Bulgarian Journal of Agricultural Science, 23 (No 2) 2017, 330–337
Agricultural Academy

EFFECTS OF CAPSULE MATERIALS ON THE TEXTURAL AND SENSORY CHARACTERISTICS OF KASHAR CHEESE RIPENED WITH ENCAPSULATED LIPASE AND PROTEASE

MUSA S. AKIN; MUTLU. B. GÜLER-AKIN*
Harran University, Agricultural Faculty, Department of Food Engineering, Sanlıurfa, 63100 Turkey

Abstract

Akin, M. S., M. B. Güler-Akın, 2017. Effects of capsule materials on the textural and sensory characteristics of Kashar cheese ripened with encapsulated lipase and protease. *Bulg. J. Agric. Sci.*, 23 (2): 330–337

In this study, lipase and protease enzymes cocktails encapsulated in k-karragenan, gellan and sodium alginate by using emulsion and extrusion techniques were added in cheese milk together with rennet. The effects of the encapsulating material and ripening period on the textural and sensory characteristics of Kashar cheese were investigated. The study demonstrated that capsule materials significantly affected textural and sensory characteristics of Kashar cheese ripened with encapsulated enzyme cocktails. Cheeses treated with k-carrageenan capsules showed poor textural properties and the lowest sensory scores. Sodium alginate capsules disrupted under cheese manufacturing conditions appeared to be more suitable in accelerating cheese ripening than gum capsules.

Key words: Kashar cheese, enzyme encapsulation, sensory, textural properties

Introduction

After White cheese, Kashar cheese is the most commonly produced and consumed cheese in Turkey, the Balkan, Peninsula and the Mediterranean region. The main problem in manufacturing Kashar cheese is the long maturation period which increases the cost of handling significantly. Maturation is very important in developing the unique flavour, aroma and texture of the cheese before marketing. However, the long maturation period increases the price of the cheese (Fox, 1993).

Several attempts have been made to reduce the ripening period by the addition of individual and mixed lipase, protease and b-galactosidase enzymes, some of which have been reported to halve the normal maturation period of cheese. Lipolysis and proteolysis play an important role in cheese ripening, and a large number of studies dealing with the acceleration of lipolysis and proteolysis through the addition of free lipolytic and proteolytic enzymes to either cheese milk or curd have been published (Kocak et al., 1996; Caglar and Cakmakci, 1998). The addition of free lipases has resulted in premature attack leading to excessive lipolysis and texture and flavour defects (Kocak et al., 1996). Direct addition of protease enzyme to the cheese milk was not successful due to loss of enzymes in the whey, poor enzyme distribution, reduced yield and poor-quality cheese. Incorporation of encapsulated enzyme eliminated the problems associated with direct enzyme addition. The use of microencapsulated enzymes has been proposed to circumvent these drawbacks. Enzyme microcapsules physically separate the enzyme from the substrate in the curd and the enzyme is only released into the curd upon capsule breakdown during ripening (Karel, 1990).

Vegetable gels such as Konjac, liposomes, milk fat, some food gums and hydrophilic hydrocolloids are used for enzyme encapsulation. Gums have been extensively used for the immobilization of living cells and, to a lesser extent, enzymes. Gum capsules are easy to prepare, and gums are rela-

**Corresponding author*: mutluakin@harran.edu.tr

tively widely available, cheap, and biologically compatible (Kailasaphaty and Lam, 2005).

Limited information is available on the accelerated ripening of Kashar cheese using encapsulated enzymes (Caglar and Cakmakci, 1998; Akin et al., 2012). In the present study an application of food gums as an alternative to liposomes for enzyme encapsulation to accelerate Kashar cheese ripening was investigated. Three gums (gellan, k-carrageenan and sodium alginate) were used to encapsulate enzymes for application to cheese milk. The objective of this work was to study the effect of encapsulated lipase and protease enzymes cocktails added to Kashar cheese milk on the textural and sensory characteristics of the cheese during storage.

Materials and Methods

Gums, enzymes and chemicals

Sodium alginate, k-carrageenan and gellan gums were supplied by Sigma Chemicals (İstanbul, Turkey). The enzyme Palatase 20000 L (LUN 00217) and Flavourzyme 1000 L was obtained from Novozymes (İstanbul, Turkey). Direct-set frozen lactic acid starter cultures (Ezal MA014) containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* were obtained from Ezal (France). Rennet (ECOREN 200) was obtained from Maysa Gıda (İstanbul). All other reagents used were of analytical grade.

Preparation of gum capsules

k-carrageenan and gellan capsules were prepared by a modified method of Audet and Lacroix (1989). Gum powders (1.5 gk-carrageenan and 0.3 gellan) was suspended in three lots of 50 mL deionized water, heated to 80°C, stirred and kept at that temperature for 20 min to completely dissolve the polymer. The solutions were cooled to 40°C and mixed with 0.133 mL of 7.5% (w/v) solution of Palatase 20000 L and 13.3 mL of 7.5% (w/v) solution of Flavourzyme 1000 L to produce of capsules. The mixture was rapidly poured into 150 mL soybean oil containing 0.2% emulsifier in a beaker maintained at 40°C while stirring with a magnetic stirrer. The water-in-oil emulsions were cooled to 25°C to allow the gum droplets to gel. The oil phase was decanted, and the resulting capsules were harvested by centrifugation (100x*g*, 2 min). The gel beads were washed twice with distilled water, and the capsules were separated from the supernatant by sieving. The beads formed were hardened by soaking in 0.07% calcium chloride solution for 2 h.

