

Effects of different coagulant enzymes used on quality of traditional Örgü cheese (Braided cheese)

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Abstract

In this study, Örgü cheese has been produced by using different coagulating enzymes (calf rennet, microbial enzymes, recombinant chymosin). The effects of different coagulating enzymes which are used on the characteristic of mineral material and cheese has been observed during 90 days ripening time. Mineral material contents of Örgü cheese have been determined with ICP-OES (inductively coupled plasma optical emission spectroscopy). Proteolysis levels of cheese have been observed with chemical analysis and help of SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The determined difference between analysis results, titratable acidity, total nitrogen, water soluble nitrogen, ripening index, total solid, fat, fat in total solid, salt, salt in total solid, ash, texture, mineral material (Ca, Fe, Cu, Al, Mg, Mn) of Örgü cheese's analysis result haven't been regarded as significant statistically. Each of enzymes which are used effects similarly on α -casein and β -casein during the ripening time and each of the ratios which are gained have been closely determined.

Key words: Örgü Cheese, coagulating enzymes, mineral material, microstructure

Introduction

Örgü (Braided) cheese is a semi hard traditional cheese which contains high level of fat, its curd is scalded, and ripened in brine after knitting. In the production period, a fresh raw milk is filtered using cheesecloth, coagulated with rennet and cutted like small cubes. After whey draining curd is fermented until acidity as applicable to knitting in ambient temperature. In optimum acidity, curd is sliced, is scalded in hot brine (72 °C; % 5 salt) and is shaped by knitting. Cheeses are consumed fresh or after ripening in brine (Figure 1) (Çelik and Türkoğlu, 2007; Hayaloğlu et al., 2008).

The main process during cheese production is coagulation of milk (Er and Sarımehmetoğlu, 2009). Milk coagulant enzymes are gained from animal products, especially calf rennet. Except with animal rennet, milk coagulation is possible to achieve with the proteolytic enzymes which are gained from

various sources. These can be coagulating enzymes which are gained from different animal species, microbial proteases which are gained from some plants (Tejada et al., 2008; Ahmed et al., 2009). Microbial based coagulators are gained from *Mucor pusillus*, *Rhizomucor miehei*, *Rhizomucor pusillus* sp. *lindt*, *Aspergillus oryzae*, *Mucor miehei* and *Endothia parasitica* (Shieh et al., 2009). Recombinant chymosine is the first enzyme which is gained by using recombinant DNA technology with genetic modification, it is transferred to *Kluyveromyces lactis* and *Aspergillus spp.* (Henriksen et al., 1999).

It has been thought that the use of different enzymes will influence on the chemical, physical qualities and the yield of cheese (Alichanidis et al., 1984; Ustunol and Hicks, 1990).

In our study, calf rennet, microbial enzyme and recombinant chymosine have been used in the production of Örgü cheese from cow milk. Within

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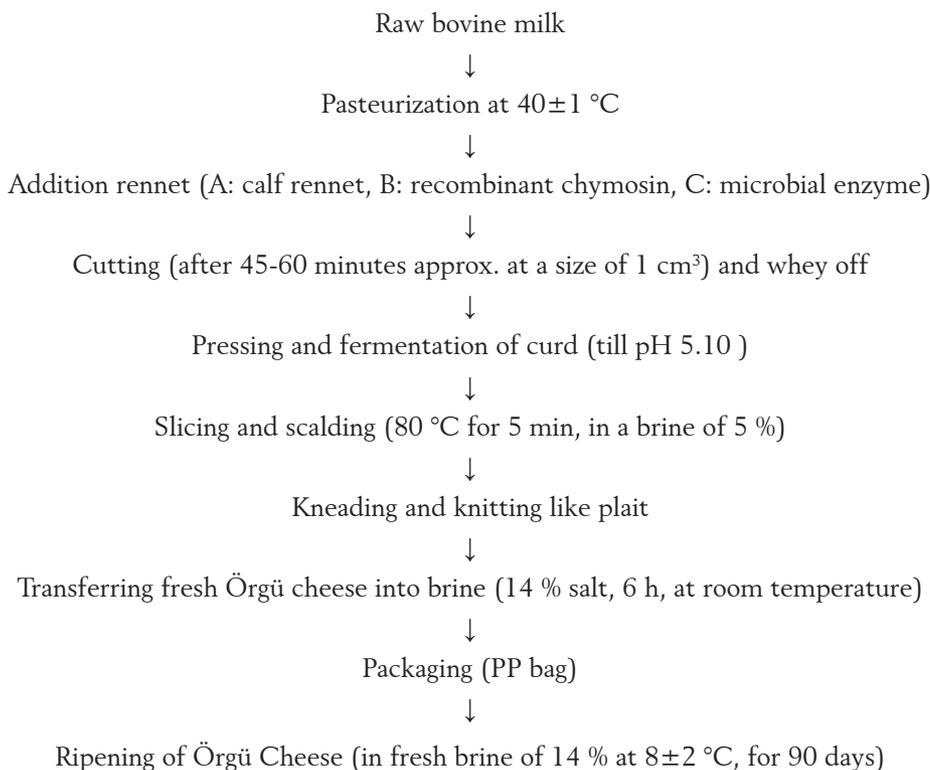


