IN THE NAME OF ALLAH,
MOST GRACIOUS, MOST MERCIFUL

J. King Saud Univ., Vol. 22, *Agric. Sci.* (2), pp. 59-69, Riyadh (2010/1431H.)

Susceptibility of Camel, Buffalo and Cow Milk Caseins to Proteolysis by Different Proteolytic Enzymes

Ali A. M. Metwalli* **and A. A. Al-Saleh**
Department of Food Science and Nutrition,
College of Food Sciences and Agriculture, King Saud University,
P. O. Box 2460, Riyadh 11451, Saudi Arabia
E-mail: ali_metwalli@yahoo.com

(Received 10/5/1430H.; accepted for publication 11/1/1431H.)

Keywords: Caseins, Proteolytic enzymes, Electrophoresis, Total nitrogen.

Abstract. The proteolytic activities of trypsin, chymotrypsin, pepsin, plasmin and fungal rennet from *Mucor miehei* on caseins from different types of milk were studied. The degree of hydrolysis was determined using different methods including measuring the soluble nitrogen in 4% trichloroacetic acid, changes in optical densities at 280 and 340 nm and using polyacrylamide-gel electrophoresis. Camel milk casein showed significant ($p < 0.05$) resistance to all proteolytic enzymes compared to cow or buffalo milk caseins. Susceptibility of cow and buffalo milk caseins to proteolytic enzymes was varied. Buffalo milk casein exhibited higher digestibility than cow casein with trypsin, plasmin and fungal rennet from *Mucor miehei*, while cow milk casein showed higher proteolysis than buffalo milk casein with pepsin and chymotrypsin. The degree of hydrolysis of different caseins with different enzymes was also different. Chymotrypsin, trypsin and pepsin showed greater proteolytic activities than plasmin and fungal rennet did. Electrophoretic patterns of different types of caseins revealed considerable differences among the enzymes with respect to the degradation of specific casein fractions.

Introduction

Compositions of casein isolated from milk of different species are different. The casein content of milk represents about 80% of cow milk proteins. The principal casein fractions are α_{s1}-, α_{s2}-, ß- and κ-casein in ratio 4:1:4:1 (Walstra *et al.*, 1984). The above ratios are substantially differing from milk to another. Kappeler *et al.* (2003) reported that cow casein contains 12, 3, 10 and 3.5 g kg^{-1} αs_1-, αs_2-, ß- and κ-casein respectively, while camel casein contains 5, 2.2, 15 and 0.8 g kg^{-1} respectively and ß-casein is the principle casein fraction in camel milk (~65%) and also in human milk. This may affect the proteolysis of casein fractions of different types of milk by proteolytic enzymes. Milk protein hydrolyzate can be suitable sources of protein for allergic infants and for individuals, and can also be added to microbial media to accelerate fermentation process (Lucas *et al.*, 2004). Casein derived peptides display an immunomodulatory role. To human and animal Immunomodulating peptides have been found to stimulate the proliferation of human lymphocytes, the phagocytic activities of macrophages and antibody synthesis (Korhonen and Pihlanto, 2001). Furthermore, some peptides derived from milk protein hydrolysis may contribute to the antitumor effects (Matar *et al.*, 2003). Antimicrobial fragments have been derived from hydrolysis of α_{s1}-, α_{s2}- and κ-casein (Lahov and Regelson, 1996). These peptides have been found to be active against a broad range of pathogenic organisms, e.g. Escherichia, Helicobacter, Listeria, Salmonella, Bucillus, Staphylococcus, yeasts and filamentous fungi (Recio and Visser, 1999; Makoto and Dong, 2007; Al-Saleh *et al.*, 2009). These peptides also inhibit angiotensin-converting enzyme (ACE) leading to regulate blood pressure (antihypertensive effect) (Otte *et al.*, 2007; FitzGerald *et al.*, 2004; Mizuno *et al.*, 2004).

