Effect of Plasmin, Milk Somatic Cells and Psychrotrophic Bacteria on Casein Fractions of Ultra High Temperature Treated Milk

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The present study examined the enzymes responsible for proteolysis of casein in milk. Reversed-phase HPLC was used to differentiate the peptide products, of each proteinase, soluble in 12% trichloroacetic acid and in pH 4.6 milk filtrates. Peptides produced by bacterial proteinase were less hydrophobic and eluted early in the RP-HPLC chromatogram, while peptides produced by plasmin and somatic cell proteinase were more hydrophobic and eluted later. β-caseins were preferential substrates for plasmin, whereas αs- and κ-caseins were hydrolysed to a lesser extent. Proteases from bacterial origin predominantly affected κ-casein, while β-casein and αs-casein were less susceptible. Somatic cell proteinase degraded mainly β-casein, followed by αs-casein and κ-casein. When milk was contaminated by bacterial proteinases, chromatograms of the TCA 12% filtrate showed early peaks, while the pH 4.6 filtrate showed early and late peaks when proteolysis was caused by either plasmin, somatic cell proteinase or bacterial proteinase.

Keywords: plasmin, somatic cell proteinase, bacterial proteinase, proteolysis, RP-HPLC, UHT milk

Introduction

Cow milk contains different proteolytic enzymes which may cause alterations of milk proteins. Some of them are naturally present in milk and some are secreted by microorganisms. Moreover, enzymes can be released during udder infection when cells and cell structures are destroyed by pathogenic bacteria. During the UHT treatment, most bacteria are inactivated but heat-stable enzymes of native or bacterial origin can survive and give rise, during storage, to both gelation and off-flavours (Burton, 1988). Gelation and bitterness are usually related to protein breakdown (Harwalkar, 1982). Proteinases from psychrotrophic microorganisms are able to degrade κ-, αs- and β-caseins (Sorhaug and Stepaniak, 1997). Plasmin, the active enzyme derived from plasminogen, is usually considered as the principal proteolytic enzyme of bovine origin in milk (Lotte et al., 2004). Plasmin hydrolyses mainly β-caseins and to a lesser extent αs-caseins (Anthony et al., 2005). De Noni et al. (2007) reported that indigenous proteolytic activity in milk was related to plasmin, and hence to the somatic cell count (SCC) of milk, and that their activity may persist after heat treatment. Barry & Donnelly (1981) reported that 90% of the protease activity in mastitic milk could be attributed to plasmin. In contrast DeRham and Andrews (1982) reported that only one-third of the proteinase activity in mastitic milk was due to plasmin. Similarly, Saeman et al. (1988), Leroux et al. (1995) and Somers et al. (2003) showed that plasmin was not the only enzyme responsible for degradation of casein in milk from cows with mastitis, and concluded that proteinases from somatic cells and blood may also play a significant role in casein hydrolysis in mastitic milk. High SCC predispose raw milk to hydrolysis of αs- and β-casein by proteinases, resulting in a reduction in the concentration of these milk proteins (Verdi et al., 1987; De Noni et al., 2007). Marino et al. (2005) reported that caseins are degraded by leukocyte proteinases in the order αs- > β > κ-casein. Comparison of low and high SCC milk samples has shown substantial proteolytic breakdown of αs- and β-caseins by proteinases associated with somatic cells (Verdi et al., 1987). In the present study, a reversed phase (RP)-HPLC method was used to characterize peptides soluble in trichloroacetic acid (TCA) 12% and

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in pH 4.6 milk filtrate, in order to differentiate between the action of plasmin, somatic cells and bacterial proteinases on milk proteins.

Materials and Methods

Somatic cell proteinase Somatic cells were recovered by centrifugation (1000 × g for 20 min at 4°C) of high SCC (1 × 10^6 cells mL^-1) bovine milk, obtained from a dairy farm in the region of Monastir Tunisia. The resultant pellet (i.e., somatic cells) was resuspended in phosphate buffered saline (PBS) at pH 6.8, as described by Verdi and Barbano (1991). The SCC of this stock cell preparation was determined, after dilution in PBS, using a Fossomatic 400 (Foss Electric, Hillerød, Denmark) to be 1.2 × 10^7 cells mL^-1. The types of somatic cells in the extract were not specifically determined, but it is likely that the somatic cell population in a milk sample of such high SCC was dominated by polymorphonuclear neutrophil leucocytes (Azzara and Dimick, 1985). The somatic cell suspension was then subjected to six freeze-thaw cycles to disrupt cells and release intracellular proteases. Microscopic examination of the freeze-thawed suspension revealed no intact cells.

