

*Full Length Research Paper*

# Accelerated ripening of Kashar cheese with encapsulated protease

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**In this study, protease enzymes were encapsulated in  $\kappa$ -carrageenan, gellan and sodium alginate using emulsion and extrusion techniques and were then added in cheese milk together with rennet. The effects of the encapsulating material and ripening period on the chemical, textural and sensory characteristics of Kashar cheese were investigated. The study demonstrated that sodium alginate, gellan and  $\kappa$ -carrageenan could successfully be used as protease carrier systems to accelerate the protein breakdown process during the ripening of Kashar cheese. Those samples treated with  $\kappa$ -carrageenan capsules showed the highest rate of proteolysis compared to those treated with the other capsules.**

**Key words:** Kashar cheese, enzyme encapsulation,  $\kappa$ -carrageenan, gellan, sodium alginate.

## 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 flavor, aroma and texture of the cheese before marketing. The time required to develop this characteristic flavor and texture varies from a few weeks for soft cheese up to 3 years for very hard cheese varieties (Gripon et al., 1991). 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  $\beta$ -galactosidase enzymes, some of which have been reported to halve the normal maturation period of cheese (Law, 2001).

Direct addition of 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. The use of liposomes as enzyme-encapsulating substances has some drawbacks. They may be expensive and are generally not regarded as safe and edible. A group of substances that exhibit excellent encapsulating abilities includes food gums or hydrophilic hydrocolloids. 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 relatively widely available, cheap, and biologically compatible (Kailasaphaty and Lam, 2005).

Limited information is available on the accelerated ripening of Cheddar cheese using encapsulated enzymes. We investigated food gums as an alternative to liposomes for enzyme encapsulation to accelerate cheese ripening. Three gums (gellan,  $\kappa$ -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 protease enzymes added to Kashar cheese milk on the physicochemical,

rheological, and organoleptic characteristics of the cheese during storage

## MATERIALS AND METHODS

### Gums, enzymes and chemicals

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

### Preparation of gum capsules

$\kappa$ -Carrageenan and gellan capsules were prepared by a modified method of Audet and Lacroix (1989). Gum powders (1.5 g  $\kappa$ -carrageenan and 0.3 gellan) was suspended in three lots of 50 ml deionized water separately, 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 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 by immersion in a water bath while stirring (2000 rpm) with a marine impeller. 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 (100 $\times$ 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 (350 $\times$ 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  $\mu$ m and 150  $\mu$ m 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<sub>2</sub>. 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 efficiency of enzyme encapsulation in three types of capsules

was measured by determining the proteolytic activity of the enzyme using casein as a substrate (Sarath et al., 1989) and was used to express the entrapment efficiency (EE) as follows:

$$EE (\%) = \frac{\text{encapsulated units}}{\text{encapsulated units} + \text{unencapsulated unit}} \times 100$$

Proteolytic activity of the enzyme was determined as trichloroacetic acid (TCA) soluble peptides and amino acids following the precipitation of intact casein with TCA. Capsules prepared by addition of 5.0 ml of a 7.5% solution of Flavourzyme 1000 L to the encapsulant solutions were used for this purpose. The total enzyme activity was determined from a bulk solution of capsules (before separation of capsules from the un-encapsulated material). The bulk solutions (10 ml) containing  $\kappa$ -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 to 24°C) until completely dissolved. Separated gum capsules were treated similarly in trisodium citrate solution.

One unit of specific enzyme activity was defined as the increase in absorbance at 280 nm across a 1 cm path length caused by a unit amount (1 mg) of enzyme (expressed as total nitrogen) under the conditions of the assay.

### Cheese making

A 7.5% (w/v) solution of Flavourzyme 1000 L (1000 LAPU/g) was encapsulated in sodium alginate,  $\kappa$ -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<sub>2</sub> (0.02%) were then added. For the experimental cheeses, enzyme capsules made of sodium alginate by emulsion techniques (Group B), sodium alginate by extrusion techniques (Group C),  $\kappa$ -carrageenan (Group D) and gellan gums (Group E), 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 1 LAPU/g cheese. Group A was the control. When the pH of the milk reached 6.2 to 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<sup>3</sup>. The cut curd was allowed to settle for 15 min. Cooking was performed by increasing the temperature from 34 to 40°C over 30 min. The heating rate was an increase of 1°C for every 4 to 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  $\mu$ m stainless steel sieve. The capsules were collected on the sieve and re-added to the curd. The curd was hand-stretched in a 6% brine at 74°C for 2 min for all the cheeses studied. Brine was strained using a 120  $\mu$ m 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 color for 24 h at 15 $\pm$ 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 to 6°C for 180 days. Cheese samples were taken for chemical, textural and sensory analyses on the 1st, 15th, 90th and 180th days of storage. Cheese was manufactured in triplicate for each group.

