek acid/rennet-curd cheese, made with four different commercial starter cultures were compared. Two mesophilic starter cultures, MA011 (containing Lactococcus lactis subsp. lactis and Lc. lactis subsp. cremoris) and Probat 222 (containing Lc. lactis subsp. lactis, L. lactis subsp. cremoris, Lc. lactis subsp. lactis biovar. diacetylactis and Leuconostoc mesenteroides subsp. cremoris), a thermophilic yoghurt culture, CH-1 (containing Lactoba-cillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus) and a mixed mesophilic/thermophilic starter culture, CHOOZIT MT 1 (containing Lc. lactis subsp. lactis, L. lactis subsp. cremoris, Str. thermophilus and Lb. delbrueckii subsp. bulgaricus) were added in the cheese milk at the suppliers’ recommended levels. The results indicated that the compositional characteristics of Galotyri cheese were not significantly (P>0.05) affected by the different starter cultures used. It was concluded that high quality Galotyri cheese could be produced by using any of the cultures MA011, CHOOZIT MT 1 or CH-1.

1. Introduction

Galotyri is one of the oldest Greek traditional cheeses. It is an acid/rennet-curd cheese made from ewe or goat milk or mixture of both. Since Galotyri has pleasant organoleptic characteristics, much appreciated by the Greek consumers, in recent years there is a demand for its production. The manufacture of Galotyri in many regions of the country with different procedures causes inconsistent product quality (Anifantakis,1991). Thus, the unstandardized processing methods used by the dairies need to be studied. Recently, Kondyli et. al. (2007) determined the effects of different processes of Galotyri cheese used by the Greek dairies on its chemical and sensory characteristics to ensure product of consistent composition and quality. They found that the use of rennet and the salting of curd, rather than the cheese milk, contributed to a more consistent cheese quality. Since the effect of starter cultures on cheese quality has been well documented, the objective of this work was to study the effects of different commercial starters on the quality of Galotyri, made using the process suggested by Kondyli et al. (2007), in order to select the starter giving the best and most consistent during storage cheese quality.

2. Materials and methods

The cheese manufacture was made with the process suggested by Kondyli et al. (2007). The chemical analyses and the sensory evaluation of cheeses were carried out using standard methods.

3. Results and discussion

There were no significant (P>0.05) differences in the compositional properties (moisture, fat, fat-in-dry matter, protein, ash, salt and salt-in-moisture) among the four cheeses (Table 1). However, the cheese made with the thermophilic CH-1 culture had significantly (P<0.05) higher and lower titratable acidity and pH values, respectively, than the cheeses made with the other cultures. Table 2 shows that the four cheeses did not differ (P>0.05) in appearance and texture. However, the cheese made with the Probat 222 culture had significantly (P<0.05) lower flavour and total assessment scores, throughout storage, than those of the other cheeses, which did not differ (P>0.05) in these sensory characteristics.

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4. Conclusions

The use of different starter cultures resulted in cheeses with similar compositional characteristics. The MA011, CHOOZIT MT 1 or CH-1 cheeses had similar sensory scores but significantly higher flavour and total scores than the Probat 222 cheese.

References

II-P109: Chemical and Sensory Characteristics of Galotyri Cheese Made Using Different Procedures

E. Kondyli¹, M.C. Katsiari¹, L.P. Voutsinas¹

Summary

Galotyri is a traditional fresh spreadable Greek cheese. Three cheesemaking methods were evaluated, namely production of cheese using salted ewes’ milk, starter culture and rennet (SM+R) and starter culture with or without rennet and salting the curd after draining (R+SC or SC, respectively). The SC cheese had significantly (P< 0.05) higher moisture and lower fat and protein contents than the other two cheeses made with rennet. No significant (P>0.05) differences in fat-in-dry matter, lactose, salt, salt-in-moisture, ash, pH, acidity and yield were observed among the cheeses studied. The R+SC cheese had the most consistent quality during storage and was the most preferred by the panelists.

1. Introduction

Galotyri is made from ewe or goat milk or mixtures of both. The milk used for its production should be of good quality, whole raw or pasteurized. The addition of traditional rennet or other enzymes as well as of harmless starter cultures is allowed. Galotyri is a white cheese with spreadable texture, without a rind and holes and is characterized by a sourish and pleasant refreshing taste and aroma. Although it is manufactured in many regions of the country, the manufacturing process is different from region to region, leading to cheeses with variations regarding the appearance and the sensory properties (Anifantakis, 1991). To date, no study to determine the effects of different manufacturing processes of Galotyri cheese currently being used in dairies on its chemical and sensory characteristics has been done. Thus, our objective was to compare different production procedures of Galotyri cheese with the main goal to determine the one leading to a consistently high quality product.

2. Materials and methods

Three cheesemaking methods were evaluated, namely production of cheese using salted ewes’ milk, starter culture and rennet (SM+R) and starter culture with or without rennet and salting the curd after draining (R+SC or SC, respectively). The cheesemaking procedures are shown in the following flow diagram.

3. Results and discussion

There were no significant differences in the physicochemical properties among the three cheeses with the exception that the SC cheese had significantly (P< 0.05) higher moisture and lower fat and protein contents than the other two cheeses made with rennet (Table 1). The cheesemaking method significantly affected cheese proteolysis as shown by the determination of water soluble nitrogen (WSN). Omission of

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rennet during cheese preparation resulted in reduced proteolysis during manufacture and storage of Galotyri. The SM+R cheese had higher levels of WSN than the other cheeses throughout storage. No bitterness or off-flavour was noted by any member of the taste panel in SC and R+SC cheeses. On the contrary, the SM+R cheese exhibited slight bitterness and unclean flavour after 15 days from manufacture. The R+SC cheese obtained higher total score than the other cheeses and was the most preferred by the panelists due to its consistent quality during storage.

Table 1: Chemical characteristics at 2 days and proteolysis during storage of Galotyri cheese made by different methods

<table>
<thead>
<tr>
<th>Component</th>
<th>Production method</th>
<th>R+SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>SM+R</td>
<td>SC</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>74.08±0.74a</td>
<td>76.35±0.13a</td>
</tr>
<tr>
<td>FDM (%)</td>
<td>10.75±0.29a</td>
<td>9.50±0.29a</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>40.25±1.08a</td>
<td>40.18±1.37a</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>3.20±0.13a</td>
<td>2.69±0.23a</td>
</tr>
<tr>
<td>S/M (%)</td>
<td>2.11±0.02a</td>
<td>2.07±0.13a</td>
</tr>
<tr>
<td>pH</td>
<td>2.19±0.01a</td>
<td>2.27±0.04a</td>
</tr>
<tr>
<td>pH</td>
<td>4.39±0.03a</td>
<td>4.41±0.02a</td>
</tr>
<tr>
<td>Acidity (%lactic acid)</td>
<td>0.73±0.01a</td>
<td>0.71±0.01a</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>52.00±0.59a</td>
<td>52.13±0.77a</td>
</tr>
<tr>
<td>WSN (%TN) at 1 day</td>
<td>9.67±0.38a</td>
<td>5.73±0.11a</td>
</tr>
<tr>
<td>WSN (%TN) at 15 days</td>
<td>7.30±0.30a</td>
<td>6.10±0.26a</td>
</tr>
<tr>
<td>WSN (%TN) at 30 days</td>
<td>8.67±0.22a</td>
<td>6.76±0.17a</td>
</tr>
<tr>
<td>ΔWSN (%TN) 30 days</td>
<td>1.70±0.37a</td>
<td>1.03±0.17a</td>
</tr>
</tbody>
</table>

a,b Means in each row bearing a common superscript did not differ significantly (P>0.05).
c Mean values ± s.e. of three trials.
* Increase in WSN (% TN) between 1 and 30 days.

Table 2: Sensory characteristics of Galotyri cheese made by different methods during storage

<table>
<thead>
<tr>
<th>Sensory characteristic</th>
<th>Age of cheese (days)</th>
<th>Production method</th>
<th>R+SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance (10)c</td>
<td>2</td>
<td>SM+R</td>
<td>SC</td>
</tr>
<tr>
<td>Body and texture(40)c</td>
<td>2</td>
<td>9.36±0.18a</td>
<td>8.12±0.11a</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>9.13±0.13a</td>
<td>8.13±0.12a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9.23±0.03a</td>
<td>8.40±0.10a</td>
</tr>
<tr>
<td>Flavour (50)c</td>
<td>2</td>
<td>36.37±0.23a</td>
<td>31.33±0.48a</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>36.13±0.81a</td>
<td>31.20±0.92a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>35.60±0.61a</td>
<td>32.27±0.27a</td>
</tr>
<tr>
<td>Total (100)c</td>
<td>2</td>
<td>44.03±0.50a</td>
<td>41.42±0.30a</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>35.83±2.10a</td>
<td>42.83±0.60a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>36.33±0.83a</td>
<td>41.25±1.52a</td>
</tr>
<tr>
<td></td>
<td>89.76±0.75a</td>
<td>80.87±0.33a</td>
<td>88.72±0.65a</td>
</tr>
<tr>
<td></td>
<td>81.0±1.14a</td>
<td>82.17±1.48a</td>
<td>89.17±1.49a</td>
</tr>
<tr>
<td></td>
<td>81.17±1.42a</td>
<td>81.92±1.83a</td>
<td>87.51±1.03a</td>
</tr>
</tbody>
</table>

a,b Means in each row bearing a common superscript did not differ significantly (P>0.05).
c Mean values ± s.e. of three trials.
* Values in parentheses are maximum attainable scores.
4. Conclusions

The results from the comparative study of different manufacturing processes of Galotyri cheese currently being used in dairies showed that the cheesemaking technology had a significant impact on cheese quality, by affecting the development of proteolysis in cheese. Addition of rennet improved the draining characteristics of the curd and increased curd firmness. Salting the cheese milk resulted in a significant deterioration of Galotyri cheese flavour during storage, due to the development of bitterness and off-flavour. The use of rennet and the salting of curd, rather than the cheese milk, contributed to a more consistent Galotyri cheese quality.

