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# Determination of free amino acids in whole-fat Turkish White Brined Cheese produced by animal and microbial milk-clotting enzymes with and without the addition of starter culture

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**Summary**

Coagulating enzymes are essential ingredients for the production of different cheese varieties. The objective of this research was to summarize the effect of rennet type (calf rennet and microbial rennet from *Rhizomucor miehei*) and starter culture on the sensory properties and free amino acids (FAA) release during the ripening of Turkish White brined cheese. The concentrations of FAA and sensory properties were similar for cheeses made with both types of coagulant and starter culture. Aminoacids Phe, Leu - Ile, Gln, Val, Pro and Ala were the principal FAAs in the White brined cheeses at all stages of ripening.

*Key words*: white brined cheese, rennet, starter culture, proteolysis

## Introduction

Brined cheeses are mainly manufactured in Mediterranean and Balkan countries. They are produced under different names, such as White Cheese (Turkey), Feta (Greece), Bjalo Salamureno Sirene (Bulgaria), Domiati (Egypt), Teleme (Greece, Romania, Turkey), Iranian White (Iran) and Beli sir u kriškama (former Yugoslavia) (Hayaloglu et al., 2002; Kamber, 2008a).

Turkish White Cheese, produced traditionally in nearly every part of the country, is the most popular cheese variety in Turkey, representing approximately 60 % of the country's total cheese production (Hayaloglu et al., 2002; Kamber 2008a,b). This cheese was originally manufactured from sheep's or goat's milk, but cow's milk or a combination of milks is now generally used for its production. White Cheese is a cheese variety that is brine-salted and ripened in brine (a 12-14 g/100 g NaCl solution). A typical White Cheese has specific characteristic flavour, soft or semi-hard texture, and very fine eyes or without eyes. It has a salty and acidic taste and it is suitable for slicing and is liable to crumble. It varies in colour from shiny white or off-white to pale yellow depending upon the source of animal milk (Kamber, 2008a,b). Generally, the cheeses are cubical or rectangular, typically 7x7x7 or 7x7x10 cm, and weigh approximately 350 to 500 g. The cheese is matured for a period of 1-3 months (Dinkci and Gonc, 2000; Hayaloglu et al., 2002, 2004).

Cheese ripening is influenced by different factors, including the endogenous or exogenous enzymes and microflora derived from the raw milk, starter cultures, coagulants, and manufacturing and ripening conditions (Fox and McSweeney 1996; Sousa et al. 2001; Wilkinson and Kilcawley 2005; Ozcan and Kurdal 2012). Proteolysis is usually regarded as the main biochemical process during cheese ripening and one of the most important factors for the development of typical cheese flavour and texture. Rennet or a similar coagulating enzyme, plasmin, the starter bacteria, and the non-starter microflora are the main proteolytic agents involved in cheese ripening. Proteins are partially hydrolyzed by rennet and other native microbial enzymes to

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produce lower-molecular-weight compounds and are further broken down by peptidases into various nitrogenous substances, such as proteose, peptone, amino acids and amines (Farkye and Fox, 1990; Fox and McSweeney, 1996; Fox et al., 1999; Martinez-Cuesta et al., 2001).

The enzymes from lactic acid bacteria are the main factors responsible for proteolysis and formation of compounds that are essential for cheese ripening (Martinez-Cuesta et al., 2001). Different mixed starter cultures, including thermophilic and/or mesophilic bacteria, are used in the production of Turkish White Cheese (Hayaloglu et al., 2002, 2004).

Commercial calf rennet is used in White Cheese manufacturing, primarily as a milk coagulant (Kamber 2008b). However, the increased consumption of cheese has led to an increase in the demand for rennet, while there has been a decrease in the number of young animals which stomach are used for rennet production after their slaughtering (leading to the investigation of alternative milk clothing enzymes of different origins). Therefore, other suitable coagulants (bovine, porcine and chicken), including proteinases from microorganisms (*Mucor miehei*, *Mucor pucillus* and *Cryphonectria parasitica* (formerly *Endothia parasitica*)), have become more popular in the production of cheeses (Guinee and Wilkinson, 1992; Jacob et al., 2011). Therefore, the objectives of this study were to investigate the effects of different rennet types (calf and microbial coagulant obtained from *Rhizomucor miehei*) together with no addition or addition of the starter on the free amino acid (FAA) content of whole-fat Turkish White brined cheese.