Bacterial proteinase In order to obtain bacterial proteinase, *Pseudomonas aureofaciens* (API 20NE V5.1 Code 1057547) was incubated in UHT skimmed milk at 30°C for three days and centrifuged at 1000 × g for 30 min, as described by Deeth et al. (2002) and Datta and Deeth (2003). The supernatant, supposed to contain the extracellular proteinase, was used as the *Pseudomonas* proteinase.

Plasmin In this trial we used bovine plasmin obtained from Sigma Chemical Company (Cat. No.P-7911)

Proteolysed UHT milk samples Fresh raw milk with low bacterial count (less than 5 × 10^4 cfu/mL), was obtained from a dairy plant located in the north east of Tunisia that received milk from 32 dairies. Milk was obtained the same day of milking. UHT treatment was applied to selected raw milk using indirect heating at 146°C for 4 s. Homogenization was realised downstream, using a two stage aseptic homogeniser operating at 35 MPa in the first stage and at 5 MPa in the second stage. Sterile containers were filled with the UHT-processed milk in a laminar flow cabinet. Fresh UHT milk samples were hydrolysed with *Pseudomonas* proteinase at 40°C for 3 h. The reaction was stopped by heating at 100°C for 10 min as described by Datta and Deeth (2003). The concentrations of *Pseudomonas* proteinase used were: (6.25, 12.5, 25, 50 and 200 mL/L UHT milk). A UHT skimmed milk sample with no added proteinase was used as a control. The experiments were performed in duplicate.

In order to investigate the effect of extensive proteolysis by plasmin, plasmin was added to freshly processed UHT milk to obtain final concentrations of 100, 500, 1000, 2000 mg/L and incubated for 3h at 40°C. The reaction was stopped by heating at 100°C for 10 min. Milk sample without added plasmin was used as a control.

Somatic cells proteolysis was investigated by adding somatic cell proteinase to freshly processed UHT milk to final concentrations of 5, 10, 50, 100 and 150 mL/L and incubated to 37°C for 1 h. The reactions were stopped by cooling to 4°C for 15 min. A UHT skimmed milk sample with no added proteinase was used as a control. All the experiments were performed in duplicate.

Preparation of 12% TCA filtrates To prepare the 12% TCA filtrate, 24% TCA was added to an equal volume of milk. After vortexing, the solution was allowed to stand at room temperature for 1 h, and then centrifuged at 24,000 × g for 15 min. The supernatant was filtered first through Whatman filter (G541) and then through 0.45µm filter.

Preparation of pH 4.6 filtrates Milk samples were adjusted to pH 4.6 by adding 10% acetic acid with vortexing. The mixture was allowed to stand for 1 h at room temperature, and then centrifuged at 24,000 × g for 15 min. The same procedure was followed as described above for TCA (12%) soluble extracts. pH 4.6-soluble extracts were filtered through 0.45µm filters before injection.

Analysis of peptides by RP-HPLC RP-HPLC analysis was carried out using a Thermo Finnigan HPLC system (Thermo Finnigan, San Jose, California, USA) composed of an autosampler, temperature control unit for the column (SpectraSystem AS3000), a degasser system (SpectraSystem SCM1000), a quaternary gradient pump (SpectraSystem P4000) and a photodiode array detector (SpectraSystem UV6000LP). A personal computer with a software package for system control and data acquisition (ChromQuest 4.0) was used for analyses. A Phenomenex Jupiter C18 wide-pore analytical column (5 mm, 300 Å, 250 × 4.6 mm, Torrance, CA, USA) was used for all separations. Chromatographic conditions were: Solvent A: 0.1% trifluoroacetic acid (TFA) in deionised water and Solvent B: 0.1% TFA in acetonitrile. A sample volume of 100µL was injected into the HPLC system, using the following gradient; 100% eluant A for 5 min, increasing linearly to 50% over 55 min, holding at 50% B for 5 min, increasing to 60% B over 5 min, and finally holding at 60% B for 5 min. The column was finally eluted with 100% eluant B for 2 min. The absorbance of the eluate was monitored at 214 nm (Kelly and Foley, 1997). All samples were analysed in duplicate.