References

II-P111: Influence of Autochthonous Starter Cultures on Physicochemical Parameters of Sardinian PDO Cheeses

N.P. Mangia, M.A. Murgia, G. Garau, M.G. Sanna, P. Deiana

Summary
The aim of this work was to investigate the influence of different autochthonous starter cultures on some physicochemical parameters of Pecorino Sardo, Pecorino Romano and Fiore Sardo PDO cheeses. These experimental cheeses (E) were compared with controls (C) manufactured with the methodologies and protocols specified by the respective regulatory boards.

A rapid accumulation of free amino acids (FAAs) was detected for Pecorino Sardo cheese during the last phase of the ripening. Any significant difference for free fatty acids (FFAs) was detected between control and experimental cheese at the end of the ripening. Fiore Sardo cheese was showing the highest amounts of FFAs compared to Pecorino Romano and Pecorino Sardo. Overall Pecorino Romano cheese was showing the lowest amount of total FAAs and FFAs compared with the other PDO cheeses.

1. Introduction
Enzymes of starter bacteria, non-starter lactic acid bacteria as well as rennet and milk are all responsible for proteolysis in cheese. This latter is important during the maturation of cheese and contributes directly to the development of the desired texture and flavour intensity (1).

Pecorino Sardo, Pecorino Romano and Fiore Sardo cheese are Protected Denomination of Origin (2) made from full cream ewe’s milk.

The use of autochthonous LAB cultures, which is allowed by the manufacturing procedures specified under the PDO, could be helpful to achieve a better management of the process and maintain the cheese “typicality“ as previously pointed out for similar cheeses (3).

In this study we evaluated the influence of different autochthonous starter cultures on some physicochemical parameters of Pecorino Sardo, Pecorino Romano and Fiore Sardo PDO cheeses. These experimental cheeses (E) were compared with controls (C) manufactured with the methodologies and protocols specified by the respective regulatory boards.

2. Materials and methods
All the starter cultures were prepared in the laboratory using selected autochthonous strains belonging to the DiSAABA microbiological collection.

Fiore Sardo: selected cocci (Lactococcus lactis subsp. lactis CFM7) and rods (Lactobacillus casei subsp. casei Lc101 and Lactobacillus plantarum Lp17) were mixed in a final ratio of 3:1. Lb. casei subsp. casei and Lb. plantarum were in the ratio of 1:1. These ratios were based on the relative abundance of the LAB species during the fermentative phase of traditional Fiore Sardo (4).

Pecorino sardo: selected cocci and rods were mixed in the final ratio of 3:1. Lc. lactis subsp. lactis LPS31 and S. thermophilus SPS31, among cocci, were in the ratio of 1:1 as well as Lb. casei subsp. casei 3PS103 and Lb. helveticus LbPS2. Mesophilic strains were grown in sterile ewes’ milk at 30 °C for 12 h, while thermophilic strains were grown at 42 °C for 12 h.

Pecorino Romano: the selected strains S. thermophilus St14, Lactobacillus helveticus Lh87 and Lactobacillus delbrueckii subsp. lactis Ld10 were mixed in a final ratio of 3:1:1 respectively, thermophilic strains were grown in sterile ewes’ milk at 42 °C for 12 h.

Free amino acids (FAAs) were extracted from the cheeses as described previously (5). Identification and quantification were achieved using an HP 1050 HPLC system with an HP 1046A fluorescence detector and the HP Chemstation Rev. A.06.03 software (Hewlett-Pack-
ard Co., Wilmington, USA). A Hypersil C18 AA column (200x2.1 mm, 5 μm) with a guard column (Agilent Technologies Company, Palo Alto, USA) were used. All of the instrumental analytic conditions of derivatisation and quantification of the amino acids were as described by Gratzfeld-Hüesgen (6).

The FFAs were extracted from the cheeses and analysed by gas chromatography according to the methods of de Jong & Badings (7) with some minor modifications. FFAs (C₄-C₁₈:₃) were separated using a Nukol capillary column (15 m, 0.53 mm I.D., 0.50 μm Df; Sigma-Aldrich Co., St. Louis, USA) using an HP 5890 series II gas chromatograph (Hewlett-Packard Co., Wilmington, USA) with an auto-sampler and a flame ionization detector. Data acquisition was carried out using the HP Chemstation Rev. A.06.03 software (Hewlett-Packard Co., Wilmington, USA).

3. Results and discussion

In table 1 we reported the content of FAA and FFA in Fiore Sardo, Pecorino Sardo and Pecorino Romano cheese.

At the end of the ripening Fiore Sardo cheese was showing the highest amounts of FFAs compared to Pecorino Romano and Pecorino Sardo. The accumulation of the FFAs in this cheese seemed to be influenced by the use of raw milk, which preserves its total content of constitutive lipases, rather than the rennet enzymes or the cheese microflora.

At 210 d of ripening the content of FFAs was 1040.46 and 1022.18 mg/100g, for the experimental and the control cheese respectively, something intermediate between Pecorino Sardo and Pecorino Romano.

A rapid accumulation of FAAs was detected for Pecorino Sardo in the last period of ripening, between 120 and 210 d. This was particularly evident for the E cheese (3077.02 mg/100g) and could be due to the superior proteolytic activity of the experimental starter. In general, the content of FFAs was not very high during the ripening as lipase-free liquid calf rennet was used for the Pecorino Sardo cheese manufacturing. As a consequence the evolution of FFAs was likely due to the cheese microflora. No significant difference for FFAs was detected between control and experimental cheese.

Overall Pecorino Romano cheese was showing the lowest amount of total FAAs (slightly more abundant in the E cheese with respect to the control) and FFAs compared with the other PDO cheeses; most likely the high content of sodium chloride and the low aw of this cheese influenced all the lipase activities, even those present in the rennet paste.

Table 1: a, b, c.: Change in Free Fatty Acids (FFA) and Free Amino Acids (FAA) of Fiore Sardo (a), Pecorino Sardo (b) and Pecorino Romano (c) cheeses value expressed as mg 100g⁻¹ of TS

<table>
<thead>
<tr>
<th>Ripening time</th>
<th>1 day</th>
<th>5 days</th>
<th>30 days</th>
<th>150 days</th>
<th>210 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>a FAA</td>
<td>51.62</td>
<td>114.09</td>
<td>301.81</td>
<td>103.49</td>
<td>1040.46</td>
</tr>
<tr>
<td>FAA</td>
<td>104.95</td>
<td>229.17</td>
<td>815.96</td>
<td>2067.06</td>
<td>2859.33</td>
</tr>
<tr>
<td>b FAA</td>
<td>109.17</td>
<td>221.36</td>
<td>851.51</td>
<td>1957.88</td>
<td>3077.02</td>
</tr>
<tr>
<td>FAA</td>
<td>190.57</td>
<td>288.95</td>
<td>370.35</td>
<td>437.29</td>
<td>683.83</td>
</tr>
<tr>
<td>c FAA</td>
<td>97.06</td>
<td>318.74</td>
<td>559.31</td>
<td>740.60</td>
<td>682.70</td>
</tr>
<tr>
<td>FAA</td>
<td>122.92</td>
<td>161.97</td>
<td>288.95</td>
<td>370.35</td>
<td>437.29</td>
</tr>
</tbody>
</table>
4. Conclusions

The use of autochthonous starters allowed improved organoleptic characteristics typical of PDO cheese, in particular the production of Pecorino Sardo and Fiore Sardo cheese with significantly content of FAAs and FFAs, respectively. Overall the accumulation of the FAAs and the FFAs in the PDO cheeses investigated appeared to be favoured by the presence of the mesophilic microflora, reduced by the use of raw milk and influenced by the elevated salt concentration.

References


II-P112: The Utilisation of the RP-HPLC Method for Determination of Free Amino Acids in the Ripening Process of Ewe Cheese from the Island Krk

N. Mikulec¹, I. Habuš², N. Antunac¹, Lj. Vitale², J. Havranek¹, S. Kalit¹, N. Brajenović²

Summary
During cheese ripening process, proteolytic enzymes degrade milk proteins to polypeptides, peptides and amino acids. Organoleptic properties of a cheese, in addition to other constituents, depend on present amino acid and their ratio. We have applied reverse phase - high performance liquid chromatography (RP-HPLC) to analyze ewe cheese from the island Krk taken at day 0, 30, 60, 90 and 120 of ripening. Amino acids and small peptides soluble in trichloroacetic acid react with 6-aminoquinolyn-N-hydroxysuccinimidyl carbamate (ACQ) to form derivatized amino acids and small peptides as highly stable ureas with Waters AccQ Tag package. Obtained derivatives were separated on Waters AccQ Tag C18 column and detected at λ 254 nm. Total content of amino acids increased during ripening process. In mature cheese dominated glutamic acid, leucine, valine, phenylalanine and proline. Those amino acids are known as characteristic for hard ewe cheeses in general, and our results corroborate with those data.