Nagaoka *et al.* (2001) identified a hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) from the tryptic hydrolysate of milk proteins. This peptide also suppressed cholesterol absorption.

* Corresponding author: Tel. +966592318950; Fax: +96614678394.

Bioactive peptides (opoid activity) like β-casomorphin, α_{s1}-casein-exorphin and casoxin derived from hydrolysis of α_s-, β- and κ-casein respectively were observed (Makoto and Dong, 2007; Meisel and FitzGerald, 2000). Antioxidant activity was derived after hydrolysis of α_s-, β- and κ-casein of ovine milk by pepsin, trypsin and chymotrypsin (Gomez *et al.*, 2008). Enzymic milk protein hydrolyzate can also be used as slurry to accelerate cheese ripening or as hydrolyzed milk proteins formulas for infants who have cow milk allergy (Moneret-Vautrin *et al.*, 2001). The aim of this study is to compare the susceptibilities of camel, buffalo and cow milk caseins to hydrolysis by different types of proteases at different incubation time.

Materials and Methods

Proteolytic enzymes and purified α_s- and β-casein were purchased from Sigma Aldrich, St. Louis, USA. The following enzymes were used: plasmin (EC3. 4. 21. 7) lyophilized powder, 0.12 units mg^{-1} solid; trypsin (EC 3. 4. 21. 4) lyophilized powder 13000-20000 BAEE unit mg^{-1} protein; pepsin (EC 3. 4. 23. 1) lyophilized powder, 3200-4500 units mg^{-1} protein; chymotrypsin (EC 3. 4. 21. 1) lyophilized powder, 40-60 units mg^{-1} protein; rennet type II from *Mucor miehei* (R-5876).

Acid casein preparation

Acid casein was prepared from different types of raw skim milk. Briefly, skimmilk was acidifed to pH 4.6 with 1 M HCl. Casein precipitate was washed three times with water, then redissolved in NaOH 1 M at pH 7.0, reprecipitated at pH 4.6, and the precipitated casein washed further for two times. The caseins were freeze dried. The lyophilisate was reconstituted in Jennes and Koops buffer (1962) to have a final casein concentration of 2.5 g 100 mL^{-1}. Before adding the enzyme, the pH of casein solution was adjusted to the desired value by adding 1 M NaOH before incubation. In all cases, 0.02% sodium azide (Avonchem, A7752-J) was added to the suspensions to prevent bacterial growth.

In vitro enzymatic digestions

The *in vitro* hydrolyses were performed as follows: Pepsin enzyme was dissolved in 50 mM acetate buffer pH 5.0, trypsin, chymotrypsin, plasmin and rennet were dissolved in 50 mM phosphate buffer at pH 7.0 except rennet was at pH 6.6. Caseins were suspended in Jennes and Koops buffer at the final concentration of 2.5 g 100 mL^{-1}. The suspended caseins were vigorously stirred by Polytron PT 3000, Brinkmann (Littau-Swizerland). The pH was adjusted to the desired values by 1M NaOH before adding the freshly prepared enzyme solution (at enzyme/protein ratio 1:100). The enzyme/casein solution was incubated under constant stirring for up to 300 minutes at 37°C. The reactions were terminated by adding trichloroacetic acid (TCA) 8% at interval times to a final concentration of 4%. The precipitated casein was filtered using filter paper Whatman No. 42 and the absorbance of the filtrate was measured without dilution at 280 nm for plasmin and rennet from *Mucor miehei* protease. The optical densities of trypsin, chymotrypsin and pepsin treated samples were measured after dilution three times with distilled water and recorded without correction. Total nitrogen (T.N.) of all samples was determined in the filtrate using Kjldahl method (IDF, 1993).