The quantification of casein fractions (α s, β, κ and γ) in samples was performed by measuring peak areas of samples, and plotting against the calibration curves of each casein fraction. Purified casein standards were obtained from Sigma.
Chemical Co. Individual casein standards were prepared before RP-HPLC analysis in the same way as described for milk samples.

The precision of the method was also evaluated. The repeatability of peak areas was assessed by replicate injections (n = 10) of individual casein standards. Relative standard deviations were lower than 3.48%.

**Appearance and gel formation of milk** Milk appearance such as clotting or gelling was controlled by gently tilting the sample. Gelation was first seen at the bottom of the container. Gelation was defined as the appearance of a thick white gel of the lower portion of the containers.

**Statistical analysis** The data were assessed by analysis of variance (ANOVA) using the general linear model procedure. (Statistical Analysis Systems Institute, 2004). The results were considered significant if the associated *p*-value was < 0.05. Student’s t-tests were employed for paired comparison of means.

**Results and Discussion**

**Proteolysis of UHT milk with added Pseudomonas proteinase and plasmin**

**Appearance** A typical visual physical appearance of the UHT treated milk was apparent due to the effects of the three types of proteinases. During incubation at 40°C for 3 h, *Pseudomonas* proteinase, at a concentration of 200 mL/L, caused the formation of a curd or a gel with custard-like consistency throughout the whole milk sample. On the other hand, UHT milk incubated with plasmin (100 mg/L) had a less dense gel that was more easily broken when stirred. UHT milk incubated with 150 mL/L of somatic cell proteinase didn’t gel but a sediment layer was observed at the bottom of the container. Concentrations of 100 mg/L of plasmin and 150 mL/L of somatic cell proteinase exhibited less proteolysis than *Pseudomonas* proteinase under the conditions used in this trial. UHT milk incubated with high concentrations of plasmin (1000 and 2000 mg/L) was completely clarified. Visser (1981) reported that clarified samples exhibited extensive breakdown of casein while the gelled samples exhibited limited proteolysis. Gels caused by bacterial proteinases have a tighter protein network with thicker strands and contain more intact casein micelles and micelle aggregates, than plasmin-initiated gels (Fox, 1981; Hardham, 1998). Datta and Deeth (2003) reported that *Pseudomonas* proteinase preferentially hydrolyses the hydrophilic glycomacropeptide from κ-casein on the outside of the micelle. This leaves the casein micelle largely intact and also reduces steric repulsion between micelles, allowing the formation of a more compact gel. By contrast, plasmin attacks β-casein located inside the micelle, thereby disrupting the micelle and inhibiting the formation of a strong gel. Aroon kamonsri (1996) showed that casein micelles treated with plasmin formed gels that were not as intact as gels formed by treatment with bacterial proteinase. The plasmin-treated samples appeared to contain partially disintegrated casein micelles and their linkages. This was also confirmed by other researchers (Visser 1981; de Koning *et al.*, 1985) who reported that the bacterial gels appeared to have more intact casein micelles, casein micelle aggregates and their linkages. Kelly and Foley (1997) and Topçu *et al.* (2006) demonstrated that UHT milk made with high SCC didn’t gel but showed sedimentation at the bottom part of the container. Auldist *et al.* (1996) reported that UHT milk samples made with high SCC (687,000 cells/mL), gelled after 6 months of storage.

**Analysis of proteolysis by RP-HPLC** RP-HPLC chromatograms of pH 4.6 filtrates of freshly processed UHT milk, proteolysed by plasmin, somatic cells and *Pseudomonas* proteinases, are shown (Fig. 1). Two different groups of peaks could be distinguished from the chromatograms. The first group eluting before 15 min, and a second group eluting between 50 to 70 min. The chromatograms of the filtrates from milk hydrolysed by the *Pseudomonas* proteinase contained only the first group of peaks, while the second group was evident for the samples hydrolysed by plasmin and somatic cell proteinase.

Fig. 2 shows the RP-HPLC profile of the corresponding 12% TCA filtrates of the three hydrolysed milk samples depicted in Fig. 1. The plasmin (100 mg/L) and the somatic cell proteinase (150 mL/L) hydrolysed samples exhibit virtually no peaks in the chromatogram. In contrast, UHT milk samples proteolysed by the *Pseudomonas* proteinase showed substantial peaks eluting before 15 min.