Figure 1. Schematic diagram of the manufacture of experimental Örgü Cheese

study, changes which occur depending on the usage of different enzyme during the three months storage time. It has been tried to determine the changes which occur depending on the usage of different enzyme during the three months storage time with the help of physical, chemical and biochemical analyses.

Material and methods

Materials

Raw cow's milk was supplied from Breeding Cattle Breeder Association Isparta, Turkey. In the production of cheese calf rennet (A) (Renna, % 100 natural calf omasum, Mayasan, Istanbul, Turkey), recombinant chymosine (B) (Renmax 600 L, % 100 Chymosine, *Aspergillus niger* spp. *awamori* with the gene technology, Mayasan, Istanbul, Turkey) and microbial enzyme (C) (Mayasan, *Rhizomucor miehei* protease, Mayasan Istanbul, Turkey) have been used.

Methods

Cheese manufacture and sampling

In this study, Örgü cheeses were produced using three different coagulants (calf rennet, recombinant chymosine and microbial enzyme). They were produced in triplicate and therefore three trials were done. A simplified flow chart for the production of Örgü cheese made from raw milk (RMC) is shown in Figure 1. The experimental cheese from raw milk was produced in Suleyman Demirel University Engineering Faculty, Department of Food Engineering Research and Practice Lab - Isparta, Turkey. After production, the cheese samples were transferred into jars, filled with fresh brine (1/1: w/w) containing 14 % salt, and ripened for 90 days at 8 ± 2 °C. Cheese samples were then analyzed on the 1st, 30th, 60th and 90th days of ripening for physical, chemical and biochemical properties.

Chemical analysis

The titratable acidity (TA, as lactic acid %, acidity of milk- AOAC Official Method 947.05), total

solid (TS %, AOAC Official Method 990.20) was determined as outlined by AOAC methods (2000a, b). Fat (%) and ash (%) of the raw milk was determined using the method of James (1999). pH values were measured using a pH meter (wtw-ph-3110). Percentage of NaCl, ash and fat of cheese was determined using the method of James (1999). NaCl content was expressed as salt concentration. Moisture contents of cheese were determined by AOAC (2000c) methods (Official Method 926.08). Total nitrogen (TN) and water-soluble nitrogen (WSN) levels were determined according to the method of Grippon et al. (1975). Protein content was calculated by multiplication of TN content with 6.38. Ripening index of Örgü cheese samples were calculated using following equation:

$$\text{Ripening index (\%)} = (\text{WSN}/\text{TN}) \times 100$$

Extraction of protein and sample preparation for PAGE

Protein degradation in the all Örgü cheeses was evaluated by electrophoresis as SDS-PAGE (12.5 % polyacrylamides gel) using the Leammli method (1970). Protein standards (SDS 7) were obtained from Sigma Chemical (St. Louis, MO) to identify degraded or breakdown products of protein molecules.