Preparation of sodium alginate capsules by using emulsion and extrusion techniques

The modified method of Sheu and Marshall (1993) was used. A 2% alginate mixture containing 2% Hi-maize resistant starch and 0.133 mL of a 7.5% (w/v) solution of Flavourzyme 1000 L were prepared. The mixture was dropped into oil containing Tween 80 (0.02%). After the dropping was completed, the mixture was stirred vigorously until it was emulsified and appeared creamy. A solution of 0.1 M calcium chloride was then added quickly along the side of the beaker; the phase separation of the oil /water emulsion then occurred. The mixture was left to stand for 30 min to allow the calcium-alginate beads to separate and settle at the bottom of the calcium chloride layer. The oil layer was drained, and beads were collected by low-speed centrifugation (350x*g*, 15 min), washed once with 0.9% saline containing 5% glycerol, and stored at 4°C. Size separation of the beads was performed using 500 µm and 150 µm steel sieves.

The extrusion technique of Krasaekoopt et al. (2003) was used. In this study, the protease in the 13.3 mL Flavourzyme 1000 L solution was mixed with 20 mL of 2% (w/v) sodium alginate solution (Sigma Aldrich Steinheim, Germany). The suspension was injected through a 0.11 mm needle into 0.05 m $CaCl_2$. The beads were allowed to stand for 30 min to gelate, then rinsed with 0.9% saline containing 5% glycerol and subsequently kept in at 4°C.

Rates of enzyme entrapment

The total enzyme activity was determined in a bulk solution of capsules (before separation of capsules from the un-encapsulated material). The bulk solutions (10 mL) containing k-carrageenan and gellan capsules were separately dispersed in 50 mL of 0.4% trisodium citrate solutions and stirred for 30 min at room temperature (23–24°C) until completely dissolved. Separated gum capsules were treated similarly in trisodium citrate solution. The bulk solutions (10 mL) containing sodium alginate capsules were separately dispersed in 50 mL of 1% sodium citrate solutions and stirred for 10 min at room temperature (23–24°C) until completely dissolved. The efficiency of lipase and protease enzyme encapsulation for the three types of capsules were measured separately for lipase and protease according to the methods of Teng and Xu (2007) and Sarath et al. (1989) respectively. The rate of EE was the percentage of enzyme encapsulated (expressed as units enzyme activity) in capsules divided by the total units of enzyme in bulk solution multiplied by 100.

The diameters of 100 randomly selected beads of each treatment were measured with an eyepiece micrometer on an optical microscope at a magnification of 100x.

Cheesemaking

A 7.5% (w/v) solution of Palatase M (20000 LU/g) and a 7.5% (w/v) solution of Flavourzyme 1000 L (1000 LAPU/g)

were encapsulated in sodium alginate, k-carrageenan and gellan gums as described above.

Cheese production was done in the Dairy Pilot Plant of the Food Engineering Department of Harran University. One hundred twenty kilograms of standardized milk was used for each batch (1 control (coded A) and 4 treatments). The fat content of the milk was standardized to 2.5%. All batches were pasteurized at 72°C for 1 min and then cooled to 34°C. Starter culture (1%) and $CaCl_2$ (0.02%) were then added. For the experimental cheeses, enzyme capsules made of sodium alginate by emulsion techniques, sodium alginate by extrusion techniques, k-carrageenan and gellan gums, were introduced into the cheese milk at 34°C, just before the addition of rennet, and the samples were coded B, C, D and E respectively. These quantities corresponded to 0.2 LU/g milk fat. When the pH of the milk reached 6.2–6.3, rennet diluted with pure water was added. Cutting was performed 30 min later. The curd was cut with a curd knife into cubes of 1 cm^3. The cut curd was allowed to settle for 15 min. Cooking was performed by increasing the temperature from 34°C to 40°C over 30 min. The heating rate was an increase of 1°C for every 4-5 min. At the end of cooking, a third of the whey content was drained from each batch. At the same time, the cheese curd was agitated. The cheese curd was fermented until it reached a pH level of 5.0. The remaining whey was then drained. Cheese whey was collected during the manufacturing and strained using a 120-µm stainless steel sieve. The capsules were collected on the sieve and re-added to the curd. The curd was hand-stretched in 6% brine at 74°C for 2 min for all the cheeses studied. Brine was strained using a 120 µm stainless steel sieve and the capsules were collected on the sieve and re-added to curd. The curds were placed into cylindrical stainless steel molds and turned 30 min later to provide a flat surface. All cheeses were cooled at room temperature, and the molds were removed. Then, the cheeses were allowed to gain their yellow colour for 24 h at 15±2°C.