The peptides produced in milk by plasmin, somatic cell proteinase and psychrotrophic bacterial proteinases were different and could be separated by RP-HPLC into two distinct groups. The peptides produced by *Pseudomonas* proteinase were the first to elute from the column, so they were considered to have the lowest time retention. The peptides produced by plasmin and somatic cell proteinase eluted later from the column and had the greatest time retention. In accordance with Datta and Deeth (2003), the retention time of a peptide characterises its size, hydrophobicity and solubility in acid. A peptide with a low retention time was considered to be the smallest, the least hydrophobic and the most acid soluble.

It can be concluded that the first group of RP-HPLC peaks in Fig. 1 (eluted in the first 15 min) represented not only the least hydrophobic peptides but also the smallest and most acid-soluble, while the second group (eluted after 50 min) represented the largest and least acid-soluble peptides as well as the most hydrophobic. On this basis,
the *Pseudomonas* proteinase produced mostly small, acid-soluble peptides, while plasmin and somatic cell proteinase produced predominantly large peptides soluble in the pH 4.6 filtrate but not in 12% TCA. The RP-HPLC patterns were similar to those reported by Lopez-Fandino *et al.* (1993). They compared the patterns for peptides soluble in 4% TCA and in the pH 4.6 milk filtrate that resulted from proteolysis of casein by plasmin, somatic cells and *Pseudomonas* proteinase. In their chromatograms, most peptides from the bacterial proteolysis eluted before 24 min, while those from indigenous proteinases eluted after this time. Kelly and Foley (1997) found that chromatograms of the pH 4.6 soluble fraction resulting from proteolysis by plasmin and somatic cell proteinase were composed of peaks eluted between 50 and 70 min. Driessen (1981) showed that bacterial contamination of UHT milk resulted in high nonprotein nitrogen values in

Fig. 1. Peptide patterns of pH 4.6 milk filtrates of proteolysed UHT milk (plasmin 100mg/L (a); somatic cell proteinase 150mL/L (b) and *Pseudomonas* proteinase, 200 mL/L (c); UHT milk control (d)).
12% TCA filtrates, while milk incubated with endogenous proteinases had a high value of noncasein nitrogen, which was measured in the pH 4.6 filtrates. Driessen (1981) also demonstrated that endogenous milk proteinase splits β-casein into larger fragments, that could be precipitated by 12% TCA and do not contribute to the NPN value. Mulvihill and Donovan (1987) reported that large peptides in the pH 4.6 milk filtrates from the endogenous proteinase digestion were derived from β-casein as these peptides were soluble in pH 4.6 filtrates and were precipitated by 12% TCA.

**Plasmin proteolysis** The effect of plasmin on casein hydrolysis was investigated by incubating UHT milk samples with increasing concentrations of exogenous plasmin (Table 1). Increasing plasmin concentrations from 100 to 2000 mg/L in the UHT milk caused a break down of β-casein, αs-casein and κ-casein at a rate of 6.29%, 0.93% and 0.13%, respectively. In contrast, γ-casein breakdown increased at a rate of 7.5% (Table 1).

It can be concluded that β-casein was most susceptible to breakdown by plasmin than α-casein, and κ-casein. κ-casein appeared to be more resistant to breakdown by plasmin. These results are in accordance with previous studies. Grieve and Kitchen (1985); Grufferty and Fox (1988); Leroux et al. (2003) and Isabelle et al. (2008) reported that αs-caseins and β-caseins are preferential substrates for plasmin, whereas κ-caseins were hydrolysed to a lesser extent. While αs-casein was susceptible to proteolysis by plasmin, αs₂-casein and β-casein are the preferred substrates (Anthony et al., 2005). Leroux et al. (2003), explained the preferential order of casein degradation, by the fact that β-caseins were the most hydrophobic peptides, characterized by a specific three-dimensional structure facilitating plasmin access. Grufferthy and Fox (1988) reported that despite the fact that κ-caseins have many potential cleavage sites for plasmin, they still were the most resistant to this proteinase action.