1. Introduction
Ewe cheese from the island Krk is highly nutritional and possesses a specific flavour and smell. Ripening, along with drying, represent the chemical changes mostly catalysed by enzymes, amongst which proteolytic enzymes hold an important role. Proteolytic enzymes are degrading milk protein to the peptides of different length and amino acids, whose ratio significantly influences the texture and organoleptic characteristics of cheese. The aim of this study was to determine the amino acid content in the ewe cheese during its ripening process.

2. Material and methods
This study was performed with the ewe cheese samples collected at the farms located on the island Krk, which are breeding Croatian autochthonous sheep. The cheese samples were taken on the 1st, 30th, 60th, 90th and 120th day of the ripening process. The content and ratio of free amino acids in the cheese samples was determined by reversed phase - high performance liquid chromatography (RP-HPLC). Amino acids and small peptides soluble in trichloroacetic acid were transformed with 6-aminoquinolyn-N-hydroxysuccinimidyl carbamates (ACQ) to form derivatized amino acids and small peptides as highly stable ureas, using Waters AccQ Tag package, separated on Waters AccQ Tag C18 column and detected at λ 254 nm.

3. Results and discussion
Main results are presented in figures 1 and 2. As with previous studies, the principal FAA at most ripening times were Glu, Pro, Leu, Phe and Val [1, 2, 3]. Similarly, the concentrations of all individual FAA increased with ripening time. The high content of Glu found in ewe cheese, originating from casein, is in accordance with the data found for other (e.g. Cheddar, Manchego, Idiazábal, Mahón) hard cheeses [1, 2, 3]. The proteolysis of β-casein contributed to the high content of free Pro found in the cheese samples. This finding is also in accordance with the literature reported data from similar studies [1]. Leu, Phe and Val as the essential amino acids are present in high content in αs1-casein. In our studies we found higher content of nonessential vs. essential amino acids, Pro and Glu, due to more intensive proteolysis of β-casein than of αs1-casein.

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² “Ruđer Bošković” Institute, Department of Physical Chemistry, Laboratory for Analytical Chemistry, Bijenička c. 54, 10002 Zagreb, Croatia.
4. Conclusions

The study was performed with ewe cheese from the island Krk, which has a specific flavour and smell due to the milk of Croatian autochthonous sheep and their typical extensive farming management which includes grazing of sheep during all seasons with Mediterranean plants. The content of total free amino acids (TFAA) was increasing during cheese ripening process. Predominant free amino acids (FAA) in the cheese during all ripening stages were Glu and Pro (nonessential amino acids) and Leu, Phe and Val (essential amino acids). Our results are in accordance with the data from similar studies, confirming that high levels of mentioned free amino acids might be a common characteristic of hard ewe cheeses.

References


II-P114: Influence of Cheese Technology on Aflatoxin M₁ Interaction with Proteins in Goat and Ewe Milk

T.M.P. Cattaneo¹, L. Monti¹, E.V. Panarelli¹, R. Giangiacomo¹

Summary

Aflatoxin M₁ (AFM₁) is a toxic compound excreted in milk when animals are fed with aflatoxin B₁ (AFB₁) contaminated feed. In cow milk, AFM₁ has been proved to bind preferentially casein and thus changes of the concentration of the contaminant caused by processing shall be taken into account.

The aim of this work was to collect information about AFM₁ affinity for goat and ewe milk proteins, for which few data are available.

We used ELISA method to investigate how cheese technology could affect AFM₁ distribution between acid and rennet curd and whey, and Ricotta cheese obtained from artificially contaminated milk. We also studied AFM₁ distribution between milk proteins in native conditions after ultracentrifugation and ultrafiltration.

AFM₁ showed great affinity for proteins in general and casein specifically and a better capacity to link goat casein in comparison with ewe casein. In Ricotta cheese, it was able to bind whey proteins, too.

1. Introduction

Aflatoxin M₁ (AFM₁) is an hydroxylated metabolite of aflatoxin B₁ (AFB₁), a toxic compound produced by Aspergillus flavus and A. parasiticus on feed and foodstuffs in particular conditions [1]. AFM₁ is excreted in milk and its maximum accepted value has been regulated at 0.050 µg/kg [2]. AFM₁ has been proved to bind preferentially casein [3] and thus, during cheese making by using contaminated milk, toxin is concentrated in the curd. Investigations on the affinity of AFM₁ towards different proteins in ewe and goat milk are very limited, even if this aspect strongly influences toxin recovery in cheese. For milk products, changes of the concentration of the contaminant caused by processing shall be taken into account [2]. So, the aim of this work was to collect information about distribution and Enrichment Factors (EFs) of AFM₁ in the acid and rennet curd produced by artificially contaminated ewe and goat milk, and Ricotta cheese obtained from residual whey, and to investigate if and how technology could affect AFM₁ distribution.

2. Materials and methods

Milk: Raw ewe and goat milk were artificially contaminated with an AFM₁-methanol solution (Sigma Chemical, St. Louis, MO, USA) to obtain three batches at different levels of contamination (50, 100, 150 ppt). Uncontaminated milk (<5 ppt) was used as control.

Coagulation processes: Three different processes in duplicate were taken into account.

-Quark type cheese: acid coagulation was performed by addition of 88% lactic acid to decrease milk pH to 4.3. Precipitated casein was separated by centrifugation at 3000 x g for 10 min.

-Enzymatic coagulation: milk was heated at 60°C for 15 min and refrigerated to 36°C; liquid rennet 1:3000 w/w (strength 1:10 000, chymosine 80%; Cagliificio Clerici, Cadorago, Como) was added. Coagulation was performed in 30 min and the curd was separated by using a suitable mesh.

-Ricotta cheese: residual whey from both coagulation processes were neutralized using 2N NaOH and heated at 90°C; whey proteins were coagulated in the final step by addition of 2N HCl up to pH 5.6. After resting for 15 min, the coagulum was separated by draining.

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Ultrafiltration (UF): UF was performed in an Amicon apparatus using a 10 kDa cut-off membrane until retentate was half the starting volume.

Ultracentrifugation (UC): native casein micelles were obtained by UC at 80 000 x g at 4°C for 45 min; before analysis, they were diluted using 50 mM phosphate buffer, pH 6.8, in order to reach a final volume equal to the starting milk volume.

Samples analysis: AFM$_1$ concentration was determined in all samples by using a commercial ELISA kit (Ridascreen aflatoxin M1, R-Biopharm, Darmstadt, Germany). Both liquid and cheese samples were analysed as recommended by the kit producer.

Data analysis: Enrichment Factor (EF) = \([\text{AFM}_1\text{ product (ppt)}] / [\text{AFM}_1\text{ raw material (ppt)}]\); Percentage distribution = \([\text{AFM}_1\text{ product (ppt)} \times \text{product quantity (kg)}] / [\text{AFM}_1\text{ raw material (ppt)} \times \text{raw material quantity (kg)}] \) * 100

3. Results and discussion

AFM$_1$-casein interaction in ewe and goat milk during cheese production

For both goat and ewe milk, AFM$_1$ concentration in curd rose proportionally to toxin concentration in raw milk, independently from the applied cheese technology. Anyway, Enrichment Factors (EFs) showed a different affinity between AFM$_1$ and casein in milk of different species (Table 1). In curd produced by ewe milk EFs were around 2.3, in good agreement with values found by Battacone et al. after applying a rennet cheese technology [4]. It seemed as if curd capacity in binding AFM$_1$ decreased when toxin level increased in raw milk. In goat curds, EFs were higher than those calculated for ewe cheese, with a clear increase for AFM$_1$ concentrations above 50 ppt. This seemed to indicate a high affinity of goat casein for AFM$_1$. Also cheese technology seemed to influence EFs. Even though at a non-significant level, EFs in rennet curd are higher than those in acid curd, probably as a consequence of the higher quantity of whey which remains trapped in curd if compared to the acid one. Moreover, acidification can produce a modification of charges which consequently affects AFM$_1$-casein binding and increments toxin release in whey.

Table 1: AFM$_1$ concentration in acid and rennet curd and whey obtained from goat and ewe milk transformation; Enrichment Factors obtained in rennet and acid curd

<table>
<thead>
<tr>
<th>Milk [ppt]</th>
<th>Rennet curd</th>
<th>Acid curd</th>
<th>Rennet whey</th>
<th>Acid whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[ppt] ± sd *</td>
<td>EFs ± sd **</td>
<td>[ppt] ± sd</td>
<td>EFs ± sd</td>
</tr>
<tr>
<td>Goat milk</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>108 ± 14</td>
<td>1.4 ± 0.3</td>
<td>74 ± 17</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>357 ± 21</td>
<td>4.6 ± 0.3</td>
<td>237 ± 37</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>535 ± 87</td>
<td>3.9 ± 0.6</td>
<td>447 ± 72</td>
</tr>
<tr>
<td>Ewe milk</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>89 ± 16</td>
<td>2.0 ± 0.4</td>
<td>120 ± 7</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>183 ± 41</td>
<td>2.0 ± 0.4</td>
<td>241 ± 45</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>370 ± 101</td>
<td>2.4 ± 0.6</td>
<td>334 ± 22</td>
</tr>
</tbody>
</table>

* = concentration [ppt] ± standard deviation.
** = Enrichment Factors ± standard deviation.

