Accelerated Ripening of Iranian UF White Cheese Using Adjunct Cultures

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ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form
1 July 2014
Accepted 31 August 2014
Available online 15 September 2014

Keywords:
White UF cheese, Acceleration, ripening, Adjuncts

ABSTRACT

The effect of an adjunct culture (R-704 containing Lactococcus lactis subsp.cremoris and Lactococcus lactis subsp.lactis) on the ripening factors in Iranian White UF Cheese was studied. It was thought to play a role in the acceleration of cheese ripening. To investigate this hypothesis UF cheese was manufactured using two adjunct systems. Adjunct system A contained a blend of a standard starter system and also the adjunct culture (R-704). System B contained only the mentioned adjunct culture (R-704). A control made using the standard procedure was also prepared. Assessment of proteolysis, as examined by polyacrylamid gel electrophoresis of cheese, pH 4.6-SN/TN and NPN during 60 days of ripening showed significant (P<0.01) differences between the control and experimental cheeses, so that experimental cheeses (particularly experimental cheese A) had consistently higher levels of soluble nitrogen in pH4.6 and hydrolysis of caseins than did the control throughout ripening.

INTRODUCTION

The ripening of UF cheese from five-fold or fully concentrated milk proceeds much more slowly[3]. Many UF cheese varieties exhibit slower flavor development than their traditional counterparts (Bastian et al,1991). This means that protein breakdown are retarded relative to traditional cheese. Many studies on this phenomenon have demonstrated that this is due mainly to a very slow breakdown of β-casein in the UF cheese because of the presence of the whey proteins that inhibit proteolytic enzymes (Bastian et al,1991., Bastian et al,1993., Bech,1993., Benfeldt, 2006). They also bind flavor compounds, dilute the casein matrix and increase aqeous phase viscosity (Bastian et al,1991). Whey proteins inhibit plasmin, chymosin, microbial enzymes and rennets and probably other proteinases and peptidases (Hesari et al,2006., Groppin e tal,1985). The primary hydrolysis of β-casein is mainly catalysed by plasmin. Much evidence suggests that the action of plasmin in UF cheese is inhibited by β-lactoglobulin (Bech,1993., Benfeldt,2006., Rao &Renner,1989) and this is on of the reasons for slower ripening of UF cheese.

With advancing technology and changing markets, new methods for accelerated ripening systems are emerging. These include: elevated temperature, high-pressure processing, addition of enzymes, attenuated starter cultures and adjunct cultures (Law,2001). An understanding of the reasons for the slower ripening of UF cheese opens up the possibility of improving flavor development in such cheeses. Studies have shown that it is possible to accelerate cheese ripening by addition of adjuncts (Bintsis & Robinson, 2004., Hannon et al,2006., Hannon et al, 2003., McSweeney, 2004., Lynch et al,1999., Michaelidou et al, 2003a., Michaelidou et al, 2003b.,Ortigosa et al, 2006., Verachia, 2005). The use of adjuncts inaddition to normal starter cultures in cheese-making can result in higher concentrations of enzyme in cheese. Many researchs have reported the inclusion of various strains of Lactobacilli and Lactococi as adjuncts to improve flavor development and accelerate cheese ripening. In general such adjuncts modified proteolysis, resulting in the formation of high concentrations of small peptids and particularly free amino acids (FFA) in the final cheese and improved flavor compared to the control cheese (Hannon et al,2003., Michaelidou et al,2003a).

The objective of the present study, was to investigate the effect of the adjunct culture R-704 on the proteolysis and accelerated ripening in Iranian White UF Cheese.
MATERIALS AND METHODS

2.1. Adjunct culture:
A commercially available mesophilic culture was used as an adjunct culture (R-704, Chr. Hansen's Laboratorium, Copenhagen, Denmark). The R-704 culture contains two *Lactococcus lactis* strains; one is subsp.cremoris and the other one is subsp lactis.

2.2. Cheese manufacture:
UF cheese was made in triplicate using three different starter systems as described in table 1 by a standard UF cheese-making procedure, in Iran Dairy Industry Inc., Pegah Co.,(Tabriz, Iran).