**Bacterial proteolysis** In order to evaluate bacterial proteolysis, Pseudomonas proteases were added to fresh UHT milk and incubated at 40°C for 3 h. Increasing the concentration of Pseudomonas proteases induced a significant decrease (P < 0.05) of casein fractions in UHT milk.

| Table 1. Casein fractions of UHT milk incubated with plasmin. |
|-----------------|-------|-------|-------|-------|
| Plasmin (mg/L UHT milk) | 100   | 500   | 1000  | 2000  |
| γ-casein%        | 3.90a | 4.52a | 6.71a | 11.40a |
| κ-casein%        | 16.82a| 16.79a| 16.72a| 16.69a |
| β-casein%        | 37.10a| 36.75b| 34.89a| 30.81a |
| αs-casein%       | 42.03a| 41.94b| 41.68a| 41.10a |

Values in the same row with different superscripts differ (P < 0.05).
The addition of somatic cell proteinase to UHT milk samples (Table 2). Increasing the concentration of Pseudomonas proteinase in UHT milk from 6.25 to 200 mL, caused a degradation of κ-casein, β-casein and α s-casein with a rate of 3.07%, 1.12% and 0.32%, respectively. These results indicate that proteases from bacterial origin predominantly affect κ-casein, while β-casein and α s-casein were less susceptible to bacterial proteases. These findings are supported by previous studies by McPhee and Griffiths (2002); Datta and Deeth (2003); Kahina et al. (2005); Leitner et al. (2006) and Isabelle et al. (2008) who reported that κ-caseins were preferential substrates for bacterial proteases, and that β- and α s-caseins were hydrolysed to a lesser extent. Sorhaug and Stepaniak (1997) reported that proteases from different species and strains of Pseudomonas differ in their substrate specificities toward milk proteins. Most proteinases from psychrotrophs were able to degrade κ-casein. Kahina et al. (2005) showed that incubation of milk with psychrotrophic bacteria caused hydrolysis of κ-caseins with a rate of 45%, while β-casein and α s-casein were degraded at a rate of 30% and 10%, respectively. They also indicated that the length of incubation of milk with psychrotrophic proteases increased the rate of casein degradation.

Somatic cell proteolysis  The addition of somatic cell extracts to UHT milk samples showed significant effects (P < 0.05) on casein fractions. Examination of casein hydrolysates identified the main patterns derived by the breakdown of caseins by somatic cell proteinase (Table 3). Increasing the concentration of somatic cell proteinase from 5 mL to 150mL/L of UHT milk samples caused a reduction of β-casein at a rate of 2.13%. A slight decrease in α s-casein was observed for all samples, however, this decrease was only significant for UHT milk samples treated with proteinase concentrations of 50, 100 and 150 mL/L. The κ-casein content remained the same despite the fact that UHT milk samples had been incubated with high concentrations of somatic cell proteinase. κ-Casein degradation did not exceed 0.04% for all concentrations of somatic cell proteinase. These results indicate that somatic cell proteinase hydrolyse mainly β-casein, followed by α s-casein and lastly κ-casein. The same findings were reported by Saeman et al. (1988), Leroux et al. (2003) and Kahina et al. (2005). Leroux et al. (1995) observed a decrease of 2% in β-caseins and 1.6% in α s-caseins when SCC exceeded 250,000 cells/mL in raw milk. They also indicated that γ-caseins increased at a rate of 3.5% but κ-caseins didn’t vary significantly (P > 0.05). According to Verdi and Barbano (1991) κ-caseins were the most resistant to proteolysis by somatic cell proteinase followed by α-casein.

Methodology proposed to identify the cause of proteolysis in UHT milk  Analysis of the peptides in 12% TCA and pH 4.6 milk extracts described above, were used to suggest a useful approach for identifying the causes of proteolysis in UHT milk.

*Proteolysis by endogenous proteinases, plasmin and somatic cell proteinase, are characterized by chromatograms, of the peptides soluble in pH 4.6 milk extracts, containing substantial and late peaks between 50 to 70 min. On the other hand, the chromatograms of the 12% TCA extracts showed

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**Table 2.** Casein fractions of UHT milk incubated with Pseudomonas proteinases.

<table>
<thead>
<tr>
<th>Pseudomonas proteinase concentration (mL/L UHT milk)</th>
<th>6.25</th>
<th>12.50</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-casein%</td>
<td>3.72⁠</td>
<td>4.18⁠</td>
<td>4.65</td>
<td>5.26⁠</td>
<td>6.37</td>
<td>9.81⁠</td>
</tr>
<tr>
<td>κ-casein%</td>
<td>16.68⁠</td>
<td>16.36</td>
<td>16.02</td>
<td>15.70</td>
<td>14.82</td>
<td>13.61⁠</td>
</tr>
<tr>
<td>β-casein%</td>
<td>37.39⁠</td>
<td>37.28</td>
<td>37.19</td>
<td>37.02</td>
<td>36.88</td>
<td>36.27⁠</td>
</tr>
<tr>
<td>α s-casein%</td>
<td>42.21⁠</td>
<td>42.18</td>
<td>42.14</td>
<td>42.02</td>
<td>41.93</td>
<td>41.89⁠</td>
</tr>
</tbody>
</table>

Values in the same row with different superscripts differ (P < 0.05).