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

Chemical parameter**	Cheese				
	A	B	C	D	E
pH	5.37±0.02 <sup>a</sup>	5.28±0.02 <sup>b</sup>	5.29±0.02 <sup>b</sup>	5.21±0.03 <sup>c</sup>	5.14±0.04 <sup>d</sup>
Titrate acidity (%l.a.)	1.15±0.021 <sup>d</sup>	1.28±0.042 <sup>c</sup>	1.28±0.031 <sup>c</sup>	1.31±0.021 <sup>b</sup>	1.35±0.020 <sup>a</sup>
Moisture (%)	45.48±0.32 <sup>a</sup>	47.75±0.33 <sup>c</sup>	46.20±0.43 <sup>b</sup>	47.18±0.53 <sup>c</sup>	47.34±0.35 <sup>c</sup>
Protein (%)	23.52±0.34 <sup>a</sup>	22.48±0.32 <sup>b</sup>	23.76±0.25 <sup>a</sup>	23.34±0.15 <sup>a</sup>	22.48±0.20 <sup>b</sup>
Fat in dry matter (F/D)	51.81±0.23 <sup>b</sup>	51.67±0.31 <sup>b</sup>	52.67±0.22 <sup>a</sup>	51.58±0.28 <sup>b</sup>	52.22±0.40 <sup>a</sup>
Salt (%)	1.76±0.05 <sup>a</sup>	1.60±0.06 <sup>c</sup>	1.71±0.04 <sup>b</sup>	1.70±0.06 <sup>b</sup>	1.55±0.05 <sup>d</sup>

A, Control cheese; B, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by emulsion techniques; C, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by extrusion techniques; D, cheeses contain flavourzyme 1000 LAPU/g in  $\kappa$ -carrageenan capsules; E, cheeses contain flavourzyme 1000 LAPU/g in gellan capsules.\*\*Different letters following numbers in the same row denote significant differences ( $p < 0.05$ ).

### 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 of the milk and cheeses were determined by the Kjeldahl method (Gripon et al., 1975). The total fat and dry matter contents of the cheese samples were determined using the method proposed by Gerber (AOAC, 1990) and gravimetric methods, respectively. The salt content of the cheeses was determined by the Mohr titration method (TSE, 1994).

### Determination of proteolysis

Cheese protein (casein) degradation during ripening was evaluated after 1st, 15th, 30th, 60th, 90th, 120th and 150th days using by mini urea polyacrylamide gel electrophoresis (Urea-PAGE). Electrophoresis was carried out on a vertical slab unit (Bio-Rad Laboratories, Inc. 1000 Alfred Nobel Drive, Hercules, California, USA) and the stacking gel system described by Craemer (1991).

### Texture measurements

The textural properties of Kashar cheese were evaluated by the textural profile analysis (TPA) method (Bourne, 1978). TPA 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 panelists. The panel was made up of staff members and postgraduate students from the Harran University, Food Engineering Department who had previous experience with cheese sensory evaluation. A 5-point hedonic scale was used to evaluate flavor, texture, odour and appearance. General acceptability was the sum of flavor, texture, odor and appearance scores.

### Statistical analyses

Each cheese experiment was repeated three times. The experiment was designed according to a 5×8×3 factorial design. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software program (version 5.0).

Statistically different groups were determined by the Least Significant Difference (LSD) test (Bek and Efe, 1995).