AFM$_1$-whey proteins interaction in Ricotta cheese production

AFM$_1$ showed a certain affinity also for whey proteins, which are the main constituents of Ricotta cheese (Figure 1). As already evidenced during cheese production, toxin was more retained in goat products, especially in Ricotta cheese produced by rennet whey. In this case, product yield was even lower because whey proteins partially co-precipitated with casein during cheese making, so these data stress the high AFM$_1$-goat proteins affinity. The reduction of the number of binding sites for AFM$_1$ could also explain the higher content of toxin found in the deproteinized whey.
AFM$_1$ distribution between milk proteins in native conditions:

**UF of ewe and goat milk:** Independently from the level of contamination, after UF less than 20% of the toxin was found in permeate, while nearly 80% was retained by the membrane (Figure 2). Considering that AFM$_1$ is a small molecule (MW 328 Da) which could pass membrane cut-off, these results indicated that the toxin was probably retained by a casein-binding with the formation of a stable complex which prevents toxin from passing through.

**UC of ewe milk:** AFM$_1$ concentration in both precipitate and soluble fractions increased proportionally to AFM1 contamination in raw milk (Table 2). Anyway, percentage distribution evidenced an increase of toxin concentration in the soluble fraction and a decrease in casein proportional to AFM$_1$ contamination in milk.

These data seemed to indicate a higher capacity of large casein micelles to bind AFM$_1$ if compared to soluble proteins, and a progressive saturation effect of hypothetical binding sites.
### Table 2: AFM\textsubscript{1} concentration and percentage of distribution in precipitate and soluble fractions obtained from UC of ewe milk

<table>
<thead>
<tr>
<th>Milk [ppt]</th>
<th>Precipitate [ppt]</th>
<th>Precipitate %</th>
<th>Soluble fraction [ppt]</th>
<th>Soluble fraction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>278</td>
<td>67</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>88</td>
<td>425</td>
<td>52</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>154</td>
<td>511</td>
<td>36</td>
<td>142</td>
<td>70</td>
</tr>
</tbody>
</table>

#### 4. Conclusion

AFM\textsubscript{1} showed great affinity for milk proteins in general and casein specifically, also in the case of ewe and goat milk and milk products. Toxin concentrated in curd and, in Ricotta cheese, it was also able to bind whey proteins. Goat casein showed a better capacity to link AFM\textsubscript{1} in comparison with ewe casein, resulting in higher EFs.

#### References


#### Acknowledgements

Authors would like to thank the Zootechnical Sciences Department, University of Sassari, for supplying milk, and Prof. Stefania Iametti and DISMA team, Faculty of Agricultural Science, University of Milan, for the scientific support in performing UC and UF processes.

This work was supported by the finalized project “SISPROLAT” (MiUR-MIPAF, Rome, Italy).
II-P116: Effect of Sodium Chloride and Some Hydrocolloids on the Rheological Properties of Rennet-induced Gels of Skim Sheep Milk

A.R. Pérez-Marqués¹, L. Matía-Merino², M. García-Castillo¹, E. Fernández-Fernández³

Summary
The objective of this research was to investigate the effect of sodium chloride and pectin (widely used hydrocolloid) on the rheological and coagulation properties of sheep milk curd using plant coagulant or chymosin. Rheological properties of the gels were measured by dynamic oscillatory rheometry. Gelation time and strength of rennet-induced sheep milk gels were affected by sodium chloride concentration and pectin. In general, plant coagulant produced less firm gels than calf extract rennet but showed a higher coagulant activity and produced firmer gels at the highest sodium chloride levels. Significant differences were found in the viscoelastic properties of the final gels when pectin (low-methoxyl and high-methoxyl) was used.

1. Introduction
Enzymatic coagulation of milk is the first step in the manufacture of cheeses and curds. Plant coagulants have traditionally been used in Southern Europe to produce the curd in artisan cheesemaking. However, systematic research on sheep milk curd properties produced by the use of plant coagulants has received less attention compared to chymosin-induced curds.

The protein aggregation process that results in curd formation can be followed through rheological measurements. This process may be influenced by various technological parameters and by the presence of added ingredients.

Sodium chloride has an important role in cheesemaking as a preservative and flavour compound. Sometimes this ingredient is added to the milk directly having consequences on coagulation process from a technological and economical point of view. Pectin is a polysaccharide widely used in food systems as a texture agent through their thickening and gelling properties.

2. Material and methods
Sodium chloride (0-0.3M) or pectin (low-methoxyl (LM) and high-methoxyl (HM)) solutions (0-0.20% w/w) were mixed with reconstituted skim sheep milk powder (10% w/w). Calf rennet extract (chymosin min. 95%) and plant coagulant (from Cynara cardunculus L.) were used as clotting agents. Milk gelation experiments were conducted at 30ºC and at the natural pH of milk (pH ~6.60). The amount of each coagulant was fixed in order to obtain a gelation time (Tg) of ~20 minutes in these conditions. Rheological properties of the gels were measured in situ by dynamic oscillatory rheometry (Paar Physica MCR301 rheometer, 4/40 cone and plate geometry). Gelation was monitored at 1 Hz and 0.5% strain at 30ºC. The tests were run for 6 hours. Large deformation properties were also studied (from 0.025 to 1200 Pa).

Statistical analysis was conducted using SPSS program.

3. Results and discussion
Gelation time was not significantly affected by NaCl in a concentration range from 0.001 to 0.1M (plant coagulant) and from 0.001 to 0.05M (chymosin) as shown in Table 1. Above these levels, NaCl severely impaired the coagulation of milk, especially in chymosin-induced gels. The reduction

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on the enzymatic rate could be related to the screening of charges on the enzyme and its substrate that slows down the reaction (Daviau et al., 2000).

**Table 1:** Effect of NaCl concentration on gelation time (Tg) and storage modulus (G’) for skim sheep milk gels induced by chymosin and plant coagulant (*Cynara cardunculus*)

<table>
<thead>
<tr>
<th>NaCl (M)</th>
<th>Chymosin</th>
<th>Plant Coagulant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tg (min)</td>
<td>G’ 6h (Pa)</td>
</tr>
<tr>
<td>0</td>
<td>20.60a</td>
<td>368b</td>
</tr>
<tr>
<td>0.001</td>
<td>25.33a</td>
<td>405a</td>
</tr>
<tr>
<td>0.01</td>
<td>25.37a</td>
<td>388ab</td>
</tr>
<tr>
<td>0.05</td>
<td>30.34ab</td>
<td>372ab</td>
</tr>
<tr>
<td>0.1</td>
<td>36.67b</td>
<td>366b</td>
</tr>
<tr>
<td>0.15</td>
<td>66.19c</td>
<td>302c</td>
</tr>
<tr>
<td>0.3</td>
<td>87.65cd</td>
<td>220d</td>
</tr>
</tbody>
</table>

*abcd* Means with different superscripts within each parameter are different (P<0.05)

A maximum in gel firmness was observed with the addition of 0.001M NaCl for chymosin and the addition of 0.05M NaCl for plant coagulant. This maximum could be explained by an increase in Ca²⁺ activity (exchange of casein-bound Ca²⁺ with Na⁺) and a lower pH. Plant coagulant produced firmer gels (higher elastic modulus G’) above 0.05M NaCl than chymosin-induced gels. Higher levels of NaCl resulted in a decrease in firmness. Changes in micellar size and solvation properties of the micelles due to the screening of charges (Jaubert et al., 1999) could lead to a decreasing number of effective collisions that may account for the weaker curds at high ionic strengths.

In general, the effect of NaCl was less significant in the plant coagulant-induced gelation, probably due to the more extensive and non-specific hydrolytic activity of the enzymes present in this coagulant.

A remarked different effect was observed between both pectins depending on their concentration (Figure 1): whereas added LM pectin resulted in a homogeneous network development up to 0.2% LM pectin, the curds with added HM pectin showed a spontaneous gel shrinkage that lead to the drop of G’ and the loss of the stress signal above 0.1% (as observed previously in acid gels) (Matía-Merino et al., 2004). For both LM and HM pectins, gelation time (Tg) decreased linearly with pectin concentration (from 0.05% to 0.2%).

The ability of LM pectin to gel in the presence of ionic calcium may account for the effects in curd formation as compared to HM pectin.

![Figure 1](image_url)
4. Conclusion

Gelation time and strength of rennet-induced sheep gels were affected by sodium chloride concentration and pectin. For both coagulants, above a critical NaCl concentration, gelation times increased and softer gels were obtained. However, plant coagulant showed a more stable coagulant activity to sodium chloride levels.

Addition of LM pectin at 0.2% level helped to form stronger curds. The same levels of HM pectin caused gel shrinkage and serum release.