The mass of one block of fresh Kashar cheese was approximately 600 g. The blocks of cheeses were surface-salted for 1 week and stored at 4–6°C for 180 days. Cheese samples were taken for chemical analyses on the 1st, 15th, 30th, 60th, 90th, 120th and 150th days of storage. Cheese was manufactured in triplicate for each group.

Cheese composition

The pH of the milk (TSE, 1994) and cheeses (TSE, 1995) was measured using a digital pH-meter (model of Orion 250 A, Orion Research Inc., Boston, USA). The protein content and water sobuble nitrogen (WSN) of cheeses were determined by the Kjeldahl method (Gripon et al., 1975). Ripening index (RI) was estimated by using the formula: (WSN/TN)x100, as proposed by Alais (1984). The total fat and dry matter contents of the cheese samples were determined using the method proposed by Gerber (TSE, 1994) and gravimetric methods, respectively. The salt content of the cheeses was determined by the Mohr titration method (AOAC, 1990). The total free fatty acids (TFFAs) were determined according to the procedure of Sukhija and Palmquist (1988).

Texture measurements

The textural properties of Kashar cheese were evaluated by the method of TPA (Bourne, 1978). Texture profile analysis was performed on cheese samples by using a double compression test (TA-XT2i Texture Analyzer; Stable Micro Systems, NY, USA).

Sensory evaluation

The samples were organoleptically assessed by ten panellists. The panel was made up of staff members and postgraduate students of Harran University Food Engineering Department having previous experience with cheese sensory evaluation. Sensory assessment was performed in standard individual tasting booths, in the morning, according to the Turkish standard TS 3272 (TSE, 1999). A 5-point hedonic scale was used to evaluate flavour, texture, odour and appearance. General acceptability was the sum of flavour, texture, odour and appearance scores.

Statistical analyses

Each cheese experiment was repeated three times, and each analysis was done in duplicate. The experiment was designed according to a 5x8x3 factorial design. All statistical analyses were performed using the SPSS statistical software program (version 5.0). Statistically different groups were determined by the LSD (Least Significant Difference) test (Bek and Efe 1995).

Results and Discussion

Enzyme encapsulation

The encapsulation efficiencies of Palatase M (20000 LU/g) in k-carrageenan, gellan gums, sodium alginate produced by emulsion techniques or sodium alginate by extrusion techniques were, respectively, 58.7, 51.0, 42.50 and 53.1% of the initial activity (mean of 3 separate trials). Encapsulation efficiencies of Flavourzyme 1000 (1000 LAPU/g) in k-carragennan, gellan gums, sodium alginate by emulsion techniques or sodium alginate by extrusion techniques were, respectively, 54.1, 47.3, 39.2 and 47.9% of the initial activity (mean of 3 separate trials). The encapsulation efficiencies of the four capsulants were statistically significantly different

from each other ($p < 0.01$). The ionic strength of the capsule-hardening solution (calcium chloride) may have had an effect on the activity of the enzymes (Kailasaphaty and Lam, 2005).

The beads were globular in shape. The capsule materials influenced on the size of the beads. The sodium alginate capsules by extrusion technique were bigger than the other capsules. The diameter of beads of k-carragennan, gellan gums, sodium alginate by emulsion technique were 1.68 mm, which was significantly lower than that of sodium alginate by extrusion technique beads (1.90 mm).

Chemical composition

The main chemical composition of the control and experimental cheeses is given in Table 1. Significant differences were observed between the control and experimental cheeses. The experimental cheese curds had a significantly higher moisture content and titratable acidity but lower pH contents as compared to the control ($p<0.01$). The high moisture content of the gum-capsule-treated cheese curds was due to the hydrophilic nature of sodium alginate, carrageenan, and gellan gums, which retained moisture in the cheeses. The protein, fat and salt contents of the experimental cheeses were close to those of the control ($p<0.05$). Similar results were reported for capsule-treated cheeses by Kheadr et al., (2003); Kailasaphaty and Lam (2005).

The total free fatty acid (TFFA) contents of the control and experimental cheeses were quantified throughout a 180-day of ripening period (Table 2). The production of TFFAs was markedly stimulated by the introduction of encapsulated lipases and proteases. Upon aging, the amount of TFFA produced increased with the ripening period; moreover, the experimental cheeses had accumulated higher quantities of TFFAs, depending on the type of capsule, as compared to control ($p<0.01$). The higher incidence of TFFAs was found in the k-carrageenan capsules-treated cheeses. The lower TFFA content of sodium-alginate-treated cheeses suggests that these capsules probably release their enzyme contents very slowly. The progressive increase in the concentration of such TFFAs established during cheese ripening, suggested a gradual release of the encapsulated lipases. Similar results were reported for ecapsulated lipase-treated cheeses by Akin et al. (2012).

There were significantly ($p<0.01$) higher levels of proteolysis in enzyme treated cheeses as compared to the control cheese (Table 2). Cheeses treated with k-carrageenan capsules showed a highest rate of increase in WSN and RI. The higher rate of WSN and RI in the k-carrageenan capsules-treated cheeses was probably due to the low stability of k-carrageenan gels in solutions with low pH values (Kailasapathy and Lam, 2005), similar to that observed in ripening cheese. The observed slower rate of increase in WSN and RI in cheeses treated with sodium alginate capsules suggests that these capsules probably release their enzyme contents very slowly.