O-phthaldialdehyde (OPA) analysis

O-phthaldialdehyde (OPA) measurements were analyzed in TCA filtrate according to the method described by Church *et al.* (1983), using a dual beam u.v. – visible spectrophotometer (Biospec 1601, Shimadzu Scientific Instruments, Japan) with quartz cuvette. The OPA reagent was prepared by combining 25 mL of 100 mmol L^{-1} sodium tetra borate, 2.5 mL 20% (w/w) SDS, 40 mg OPA dissolved in 1 mL methanol, and 100 µL 2-mercaptoethanol, then adjusting the volume to 50 mL with distilled water. A 50 µL of TCA filtrate (in the case of trypsin, chymotrypsin and pepsin treated samples, TCA filtrate was diluted with water 1:3 and the recorded optical densities were not corrected) was mixed with 1 ml OPA reagent in a 1 ml quartz cuvette and the absorbance was read at 340 nm after 2 minutes. An average of two readings for each sample was recorded.

Polyacrylamide gel electrophoresis (SDS-PAGE)

Treated and untreated casein samples were separated using a polyacrylamide gel with the following characteristics. Separation gel (final concentration): 12.5% acrylamide; 0.3% bisacrylamide; 0.37 M Tris-HCl buffer, pH 8.8; 0.1% SDS; 0.03% ammonium persulfate; and 0.1% N,N,N′,N′-tetramethylenediamine. Stacking gel (final concentration): 4.5% acrylamide, 0.12% bisacrylamide, 0.125 M Tris-HCl buffer pH 6.8; 0.1% SDS; 0.09% ammonium persulfate; and 0.1% N,N,N′,N′-tetramethylenediamine. Running buffer: 0.125 M Tris, 0.196 M glycine and 0.1% SDS (wt/vol), pH 8.3. After the electrophoresis run (18 mA / 1.5 mm thickness gel at 10°C) for approximately 6 hours, the gels were marked with

Coomassie brilliant blue R-250 (0.2% in 45:45:10 methanol:water:acetic acid). Prestained molecular weight marker solution (broad range, Sigma) was used contained: bovine albumin (66-kDa), egg albumin (45 kDa), glyceraldehydes-3-p-dehydrogenase (36 kDa), carbonic anhydrase, bovine (29 kDa), trypsinogen, bovine pencrease (24 kDa), soybean trypsin inhibitor (20 kDa), Bovine milk α-lactalbumin (14.2 kDa). Purified αs and β-caseins were suspended in sample buffer at a final concentration of 1 mg mL^{-1}.

Statistical analysis

Data from each incubation time were tested by ANOVA two-way factorial analyses of variance using Minitab Version 10 for Windows. Least significant differences (LSD, $P < 0.05$) for comparisons between any pair of data were calculated using Tukey's t (Zar, 1996).

Results and Discussion

Effect of trypsin

The degree of hydrolysis of casein samples was expressed as increase in optical density at 280 nm with a similar concentration of trypsin enzyme for different periods. This reading (280 nm) determines the released peptides containing tyrosine and/or tryptophan residues in their sequences but not the whole proteolysis (Gaucheron *et al.*, 2001). The degree of hydrolysis of different types of casein by trypsin enzyme measured at 280 nm is shown in Fig. 1. The results indicated that the degree of hydrolysis significantly increased with increasing incubation time for all types of caseins. Moreover, it has been observed that the optical density of different types of casein hydrolyzate increased rapidly at the beginning of incubation period (60 minutes) and then increased gradually till the end of incubation time (300 minutes) (Fig. 1). Buffalo's casein was significantly ($p < 0.05$) susceptible to hydrolysis than cow's and camel's caseins. However, camel casein's showed resistance to trypsin enzyme.

These results are in good agreements with that obtained by Salami *et al.* (2008) who found that bovine caseins were more susceptible to hydrolysis by trypsin than camel caseins. Primary structural of the four casein fractions (α_{s1}-, α_{s2}-, β- and κ-caseins) in three types of milk are different (Ferranti *et al.*, 1998). Tezcucano *et al.* (2007) reported that the number of covalently attached phosphate groups to serine and therionin residues greatly influence functional properties of these proteins including their digestibility. Moreover, Beg *et al.* (1986) reported that camel milk protein is likely to have a rigid highly cross-linked polypeptide chain. All these factors affect the rate of proteolysis. The total nitrogen (T. N.) content in 4% TCA filtrate of cow, buffalo and camel caseins after treatment with trypsin enzyme was determined. All three types of caseins showed high susceptibility to hydrolysis by trypsin.