## RESULTS AND DISCUSSION

### Enzyme encapsulation

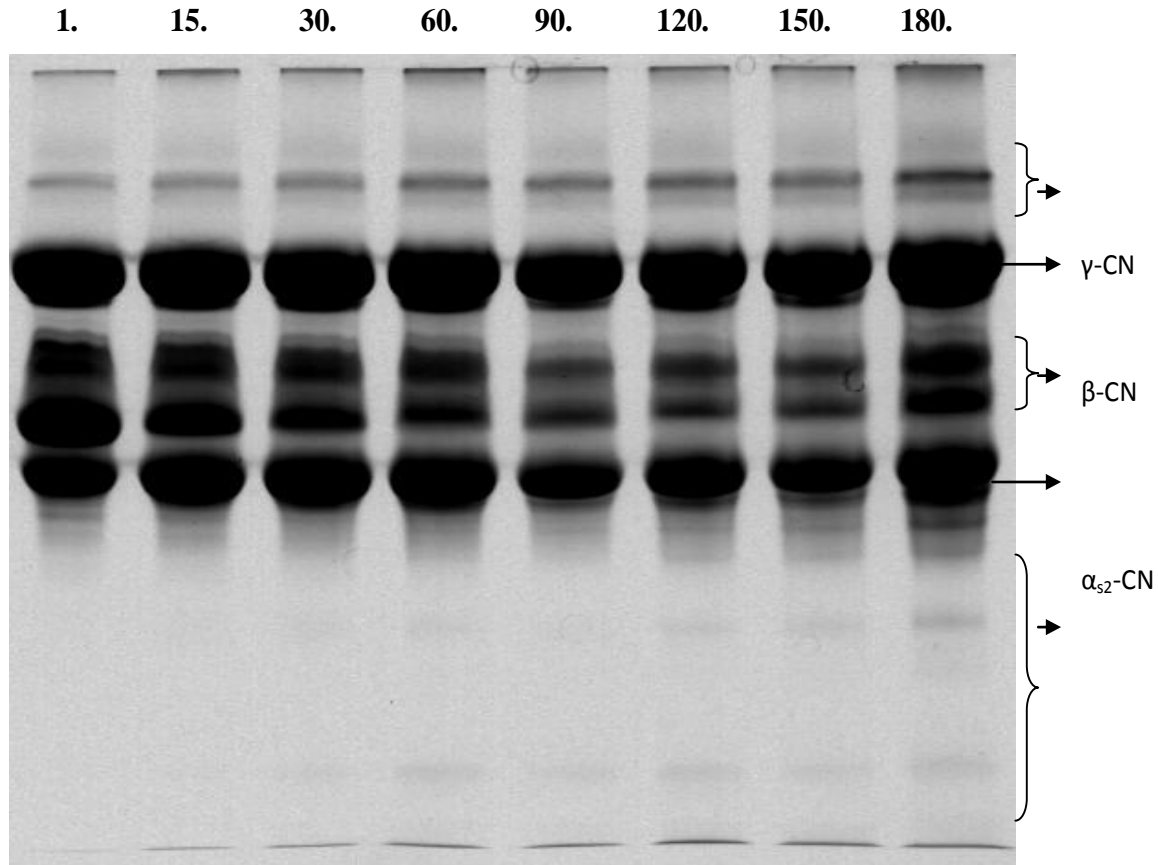
Encapsulation efficiencies of Flavourzyme 1000 (1000 LAPU/g) in  $\kappa$ -carrageenan, gellan gums, sodium alginate by emulsion techniques or sodium alginate by extrusion techniques were respectively, 55.5, 48.23, 40.89 and 49.39% of the initial activity (mean of 3 separate trials). The encapsulation efficiencies for the four capsulants were 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 enzyme (Kailasaphaty and Lam, 2005).

### Chemical composition

The gross chemical composition of the control and experimental cheeses is given in Table 1. Significant differences were observed between the control and experimental cheeses. The cheese curds had a significantly higher moisture content and titrate acidity but lower pH contents as compared to the control ( $p < 0.01$ ). The high moisture content of the 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 and fat contents of the experimental cheeses were close to each other ( $p > 0.05$ ). Salt content of the experimental cheeses were slightly lower than those of the control ( $p < 0.05$ ). Similar results were reported for capsule-treated cheeses by Kheadr et al. (2000) and Kailasaphaty and Lam (2005).

### Proteolysis

There were significantly ( $p < 0.01$ ) higher levels of



**Figure 1.** Electrophoretograms of control cheeses during 180 days of ripening.

proteolysis in treated cheeses as compared to the control cheese (Figures 1 to 5). Cheeses treated with  $\kappa$ -carrageenan capsules showed a high rate of increase in  $\beta$ -,  $\alpha_{s1}$ -casein degradation. The higher rate of  $\beta$ -,  $\alpha_{s1}$ -casein hydrolysis in the  $\kappa$ -carrageenan capsules-treated cheeses was probably due to the low stability of  $\kappa$ -carrageenan gels in solutions with acidic pH (Kailasaphaty and Lam, 2005), similar to that observed in ripening cheese. On the other hand, no report is available in the literature about the effect of the protease enzyme encapsulated in sodium alginate on cheese properties, to which a part of this work has been addressed. The observed slower rate of  $\beta$ - and  $\alpha_{s1}$ -casein degradation in cheeses treated with sodium alginate capsules suggests that these capsules probably release their enzyme contents very slowly. This implies that gellan capsules remain relatively stable within the cheese curd.