An increasing trend for WSN and RI values was observed during the 180-day of ripening period for all cheeses ($p < 0.01$). This was expected because amino groups are produced in cheese as a consequence of protein breakdown during ripening (Fox et al., 1993). An increase in proteolysis in enzyme treated cheese compare to control at any given time during ripening indicated that an acceleration of ripening.

Textural properties

Introduction of capsules into the cheese matrix, as well as the ripening process, affected the textural properties of experimental cheeses (**$P<0.01$). Enzyme-treated cheeses exhibited noticeable differences in their textural properties from the day of manufacture compared to control cheeses. The changes in textural properties (the parameters studied were: hardness, cohesiveness, springiness, gumminess and chewiness) during ripening of control and experimental cheeses are shown in Figures 1, 2, 3, 4 and 5.

Table 1
Chemical composition of control and encapsulated lipase and protease-treated cheeses on day of manufacture

Chemical parameter	Cheeses*				
	A	B	C	D	E
pH	5.37±0.02^{a}	5.29±0.04^{b}	5.32±0.04^{b}	5.17±0.02^{c}	5.08±0.04^{c}
Titratable acidity (%l.a.)	1.15±0.021^{d}	1.278±0.021^{c}	1.170±0.021^{d}	1.332±0.031^{b}	1.404±0.021^{a}
Moisture (%)	45.48±0.32^{a}	47.84±0.38^{d}	46.40±0.35^{b}	47.13±0.37^{c}	47.88±0.37^{d}
Protein (%)	23.52±0.34^{b}	22.38±0.22^{d}	23.88±0.27^{a}	23.52±0.24^{b}	22.77±0.19^{c}
Fat in dry matter (F/D)	51.81±0.23^{b}	51.77±0.3^{b}	52.54±0.11^{a}	51.86±0.42^{b}	51.48±0.08^{b}
Salt (%)	1.76±0.05^{a}	1.66±0.07^{b}	1.76±0.05^{a}	1.70±0.05^{b}	1.40±0.06^{c}

*__A;__ control cheese, __B; C; D;__ and __E__ cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

** Different letters following numbers in the same row denote significant differences ($p < 0.05$)

Table 2
Lipolysis and proteolysis of control and enzyme-treated cheeses during 180 days ripening (n = 3)

Cheeses	Ripening period (day)	TFFA, (%)	TN, (%)	WSN, (%)	RI, (%)
	1	34.87±0.356^{m5}	3.687±0.053^{g3}	0.321±0.014^{l5}	8.70±0.27^{n5}
	15	45.83±0.373^{m4}	4.063±0.154^{e2}	0.424±0.076^{k4}	10.44±0.32^{m4}
A	90	95.41±1.562^{k3}	4.417±0.114^{a1}	0.665±0.077^{i3}	15.06±0.82^{k3}
	120	115.40±1.882^{k2}	4.437±0.106^{a1}	0.744±0.066^{h2}	16.77±0.69^{i2}
	180	161.87±1.997^{j1}	4.459±0.125^{a1}	0.864±0.095^{g1}	19.38±1.54^{h1}
	1	50.24±0.455^{m5}	3.508±0.034^{h3}	0.347±0.020^{l5}	9.88±0.462^{m5}
	15	68.64±1.020^{l4}	3.992±0.074^{e2}	0.651±0.094^{i4}	16.31±0.71^{i4}
B	90	171.63±2.888^{i3}	4.298±0.107^{b1}	1.031±0.094^{f3}	23.99±0.38^{g3}
	120	222.61±3.014^{h2}	4.315±0.086^{b1}	1.162±0.098^{d2}	26.93±0.38^{e2}
	180	328.04±4.314^{d1}	4.313±0.102^{b1}	1.341±0.099^{b1}	31.09±1.6^{c1}
	1	50.81±0.389^{m5}	3.743±0.043^{g3}	0.452±0.014^{k5}	12.07±0.25^{l5}
	15	72.41±0.867^{l4}	3.966±0.088^{f2}	0.634±0.112^{i4}	15.96±0.59^{j4}
C	90	191.06±2.669^{i3}	4.285±0.112^{b1}	1.031±0.088^{f3}	24.06±0.88^{g3}
	120	250.23±2.974^{g2}	4.309±0.121^{b1}	1.172±0.094^{d2}	27.20±0.94^{e2}
	180	365.03±4.17^{c1}	4.317±0.115^{b1}	1.350±0.121^{b1}	31.27±2.02^{c1}
	1	53.86±0.752^{m5}	3.687±0.037^{g3}	0.433±0.014^{k5}	11.74±0.26^{l5}
	15	81.13±0.981^{l4}	3.930±0.212^{f2}	0.536±0.056^{i4}	16.24±0.54^{i4}
D	90	229.71±2.932^{h3}	4.213±0.084^{c1}	1.120±0.090^{d3}	26.58±0.63^{e3}
	120	299.81±3.213^{e2}	4.232±0.079^{b1}	1.271±0.108^{c2}	30.03±0.88^{d2}
	180	436.28±3.470^{a1}	4.232±0.111^{b1}	1.458±0.121^{a1}	34.45±2.0^{a1}
	1	52.16±0.646^{m5}	3.569±0.030^{h3}	0.437±0.016^{k5}	12.24±0.33^{l5}
	15	76.96±0.897^{l4}	3.793±0.090^{g2}	0.638±0.049^{i4}	16.82±0.62^{i4}
E	90	211.64±2.885^{h3}	4.141±0.097^{d1}	1.094±0.093^{e3}	26.42±0.4^{f3}
	120	275.21±3.055^{f2}	4.160±0.117^{d1}	1.234±0.101^{c2}	29.66±1.0^{d2}
	180	394.46±4.317^{b1}	4.161±0.110^{d1}	1.377±0.123^{b1}	33.07±2.12^{b1}