## Materials and methods

### *Materials*

Raw bovine milk supplied by the Dairy Company (Bursa, Turkey) was used in the production of cheese samples. Rennet of animal (1/18 000: Mayasan Food Industries A.S. Istanbul, Turkey) and microbial (1/16 000: Mayasan Food Industries A.S. Istanbul, Turkey) origin was used to coagulate the milk. The starter cultures were obtained freeze-dried from Danisco (Brugge, Belgium). The mesophilic starter was a blend of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*, and the thermophilic starter was a blend of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in equal proportions.

Table 1. Experimental design of White pickled cheese

| Cheese trials | Culture[a] | Animal rennet | Microbial rennet |
|---|---|---|---|
| **A** | –[b] | + | – |
| **$A_5$** | + | + | – |
| **$A_{10}$** | + | + | – |
| **$A_{20}$** | + | + | – |
| **M** | – | – | + |
| **$M_5$** | + | – | + |
| **$M_{10}$** | + | – | + |
| **$M_{20}$** | + | – | + |

[a]Starter culture, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*. *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*, 5 DCU/tons, 10 DCU/tons and 20 DCU/tons (DCU: Danisco Culture Unit)
[b]+, addition; –, no addition.

### *Methods*

#### *Experimental design*

White cheeses were made from either animal or microbial rennet using different ratios of starter culture, with three experiments, each repeated three times on different days. The cheese-making trials were denoted as A, A5, A10, A20, M, M5, M10 and M20. The experimental design of white pickled cheeses is detailed in Table 1.

### *Cheese production*

White Cheeses were produced by the modified traditional method of Hayaloglu et al. (2002) (Figure 1).

## Cheese analyses

### *FAAs content*

#### *Reagents*

Amino acid standards were obtained from Sigma Chemicals (Milwaukee, WI, USA), and all other chemicals used were of analytical grade (99 %).