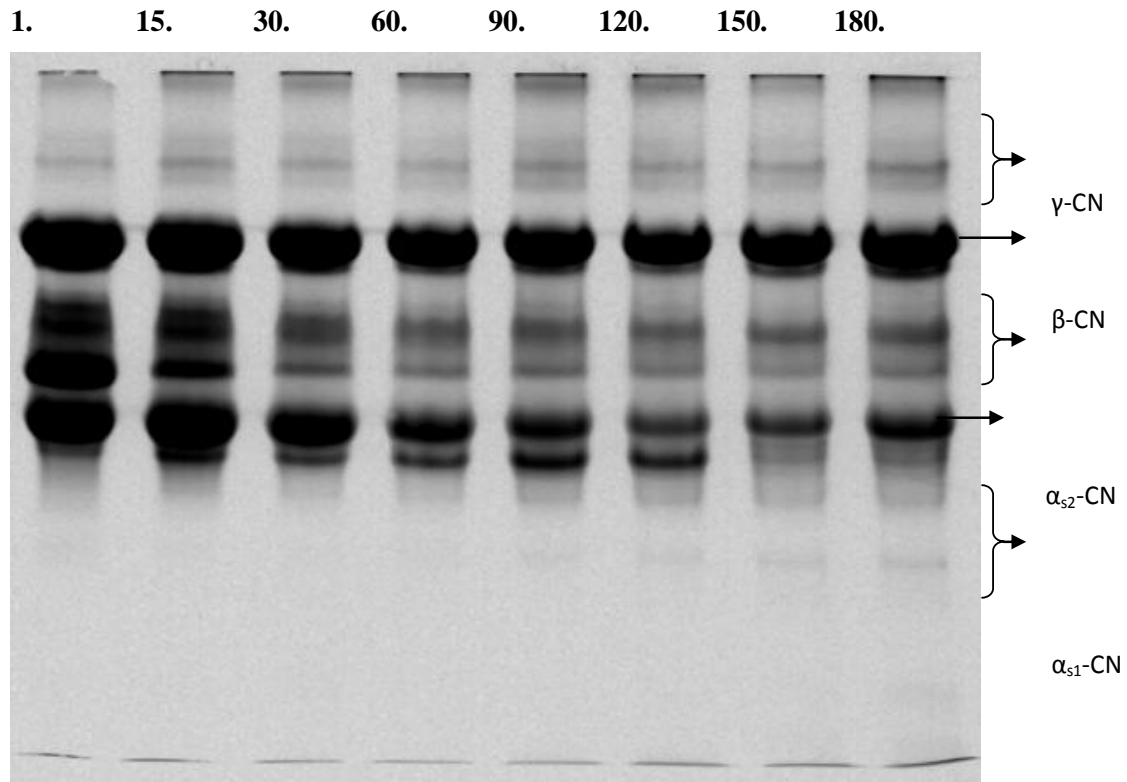
An increasing trend for  $\beta$ - and  $\alpha_{s1}$ -casein degradation was observed during the 180-day of ripening period for all cheeses ( $p < 0.01$ ). This was expected because amino groups are produced in cheese as a consequence of protein breakdown during ripening (Fox et al., 1993). Thus an increase in proteolysis in treated cheese over that of control at any given time during ripening indicates an acceleration of ripening.

### Textural properties

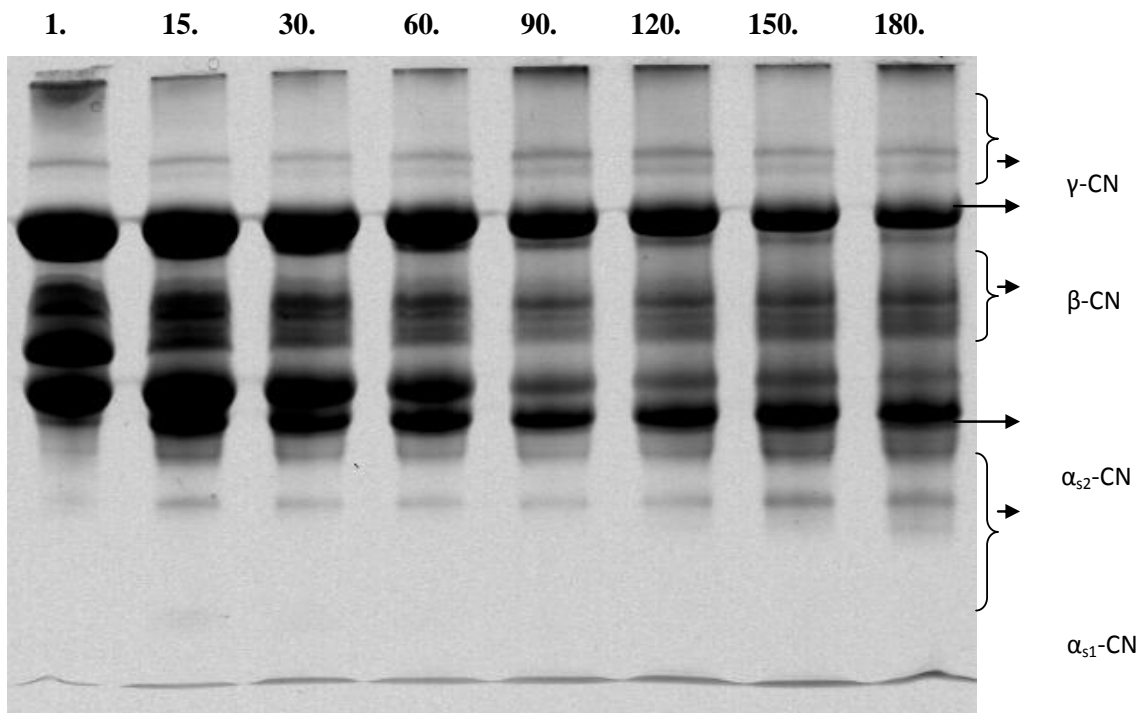
The introduction of capsules into the cheese matrix, as well as the ripening process, affected the textural properties of experimental cheeses ( $p < 0.01$ ). Protease-treated cheeses exhibited noticeable differences in their textural properties beginning from the day of manufacture compared to the control cheeses. The changes in textural properties (the parameters studied were hardness, cohesiveness, springiness, gumminess and chewiness) during the ripening of the control and experimental cheeses are shown in Table 2.