***A;** control cheese, **B; C; D;** and **E** cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carragennan capsules, in gellan capsules, respectively
** Different letters in the same column denote significant differences for capsule materials ($p < 0.01$)
***different numbers in the same column denote significant differences for storage period ($p < 0.01$)

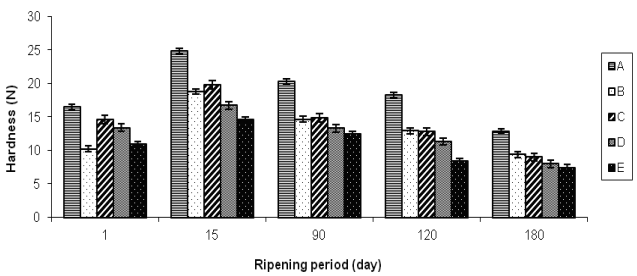

Fig. 1. Changes in the hardness of Kashar cheeses during 180 days of ripening
*A; control cheese, B; C; D; and E cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

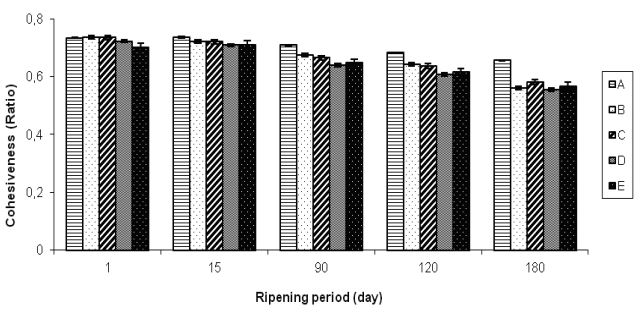

Fig. 2. Changes in the cohesiveness of Kashar cheeses during 180 days of ripening
*A; control cheese, B; C; D; and E cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

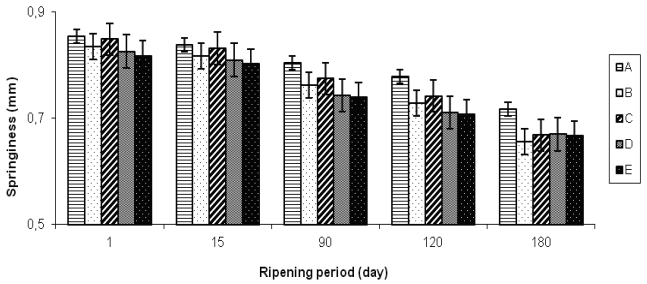

Fig. 3. Changes in the springiness of Kashar cheeses during 180 days of ripening

*A; control cheese, B; C; D; and E cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

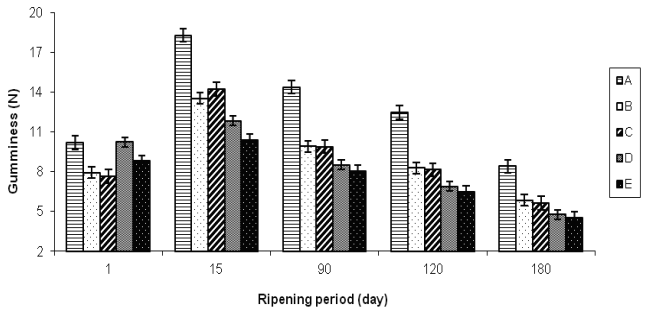

Fig. 4. Changes in the gumminess of Kashar cheeses during 180 days of ripening

*A; control cheese, B; C; D; and E cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

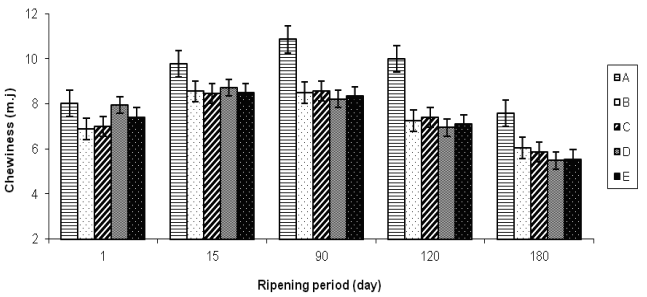

Fig. 5. Changes in the chewiness of Kashar cheeses during 180 days of ripening

*A; control cheese, B; C; D; and E cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carrageenan capsules, in gellan capsules, respectively

At the day of manufacture, experimental cheeses had significantly (**P < 0.01) lower values for the hardness as compared to control. This effect was found to be correlated to higher moisture content of capsule-treated cheeses. The lowest hardness was in the k-carrageennan capsules-treated cheeses. Based on the TPA hardness, two distinct regions occured including initial hardening up to 30 days and softening period after 30 days.