The completed electrophoresis gel was destined and preserved in the destining buffer solution. The developed gel pictures were used to quantify degradative products of casein fractions by an advanced computerized optical densitometer (OD) (UV transilluminator 2000, Bio Rad, Italy) located at the Laboratory of Research Centre in Suleyman Demirel University, Isparta-Turkey. The final numerical values of each breakdown product of protein molecules were quantitatively analyzed.

Texture profile analysis (TPA)

Texture profile Analysis has been practiced by cutting the curd in the shape of 4x4x4 cm. In all recurrences, texture measurements have been practiced from eight different areas of product, which are selected randomly, as three parallels. In the room temperature, texture measurements have been practiced with Lloyd LF Plus Texture Analysis Gadget. In the analysis, 5 mm probe has been used and probe

has been practiced with the force in the 1 N values. In the practiced analysis, the maximum load which probe has showed to pierce the samples has measured (Kaya, 2002).

Mineral material and the determination of trace elements with ICP-OES

Ca, Fe, Mg, Mn, Cu, Al, Zn content of Örgü Cheese has been determined. The samples were prepared as follows: approximately 1.0 g of sample, in the microwave system, has been lysed with 6 ml HNO₃ and 2 mL H₂O₂. Temperature programme has been applied in 400 W two minutes, in 400 W two minutes in 400 W six minutes, in 400 W five minutes, in 800 W eight minutes. The last obtained solution has been diluted to 10 ml with distilled water by cooling. The obtained solution has been analyzed with ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer; Perkin Elmer Inc. Optima 5300 DV; Massachusetts USA) (Bakırcıoğlu et al., 2011).

Aliquots of an ICP multielement standard solution (100 mg/L Merck) containing the analyzed elements was used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 1 % HNO₃. The ranges of the calibration curves (6 points) were selected to match the expected concentrations (0-30 µg/g) for all the elements of the sample studied by ICP-OES. The correlation coefficient r² obtained for all cases was 0.9999.

Scanning electron microscopy of Örgü cheese

Samples for scanning electron microscopy (SEM) were cut with a razor from the interior of the cheese and then diced into rectangular blocks approximately 5x2x2 mm these were immersed in a solution of 1 % glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2) at room temperature for 1-2 h and then stored at 4 °C. Groups of samples were subsequently washed in cacodylate buffer, dehydrated in a graded series of ethanol solutions, extracted with three changes of chloroform, transferred into ethanol, freeze-fractured in liquid nitrogen, thawed into ethanol, and finally dried at the critical point in carbon dioxide. The dried blocks were mounted on aluminium stubs, coated with a thin layer of gold in

Table 1. The results of some parameters of Örgü cheese (n=3)