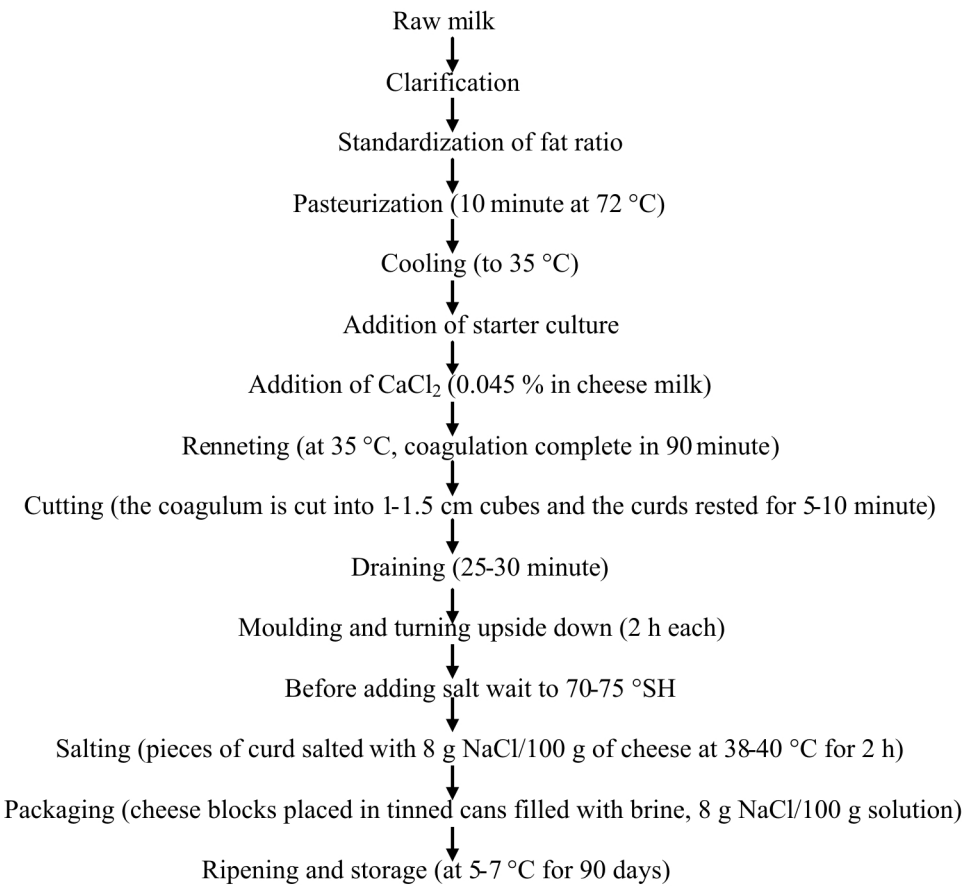


Figure 1. Protocol of White Cheese Production

Simultaneous determination of underivatized amino acids was carried out by a liquid chromatography-mass spectrometry (LC/MS; Waldbronn, Germany). Anarrow-bore column allowed rapid screening and quantitative analysis by positive LC/atmospheric pressure chemical ionization (APCI) MS with only acidified mobile phase (Ozcan and Senyuva, 2006).

*Sample preparation*

Stock solutions of 1000 $\mu$g/mL amino acids were prepared by dissolving 25 mg of each in 25 mL of distilled water. Working standards were prepared by diluting the stock solution of amino acids to concentrations of 0.05-5.00 $\mu$g/mL with 0.2 mM acetic acid. Stock solutions were kept at 4 °C for a week for daily use and kept at -18 °C for longer term storage. Working standards were prepared daily before analysis. According to the sample matrix, the sample was ground (by a blender, mesh size 2 mm) or mixed (by Ultra Turrax). The pH of each homogenized sample was measured before sample preparation. Sub-samples of the homogenate were stored at -20 °C in high density polyethylene bottles with plastic screw-capped lids. Finely, ground or homogenized sample (1 g) was weighed [fresh weight (FW)] into a 10 mL glass centrifuge tube with a cap. Ten millilitres of 0.2 mM acetic acid was added to the samples. After mixing in a vortex mixer for 2 min, the sample was centrifuged at 5000 rpm for 10 min at -5 °C. The clear supernatant was quantitatively transferred into a vial avoiding the top fat layer (if present). The supernatant was filtered through a 0.45 $\mu$m nylon syringe filter prior to LC/MS analysis.

*Chromatographic conditions*

Glass vials with septum screw caps and Zorbax Bonus-RP, Narrow-Bore RP (100 mm×2.1 mm, 3.5

$\mu$m), Zorbax SB Aq (150 mm×4.6 mm, 3.5 $\mu$m) and Zorbax Eclipse XDB C18 (75 mm×4.6 mm, 5 $\mu$m) analytical columns were supplied by Agilent Technologies (Wilmington, DE, USA). The Ace 3 C18 (100 mm×2.1 mm, 3 $\mu$m) was supplied by ACE-HPLC (Reading, UK). The Hichrom Inertsil ODS 3 (250 mm×4.6 mm, 3.5 $\mu$m) was purchased from Hi Chrom (Berkshire, UK). The Silent Crusher M homogenizer was purchased from Heidolph (Donau, Germany) and the MP220 digital pH meter was purchased from Mettler Toledo (Leicester, UK). The LC/APCI-MS analyses for the screening and quantification of 22 free amino acids was performed by the Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler and a temperature-controlled column oven coupled to an Agilent 1100 MS detector equipped with an APCI interface. The analytical separation was performed on a Zorbax Bonus-RP, Narrow-Bore (100 mm×2.1 mm, 3.5 $\mu$m) using the isocratic mixture of 0.01 mM acetic acid in a 0.2 % aqueous solution of formic acid at a flow rate of 0.2 mL/min. Data acquisition was performed in selected ion monitoring (SIM) mode using the following interface parameters: a drying gas ($N_2$) flow of 4 L/min, a nebulizer pressure of 55 psig, drying gas and vaporizer temperatures of 320 °C, a capillary voltage of 3 kV, a corona current of 8 $\mu$A and a fragmentor voltage of 55 eV. The first step