Table 1: Summary of starter culture systems used in the manufacture of UF cheese

<table>
<thead>
<tr>
<th>System</th>
<th>Starter</th>
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<tbody>
<tr>
<td>A</td>
<td>R-704 and Normal starter system</td>
</tr>
<tr>
<td>B</td>
<td>R-704</td>
</tr>
<tr>
<td>C(control)</td>
<td>Normal starter system</td>
</tr>
</tbody>
</table>

R-704=L.lactissubsp. lactis and L lactis subsp. cremoris
Normal starter = mesophilic and thermophilic starters in the ratio of 7:1 respectively(by Laboratorium Visby, Tender Asp, Denmark)

Raw milk was standardized to 3.5% fat, bactofugated in two steps, pasteurized at 72°C for 15s and then ultrafiltered at 50°C. The membrane cartridges were of the spiral wound type (NoUFPH InvensysAPV, Silkeborg, Denmark) and had a nominal molecular weight cut-off of approximately 20 kg.mol$^{-1}$ with a surface area of 16.9m$^2$. The ultrafiltration unit was operated at an inlet pressure of 5.3 bar and an outlet pressure of 1.7 bar. The retentate was pasteurized at 78°C for 60s and then cooled to 35°C inorder to add starter culture (3%). After adding fugi rennet (Renco, Eltham, New Zealand) (30 mg.kg$^{-1}$), retentate was filled (450g) into containers and passed through the 30°C room for 20min to be coagulated. After putting parchment papers on the top of the coagulum, dry salt (3%) was added. The containers were sealed with aluminum foils and held at 26-28°C for 24h and then transferred to a cool room (8°C). The next day was considered as the first day of ripening. The production was repeated in 3 different days. The three types of UF-cheese were sampled at 1, 15, 30, 45 and 60 days after ripening.

2.3. Compositional analysis:
Moisture content of cheeses (IDF, 1982), fat (Marshal,1992), salt (Fox,1963) and total protein by the macro-kejeldal method (IDF,1964) was measured. pH was determined by direct inserting of pH meter probe(Model No.209, Hanna, Portugal) into cheese.

2.4. Proteolysis assessment:
Proteolysis was assessed on 1-, 15-, 30-, 45- and 60-day old cheeses by techniques detailed below.

2.4.1. Soluble nitrogen (SN):
Proteolysis was monitored throughout ripening by determinations of the levels of soluble nitrogen at pH 4.6 (pH 4.6 –SN) (Kuchroo and Fox,1982).

2.4.2. Non protein nitrogen (NPN):
NPN fraction was measured as described by Kuchroo and Fox (1982). The levels of SN and NPN expressed as a percentage of total nitrogen (%SN/TN and %NPN/TN).

2.4.3. Electrophoresis:
Cheese N fraction insoluble at pH 4.6 was analysed by Urea-Polyacrylamide Gel Electrophoresis (Urea-PAGE) in a Protean II XI vertical slabgelunit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method of Shala\-bi and Fox (1987). Proteins were stained with Coomassie Blue G-250.

2.5. Statistical Analysis:
The experiment was conducted to evaluate the influence of the following three treatments: A, cheese made with the both adjunct culture and normal starter; B, cheese made with only adjunct and C, control cheese made with out adjunct culture. There were three replicate trials for each treatment. A randomized complete block design which incorporated the three treatments and three blocks (trials) was employed for this study. The analysis of variance by 1-way ANOVA (significant level($P<0.01$)) was performed using the SPSS program.
Differences between the means were compared at the 5% level of significance using least significant difference (LSD) test.

RESULTS AND DISCUSSION

3.1. Compositional analysis:

The composition of control and experimental cheeses was similar (Table 2), with the only exception of pH value. No significant differences ($P<0.01$) existed between the cheeses for fat, moisture, salt and protein. However, inclusion of the adjunct culture resulted in significantly lower pH values ($P<0.05$) in the experimental cheeses made with starter system A compared to B and C. Rodriguez et al. (1996) and Verachia (2005), concluded that pH decreased in experimental cheeses manufactured by adjunct cultures.

Table 2: Composition of 1-day old experimental ultrafiltered (UF) Iranian White Cheeses using starter systems A, B or C. Values presented are the means from three replicate trials ± standard deviations.

<table>
<thead>
<tr>
<th>Cheese code</th>
<th>Moisture(%)</th>
<th>Protein(%)</th>
<th>Salt(%)</th>
<th>Fat(%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64.75±0.07</td>
<td>12.35±0.87</td>
<td>3.73±0.12</td>
<td>17.82±0.32</td>
<td>4.47±0.03</td>
</tr>
<tr>
<td>B</td>
<td>65.23±0.23</td>
<td>11.27±0.77</td>
<td>3.09±0.10</td>
<td>18.01±0.42</td>
<td>4.67±0.01</td>
</tr>
<tr>
<td>C</td>
<td>63.56±0.36</td>
<td>12.20±0.52</td>
<td>2.99±0.08</td>
<td>17.91±0.44</td>
<td>4.53±0.07</td>
</tr>
</tbody>
</table>

F value: NS NS NS NS *

Cheese code: A, both adjunct and normal starter; B, only adjunct culture; C, with out adjunct culture

3.2. Proteolysis Assessment:

3.2.1. Soluble Nitrogen:

Levels of SN-pH 4.6 increased during ripening, as a consequence of proteolysis, in all UF-cheeses (figure 1).