Applied analysis	1. day			90. day		
	A	B	C	A	B	C
pH	5.26±0.00 ^{cA}	5.24±0.02 ^{bA}	5.22±0.04 ^{bA}	5.78±0.16 ^{aA}	5.66±0.15 ^{aA}	5.48±0.01 ^{aA}
Titrateable acid (% LA)	1.31±0.02 ^{aA}	1.36±0.02 ^{aA}	1.30±0.03 ^{aA}	1.06±0.00 ^{bcA}	1.02±0.05 ^{bA}	1.11±0.07 ^{bA}
Total nitrogen (%)	3.39±0.03 ^{aA}	3.51±0.00 ^{aA}	3.66±0.07 ^{aA}	3.37±0.01 ^{aA}	3.49±0.05 ^{abA}	3.43±0.03 ^{bA}
Water-soluble nitrogen (%)	0.42±0.02 ^{bA}	0.50±0.04 ^{bA}	0.47±0.03 ^{bA}	0.66±0.03 ^{aA}	0.67±0.04 ^{aA}	0.75±0.12 ^{aA}
Ripening index	12.58±0.74 ^{bcA}	14.48±1.15 ^{bcA}	13.04±0.56 ^{bcA}	19.57±0.79 ^{aA}	19.23±1.49 ^{aA}	21.89±3.31 ^{aA}
Hardness (N)	15.12±0.10 ^{aA}	15.94±0.92 ^{aA}	16.03±1.01 ^{aA}	14.58±1.96 ^{cA}	12.58±2.13 ^{cA}	13.03±0.23 ^{cA}
Dry-matter (%)	51.47±1.14 ^{aA}	49.45±0.53 ^{aA}	50.42±0.35 ^{aA}	46.06±2.15 ^{bA}	45.03±1.50 ^{bA}	44.52±1.44 ^{cA}
Fat (%)	18.50±0.00 ^{aA}	16.75±0.25 ^{abB}	16.25±0.25 ^{abB}	18.75±0.25 ^{aA}	17.12±0.12 ^{aB}	16.75±0.001 ^{aB}
Salt (%)	5.86±0.35 ^{dA}	6.28±0.29 ^{dA}	6.46±0.23 ^{dA}	8.25±0.11 ^{aA}	8.55±0.17 ^{aA}	8.31±0.53 ^{aA}
Ash (%)	7.18±0.47 ^{cA}	7.58±0.43 ^{cA}	7.30±0.13 ^{cA}	7.60±0.19 ^{cA}	8.07±0.03 ^{cA}	6.94±0.10 ^{cA}
Calcium (mg/g)	5.19±0.23 ^{aA}	4.50±0.31 ^{aA}	4.39±0.54 ^{aA}	3.92±0.50 ^{bA}	3.51±0.28 ^{bA}	3.95±0.29 ^{bA}
Magnesium (mg/g)	0.24±0.02 ^{aA}	0.20±0.04 ^{aA}	0.21±0.02 ^{aA}	0.17±0.02 ^{bA}	0.16±0.01 ^{bA}	0.16±0.01 ^{bA}
Zinc (mg/g)	0.03±0.00 ^{aA}	0.03±0.00 ^{abB}	0.03±0.00 ^{bbB}	0.03±0.00 ^{aA}	0.03±0.00 ^{aA}	0.03±0.001 ^{aA}
Iron (mg/g)	0.002±0.001 ^{abA}	0.002±0.001 ^{abA}	0.001±0.001 ^{abA}	0.002±0.001 ^{abA}	0.002±0.001 ^{abA}	0.001±0.001 ^{abA}
Copper (mg/g)	0.003±0.002	0.0005±0.001	0.006±0.0003	ND	ND	ND
Manganese (mg/g)	ND	ND	ND	ND	ND	ND

* difference between groups have determined that significant showed as capital letter. Difference between times have demonstrated that significant showed as small letter ($p < 0.01$), A - calf rennet; B - recombinant chymosin; C - microbial enzyme; ND - not determined; n - number of sample analyzed

a DSM-5 and examined by secondary electron imaging in a scanning electron microscope (Tunick et al., 2002).

Statistical analyses

Data were analysed by Statistica 7.0. The effect of heat treatment of cheese milk on the composition and same biochemical properties of Örgü cheese and the changes in studying parameters during ripening were investigated. Means with significant differences were compared by Tukey's multiple range tests (Winer et al., 1991).

Results and discussion

Analysis of raw milk

It has been found that raw milk which is used in the cheese production of pH value, titrateable acidity, dry matter, fat and total nitrogen contents respectively; 6.63 ± 0.01 , 0.154 ± 0.010 %, 11.431 ± 0.394 %, 3.299 ± 0.333 %, 4.269 ± 0.210 %. The dry matter (%), fat (%) and total nitrogen (%) contents of raw milk which is used in cheese production are the same as Aydemir's (2000) findings.

Chemical analyses of cheeses

pH values of cheeses have shown (Table 1) an alteration between 5.22 ± 0.04 (first day, C sample) and 5.78 ± 0.16 (90th day A sample) during ripening

time. Between samples of Örgü cheese, on the 90th day, there was no significant difference. pH values of the cheeses which were produced with calf rennet was higher than the pH the other cheeses. Researchers have indicated differences in pH values of cheeses which are produced using different coagulating enzymes (Yun et al., 1993; Yaşar, 2007).

In the 90th days of ripening, sample B which was produced with recombinant chymosin gave the lowest value of lactic acid, while in C sample which is produced with microbial enzyme the highest value has been determined. Çelik and Turkoglu, (2007) demonstrated that acidity of Örgü cheese increased until the 60th day of ripening period, while acidity decreased in the 90th day.