Table 2. The free amino acid (FAA) concentration of Turkish White Cheeses (mg/100 g of cheese dry matter

| Amino Acid | A | A5 | A10 | A20 | M | M5 | M10 | M20 | Signi-ficance+ |
|---|---|---|---|---|---|---|---|---|---|
| Asparagine (Asn) | 0.910 | 0.930 | 0.510 | 0.585 | 0.625 | 0.670 | 0.905 | 0.625 | ns |
| Aspartic acid (Asp) | 0.120 | 0.110 | 0.050 | <LOD | 0.120 | 0.120 | 0.125 | 0.120 | * |
| Serine (Ser) | 0.165 | 0.200 | 0.165 | 0.200 | 0.100 | 0.215 | 0.195 | 0.200 | ns |
| Glycine (Gly) | 0.245 | 0.280 | 0.165 | 0.250 | 0.115 | 0.270 | 0.220 | 0.185 | ** |
| Glutamine (Gln) | 1.895 | 1.945 | 1.160 | 1.890 | 0.610 | 1.970 | 1.360 | 1.840 | ** |
| Lysine (Lys) | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | - |
| Cystine (Cys-Cys) | 0.260 | 0.185 | 0.155 | 0.160 | 0.145 | 0.155 | 0.21 | 0.245 | ns |
| Glutamic acid (Glu) | 0.615 | 0.615 | 0.555 | 0.540 | 0.375 | 0.495 | 0.525 | 0.533 | ns |
| Threonine (Thr) | 0.195 | 0.245 | 0.185 | 0.190 | 0.110 | 0.265 | 0.230 | 0.215 | ns |
| Alanine (Ala) | 2.065 | 1.655 | 0.895 | 1.270 | 0.785 | 2.020 | 1.295 | 1.585 | ns |
| Proline (Pro) | 2.350 | 2.250 | 1.310 | 2.070 | 1.270 | 2.745 | 2.065 | 2.080 | ns |
| Valine (Val) | 3.975 | 2.635 | 1.385 | 1.610 | 2.205 | 4.075 | 2.375 | 2.005 | ns |
| Methionine (Met) | 0.415 | 0.580 | 0.095 | 0.370 | 0.250 | 0.720 | 0.620 | 0.655 | ns |
| Tryptophan (Trp) | 0.070 | 0.060 | <LOD | 0.100 | <LOD | 0.030 | 0.020 | 0.070 | ** |
| Arginine (Arg) | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | - |
| Cysteine (Cys) | 0.075 | 0.075 | 0.415 | 0.650 | 0.520 | 0.115 | 0.065 | 0.445 | ** |
| Tyrosine (Tyr) | 0.035 | 0.025 | 0.010 | 0.190 | 0.010 | 0.030 | 0.015 | 0.030 | ** |
| Phenylalanine (Phe) | 6.850 | 6.155 | 3.525 | 4.650 | 3.725 | 8.450 | 4.270 | 4.595 | ns |
| Hydroxyproline (Hyp) | 0.290 | 0.210 | 0.190 | 0.180 | 0.100 | 0.270 | 0.185 | 1.175 | * |
| Leucine- Isoleucine (Leu - Ile) | 7.880 | 4.785 | 2.845 | 3.885 | 1.975 | 6.7000 | 3.330 | 3.105 | ns |
| Histidine (His) | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | - |
| **Total** | 28.410 | 22.940 | 13.615 | 18.790 | 13.040 | 29.310 | 18.010 | 19.708 | |

A: Animal rennet. A5: Animal rennet+5DCU starter culture. A10: Animal rennet+10DCU starter culture.
A20: Animal rennet+20DCU starter culture. M: Microbial rennet. M5: Microbial rennet+5DCU starter culture.
M10: Microbial rennet+10DCU starter culture. M20: Microbial rennet+20DCU starter culture
LOD: limit of detection
+Significance level: differences in the concentration of FAAs during ripening period
ns, not significant; *P-value<0.05; **P-value<0.01

of method development involved an infusion study to determine the fragmentation pattern for the 22 amino acids in both electrospray (ESI) and APCI (in both positive and negative ionization mode to generate [M+H]+ or [M–H]+ ions). The ions of 22 amino acids were monitored for the screening and quantification of amino acids in the samples. Full scan analyses were performed in the mass range of 50–500 da for the spectral identification of amino acids and sample co-extractives. Characteristic fragmentation of the amino acids was observed using a single ion monitoring (SIM) mode.