References


II-P117: Effect of Milk Cream Homogenization on the Beneficial Fatty Acids in PDO Pecorino Sardo and Ricotta Cheese

A. Pirisi¹, A. Cabiddu¹, M. Pes¹, S. Furesi¹, M. Decandia¹, G. Molle¹, G. Piredda¹, M. Addis¹

Summary

With the aim to develop CLA-rich dairy functional foods, PDO Pecorino Sardo and Ricotta cheeses were manufactured from milk with unhomogenized cream (MUC, control milk) and from milk with homogenized cream at 100 bar (MHC, milk with homogenized cream). Free fatty acids content, vaccenic and rumenic acids partitioning into different products (1 day and 30, 60, 90, 180 days old cheese) and by-products (Ricotta cheese, whey and scotta whey) were determined. Fatty acid (FA) composition was not significantly influenced by cream homogenisation. The vaccenic acid partitioning into different products and by-products was also not significantly influenced by cream homogenisation whilst CLA resulted significantly lower (P<0.05) in whey obtained from MHC. In conclusion the results demonstrate that the level of beneficial FAs in cheese and in particular the content of vaccenic and rumenic acids, depends on that of the milk. Homogenization of the milk cream did not enhance the level of beneficial FAs in the final products.

1. Introduction

CLA refers to a mixture of positional and geometric isomers of linoleic acid with a conjugate double bond system. Conjugate linoleic acid (CLA) and in particular the cis-9, trans-11 isomer (rumenic acid) has been associated with several health promoting activities. Rumenic acid and the trans-11 C18:1 (vaccenic acid, VA) derive by biohydrogenation process of the linoleic acid in the animal rumen. The vaccenic acid is transformed in rumenic acid at the mammary gland level by the Δ-9 desaturase enzyme action. CLA and in particular rumenic acid are unusual but they are abundant in products from ruminant animals. Several studies reported higher levels of CLA in dairy products than in milk, suggesting that processing could increase CLA concentrations. However these did not report the CLA concentration of unprocessed milk from which products were manufactured in order to directly validate how processing may influence CLA concentration. The aim of this work was to verify the influence of cream homogenization on the concentration of rumenic acid and its precursors in the intermediate and in the final-products of the cheese making.

2. Material and methods

Three experimental cheese-making in three different days were conducted. In each experiment, a batch of ewe’s bulk milk was divided into two parts: control milk (CM) and milk with homogenized cream (100 bar, MHC). Both milks were processed to obtain a semi-hard cheese. Ricotta cheese also was produced from whey deriving from the cheese making process. All intermediates and final products were collected and sampled for analytical purpose: milk; cheese at 1 day and 30, 60, 90, 180 days; whey; scotta-whey and Ricotta cheese. Samples were analysed by gas-chromatography in order to determine the concentration of CLA and of vaccenic acid on fat. Fat was extracted according to the method described by Jiang et al. 1996. The lipid transesterification was conducted as reported by Chin et al. 1992.

3. Results and discussion

Fatty acid (FA) composition of cheese, Ricotta cheese and intermediate products of the cheese making, was not significantly influenced by cream homogenisation (Figures 1 and 2). The vaccenic acid partitioning into different products and by-products was also not significantly influenced

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by cream homogenisation (Figure 3) whilst CLA resulted significantly lower (P<0.05) in whey obtained from MHC (Figure 4). However, even if Ricotta cheese is produced from whey, no significant differences were found in its composition with reference to the CLA content.
**Figure 3.** Effect of cream homogenization on partitioning of C18:1 trans 11.

<table>
<thead>
<tr>
<th>Product</th>
<th>VA (mg/g of fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk milk</td>
<td>32.30</td>
</tr>
<tr>
<td>Control milk</td>
<td>32.30</td>
</tr>
<tr>
<td>Cheese (24h)</td>
<td>31.03</td>
</tr>
<tr>
<td>Whey</td>
<td>31.26</td>
</tr>
<tr>
<td>Ricotta cheese</td>
<td>31.02</td>
</tr>
<tr>
<td>Scotta whey</td>
<td>30.75</td>
</tr>
<tr>
<td>Milk after</td>
<td>32.79</td>
</tr>
<tr>
<td>Cream homogenized</td>
<td></td>
</tr>
<tr>
<td>Cheese (24h)</td>
<td>30.66</td>
</tr>
<tr>
<td>Whey</td>
<td>31.72</td>
</tr>
<tr>
<td>Ricotta cheese</td>
<td>30.68</td>
</tr>
<tr>
<td>Scotta whey</td>
<td>31.55</td>
</tr>
</tbody>
</table>

**Figure 4.** Effect of cream homogenization on partitioning of CLA c 9, t 11.

<table>
<thead>
<tr>
<th>Product</th>
<th>CLA (mg/g of fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk milk</td>
<td>15.45</td>
</tr>
<tr>
<td>Control milk</td>
<td>15.45</td>
</tr>
<tr>
<td>Cheese (24h)</td>
<td>15.18</td>
</tr>
<tr>
<td>Whey</td>
<td>13.40</td>
</tr>
<tr>
<td>Ricotta cheese</td>
<td>15.43</td>
</tr>
<tr>
<td>Scotta whey</td>
<td>15.50</td>
</tr>
<tr>
<td>Milk after</td>
<td>15.58</td>
</tr>
<tr>
<td>Cream homogenized</td>
<td></td>
</tr>
<tr>
<td>Cheese (24h)</td>
<td>15.07</td>
</tr>
<tr>
<td>Whey</td>
<td>12.89</td>
</tr>
<tr>
<td>Ricotta cheese</td>
<td>15.60</td>
</tr>
<tr>
<td>Scotta whey</td>
<td>13.88</td>
</tr>
</tbody>
</table>

* p<0.05
4. Conclusion

In conclusion the results demonstrate that the level of beneficial FAs in cheese and in particular the content of vaccenic and rumenic acids, depends from that of the milk. Homogenization of the milk cream did not enhance the level of beneficial FAs in the final products.

Acknowledgements

The authors gratefully acknowledge the technical assistance of S Spada and M Fiori, as well as collaboration of all technical staff working at the laboratories of IZCS. This work was partially funded by the BIOCLA (QLK1-2002-02362) project.

References

II-P118: The Effect of Milk Fat Standardization on Pecorino Sardo Cheese Yield and its Prediction

A. Pirisi¹, A.F. Mulargia², M. Pes¹

Summary

This study investigate the effect of milk fat content, on actual cheese yield and predicted cheese yield, as determined by the Pirisi et al., 2002 formula. Pecorino Sardo cheese was manufactured by the traditional procedure from sheep milk containing about 7.0 (full), 4.6 (medium), 3.8% (low) fat. Least squares analyses of data indicated that higher actual yield (AY) and moisture-adjusted cheese yield (MACY) were obtained from higher fat contents milk (P<0.01). Thus, AY and MACY values decreased as the fat content in cheese milk decreased. The cheese yields predicted using the Pirisi et al., 2002 formula were significantly lower than the corresponding AY for the medium and low fat milk. Fat recovery was higher (P<0.05) for medium and low fat milk than full fat milk. As for the losses in cheese whey, milk fat content did not significantly influence the amount of protein lost in cheese whey. In contrast, fat losses in the bulk whey increased significantly with increasing milk fat content (P<0.01). In conclusion cheese yields were directly related to the fat level of cheese milks. An overall reduction in cheese yield is inevitable in the production of cheese from low-fat milk, since the sum of the casein and fat contents of the milk, the principal components that determine cheese yield, is reduced. Nevertheless an higher yield efficiency was found for low fat milk in relation to lower losses of fat in whey and higher fat recovery in cheese.

1. Introduction

In the past 20 years the commercialization of low fat cheese production around the world has significantly accelerated. Even though the concept of low fat cheese manufacture is not a new idea per se, the emphasis on control of caloric intake, especially in developed countries, in the past years has largely been responsible for the growth in low fat cheese markets. The term low fat cheese generally refers to cheeses whose fat content is lower than its corresponding full fat variety. The composition of milk for manufacturing low fat cheeses differs markedly from that of full fat cheeses in a number of ways. The total fat content of milk is obviously lower, therefore, the percentage of total protein in milk is slightly higher. The net result is lower total solids in the milk. The ratio of casein to fat will also be much higher in milk for low fat cheese making. While it is true that fat in cheese is replaced by moisture, the total yield of cheese (kg cheese per kg milk) is lower for low fat cheeses because the total amount of fat removed is not equal to the amount of moisture added. This study investigate the effect of milk fat content, in the range 3.87 to 7.04%, on actual cheese yield and predicted cheese yield, as determined by the Pirisi et al., 2002 formula.

2. Material and methods

Pecorino Sardo cheese was manufactured by the traditional procedure from Sarda sheep milk containing about 7.0 (full), 4.6 (medium), 3.8% (low) fat (Figure 1). Milk and whey were analysed for fat (Gerber method); dry matter (IDF, 1987); total nitrogen (Rowland). Cheese was analysed after 24 h of its manufacture for fat (Soxhlet extraction) and total nitrogen (Gripon et al. 1975). Data were analysed statistically by SPSS 12.0 for Windows software.