When examining ratio of total nitrogen of Örgü cheeses it has been determined that the difference between ripening time is important but the difference between enzymes is not significant. In Aydemir's (2000) study, it was found total nitrogen ratio control group of kashar cheese in the first day as 3.747 ± 0.053 %.

At the end of ripening, in C sample (0.752 ± 0.122 %) water soluble nitrogen content has been observed in the highest level. In terms of ripening time, the statistical difference has been identified as important ($p < 0.01$). Mutluer (2007) has found the ratio of water soluble nitrogen between 0.730 ± 0.096 and 0.507 ± 0.109 Yun et al. (1993), have determined that the usage of recombinant chymosin, *Cryphonectria parasitica* protease and *Rhizomucor miehei* protease effect on the ratio of water-soluble nitrogen of cheeses in the mozzarella cheeses production.

The difference of values of ripening index in the storage period has been found as important statistically ($p < 0.01$). In the Kashar cheese which he produced by using calf rennet, recombinant chymosin, protease enzymes of *Rhizomucor miehei*, Yaşar (2007), has determined the highest ripening index in the Kashar cheese which is produced with recombinant chymosine. Also in our study, the lowest ripening index has been established. In B sample which is produced by using recombinant chymosin. The highest ripening index has been determined in C sample which is produced by using the protease of *Rhizomucor miehei*.

As from the first day of ripening to the 90th in the dry-matter values decrease have been seen. In

the first day, B sample which is produced with recombinant chymosin has the lowest dry-matter value, in the 90th day. The highest dry-matter value has been found in the A sample which is produced with calf rennet. The dry-matter values of cheeses has demonstrated similar values of the other researchers findings (Çelik and Türkoğlu, 2007; Türkoğlu et al., 2003).

At the end of the ripening although A sample which is produced with calf rennet has the highest fat ratio (18.75 ± 0.250 %), it has been determined that C sample which is produced with *Rhizomucor miehei* protease (16.250 ± 0.001 %) has the lowest fat ratio. B and C cheese samples demonstrated statistically similar values. The values which were found were atoned to the values which were given by Koçak et al. (1998).

In the cheeses salt ratios have shown regular increase during ripening. As cheese sample (A sample) which is produced with calf rennet has the lowest salt ratio, it has been notified that cheese sample (C sample) which is produced with microbial enzyme (*Rhizomucor miehei* protease), has the highest salt ratio. It has been reported that the usage of different coagulating enzyme has not an effect on the salt ratio (Yaşar, 2007; Johnston et al., 1994).

Although the differences which are seen between times in the ripening period, have been found as significant ($p < 0.01$), between 6.947 ± 0.102 % - 8.076 ± 0.033 % (Table 1) values of ash ratio of cheese samples have similar values in the 90th day. Çelik and Türkoğlu (2007), have found the ratio of ash between 7.09 ± 0.04 % and 8.22 ± 0.04 % values in the cheese which is produced with raw milk.

In the first day of ripening, the hardness of Örgü cheese has been found between 15.128 ± 0.105 Newton (N) and 16.036 ± 1.014 N values. Kaya (2002), has determined that the hardness of cheeses which ripened in the brines which have 5 % salt concentration is 3.45 N, the hardness of cheeses which are ripened in the brines which have 15 % salt concentration is 10.75 N, the hardness of cheeses which are stored in the brine which has 25 % salt concentration is 38.36 N.

Mineral material contents of cheeses

It has been found that the lowest calcium quantity 3.518 mg/g is seen in Örgü cheese (A) which

is gained from *Aspergillus niger* spp. *awamori* with recombinant chymosin, the highest quantity 3.959 mg/g is seen in the Örgü cheese which is produced with *Rhizomucor miehei* protease. It has been observed that amount of calcium decrease during ripening period in brine. Tejada et al. (2008), Murcia al Vino has found the value of Ca quantity between 1123 mg/100 g and 895 mg/100 g in the goat cheese which is produced with animal rennet. The values which we found in the Örgü cheese are low a little in comparison to Tejada et al. (2008), findings. It has been thought that its reason can be derived from the differences in the cheese production process.