*Quality assurance*

Quality assurance measures were employed for amino acids, which involved inclusion in each sample, duplicate samples spiked at 5, 10, 50 mg/100 g and a reagent blank. The amounts of FAAs in the various cheese samples were calculated using peak area values from duplicate analytical samples, and the peak areas were converted to concentrations using calibration curves of amino acid standards.

***Sensory analyses***

Sensory properties were analyzed as described by Ayad et al. (2000, 2004). Control and experimental cheeses were sensory graded after 1, 15, 30, 60 and 90 days of ripening. The cheeses were coded with random four-digit numbers, and approximately 100 g was partitioned into 5 g cubes. Coded samples were removed from the refrigerator (8 °C) 1 h prior the evaluation, kept at room temperature (22±1 °C), and presented to the panel. Water was provided for mouth washing between samples. The panel was made up of 12 staff of the Department of Food Engineering that had previous experience with cheese sensory evaluation. The trial cheese batches were analyzed for sensory attributes of structure and appearance, texture, taste, saltiness, aroma and general acceptability using a hedonic scale of 1-4 (Table 3).

***Statistical analyses***

The experimental data were analyzed using the ANOVA test, and significance was indicated by $P<0.01$; 0.05, using the SAS statistical software (version 8.02, SAS Institute Inc., Cary, NC, USA). All chemical measurements were conducted in triplicate.

**Results and discussion**

Milk clotting enzymes contribute to proteolysis in Turkish White brined cheese. This is due to the high retention level of the coagulant in cheese curd with a high moisture content, and storage of the cheese in salted whey that contains residual coagulant. The use of calf rennet and microbial rennets (e.g., *R. miehei*) and their implications for proteolysis during cheese production and ripening have been demonstrated by Yesilyurt (1992), Uysal (1996), Saldamli and Kaytanli (1998) and Yetismeyen et al. (1998).

The average free amino acid (FAA) concentration of White Cheeses is presented in Table 2. The FAAs of the cheeses were significantly affected by the ripening period ($P<0.01$, $P<0.05$). The M5 cheeses had the highest amino acid content, with a maximum of 29.31 mg/100 g, and were followed closely by the group A cheeses, with an amino acid content of 28.41 mg/100 g.

Proteolysis in Turkish White Cheese continues during storage in brine. Starter peptidases are responsible for the production of amino acids. The addition of lactic acid bacteria (LAB) as a starter culture, produced a higher content of short-chain peptides and FAAs during cheese ripening (Lee et al., 1990; Lane and Fox, 1996). However, different starter bacteria release different levels of individual FAA (Dráb et al., 1999) based on their enzyme system and the degree of autolysis in the cheese (Brome and Limsowtin, 1998). The total concentration of FAAs increased during ripening and Phe, Leu - Ile, Gln, Pro, Ala and Val were the principal FAAs in the cheeses at all stages of ripening (Table 2, Figure 2).

The principal FAAs including Leu, Glu, Phe, Val and Lys were present in the 60-day old Turkish White Brined Cheese made from pasteurized cow's milk (Ucuncu, 1981; Kaymaz, 1982; Hayaloğlu et al., 2004). Previous authors (Alichanidis et al., 1984; Katsiari et al., 2000; Michaelidou et al., 2003) have shown that Leu, Glu, Val and Lys were major FAAs in Feta cheese made from cow's milk. Moreover, the same FAAs were present in Iranian brine cheeses after ripening for 50 days. This did not