3. Results and discussion

Milk composition for manufacturing low fat cheeses differed markedly from that of full fat cheeses, in particular the ratio of casein to fat was much higher in low fat milk (1.16, 0.93 and

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0.62% respectively for low, medium and full fat milk). Least squares analyses of data (Table 1) indicated that higher actual yield (AY) and moisture-adjusted cheese yield (MACY) were obtained from higher fat contents in milk (P<0.01). Thus, AY and MACY values decreased as the fat content of cheese milk decreased. The yields predicted using the Pirisi et al., 2002 formula (CY=1.31F+1.58P) were significantly lower than the corresponding actual yields for the medium and low fat milk. Plot of residuals clearly indicates that in the case of low fat cheese, yields can be correctly predicted using the Pirisi et al., 2002 formula.

Fat and nitrogen recoveries in cheese are also important in cheese yield. Fat recovery rate was higher (P<0.05) for medium and low fat cheese (Table 1).

As for the matter losses in cheese whey (Table 2), the milk fat content did not significantly influence the content of protein lost in the bulk cheese whey. In contrast, fat losses in the bulk whey increased significantly with increasing milk fat content (P<0.01).

---

**Figure 1.** Scheme of Pecorino Sardo cheese making.
Figure 2. Plot of residuals.

Table 1: Cheese yield parameters (%) calculated on 1-day old cheese

<table>
<thead>
<tr>
<th></th>
<th>Full fat</th>
<th>Medium fat</th>
<th>Low fat</th>
<th>SEM</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat in milk</td>
<td>6.96</td>
<td>4.63</td>
<td>3.83</td>
<td>0.30</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Cheese yield (CY)</td>
<td>18.16</td>
<td>16.27</td>
<td>15.62</td>
<td>0.28</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Adjusted CY(^1)</td>
<td>19.98</td>
<td>17.17</td>
<td>16.19</td>
<td>0.41</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>CY on non-fat cheese</td>
<td>13.18</td>
<td>12.79</td>
<td>12.73</td>
<td>0.11</td>
<td>P&lt;0.24</td>
</tr>
<tr>
<td>Estimated CY(^2)</td>
<td>18.05</td>
<td>15.03</td>
<td>14.20</td>
<td>0.41</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Fat recovery rate</td>
<td>71.59</td>
<td>75.30</td>
<td>75.50</td>
<td>0.70</td>
<td>P&lt;0.03</td>
</tr>
<tr>
<td>Protein recovery rate</td>
<td>74.95</td>
<td>74.60</td>
<td>75.42</td>
<td>0.84</td>
<td>P&lt;0.92</td>
</tr>
</tbody>
</table>

\(^1\)Cheese yield adjusted for moisture to 47%.
\(^2\)Cheese yield estimated by Pirisi et al., 2002 formula.

Table 2: Losses in cheese whey (%)

<table>
<thead>
<tr>
<th></th>
<th>Full fat</th>
<th>Medium fat</th>
<th>Low fat</th>
<th>SEM</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat in milk</td>
<td>6.96</td>
<td>4.63</td>
<td>3.83</td>
<td>0.30</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Total solids</td>
<td>6.60</td>
<td>6.07</td>
<td>6.06</td>
<td>0.07</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Fat</td>
<td>1.68</td>
<td>1.06</td>
<td>0.89</td>
<td>0.08</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>1.14</td>
<td>1.17</td>
<td>1.18</td>
<td>0.02</td>
<td>P&lt;0.94</td>
</tr>
</tbody>
</table>
4. Conclusion

In conclusion cheese yields were directly related to the fat level of cheese milks. An overall reduction in cheese yield is inevitable in the production of cheese from low-fat milk, since the sum of the casein and fat contents of the milk, the principal components that determine cheese yield, is reduced. Nevertheless an higher yield efficiency was found for low fat milk in relation to lower losses of fat in whey and higher fat recovery in cheese.

References

II-P119: Characterization of Goats’ Milk Cheeses Manufactured with the Addition of Adjunct Cultures

M.B. Pisano\textsuperscript{1}, M. Casula\textsuperscript{2}, V. Serci\textsuperscript{1}, A. Corda\textsuperscript{1}, M. Deplano\textsuperscript{1}, M.E. Fadda\textsuperscript{1}, S. Cosentino\textsuperscript{1}

Summary

The microbiological and sensory characteristics of four cheese batches (1, 2, 3 and 4), elaborated using pasteurized milk inoculated with different combinations of cultures consisting of wild strains of \textit{L. lactis} subsp. \textit{lactis} and \textit{L. paracasei}, were monitored throughout ripening. Mean counts of aerobic mesophilic bacteria and LAB were similar in all batches throughout ripening. Levels of coagulase positive staphylococci were below the detection limit of 100 cfu ml\textsuperscript{-1} in all cheese batches; coliforms decreased over the course of ripening and were not detectable after 90 days except in cheeses from batch 2. As for sensory analysis, the cheeses receiving the highest scores for texture, taste and aftertaste throughout ripening were those from batch 4.

This work emphasizes the technological significance of these selected strains and support their use as adjunct cultures in the manufacturing of goats’ cheeses.

1. Introduction

In Sardinia, goats’ cheeses are produced from raw or pasteurized milk on both small and medium scale. The medium scale production techniques generally make use of pasteurized milk inoculated with non-specific commercial starter cultures which often results in the loss of typical characteristics in the finished product. The addition to pasteurized milk of autochthonous cultures would permit the manufacture of a uniform and safe product, and would preserve the quality characteristics of the original product.

As a part of a research project aiming to improve the quality of goats’ milk cheeses in Sardinia, the objective of this study was to study the microbiological and sensorial characteristics of goats’ milk cheeses prepared with the addition of selected autochthonous cultures.

2. Material and methods

Four cheese batches (1, 2, 3, 4) were manufactured, in two dairy plants, using pasteurized goats’ milk inoculated with autochthonous cultures. The cheese batches were prepared using 4 different combinations of cultures consisting of wild strains of \textit{Lactococcus lactis} subsp. \textit{lactis} and \textit{Lactobacillus paracasei} subsp. \textit{paracasei}. The strains were isolated from the native microflora of raw goats’ milk and selected on the basis of their physiological and biochemical properties relevant to their technological performance.

Pasteurized milk and 2, 15, 30 and 90 day-old cheese samples, were analyzed. Cheese homogenate, decimal dilutions, plating procedures and enumeration of total mesophilic bacteria, coliforms, coagulase positive staphylococci, lactococci, enterococci and lactobacilli were performed as previously described [3].

Cheese samples at 30 and 90 days of ripening were subjected to sensory evaluation by a 6-members panel. The qualities judged were: cheese shape, cheese rind, colour, interior openings, texture, smell, taste and aftertaste, scoring on a scale from 1 to 10 (1: very poor, 10: very good), as previously described [2].

3. Results and discussion

The changes in the microbial counts throughout ripening of the cheeses are shown in figure 1.

Total mesophilic bacteria increased in the first 15 days of ripening and remained constant throughout ripening in all batches, showing an evolution similar to the total Lactic Acid Bacteria (LAB). This reflects the fact that LAB were the predominant flora during the ripening of the cheeses.

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\textsuperscript{2} Labam, Laboratorio di Analisi Chimiche e Microbiologiche, Dolianova, Cagliari, Italy.
Maximum counts of coliforms were observed on the first days of ripening for all batches then they rapidly declined throughout ripening and only in batch 2 were detected after 90 days of ripening. The levels of coagulase positive staphylococci were below the detection limit of 100 cfu ml⁻¹ in all cheese batches.

All batches had high counts of presumptive lactobacilli throughout ripening with values around 9-10 log cfu g⁻¹ being reached after 15 days of ripening. The permanence of a lactobacilli population close to 10 log cfu g⁻¹ at the end of ripening indicates that the lactobacilli strains selected for use as adjunct cultures survived well in the cheeses and confirmed their importance during ripening.

Presumptive lactococci attained their maximum values in the first days of ripening then they decreased in a similar way in all batches up to 30 days of ripening.

Starting out at similar levels (3 log cfu g⁻¹) enterococci counts increased during the first 15 days of ripening then gradually decreased till the end of ripening in all batches. Similar changes in LAB counts were reported by Herreros et al., [1] in a study on the effect of adjunct cultures on microbiological characteristics of Armada goats’ milk cheese.

Figure 2 shows the sensory characteristics of the four batches of goats’ milk cheese. In general, all batches received favourable scores particularly for cheese shape, cheese rind, texture and cut appearance. The cheeses receiving the highest scores for texture, taste and aftertaste throughout ripening were those from batch 4.

**Figure 1.** Changes in microbial counts (log cfu g⁻¹) throughout ripening of goats’ milk cheeses in the four batches (1-4).
4. Conclusion

Based on the preliminary results obtained from the microbiological and sensorial analyses, the wild strains used to manufacture batches 1, 3 and 4 produced cheeses with good microbiological and sensorial characteristics. In line with the observations of several authors, the utilization of natural adjunct cultures in the manufacturing of typical cheeses appears to be a promising tool in responding to the increasing demand for products with improved quality, safety and sensory characteristics.

References


II-P120: Effect of Clarification on Chemical Composition of Caprine Whey Protein Concentrates Produced by Ultrafiltration

B. Sanmartín, O. Díaz, L.R. Turienzo, A. Cobos

Summary

The purpose of this study was to evaluate the effect of clarification by thermocalcic precipitation on chemical composition of caprine whey protein concentrates produced by ultrafiltration followed by diafiltration. Sweet caprine whey was ultrafiltrated followed by diafiltration or was submitted to thermocalcic precipitation. The aggregates were separated, and the clarified whey was ultrafiltrated and diafiltrated. The clarification procedure improved the protein content of the powders. In this way, the protein content of the clarified powders was 56.73%, and the content of untreated powders was 29.88%, whereas the aggregates only have 5.35%. The amount of lipids decreased from 57.35% in powders without clarification to 8.81% in clarified powders, while the lipid content of aggregates was 40.95%. The levels of ash were higher in aggregates (10.72%) than those observed in clarified and unclarified powders (1.15 and 0.65% respectively).