The levels of pH 4.6-SN/TN in the cheese A were significantly higher than the cheeses with out adjunct culture ($P<0.01$).

At all ripening times the differences in pH 4.6-SN/TN between A and C were significant, while those between B and C, and A and B were not (figure 2).

Michaelidou et al. (2003a), found that proteolysis was significantly enhanced when Lc.lactis and Lc.cremoris was used as an adjunct in low fat feta cheese.

Study on low fat kefalograviera-type cheese using this adjunct culture showed the same results (Michaelidou et al., 2003b).

Fig. 1: Formation of pH 4.6-soluble nitrogen as a percentage of total nitrogen(SN/TN) in UF Iranian white cheeses with both adjunct and normal starter(A), only adjunct culture(B) and with out adjunct culture(C), during ripening.
Fig. 2: Comparisssion of means of levels of pH 4.6-soluble nitrogen as a percentage of total nitrogen (SN/TN) in UF Iranian white cheeses with both adjunct and normal starter (A), only adjunct culture (B) and with out adjunct culture (C), during ripening ($P<0.05$).

3.2.2. Non protein nitrogen (NPN):

Non protein nitrogen fraction contains medium and small-sized peptides, amino acids and smaller N compounds such as amins, urea and ammonium which are the products of secondary proteolysis of cheese and produced mainly by the action of enzymes from starter system.

%NPN/TN showed a continuous increase throughout ripening. The results of the significantly ($P<0.01$) high levels of NPN in cheeses A and B, suggest that the adjunct culture in particular has a more active peptidilytic system. This enzymatic ability significantly influenced the production of small peptides and FFA during ripening. After 60 days of ripening, levels of NPN/TN in cheeses A and B were similar (22.25 and 20.49%) while levels in C were 17% (figure3).

Fig. 3: Comparisssion of means of levels of non protein nitrogen as a percentage of total nitrogen (NPN/TN) in UF Iranian white cheeses with both adjunct and normal starter (A), only adjunct culture (B) and with out adjunct culture (C), during ripening ($P<0.05$).

The use of *Lactococcus lactis* subsp. cremoris and *Lactococcus lactis* subsp. lactis resulted in an increased production of FFA and small peptides in feta cheese (Michaelidou et al, 2003a), Lynch et al(1999) and Cagno et al(2006), reported similar results about cheddar and caciotta cheeses.

3.2.3. Electrophoresis:

Electrophoretic profiles of pH 4.6-insoluble N fraction of control and experimental cheeses of trial 1 are shown in Fig.4.
Fig. 4: Urea polyacrylamide gel electrophoretograms of experimental UF Iranian white cheeses with both adjunct and normal starter(A), only adjunct culture(B) and with out adjunct culture(C), after 1,15,30,45 and 60 days of ripening.

There were notable differences in electrophoretograms of the three cheese types. In general, degradation of β-CN is slower than αs1-CN in all kinds of cheese specially in UF-cheeses(Bech,1993). Proteolysis of αs1-CN is similar in traditional and UF cheese but of β-CN is slower in UF cheeses(Bastian,1993).

According to the electrophoretograms, β-CN remained almost intact in control cheeses during ripening, however the two experimental cheeses showed more degradation of β-CN, specially it was notable in cheeses A during the first 15 days of ripening which agreed with the similarity observed in the levels of SN-pH 4.6 fraction. Plasmin that has the important part in proteolysis of β-CN is affected by the inhibitors of whey proteins and this is responsible for the decreased degradation of β-CN in these cheeses(Bech,1993., Benfeldt,2006). It seemed that, the applied adjunct culture could be effective, to some extent, on the proteolysis of β-CN.

Proteolysis of αs1-CN was less affected by the applied starter systems and there were no sensible differences among the 3 kinds of cheeses. Coagulants have more important role than starter enzymes in the primary proteolysis of αs1-CN in Iranin white UF cheese (Hesari et al., 2006).

Michaelidou et al.(2003a), reported the similar results when using the Lactococcus lactis subsp.cremoris and Lactococcus lactis subsp.lactis as an adjunct culture in low fat feta cheese.

Conclusions:
From the results of this study it can be concluded that a commercially defined strain culture (R-704, Chr.Hansen Laboratorium, Copenhagen, Denmark) added as an adjunct in Iranian white UF cheeses had a notable effect on primary and secondary proteolysis as assessed by determination of %SN/TN, urea-PAGE and %NPN/TN of cheese.

REFERENCES