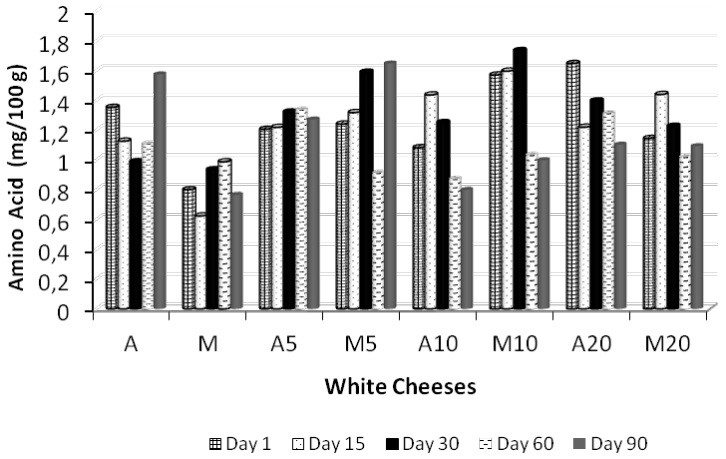


Figure 2. Free amino acid content (mg/100 g of cheese dry matter) of White Cheeses at different ripening periods

continue to the end of ripening in Feta; the amino acid Lys, Arg and Glu were predominant at the end of ripening in the cheese (Azarnia et al., 1997). According to these authors the decrease in FAA could be due to amino acid catabolism or as explained by Caric (1987) the amino acids diffused into the brine.

Lactic acid bacteria (LAB) play a major role in generating flavour compounds from amino acids in many types of cheeses. A number of enzymes involved in amino acid conversion have been identified in starter cultures. Generally these enzymes are involved in various reactions including deamination, transamination, decarboxylation and cleavage of the amino acid side chains (Yvon et al., 1997; Yvon et al., 1998; Ayad et al., 2001).

FAA composition and concentration generally depend on milk source, season, manufacturing technology, presence of natural bacteria strains and other sources of enzymes, amount of fatty acids and ripening conditions and duration (Freitas et al., 1999; Fenelon et al., 2000; Mendia et al., 2000; Vicente et al., 2001; Muňoz et al., 2003; Kenny et al., 2006; Hickey et al., 2007; Irigoyen et al., 2007; Mangia et al., 2008; Milesi et al., 2009; Sihufe et al., 2010). Table 3 shows the values of amino acids according to the concentration in unripened and ripened cheeses. The first two amino acids were Phe and Leu-Ile and they did not change based on the origin of the rennet (microbial or animal).

The content and ratio of FAAs significantly influence the texture and sensory characteristics of cheese. The relationship between the release of amino acids and flavour formation in cheese has been assumed by many researchers. Amino acids may contribute to flavour either directly or indirectly by serving as precursors for volatile aroma compounds such as aldehydes, acids, alcohols, esters and sulphur compounds (Visser, 1993; Engels and Visser, 1996; Ayad et al., 2001).

The sensory properties of White Cheeses are presented in Table 4. The maximum taste and aroma scores were found in cheese made using a 10DCU culture and animal rennet (A10) while the minimum structure, appearance and texture scores were found in cheeses with no culture addition (A and M). Because both rennet cheeses with starter culture were ripened for a certain period of time, non-textural defects were also determined. The general acceptability of cheese samples decreased as the rate of starter culture addition increased for cheese making. This may be due to a higher proteolysis in cheese prepared using higher levels of starter culture. However cheeses without starter culture (A and M) had the lowest general acceptability scores.

Flavours and aroma compounds are related to amino acid metabolism. Flavour descriptions and definition of threshold values of amino acids such as Gly, Ser, Thr, Ala and Pro amino acids have been proposed to have an effect on tastes such as sweetness (Urbach, 1995). This is especially true for not

only A5 and M5 cheeses but also cheeses made with only animal rennet (A) as these amino acid ratios were higher than in other cheeses (Table 2). The glutamic acid presence in cheese is responsible for their pleasant flavour (Ardö, 2006). The Phe, Met, Tyr and Thr amino acids are also the source of 70 odorants (Yvon et al., 2001; Ardö, 2006). Glu production from α-ketoglutarate, Gln,Val, Leu and Ile may also contribute to its increased concentration during cheese ripening.

Degradation of Leu, Ile and Val results in the formation of isovaleric, 2-methylbutanoic and isobutyric acid respectively and they are responsible for cheesy, sweaty, sour, rancid or putrid odors (Poveda et al., 2004; Ardö, 2006).