The cohesiveness and springiness of the control cheeses was slowly higher than capsule-treated cheeses throughout ripening ($p < 0.01$). The higher cohesiveness and springiness of control cheeses might be due to lower moisture content. Zisu and Shah (2004) reported that cheeses with the lower moisture content showed greater cohesiveness. As with cohesiveness, the relationship between moisture and hardness and their effects on the protein microstructure existed for springiness and are responsible for the loss in the ability of the cheese to recover to its original state (Zisu and Shah, 2004). The springiness values of all the cheeses studied continuously decreased as the storage time increased for the entire testing period of 180 days.

The highest gumminess and chewiness value was observed in control cheeses ($p<0.01$) due to its lower moisture content compare to enzyme treated cheeses. A decreasing trend in gumminess and chewiness was observed during180 days of ripening period for all cheeses.

Sensory evaluation

The mean sensory scores of the cheeses are shown in Table 3. The control cheese obtained highest scores for appearance (**P < 0.01). k-carrageennan capsules-treated cheeses gained the lowest score for appearance. This could be due to the soft and crumbly texture in this cheese as compared to the other experimental cheeses. The appearance scores of all the cheeses studied increased up to 120 days and decreased after 150 days.

The addition of encapsulated enzyme had a significant effect on the organoleptic texture scores (**P < 0.01). Control cheeses were ranked higher for texture during ripening period. This could be due to the lower moisture content and lower level of proteolysis in the cheese as compared to the experimental cheeses. The lowest texture score was in the k-carrageennan capsules-treated cheeses probably because of higher moisture content and higher level of proteolysis as compared to the other cheeses. The texture scores of all cheeses increased up to 120 days and decreased after 150 days. When the elasticity of the cheese has been reduced by proteolytic cleavage of the α_{s1}-casein (mainly caused by chymosin), which is regarded as a link in the protein network (McSweeney et al., 1993), the cheese might be perceived by

the sensory panel as harder, more brittle and less elastic. This was observed for all cheeses up to 90 d of ripening. After 150 d of ripening (data not shown) this was not observed probably because of high level of proteolysis.

Although the use of enzyme capsules resulted accelerated ripening, the lower mean score for textural parameters of the experimental cheeses as compared to the control cheese may cause a problem that affects the product acceptance. The lower mean score for textural parameters for experimental cheeses could have also been as a result of moisture retention in capsule-treated cheese. Excessive moisture retention in cheese during manufacture is known to result in soft and crumbly texture (Manning, 1985).

The main differences among treatments were found in odour and flavour scores are given below. k-carrageennan capsules treated cheeses had the lowest odour and flavour scores. In addition, the soapy off-flavour was noted in cheeses treated with k-carrageennan capsules probably due to the low stability of k-carrageennan gels in solutions with low pH similar to that reported by Kailasaphaty and Lam (2005) and Akin et al. (2012). It appears from Table 3 that the addition of encapsulated enzyme slightly improved odour and flavour intensities of the experimental cheeses depending on cheese age. There were no significant differences observed in odour and flavour characteristics between the experimental cheeses and the control cheese up to 90 days ripening. After 90 days the experimantal cheeses had lower odour and flavour scores than control cheese and this was because of the excessive rancidity.

There were significant differences in general acceptibility (**$P < 0.01$) for the experimental and the control cheeses. The most acceptable cheeses were control cheeses, followed by sodium alginate produced by emulsion, sodium alginate produced by extrusion, gellan and k-carrageennan capsules-

Table 3
Sensory scores of control and enzyme-treated cheeses during 180 days ripening (n = 3)