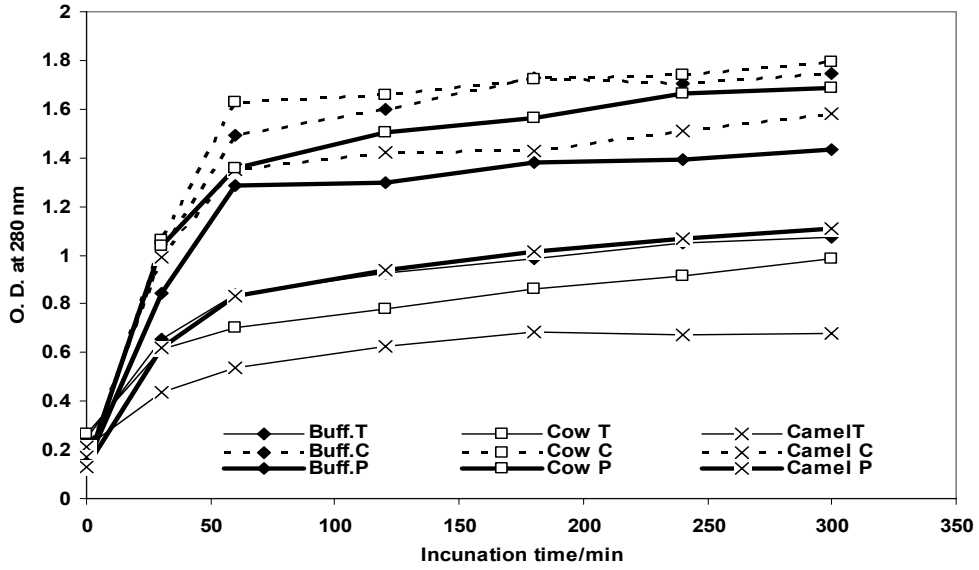

Fig. 1. Proteolytic activity of trypsin (T), chymotrypsin (C) and pepsin (P) on different types of caseins measured at 280 nm.

No significant difference ($p < 0.05$) was observed between the degree of hydrolysis of cow and buffalo caseins. However, there was a significant difference between the rate of hydrolysis of camel casein and buffalo or cow caseins. Most of liberated nitrogen occurred during the first 60 minutes of incubation. With increasing the incubation time the more total nitrogen was observed but the susceptibility of camel casein showed lower proteolysis than those of cow and buffalo caseins. Proteolysis of cow and buffalo caseins was almost comparable (data not shown).

OPA analysis of representative caseins showed a range of proteolytic activity (Fig. 2). The obtained results confirmed the above results, where the camel casein revealed the lowest hydrolysis in comparison with cow and buffalo caseins, but the extent of cow and buffalo caseins hydrolysis was almost identical after 180 min incubation.

OPA analysis has been used specifically to measure the overall proteolytic capability of proteolytic enzymes in milk. Unfortunately, this method does not provide information concerning specific milk proteins hydrolyzed or protein cleavage patterns. However, SDS-PAGE technique was used to investigate the degree of casein hydrolysis, where this method can detect low level of proteolysis and gives insight into the nature of the casein hydrolyzate. The results of proteolytic activity of trypsin enzyme on the whole casein of camel and cow milk with SDS-PAGE are shown in Fig. 3. The results showed that both α_s- and β-caseins were fully degraded by trypsin enzyme after 1-minute incubation with the formation of several low molecular weight bands. Most of these bands disappeared with increasing incubation time to 10 minutes. These results are in agreement with those obtained by Salami *et al.* (2008), who found that αs- and β-caseins of cow and camel milk were completely degraded with the addition of trypsin enzyme and incubation for 15 minutes. There is no peptide fragments were detected on SDS-PAGE.