1. Introduction

Bovine whey is usually transformed into whey protein concentrates and whey protein isolates, due to the high nutritional and functional properties of its proteins. However, the information about caprine cheese whey transformation is not abundant. Casper et al. [1] reported the superiority of caprine whey protein concentrates in what concerns functional properties. There are no studies about clarification of caprine whey before ultrafiltration treatment.

The purpose of this study was to evaluate the effect of the clarification by thermocalcic precipitation on the chemical composition of caprine whey protein concentrates produced by ultrafiltration (UF) followed by diafiltration (DF).

2. Material and methods

Sweet caprine whey was obtained from a local industry. Approximately 80 l of whey were used in each trial. 30 l were submitted to UF and DF, and 50 l, after determining the calcium content by Spinreact Diagnostics Kit, were used for thermocalcic precipitation which was performed according to the method described by Fauquant et al. [2]. The calcium content of the whey was adjusted to 1.2 g/l with CaCl₂, the pH adjusted to 7.3 with NaOH 10N and the temperature was quickly raised to 50ºC, and maintained at this value for 8 minutes. The whey was cooled to 4-6 ºC and kept overnight. The next day, the aggregates were separated and the clarified whey was submitted to UF and DF using a Centramate lab tangential flow system equipped with an Omega (polyethersulfone) membrane cassette (0.09 m² surface area, 10 kDa MW cut-off) (Pall Corporation, Ann Arbor, MI, USA). The membrane retentates and the aggregates were lyophilized using a freeze drier. All experiments were made in triplicate. The chemical compositions of these powders and of the original cheese whey were determined as follows: dry matter by over drying at 105ºC for 12 h [3], ash by incineration at 550ºC for 6 h [3], protein by the Bradford method [4] and lipids according to the method described by Hanson and Olley [5]. The lactose was determined by difference. The results were expressed in % of wet matter.

3. Results and discussion

The chemical composition of caprine cheese whey is shown in Table 1. The mean value for pH of the caprine whey was 6.33. The chemical composition of goat cheese whey was: dry matter 7.05%, lipid 2.03%, ash 0.57% and protein 0.44%. The protein, lactose and ash contents were

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lower and the lipid and dry matter contents were higher than those reported by Casper et al. [6] in caprine wheys. These different results are probably due to the chemical composition of whey depends on chemical composition of the milk and cheese-making parameters.

The chemical composition of dry products obtained from caprine cheese whey is also shown in Table 1. The dry matter contents of the powders were higher than 95%. The three types of powders showed differences in their chemical composition. The most relevant result was that the protein content of the clarified powders (56.73%) was higher than those of unclarified and clarification aggregates powders (29.88 and 5.35%, respectively). This result indicates that the clarification procedure improves the protein content of the powders. This level of protein was lower than that observed by Pereira et al. [7] in ovine cheese whey when it was submitted to thermocalcic precipitation and microfiltration through a 0.65 μm pore size membrane before ultrafiltration+diafiltration and higher when a 0.20 μm pore size membrane was used. The amount of lipids decreased from 57.35% in unclarified powders to 8.81% in clarified powders while the level in aggregates was 40.95%. The levels of ash were higher in aggregates (10.72%) than those in clarified and unclarified retentate powders (1.15 and 0.65% respectively). The lactose content was higher in clarified powders (28.69%) than in the unclarified powders (9.68%), whereas the aggregates had the highest content (38.11%).

<table>
<thead>
<tr>
<th></th>
<th>Whey</th>
<th>Clarification Aggregates Powders</th>
<th>Clarified Powders</th>
<th>Unclarified Powders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>7.05 ± 0.27</td>
<td>95.12 ± 1.23</td>
<td>95.38 ± 1.13</td>
<td>97.56 ± 0.68</td>
</tr>
<tr>
<td>Lipids</td>
<td>2.03 ± 0.27</td>
<td>40.95 ± 6.65</td>
<td>8.81 ± 2.65</td>
<td>57.35 ± 9.71</td>
</tr>
<tr>
<td>Ash</td>
<td>0.57 ± 0.02</td>
<td>10.72 ± 1.23</td>
<td>1.15 ± 0.10</td>
<td>0.65 ± 0.27</td>
</tr>
<tr>
<td>Protein</td>
<td>0.44 ± 0.03</td>
<td>5.35 ± 0.54</td>
<td>56.73 ± 2.75</td>
<td>29.88 ± 5.86</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.01 ± 0.53</td>
<td>38.11 ± 5.25</td>
<td>28.69 ± 1.43</td>
<td>9.68 ± 3.21</td>
</tr>
</tbody>
</table>

4. Conclusion

The clarification by thermocalcic precipitation increases the protein content and decreases the lipid content of caprine whey protein concentrates.

References

II-P121: Effect of Ultra-high Pressure Homogenisation on Microbial and Rennet Coagulation Properties of Goats Milk

A.J. Trujillo¹, A. Zamora¹, J. Pereda¹, J.M. Quevedo¹, B. Guamis¹

Summary

The effect of ultra-high pressure homogenisation (UHPH) on microbial and rennet coagulation properties of goat milk was studied. Milk was subjected to single or two-stage UHPH (100/200/300 MPa and 30 MPa on the primary and secondary valves, respectively) using a Stansted High Pressure homogeniser with inlet temperatures of 30ºC and 40ºC. The microbiological quality of raw and UHPH-treated milks was studied by enumerating total bacteria, psychrotrophic bacteria, coliforms, lactococci, lactobacilli and enterococci. Particle size distributions and pH of UHPH-treated milks were compared to raw and conventionally treated milks. Studied coagulation properties were rennet clotting time (RCT), rate of curd firming (RCF) and curd firmness (CF).

From the analyses performed, it can be deduced that single-stage UHPH at 200 MPa was the best treatment to enhance the microbial and technological properties of goat milk.

1. Introduction

Ultra-high pressure homogenisation is based on the same principle that of conventional ball-and-seat homogenisers, but current developments in the valve design and materials allow to reach pressures of 350 MPa. There has been an increasing interest in the application of UHPH in food technology as a minimal process for the production of a wide variety of safe and nutritious foods. Possible uses of this technology for the dairy industry include reduction of fat globule size, inactivation of enzymes, and destruction of bacteria (Briñez et al., 2006; Pereda et al., 2007; Zamora et al., 2007).

The aim of this work was to determine the effect of UHPH on the microbiology and rennet coagulation properties of goat's milk.

2. Material and methods

Ultra-high pressure homogenisation of raw goat milk was performed with a Stansted high-pressure homogeniser (model FPG11300, Stansted Fluid Power Ltd., Essex, UK). Milk was UHPH-treated under the following conditions: single or two-stage UHPH (100/200/300 MPa and 30 MPa on the primary and secondary valves, respectively) with inlet temperatures of 30ºC and 40ºC. Microbial and rennet coagulation properties of treated samples were compared to those of raw and conventionally treated milks (pasteurisation at 72ºC for 15 s and homogenisation-pasteurisation at 18 MPa and ~60ºC, and 15 s at 72ºC).

Microbiological analysis

The microbiological quality of treated and untreated milk was assessed by enumerating total bacteria, psychrotrophic bacteria, coliform, lactobacilli, lactococci and enterococci as Pereda described elsewhere (2007).

Particle size distributions and rennet coagulation properties

The particle size distribution in milk samples was determined using a Beckman Coulter laser diffraction particle size analyser (LS 13 320 series, Beckman Coulter, Fullerton CA, USA). The coagulation of warmed milks by recombinant rennet was carried out at 32ºC for 30 min. Rennet coagulation properties (rennet coagulation time (RCT), rate of curd firming (RCF) and curd firmness at 30 min (CF)) were assessed in triplicate by the Optigraph® system (Ysebaert Inc, Frepillon, France).

¹ Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA), CeRTA, XIT, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra.
3. Results and discussion

UHPH treatments at 100 MPa were able to obtain a significant reduction in the counts of all microorganisms studied compared to raw milk, which initial counts were 6.5 log cfu/ml for total bacteria, psychrotrophic and lactococci, 3.6 for lactobacilli and 5 for coliforms and enterococci. However, the reductions were small (~3 log cfu/ml concerning coliforms and <1 log cfu/ml for the other microorganisms). Above 200 MPa, important reductions were achieved (4.6 – 5.2 log cfu/ml) in relation to psychrotrophic, lactococci and total bacteria. In addition, coliforms, lactobacilli and enterococci were completely destroyed.