Cheeses	Ripening period (day)	Appearance (point)	Texture (point)	Odour (point)	Flavour (point)	General acceptability (point)
	1	4.14 ± 0.48^{a2}	4.10 ± 0.40^{a2}	4.51 ± 0.45^{a2}	4.01 ± 0.36^{a3}	21.28 ± 0.52^{a3}
	15	4.64 ± 0.45^{a1}	4.74 ± 0.26^{a1}	4.60 ± 0.40^{a1}	4.50 ± 0.25^{a2}	23.19 ± 0.48^{a2}
A	90	4.78 ± 0.22^{a1}	4.88 ± 0.12^{a1}	4.75 ± 0.25^{a1}	4.80 ± 0.20^{a1}	24.02 ± 0.78^{a1}
	120	4.84 ± 0.16^{a1}	4.90 ± 0.10^{a1}	4.82 ± 0.18^{a1}	4.87 ± 0.13^{a1}	24.28 ± 0.62^{a}
	180	4.74 ± 0.20^{a1}	$4,72\pm0,20^{a1}$	4.68 ± 0.32^{a1}	4.86 ± 0.14^{a1}	23.87 ± 0.78^{a1}
	1	4.02 ± 0.42^{a2}	3.75 ± 0.35^{b3}	4.46 ± 0.26^{a2}	3.90 ± 0.35^{a3}	20.14 ± 0.64^{b3}
	15	4.38 ± 0.45^{b1}	4.30 ± 0.35^{b2}	4.68 ± 0.26^{a1}	4.45 ± 0.23^{a2}	22.25 ± 0.62^{b2}
B	90	4.66 ± 0.24^{a1}	4.61 ± 0.31^{b1}	4.90 ± 0.10^{a1}	4.92 ± 0.10^{a1}	23.69 ± 0.65^{a1}
	120	4.67 ± 0.32^{a1}	4.44 ± 0.34^{b1}	4.71 ± 0.26^{a1}	4.48 ± 0.38^{b2}	22.74 ± 0.54^{c2}
	180	3.74 ± 0.31^{b3}	$3,06\pm0,26^{b4}$	2.83 ± 0.24^{c3}	2.66 ± 0.32^{b4}	16.17 ± 0.51^{b4}
	1	4.00 ± 0.35^{a3}	3.74 ± 0.36^{b3}	4.45 ± 0.40^{a2}	3.92 ± 0.38^{a3}	19.98 ± 0.58^{b2}
	15	4.30 ± 0.35^{b2}	4.30 ± 0.45^{b2}	4.58 ± 0.22^{a2}	4.44 ± 0.27^{a2}	22.01 ± 0.48^{b2}
C	90	4.63 ± 0.27^{a1}	4.61 ± 0.33^{b1}	4.88 ± 0.12^{a1}	4.92 ± 0.08^{a1}	23.59 ± 0.66^{b1}
	120	4.60 ± 0.35^{b1}	4.40 ± 0.30^{b2}	4.71 ± 0.28^{a1}	4.45 ± 0.40^{b2}	22.59 ± 0.71^{c2}
	180	3.65 ± 0.32^{b4}	$3,00\pm0,25^{b4}$	2.84 ± 0.27^{b3}	2.63 ± 0.32^{b4}	16.05 ± 0.49^{b3}
	1	3.91 ± 0.35^{a2}	3.60 ± 0.25^{b3}	4.50 ± 0.30^{a2}	3.75 ± 0.35^{b4}	19.51 ± 0.48^{c3}
	15	4.09 ± 0.45^{c2}	4.17 ± 0.25^{b2}	4.54 ± 0.37^{a2}	4.40 ± 0.21^{a2}	21.53 ± 0.44^{c2}
D	90	4.50 ± 0.25^{b1}	4.50 ± 0.35^{b1}	4.84 ± 0.16^{a1}	4.88 ± 0.12^{a1}	23.24 ± 0.58^{b1}
	120	4.10 ± 0.35^{c2}	4.20 ± 0.35^{c2}	4.60 ± 0.35^{b1}	4.02 ± 0.38^{c3}	21.35 ± 0.55^{d2}
	180	3.05 ± 0.25^{c3}	$3.10\pm0,27^{b4}$	2.43 ± 0.25^{c3}	2.16 ± 0.25^{c5}	14.53 ± 0.47^{c4}
	1	3.95 ± 0.38^{a2}	3.59 ± 0.24^{b3}	4.44 ± 0.28^{a2}	3.78 ± 0.24^{b3}	19.60 ± 0.60^{b2}
	15	4.17 ± 0.38^{b2}	4.21 ± 0.44^{b2}	4.55 ± 0.21^{a2}	4.46 ± 0.24^{a2}	21.74 ± 0.50^{b2}
E	90	4.52 ± 0.24^{b1}	4.54 ± 0.36^{b1}	4.87 ± 0.13^{a1}	4.90 ± 0.10^{a1}	23.37 ± 0.55^{b1}
	120	4.51 ± 0.27^{c1}	4.31 ± 0.26^{b2}	4.65 ± 0.25^{a1}	4.20 ± 0.40^{d2}	22.07 ± 0.71^{d2}
	180	3.53 ± 0.28^{b3}	$2,71\pm0,30^{c4}$	2.63 ± 0.27^{b3}	2.24 ± 0.24^{c4}	14.78 ± 0.50^{c3}

***A;** control cheese, **B; C; D;** and **E** cheeses contain Palatase M and Flavourzyme 1000 L in sodium alginate capsules produced by emulsion techniques, in sodium alginate capsules produced by extrusion techniques, in k-carragennan capsules, in gellan capsules, respectively

** Different letters in the same column denote significant differences for capsule materials ($p<0.01$)

treated cheeses. General acceptability of experimental cheeses decreased after 90 days and this was probably because of high level of lipolysis and proteolysis and poor textural properties.