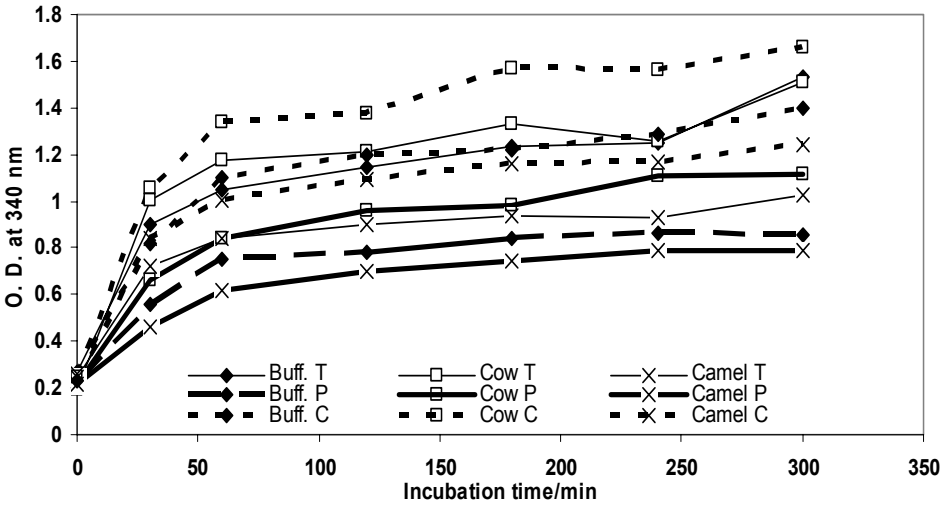

Fig. 2. Proteolytic activity of trypsin (T), chymotrypsin (C) and pepsin (P) on different types of casein measured at 340 nm.

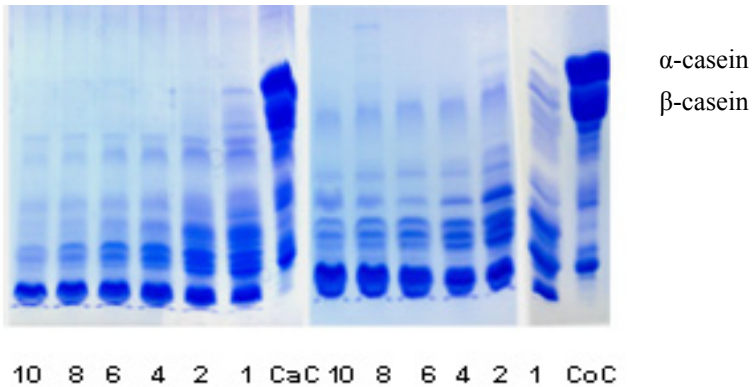

Fig. 3. SDS-PAGE profiles of caseins and of their hydrolysis with trypsin enzyme. CaC and CoC are camel and cow caseins respectively. Lanes 1, 2, 4, 6, 8 and 10 are incubation times in minutes at 37°C.