On the day of manufacture, the experimental cheeses had significantly ( $p < 0.01$ ) lower hardness values compared to the control. This was found to be correlated to the higher moisture content of the capsule-treated cheeses. Based on the TPA hardness, two distinct phases were observed: an initial hardening period up to 30 days and a softening period after 30 days.

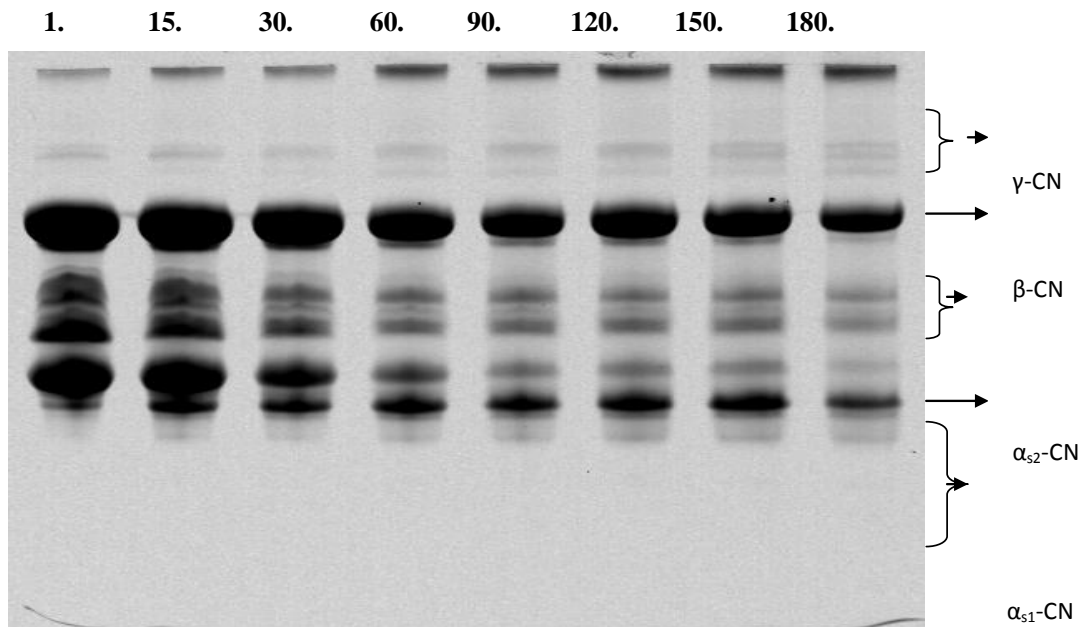
The cohesiveness and springiness of the control cheeses increased slower than that of the capsule-treated cheeses throughout the ripening period ( $p < 0.01$ ). The higher cohesiveness and springiness of the control cheeses might be due to a lower moisture content. The springiness values of all cheeses studied continuously



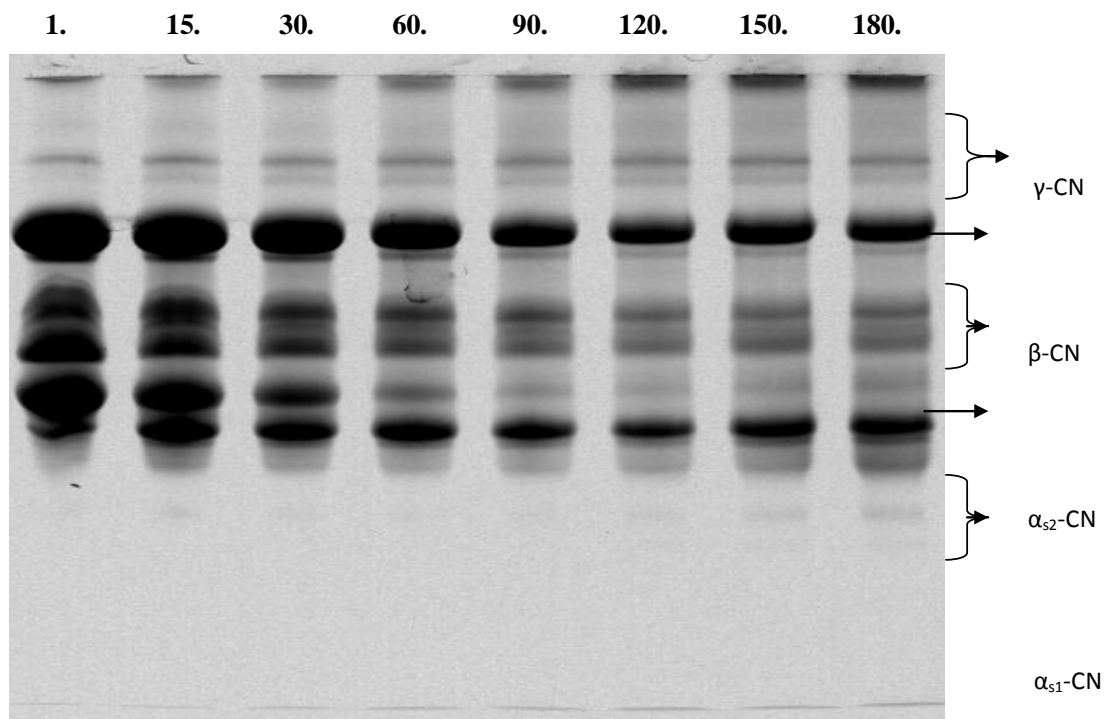
**Figure 2.** Electrophoretograms containing Flavourzyme 1000 LAPU/g in sodium alginate capsules produced by emulsion techniques cheeses during 180 days of ripening.