The changes of the magnesia contents of cheese samples in the storage period has been found as important statistically ($p < 0.01$). But, difference between cheese groups has not been determined ($p > 0.01$). Cichoscki et al. (2002), have examined mineral contents in semi-hard Prato cheese. They have designated the quantity of magnesia values between 0.50 ± 0.04 mg/g and 0.55 ± 0.09 mg/g, but magnesia values in Örgü cheeses are lower than that study.

The amounts of zinc of the cheeses have been determined between 0.028 ± 0.001 mg/g (cheese

sample which is produced with recombinant enzyme) and 0.035 ± 0.001 mg/g (with microbial enzyme). Sanjuán et al. (1998), have examined mineral material contents of Los Pedroches cheeses. They have determined zinc quantities of cheeses which are produced with animal rennet as being between 2909 $\mu\text{g}/100\text{g}$ and 3800 $\mu\text{g}/100\text{g}$ values.

At the end of the storage the highest values of iron amounts of cheeses have been notified as 0.002 ± 0.001 mg/g. Mendil (2006), has found the iron quantity in the Kars kashar cheese obtained from market 7.5 $\mu\text{g}/\text{g}$.

While copper in the samples in the beginning of storage is found, it has not been found once more during storage periods. Bontinis et al. (2008), have found copper quantity in Xinotyri cheese which is a traditional Greek cheese between 1.19 mg/kg and 1.59 mg/kg values during the storage period.

Merdivan et al. (2004), have informed that the lowest quantity in cheeses belongs to manganese values between cheese groups which they examined comparing to other minerals. They have not encountered manganese in a lot of cheese sample. In our study it has not run across manganese in the samples.

Table 2. Caseine ratios have determined that Örgü cheeses produced with different coagulant enzyme in ripening periods (%) (n=3)

*Sample	Caseines (%)	Ripening times (day)			
		1	30	60	90
A	α -caseine	35.405 ± 0.385^{aA}	34.095 ± 0.585^{aA}	32.420 ± 0.150^{aB}	28.315 ± 0.035^{aC}
	β -caseine	33.820 ± 0.460^{aA}	30.935 ± 0.665^{aB}	31.450 ± 0.120^{aBC}	27.620 ± 0.300^{aC}
	γ -caseine and peptids	30.775 ± 0.075^{aA}	34.970 ± 0.080^{aB}	36.130 ± 0.270^{aC}	44.065 ± 0.335^{aD}
B	α -caseine	34.060 ± 0.810^{aA}	32.000 ± 1.720^{aA}	30.285 ± 0.135^{aB}	28.715 ± 0.795^{aC}
	β -caseine	34.995 ± 0.425^{aA}	33.710 ± 1.160^{aB}	30.200 ± 0.550^{aBC}	29.285 ± 1.865^{aC}
	γ -caseine and peptids	30.945 ± 1.235^{aA}	34.290 ± 2.880^{aB}	39.515 ± 0.415^{aC}	42.000 ± 1.070^{aD}
C	α -caseine	35.565 ± 0.155^{aA}	33.120 ± 0.630^{aA}	30.660 ± 0.050^{aB}	28.475 ± 1.095^{aC}
	β -caseine	34.540 ± 0.040^{aA}	31.475 ± 1.205^{aB}	29.815 ± 0.105^{aBC}	29.450 ± 0.180^{aC}
	γ -caseine and peptids	29.895 ± 0.195^{aA}	35.405 ± 1.835^{aB}	39.525 ± 0.155^{aC}	42.075 ± 0.915^{aD}

* difference between groups have determined that significant showed as capital letter. Difference between times have demonstrated that significant showed as small ($p < 0.01$), A - calf rennet; B - recombinant chymosin; C - microbial enzyme; n - number of sample analyzed