Conclusion

According to the results, capsule materials had significant effect on the textural and sensory characteristics of Kashar cheese. Cheeses treated with k-carrageenan capsules showed the poor textural properties and the lowest sensory scores because of the highest rate of lipolysis and proteolysis compared to those treated with gellan or sodium alginate capsules. The conditions, to which cheeses are subjected, such as the presence of ions and lactic acid, appeared to influence the stability of gum capsules in the cheeses. The interaction between milk proteins and k-carrageenan gum capsules most likely enhanced the interaction between the encapsulated enzyme and milk proteins. Sodium alginate capsules disrupted under cheese manufacturing conditions appeared to be more suitable in accelerating cheese ripening than gum capsules. However, the easily ruptured gum capsules under cheese manufacturing conditions may lead to the rapid release of enzymes and excessive lipolysis during early ripening.

Acknowledgements

This study was supported financially by the TUBITAK (Project No: 106O409). Author thanks *Prof. Dr. Ahmet KAYA* for laboratory supplies. Author also thanks Novozymes for enzyme supplies.

References

Akin, M. S., M. B. Guler-Akin, H. A. Kirmaci, A. F. Atasoy and H. Turkoglu, 2012. The effects of lipase-encapsulating carriers on the accelerated ripening of Kashar cheese. *International Journal of Dairy Technology,* **65** (2): 243-249.

Alais, C., 1984. Science du Lait. 4th Ed., *Edition Sepaic*, Paris, 814 pp.

AOAC, 1990. Official Methods of Analysis, 15th ed. VA: *Asssociation of Official Analytical Chemists*, Arlington, pp. 840-850.

Audet, P. and C. Lacroix, 1989. Two-phase dispersion process for the production of biopolymer gel beads; effect of various parameters on bead size and their distribution. *Process Biochemistry,* **24:** 217-223.

Bek, Y. and E. Efe, 1995. Araştırma ve Deneme Metotları. *Ç.Ü.* Ziraat Fakültesi. Ders Notları, No: 71 Adana. 200 pp.

Bourne, M. C., 1978. Texture profile analysis. *Food Technology-Chicago*, **32:** 62-66.

Caglar, A. and S. Cakmakci, 1998. Kaşar Peynirinin Hızlı Olgunlaştırılmasında Proteaz ve Lipaz Enzimlerinin Farklı Metotlarla Kullanımı. 1. Peynirlerin Fiziksel ve Kimyasal Özellikleri. *Gıda*, **23** (4): 291-301.

Fox, P.F., 1993. Exogenous enzymes in dairy technology: a review. *Journal of Food Biochemistry*, **17:** 173-199.

Gripon, J. C., M. Desmazeaud, D. Le Bars and J. Bergere, 1975. Étude du rôle des microorganismes et des enzymes au cours de la maturation des fromages. II. Influence de la présure commerciale. *French Le Lait*, **548:** 502-516.

Kailasaphaty, K. and S. H. Lam, 2005. Application of encapsulated enzymes to accelerate cheese ripening. *International Dairy Journal*, **15:** 929-939.

Karel, M., 1990. Encapsulation and controlled release of food components. In: G. Schwartzberg and M. A. Rao (Eds.). *Biotechnology and Food Process Engineering*, *Marcel Dekker*, New York, pp. 277-294.

Kheadr, E. E., J. C. Vuillemard and A. El Deeb, 2003. Impact of liposome-encapsulated enzyme cocktails on Cheddar cheese ripening. *Food Research International*, **36:** 241-252.

Kocak, C., A. Bitlis, A. Gursel and Y. Avsar, 1996. Effect of added fungal lipase on ripening of Kasar cheese. *Milchwissenschaft*, **51** (1): 13-16.

Krasaekoopt, W., B. Bhandari and H. Deeth, 2003. Evaluation of encapsulation techniques of probiotics for yoghurt: a review, *International Dairy Journal,* **13** (1): 3-23.

Manning, Jr. D. W., 1985. Increasing cheese yields with carrageenan. The Cheese Reporter, (October), pp. 1-25.

Sarath, G., R. S. De la Motte and F. W Wagner, 1989. Protease assay methods. In: R. Beynon (Ed.). Proteolyic Enzymes: a Practical Approach, *Bond J IRL*, Oxford, pp. 25-56.

Sheu, T. Y. and R. T. Marshall, 1993. Micro-encapsulation of *Lactobacilli* in calcium alginate gels. *Journal of Food Science*, **54:** 557-561.

Teng, Y. and Y. Xu, 2007. A modified para-nitrophenyl palmitate assay for lipase synthetic activity determination in organic solvent. *Analytical Biochemistry*, **363:** 297-299.

TSE, 1994. TS 1018, Çiğ İ*nek Sütü Standardı*, Türk Standartları Enstitüsü, Ankara, 15 p.

TSE, 1995. TS 591. *Beyaz Peynir Standardı*, Türk Standartları Enstitüsü, Ankara, 9p.

TSE, 1999. TS 3272, *Kaşar Peyniri Standardı*. Türk Standartlar Enstitüsü, Ankara, 13 p.

Zisu, B. and N. P. Shah, 2004. Textural and functional changes in low fat Mozzarella cheeses in relation to proteolysis and microstructure as influenced by the use of fat replacers, pre-acidification and EPS starter. *International Dairy Journal*, **15:** 957-972.

Received April, 15, 2016; accepted for printing Januar y, 9, 2017