**Figure 3.** Electrophoretograms containing Flavourzyme 1000 LAPU/g in sodium alginate capsules produced by extrusion techniques cheeses during 180 days of ripening.



**Figure 4.** Electrophoretograms containing Flavourzyme 1000 LAPU/g in  $\kappa$ -carrageenan capsules cheeses during 180 days of ripening.



**Figure 5.** Electrophoretograms containing Flavourzyme 1000 LAPU/g in gellan capsules cheeses during 180 days of ripening.

decreased as the storage time increased throughout for entire testing period of 180 days.

The highest gumminess and chewiness values were

observed in the control cheeses ( $p < 0.01$ ). The decreasing level of moisture in the cheeses may have produced the higher chewiness values in these

**Table 2.** Textural properties of control and protease-treated cheeses during 180 days ripening (n=3).

Cheese	Ripening period (day) <sup>***</sup>	Properties**				
		Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
A	1	16.51±0.39 <sup>a1</sup>	0.734±0.006 <sup>a1</sup>	0.854±0.013 <sup>a1</sup>	10.17±0.28 <sup>a3</sup>	8.01±0.36 <sup>a3</sup>
	15	24.81±0.84 <sup>a1</sup>	0.736±0.021 <sup>a1</sup>	0.837±0.043 <sup>a1</sup>	18.26±0.84 <sup>a1</sup>	9.77±0.59 <sup>a2</sup>
	90	20.30±0.77 <sup>a1</sup>	0.708±0.028 <sup>a2</sup>	0.804±0.038 <sup>a2</sup>	14.37±0.78 <sup>a2</sup>	10.85±0.59 <sup>a1</sup>
	180	12.87±0.45 <sup>a2</sup>	0.656±0.016 <sup>a3</sup>	0.716±0.021 <sup>a3</sup>	8.40±0.53 <sup>a4</sup>	7.57±0.41 <sup>a3</sup>
B	1	10.80±0.44 <sup>a1</sup>	0.739±0.009 <sup>a1</sup>	0.839±0.005 <sup>a1</sup>	8.01±0.24 <sup>c3</sup>	6.73±0.48 <sup>c2</sup>
	15	18.50±0.67 <sup>a1</sup>	0.717±0.018 <sup>b2</sup>	0.822±0.038 <sup>b1</sup>	13.26±0.73 <sup>c1</sup>	8.34±0.51 <sup>c1</sup>
	90	14.13±0.49 <sup>a1</sup>	0.641±0.019 <sup>b3</sup>	0.751±0.034 <sup>b2</sup>	9.06±0.66 <sup>c2</sup>	8.40±0.43 <sup>b1</sup>
	180	7.65±0.26 <sup>a2</sup>	0.570±0.010 <sup>b4</sup>	0.648±0.011 <sup>b3</sup>	4.80±0.29 <sup>b4</sup>	5.76±0.32 <sup>b3</sup>
C	1	14.26±0.25 <sup>a1</sup>	0.724±0.004 <sup>b1</sup>	0.841±0.010 <sup>a1</sup>	8.59±0.29 <sup>b3</sup>	7.22±0.13 <sup>b2</sup>
	15	19.39±0.70 <sup>a1</sup>	0.709±0.016 <sup>c2</sup>	0.824±0.042 <sup>a1</sup>	13.75±0.81 <sup>b1</sup>	8.70±0.49 <sup>b1</sup>
	90	14.97±0.64 <sup>a1</sup>	0.635±0.022 <sup>b3</sup>	0.753±0.033 <sup>b2</sup>	9.51±0.44 <sup>b2</sup>	8.37±0.56 <sup>b1</sup>
	180	8.10±0.27 <sup>a2</sup>	0.556±0.012 <sup>c4</sup>	0.643±0.015 <sup>b3</sup>	4.91±0.42 <sup>b4</sup>	5.68±0.32 <sup>b3</sup>
D	1	13.66±0.32 <sup>a1</sup>	0.714±0.006 <sup>c1</sup>	0.828±0.010 <sup>b1</sup>	10.11±0.21 <sup>a2</sup>	8.06±0.37 <sup>a2</sup>
	15	17.02±0.61 <sup>b1</sup>	0.704±0.021 <sup>c2</sup>	0.811±0.035 <sup>b1</sup>	11.98±0.88 <sup>d1</sup>	8.86±0.44 <sup>b1</sup>
	90	13.03±0.68 <sup>b1</sup>	0.617±0.024 <sup>c3</sup>	0.711±0.031 <sup>c2</sup>	8.04±0.48 <sup>d3</sup>	8.05±0.64 <sup>b2</sup>
	180	6.87±0.35 <sup>a2</sup>	0.523±0.010 <sup>e4</sup>	0.641±0.09 <sup>b3</sup>	3.92±0.30 <sup>c4</sup>	5.19±0.31 <sup>c3</sup>
E	1	11.70±0.13 <sup>a1</sup>	0.711±0.007 <sup>c1</sup>	0.822±0.010 <sup>b1</sup>	8.31±0.17 <sup>b2</sup>	6.83±0.36 <sup>c2</sup>
	15	15.46±0.64 <sup>b1</sup>	0.708±0.022 <sup>c1</sup>	0.806±0.041 <sup>b1</sup>	10.95±0.68 <sup>e1</sup>	8.28±0.62 <sup>c1</sup>
	90	12.02±0.44 <sup>b1</sup>	0.624±0.018 <sup>c2</sup>	0.721±0.041 <sup>c2</sup>	7.50±0.55 <sup>e3</sup>	8.15±0.55 <sup>b1</sup>
	180	6.78±0.23 <sup>a2</sup>	0.534±0.022 <sup>d3</sup>	0.639±0.017 <sup>b3</sup>	3.95±0.39 <sup>c4</sup>	5.40±0.30 <sup>b3</sup>