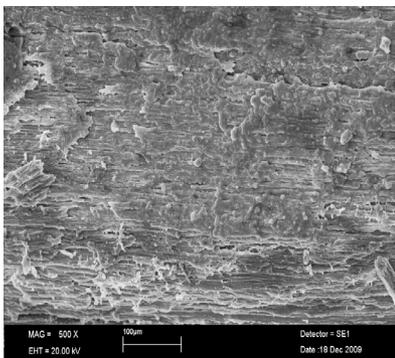
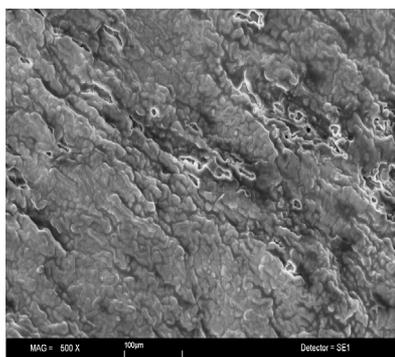
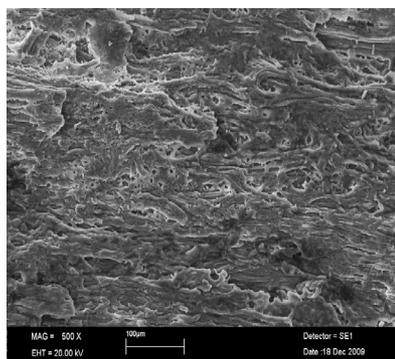


Figure 2. Scanning electron micrographs of the sample cheese (A) Renna (B) Renmax (C) Mayasan

SDS-PAGE findings of cheeses

α -casein quantity of cheeses have been determined 35.565 ± 0.155 % and 34.060 ± 0.810 % levels in the first day of ripening. α -casein quantity has been demonstrated between 28.315 ± 0.035 % (A Sample) and 28.715 ± 0.795 % (B sample) levels in the 90th day of ripening. β -casein quantities of cheeses are between 33.820 ± 0.460 % (A Sample) and 34.995 ± 0.425 % (B sample) (Table 2) levels in

the first day of ripening. As to the end of ripening they have been found between 27.620 ± 0.300 % (A sample) and 29.450 ± 0.180 % (C sample) levels.

Yaşar (2007), reported that the lowest α -casein ratio at the end of ripening was observed when the Kashar cheeses was produced with calf rennet, while the highest α -casein ratio was observed when the cheese was produced with microbial enzyme.

SEM findings

Microstructure studies provide strategic information to understand and to control cheese properties. Representative scanning electron micrographs of the sample cheese at 500x magnifications are presented in Figure 2.

In all sample cheeses, the microstructure is characterized by networks of parallel-oriented protein fibres occupied by serum and fat droplets. But, the protein matrix of B sample was more uniform than the others. This is in agreement with the findings of Lobato-Calleros et al. (2007), Dinkçi et al. (2011), Kindstedt and Guo (1997).

Conclusion

The use of three enzymes (calf rennet, microbial enzymes, and recombinant chymosin) in the coagulation of milk for production of Örgü cheese demonstrated to have similar characteristics regarding pH, titratable acidity, total nitrogen, ripening index, hardness, mineral materials and casein ratios of the cheese.

Učinak različitih koagulirajućih enzima na kvalitetu tradicionalnog Örgü sira (pleteni sir)

Sažetak

Örgü sir je proizveden korištenjem nekoliko različitih enzima (teleće sirilo, mikrobnii enzim, rekombinantni himozin) te je praćen utjecaj različitih enzima zgrušavanja na osobine mineralnih tvari i sira tijekom 90 dana zrenja. Sadržaj mineralnih tvari Örgü sira utvrđen je ICP-OES (induktivno atomska

emisijnska spektroskopija). Stupanj proteolize sira utvrđen je kemijskom analizom pomoću SDS-PAGE (sodijum dodecil sulfat poliakrilamid gel elektroforeza). Utvrđene razlike između rezultata, titracijske kiselosti, ukupnog dušika, dušika topivog u vodi, indeksa zrenja, ukupne suhe tvari, masti, masti u suhoj tvari, soli, soli u suhoj tvari, pepela, teksture, mineralnih tvari (Ca, Fe, Cu, Al, Mg, Mn) u Örgü siru nisu statistički značajne. Svaki od korištenih enzima ima sličan utjecaj na α -kazein i β -kazein tijekom dozrijevanja te su svi izračunati omjeri bili vrlo slični.

Ključne riječi: Örgü sir, koagulirajući enzimi, mineralne tvari, mikrostruktura

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