## Conclusion

Milk coagulants are essential for cheese making and one of the most important enzymes in the food industry. Many studies have focused on the proteolytic activity of enzymes in *rennet* during cheese making, and FAA content is often used as an index for cheese maturity. This study showed that the degrees of proteolysis, in terms of NPN or its main component amino acids, and sensory properties were similar in cheeses either produced using animal or microbial rennets and starter culture. The concentration of total FAAs in White pickled cheeses increased with ripening. The dominating free amino acids present in the various experimental cheeses throughout the ripening period were Phe, Leu - Ile, Gln, Pro, Ala and Val. Finally rennet and starter cul-

Table 3. Values of amino acids in unripened and ripened White Cheeses

| Number | Unripened cheeses | | Ripened Cheeses | |
|---|---|---|---|---|
| | Animal rennet | Microbial rennet | Animal rennet | Microbial rennet |
| 1 | Phe | Phe | Phe | Phe |
| 2 | Leu - Ile | Leu - Ile | Leu - Ile | Leu -Ile |
| 3 | Val | Val | Gln | Gln |
| 4 | Pro | Pro | Pro | Pro |
| 5 | Gln | Gln | Val | Ala |
| 6 | Ala | Ala | Ala | Val |

Table 4. Evaluation of sensory properties of White Cheeses

| Treatment | Structure and appearance | Texture | Taste | Salt | Aroma | General acceptability |
|---|---|---|---|---|---|---|
| A | 1.63±0.43 | 1.80±0.73 | 3.13±0.37 | 2.88±0.79 | 2.33±1.12 | 1.94±0.64 |
| A5 | 1.87±0.26 | 2.73±0.56 | 3.09±0.41 | 3.20±0.48 | 2.72±0.57 | 3.72±0.41 |
| A10 | 2.14±0.33 | 3.06±0.32 | 3.21±0.31 | 3.75±0.18 | 2.82±0.43 | 3.31±0.36 |
| A20 | 2.51±0.39 | 2.92±0.29 | 2.90±0.36 | 3.78±0.12 | 2.67±0.42 | 3.00±0.35 |
| M | 1.58±0.49 | 1.74±0.70 | 3.04±0.54 | 2.84±0.72 | 2.33±0.98 | 1.92±0.61 |
| M5 | 2.09±0.31 | 2.89±0.52 | 3.11±0.25 | 3.28±0.33 | 2.70±0.66 | 3.70±0.45 |
| M10 | 2.29±0.20 | 3.04±0.23 | 3.02±0.28 | 3.74±0.11 | 2.78±0.44 | 3.22±0.28 |
| M20 | 2.53±0.42 | 3.02±0.17 | 2.96±0.28 | 3.76±0.14 | 2.67±0.44 | 3.00±0.37 |
| Scoring | 1: soft<br>2: normal<br>3: hard<br>4: very hard | 1: broken<br>2: medium<br>3: good<br>4: very good | 1: bad<br>2: sufficient<br>3: good<br>4: very good | 1: insufficient<br>2: very salty<br>3: slightly salty<br>4: normal | 1: very or less<br>2: medium<br>3: strong<br>4: very strong | (1:4)<br>unsatisfactory/ excellent |

ture type contributes to White Brined Cheese proteolysis. Microbial enzymes which are accepted by vegetarians, can also be used in organic cheese making.

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## *Određivanje slobodnih aminokiselina u punomasnom turskom bijelom siru u salamuri proizvedenom od životinjskih i mikrobnih enzima grušanja s dodatkom ili bez dodatka starter kulture*

Enzimski preparati za grušanje mlijeka bitni su sastojci za proizvodnju različitih vrsta sira. Cilj ovog istraživanja bio je sažeti učinak tipa sirila (teleće sirilo i mikrobno sirilo od *Rhizomucor miehei*) i starter kulture na senzorska svojstva i slobodne aminokiseline (FAA) koje nastaju tijekom zrenja turskog bijelog sira u salamuri. Koncentracije FAA i senzorska svojstva slični su za sireve proizvedene s obje vrste koagulanta i starter kulture. Aminokiseline Phe, Leu - Ile, Gln, Val, Pro i Ala bile su glavne slobodne aminokiseline (FAA) u bijelim sirevima u salamuri u svim fazama zrenja.

*Ključne riječi*: bijeli sir u salamuri, sirila, starter kultura, proteoliza

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