A, Control cheese; B, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by emulsion techniques; C, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by extrusion techniques; D, cheeses contain flavourzyme 1000 LAPU/g in  $\kappa$ -carrageenan capsules; E, cheeses contain flavourzyme 1000 LAPU/g in gellan capsules; \*\*, 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 ( $p < 0.01$ ).

treatments. A decreasing trend for gumminess and chewiness was observed during the 180-day of ripening period for all cheeses.

### Sensory evaluation

The mean sensory scores of the cheeses are shown in Table 3. The control cheese scored highest in appearance ( $p < 0.01$ ).  $\kappa$ -carrageenan-capsules-treated cheeses gained the lowest score in appearance. This could be due to the soft and crumbly texture of this cheese compared to the other experimental cheeses. The appearance scores of all of the cheeses studied (except control) increased up to 90 days and decreased after 120 days.

The addition of encapsulated protease have a significant effect on the organoleptic texture scores ( $p < 0.01$ ). Nonetheless 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  $\kappa$ -carrageenan capsules-treated cheeses. Kailasaphaty and Lam (2005) reported that  $\kappa$ -carrageenan capsules-treated cheeses had lowest sensory texture score. The texture scores of all cheeses increased up to 90 days and decreased after 120 days. Although the use of enzyme capsules resulted in accelerated ripening, the lower mean score for the textural parameters of the experimental cheeses compared to the control cheeses may indicate a problem that affects product acceptance. The lower mean score for the textural parameters of the experimental cheeses may have also been a result of moisture retention in the capsule-treated cheeses. Excessive moisture retention in cheese during manufacture is known to result in a soft and crumbly texture (Manning, 1985).

The main differences among treatments were found in the odor and flavor scores, given below.  $\kappa$ -carrageenan-capsules treated cheeses had the lowest odor and flavor scores. In addition, the strongest flavor was noted in cheeses treated with  $\kappa$ -carrageenan capsules. The