Although pasteurised milk showed similar RCF and CF to that of raw milk, its RCT was higher than that of raw milk. Both milks showed similar pH and particle distributions. Conventionally homogenised-pasteurised milks showed the lowest CF. Concerning UHPH, the difference in inlet temperature affected only the distribution of milks treated at 100 MPa. The results of RCF showed a similar pattern to those of CF. Even if milks treated at 100 MPa showed lower pH, their rennet coagulation properties were similar to those of raw milk. Strongest gels were obtained by single-stage UHPH-treated milks at 200 MPa. However, two-stage UHPH diminished both RCF and CF. Particle distributions showed that the secondary stage at 200 MPa enhanced coalescence. Although UHPH-treated milks at 300 MPa showed similar RCT to that of pasteurised milk, their CF were much lower.

These results are similar to those obtained in previous studies with UHPH-treated cow milk (Pereda et al., 2007; Zamora et al., 2007).

4. Conclusion

Single-stage UHPH at 200 MPa was efficient in reducing the studied bacterial populations and enhanced the rennet coagulation properties suggesting possible application of UHPH technology for cheesemaking from goat milk.

References

II-P123: Design of Two Different Technologies for the Production of Argentinean Sheep Cheeses

C. Bergamini\textsuperscript{1}, C. Meinardi\textsuperscript{1}, S. Bernal\textsuperscript{1}, V. Wolf\textsuperscript{1}, M. Busetti\textsuperscript{2}, C. Zalazar\textsuperscript{1}

Summary

Two different technologies for the production of sheep cheeses with milk from Pampinta breed were developed. A first protocol was focused in the production of a cheese characterised by a soft flavour and low proteolysis level, a second protocol was directed to obtain an extra-ripened product with intense flavour and high level of proteolysis.

1. Introduction

Ovine milk farm activity was introduced in the country by European immigrants around 1940, but this activity is quite important only since 1980. (McCormick and Lynch, 2003).

Produced cheeses have no national identity and generally they belong to semi-hard type. Pampinta breed is an Argentinean development (INTA) from Frisona (75%) and Corridale (25%) with good meat and milk yields. (McCormick et al 2004)

The lack of an Argentinean typical sheep’s cheese and the scarce information about the making technology and ripening, were the reasons to develop this work, using milk from Pampinta sheep as raw material. The objective of the present work was to develop different cheese making protocols in order to produce cheeses of standardised and constant features.

2. Material and methods

40L of Pampinta (INTA, Anguil, La Pampa Argentina) bred milk (Fat 4.15\% ± 0.7; Protein 5.37\% ± 0.35) was batch pasteurised at 65\(^\circ\)C during 20 minutes. Calcium chloride (0.02\%w/v) was added after cooling at 38\(^\circ\)C. The milk was finally divided in two portions in order to produce two different cheese types (S and L). Seven manufactures were made for each type of cheese. Technological characteristics of S and L cheeses can be seen in Table 1.

Curd was then put in forms about 700g each one, pressed during 18 h and salted during 7 h in brine (20\% w/v) at 12\(^\circ\)C. Cheeses were ripened until six months (12\(^\circ\)C; 80\% RH).

\begin{itemize}
\item \textbf{ANALYSIS SCHEDULE}
\item 180 days
\item Informal sensory evaluation
\item 2 and 180 days
\item Humidity
\item 2 days
\item Fat and proteins
\item 2, 45, 90 and 180 days
\item Starter population
\item Electrophoresis
\item Nitrogen fractions and pH
\end{itemize}

\textsuperscript{1} Instituto de Lactología Industrial, Facultad de Ingeniería Química (UNL), Santa Fe, Argentina.
\textsuperscript{2} Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Anguil, La Pampa, Argentina.
3. Results and discussion

Technological differences between S and L cheeses did not influence the humidity values in 2 days, 42.8 and 42.7% respectively.

In both cheeses, S and L, the humidity diminished significantly during ripening. In 180 days the humidity decreased about 32%. (28.8 and 29.1% respectively)

Fat and total proteins do not showed differences between cheeses S and L in 2 days. (30.2 and 21.8% for S cheeses and 30.0 and 23.1% for cheeses L)

The high acidifying activity of lactobacilli was responsible of the low pH in cheeses L (5.47 versus 5.00), but no differences in pH were observed during ripening both for S and L cheeses.

Yield expressed as kg of cheeses produced by 100L of milk was about 17%.

Streptococcus thermophilus counts were not different between cheeses S and L during ripening, but a decrease of about two orders was observed in 180 days.

A decrease of four orders was observed in lactobacilli count (L. bulgaricus + L. helveticus) for L cheeses between 2 and 90 days. In 180 days these microorganisms were not detected.

Soluble nitrogen at pH 4.6 was not different for cheese S and L at any ripening time.

TCA and PTA soluble nitrogen fractions were however, significantly different (p<0.05) for S and L cheeses at any ripening time. L cheeses showed the highest values.

Electrophoretic patterns (Fig. 1) showed that β-casein was not hydrolysed during ripening, both in S and L cheeses, and αs-1 casein was more degraded in L cheeses through the ripening. As a consequence, the concentration of αs-1 casein was higher in cheeses L than in cheeses S. S and L cheeses were informally evaluated in 180 days of ripening by a non trained panel.

Table 1: Technological characteristics of S and L cheeses

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<tr>
<th></th>
<th>Cheeses S</th>
<th>Cheeses L</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVS Starter</td>
<td>Streptococcus thermophilus 100%</td>
<td>Streptococcus thermophilus 60%</td>
</tr>
<tr>
<td>(Added in order to</td>
<td></td>
<td>Lactobacillus bulgaricus 20%</td>
</tr>
<tr>
<td>reach in milk 10⁶ UFC mL⁻¹)</td>
<td></td>
<td>Lactobacillus helveticus 20%</td>
</tr>
<tr>
<td></td>
<td>(Chr. Hansen, Argentina)</td>
<td></td>
</tr>
<tr>
<td>Milk coagulant</td>
<td>Corn-size</td>
<td>Rice-size</td>
</tr>
<tr>
<td></td>
<td>Maxiren 150 (0.014 g L⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Curd size particles</td>
<td>10% of whey was replaced by hot water (60ºC)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curd washing</td>
<td>1ºC min⁻¹; 43ºC</td>
<td>1ºC min⁻¹; 47ºC</td>
</tr>
<tr>
<td>Rate and temperature of heating</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Electrophoretic patterns for L and S cheeses during ripening
All of the panel members agree that L cheeses had more intense flavour than cheeses S. In relation to S cheeses, as a consequence of its low humidity level and its light sensory characteristics, the conclusion of the panel was these cheeses must be consumed with a minor time of ripening.

Parameters evaluation during cheese ripening showed a higher ripening degree and a lower pH for cheeses L than for cheeses S.

Despite of no differences were found between S and L cheeses for the soluble nitrogen fraction at pH 4.6, electrophoresis of insoluble fraction at pH 4.6 showed a high degradation of $\alpha_s$ casein for L cheeses. As a consequence, a high production of $\alpha_s$I casein was also observed for these cheeses.

The greater availability of $\alpha_s$I casein in cheeses L, in association with proteases from the lysis of lactobacilli, totally disappeared in 90 days, were probably the reasons of the high level of TCA and PTA soluble nitrogen fractions in these cheeses.

4. Conclusions

In the present work a standardised method for the production of sheep cheeses was developed. This method allows the manufacture of cheeses with a constant quality.

Two cheeses types with different characteristics were obtained. The first one had a high proteolysis level with a more intense flavour and long time of ripening. The second one had less flavour and short ripening time.

The results of this work are a contribution to the best knowledge of the production and ripening of sheep cheeses in Argentina.

Another remark was the utilisation of milk form Pampinta, a typical national bred, as raw material. This milk allows the production of good quality cheeses with satisfactory yields.

References

The Challenge to Sheep and Goats Milk Sectors
Posters of an International Symposium, April 18-20, 2007, Alghero - Sardinia, Italy

**Foreword**
(appears in every part)

**Part 1: Posters I-P001 to I-P043**

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ABSTRACT

Scientific posters presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors, 18-20 April 2007, Alghero, Italy. Presented in 4 sessions related to (1) raw milk, (2) processing and product, (3) characteristics of ewe’s and goat’s milk products and (4) market and perspectives.

Keywords: Acids; Artisanal; Breeding; Casein; Cheese; CLA; Ewe; Fat; Functional; Goat; Lactation; Lamb; Livestock; Market; Marketing; Milk; Non-bovine; Nutrition; Processing; Production; Protein; Quality; Raw; Rennet; Separation; Sheep; Technology; Udder; Vitamin; Yield

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* .................................................. Usually double quotes and not single quotes
? ! .................................................. Half-space before and after question marks, and exclamation marks
± .................................................. Half-space before and after microorganisms
Infra-red ...................................... Without a hyphen
et al. .............................................. Not underlined nor italic
e.g., i.e., ......................................... Spelled out in English - for example, that is
litr .............................................. Not liter unless the author is American
ml, mg, .......................................... Space between number and ml, mg,
sulfuric, sulfite, sulfate ...................... Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC international ........................ Not AOAC
programme ................................... Not program unless a) author is American or b) computer program
milk and milk product ..................... rather than “milk and dairy product” - Normally some latitude can be allowed in non scientific texts
Decimal comma ................................ in Standards (only) in both languages (as agreed by ISO)
No space between figure and % - i.e. 6%, etc.
Milkfat ......................................... One word
USA, UK, GB .................................. No stops
Figure ......................................... To be written out in full
1000-9000 ...................................... No comma
10 000, etc. ................................... No comma, but space
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second .......................................... ø s
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