**Table 3.** Casein fractions of UHT milk incubated with somatic cell proteinases.

<table>
<thead>
<tr>
<th>Somatic cell proteinase concentration (mL/L UHT milk)</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-casein%</td>
<td>3.82⁠</td>
<td>4.11</td>
<td>5.01</td>
<td>6.34</td>
<td>7.15⁠</td>
</tr>
<tr>
<td>κ-casein%</td>
<td>16.85⁠</td>
<td>16.84</td>
<td>16.82</td>
<td>16.83</td>
<td>16.81⁠</td>
</tr>
<tr>
<td>β-casein%</td>
<td>37.15⁠</td>
<td>36.94</td>
<td>36.26</td>
<td>35.46</td>
<td>35.02⁠</td>
</tr>
<tr>
<td>α s-casein%</td>
<td>42.18⁠</td>
<td>42.11</td>
<td>41.91</td>
<td>41.37</td>
<td>41.02⁠</td>
</tr>
</tbody>
</table>

Values in the same row with different superscripts differ (P < 0.05).
no peaks. Compared to peaks generated by plasmin proteolysis, peaks of somatic cell proteolysis have lower amplitude.

This situation may be attributed to residual activity of the plasmin and somatic cell proteinase after UHT treatment, especially when high SCC milk is used. During storage, plasmin is generated by the action of a plasminogen activator on the precursor plasminogen (Datta and Deeth, 2003). Proteolytic damage to casein and an increase in conversion of plasminogen to plasmin has been observed in milk with high SCC (Verdi and Barbano, 1991).

*Proteolysis by Pseudomonas proteinases is characterized by a chromatogram of the peptides soluble in either pH 4.6 milk extracts or in 12% TCA containing early peaks within 15 min. This situation can be encountered in the case of use of raw milk with high bacterial count.

Practical significance *If proteolysis is caused by plasmin, it is likely that the conditions of the UHT processing are too mild. Such conditions cause less inhibition of plasmin action on the casein (Datta and Deeth 2003). Manji and Kakuda (1988) reported that direct UHT processes, using steam infusion or injection, cause less denaturation of plasmin. To increase plasmin inactivation in UHT milk, Pereda et al. (2008) suggested the use of ultra-high pressure homogenisation.

*If proteolysis is caused by somatic cell proteinase and or bacterial proteinase, this indicates that raw milk quality is compromised. In the case of bacterial proteinase, proteolysis is mainly attributed to psychrotrophs which constitute the predominant micro-organisms in raw milk (Sorhaug and Stepaniak, 1997). Significant contaminations by psychrotrophic bacteria occur due to inadequately sanitized surfaces of milking, storage and transporting equipments (Mc Phee and Griffiths, 2002).

*Proteolysis caused by somatic cells indicates that the SCC allowed by the manufacturer in raw milk is too high. Leroux et al. (1995) reported that in milk with a SCC > 2.5 × 10⁶ cells/mL, almost 40% of proteolytic activity can be due to non-plasmin enzymes.

Conclusion

The results of this study show that peptides produced by bacterial proteinase are less hydrophobic and elute within 15 min in the RP-HPLC chromatogram, while the peptides produced by plasmin and somatic cell proteinase are more hydrophobic and elute between 50 to 70 min. The 12% TCA filtrate showed substantial peptide peaks if the milk was contaminated by bacterial proteinases, while the pH 4.6 milk filtrate showed peptide peaks when proteolysis was caused by either plasmin, somatic cell proteinase or bacterial proteinase. β-caseins are preferential substrates for plasmin, whereas α-casein and κ-casein are hydrolysed to a lesser extent. Proteases from bacterial origin predominantly affect κ-casein, while β-casein and α s-casein are less susceptible to bacterial proteases. Somatic cell proteinase hydrolyses mainly β-casein, followed by α s-casein and lastly κ-casein. Findings of this work highlight the possibility of use of the RP-HPLC to distinguish between proteolysis in UHT milk caused by native milk plasmin, somatic cell proteinase and bacterial proteinase, in order to facilitate remedial actions to prevent or improve proteolysis problems during UHT treatment of milk.

References