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

Cheese	Ripening period (day) <sup>***</sup>	Properties**				
		Appearance	Texture	Odor	Flavor	General acceptability
A	1	4.14±0.48 <sup>a2</sup>	4.10±0.40 <sup>a2</sup>	4.51±0.45 <sup>a1</sup>	4.01±0.36 <sup>a3</sup>	21.28±0.52 <sup>a3</sup>
	15	4.64±0.45 <sup>a1</sup>	4.74±0.26 <sup>a1</sup>	4.60±0.40 <sup>a1</sup>	4.50±0.25 <sup>a2</sup>	23.19±0.48 <sup>a2</sup>
	90	4.78±0.22 <sup>a1</sup>	4.88±0.12 <sup>a1</sup>	4.75±0.25 <sup>a1</sup>	4.80±0.20 <sup>a1</sup>	24.02±0.78 <sup>a1</sup>
	180	4.74±0.20 <sup>a1</sup>	4.72±0.20 <sup>a1</sup>	4.68±0.32 <sup>a1</sup>	4.86±0.14 <sup>a1</sup>	23.87±0.78 <sup>a1</sup>
B	1	4.02±0.33 <sup>a2</sup>	3.80±0.25 <sup>a3</sup>	4.50±0.30 <sup>a2</sup>	3.95±0.25 <sup>a3</sup>	20.14±0.46 <sup>b3</sup>
	15	4.36±0.33 <sup>b2</sup>	4.28±0.24 <sup>b2</sup>	4.61±0.32 <sup>a2</sup>	4.52±0.37 <sup>a2</sup>	22.25±0.42 <sup>b2</sup>
	90	4.83±0.17 <sup>a1</sup>	4.70±0.30 <sup>a1</sup>	4.95±0.06 <sup>a1</sup>	4.97±0.04 <sup>a1</sup>	24.16±0.64 <sup>a1</sup>
	180	3.78±0.31 <sup>b3</sup>	2.21±0.21 <sup>b4</sup>	3.04±0.21 <sup>b3</sup>	2.33±0.31 <sup>b4</sup>	15.12±0.55 <sup>b4</sup>
C	1	3.98±0.30 <sup>a3</sup>	3.80±0.26 <sup>a3</sup>	4.60±0.35 <sup>a2</sup>	3.87±0.28 <sup>a3</sup>	20.00±0.52 <sup>b3</sup>
	15	4.28±0.50 <sup>b2</sup>	4.26±0.26 <sup>b2</sup>	4.61±0.35 <sup>a2</sup>	4.50±0.27 <sup>a2</sup>	22.07±0.42 <sup>b2</sup>
	90	4.80±0.20 <sup>a1</sup>	4.68±0.28 <sup>a1</sup>	4.95±0.05 <sup>a1</sup>	4.96±0.06 <sup>a1</sup>	24.10±0.80 <sup>a1</sup>
	180	3.66±0.29 <sup>b4</sup>	2.17±0.25 <sup>b4</sup>	3.02±0.27 <sup>b3</sup>	2.26±0.24 <sup>b4</sup>	14.65±0.47 <sup>b4</sup>
D	1	3.84±0.27 <sup>b3</sup>	3.51±0.37 <sup>b3</sup>	4.45±0.25 <sup>a2</sup>	3.74±0.29 <sup>b3</sup>	19.32±0.56 <sup>c3</sup>
	15	4.15±0.27 <sup>b2</sup>	4.05±0.37 <sup>b2</sup>	4.51±0.21 <sup>a2</sup>	4.48±0.29 <sup>a2</sup>	21.43±0.58 <sup>c2</sup>
	90	4.60±0.30 <sup>a1</sup>	4.58±0.32 <sup>a1</sup>	4.88±0.12 <sup>a1</sup>	4.90±0.10 <sup>a1</sup>	23.56±0.77 <sup>b1</sup>
	180	3.39±0.22 <sup>c4</sup>	1.93±0.23 <sup>b4</sup>	2.61±0.25 <sup>c3</sup>	1.99±0.25 <sup>c4</sup>	14.13±0.51 <sup>c4</sup>
E	1	3.94±0.31 <sup>a3</sup>	3.62±0.39 <sup>b3</sup>	4.48±0.38 <sup>a2</sup>	3.80±0.35 <sup>b3</sup>	19.67±0.53 <sup>b3</sup>
	15	4.25±0.21 <sup>b2</sup>	4.12±0.39 <sup>b2</sup>	4.62±0.35 <sup>a2</sup>	4.50±0.22 <sup>a2</sup>	21.79±0.43 <sup>c2</sup>
	90	4.71±0.23 <sup>a1</sup>	4.66±0.24 <sup>a1</sup>	4.92±0.08 <sup>a1</sup>	4.92±0.10 <sup>a1</sup>	23.86±0.74 <sup>a1</sup>
	180	3.30±0.30 <sup>c4</sup>	2.04±0.27 <sup>b4</sup>	2.45±0.24 <sup>d3</sup>	2.06±0.27 <sup>c4</sup>	13.46±0.41 <sup>d4</sup>

A, Control cheese; B, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by emulsion techniques; C, cheeses contain flavourzyme 1000 LAPU/g in sodium alginate capsules produced by extrusion techniques; D, cheeses contain flavourzyme 1000 LAPU/g in  $\kappa$ -carrageenan capsules; E, cheeses contain flavourzyme 1000 LAPU/g in gellan capsules; \*\*, 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 ( $p < 0.01$ ).

addition of encapsulated protease slightly improved the odor and flavor intensities of the experimental cheeses, depending on cheese age. After 90 days, the experimental cheeses had higher odor and flavor scores than the control cheese due to higher proteolysis.

There were significant differences in the general acceptability ( $p < 0.01$ ) of the experimental and control cheeses. The most acceptable cheeses were the control cheeses, followed by sodium alginate produced by emulsion, sodium alginate produced by extrusion, gellan and  $\kappa$ -carrageenan-capsule-treated cheeses. The general acceptability of the experimental cheeses decreased after 90 days, probably due to the high level of proteolysis and poor textural properties.

## Conclusion

This study demonstrates that sodium alginate, gellan and  $\kappa$ -carrageenan can be successfully used as protease carrier systems to accelerate ripening of Kashar cheese. The use of this system could be considered in the production of Kashar cheese with high flavor intensity in a

relatively short time (90 days). Experimental cheeses ripened for 90 days, exhibited textural and sensory characteristics similar to those of control cheeses ripened for 180 days.

This study confirms that the impact of encapsulated enzymes in cheese ripening is influenced greatly by the nature of the encapsulating gums themselves. Cheeses treated with  $\kappa$ -carrageenan capsules showed the highest rate of proteolysis compared to those treated with gellan or sodium alginate capsules. 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 proteolysis during early ripening.

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