Effect of pepsin

Pepsin is belonging to acid proteinases whose optimal action on protein substrate is in the pH range 2-5 and attacks the hydrophobic amino acid units flanking the sensitive peptide bonds at Tyr-x, Leu-x, Phe-x and Try-x (Voynick and Fruton, 1971). The pH of the stomach contents at the height of digestion in the breast fed infant ranges from about 2.8 to 6 with an average of 3.6. In infants fed cow's milk the pH is higher, ranging between 4.5 and 6.0 and in adults the pH ranges from 1 to 6 for food going out of the stomach (Kennedy *et al.*, 1955). In this study, the caseins were suspended in Jennes and Koops (1962) buffer at pH 5.0. The results in Fig. 1 showed the proteolytic activity of pepsin on different types of casein measured at 280 nm. The data revealed that buffalo and cow caseins showed a significant proteolysis ($p < 0.05$) than did camel casein. Mickelsen and Fish (1970) reported that pepsin does not hydrolyze β-casein extensively enough to release significant amounts of non-protein nitrogen soluble in 12% trichloroacetic acid. It is noteworthy to mention that β-casein is the main casein in camel milk (represents about 65% of the total casein) (Zhang *et al.*, 2005). Moreover Kennedy *et al.* (1955) found that human casein is more slowly digested than bovine casein by commercial pepsin (the principle casein in human milk is β-casein (Kappeler *et al.*, 2003). Ganguli *et al.* (1963) found that pepsin hydrolysis was slower for buffalo milk casein than it was for cow milk casein, regardless of the period of incubation, substrate concentration, and enzyme concentration. The assessment of liberated nitrogen (T.N.) showed a significant difference among the three different types of milk caseins. Cow casein exhibited higher peptic activity than buffalo casein; however, camel milk casein had the lowest peptic activity (data not shown).

The same trend was observed when the rate of protein hydrolysis measured at 340 nm using OPA reaction (Fig. 2). The results in Fig. 4 show SDS-PAGE profile of bovine and camel casein hydrolysis with pepsin enzyme. The results demonstrated that the degree of α_s- and β-casein hydrolysis in both types of milk were significantly different. The intensity of α_s- and β-casein bands of whole cow casein greatly decreased with the addition of pepsin and with increasing incubation time, while those of whole camel casein slightly decreased. Two main bands were appeared in whole cow casein with the addition of pepsin and incubation for 1 minute at 37°C. The first band appeared and moved a head of β-casein band and the other moved and appeared at the end of the gel (Fig. 4). When pepsin was added to whole camel casein, only one low molecular band appeared at the end of the gel similar with that appeared with cow casein.

Effect of chymotrypsin

Chymotrypsin is a serine protease acting in the digestive systems of mammals and other organisms. The main substrates of chymotrypsin include tryptophan, tyrosine, phenylalanine and methionine, which are cleaved at the carboxyl terminal (Ma *et al.*, 2005). Like many proteases, chymotrypsin will also hydrolyze ester bonds *in vitro*. The hydrolysis rate of camel, cow and buffalo caseins by chymotrypsin enzyme measured at 280 nm are presented in Fig. 1.

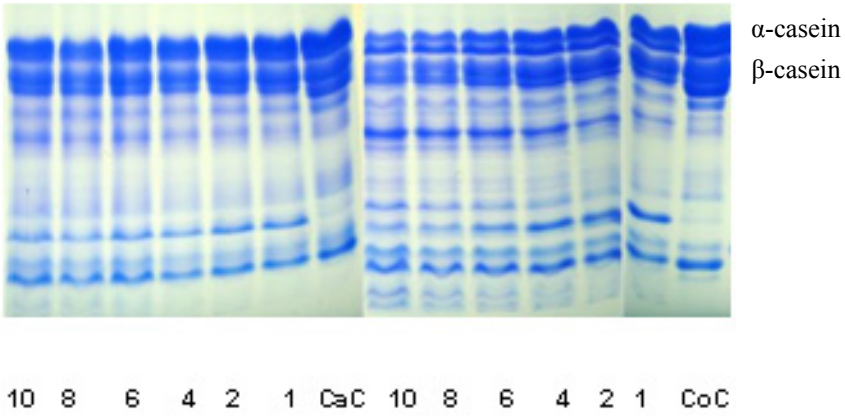

Fig. 4. SDS-PAGE profiles of caseins and of their hydrolysis with pepsin enzyme. CaC and CoC are camel and cow caseins respectively. Lanes 1, 2, 4, 6, 8 and 10 are incubation times in minutes at 37°C.

It is presumed that the rate of caseins hydrolysis linearly increased at incubation for 60 minutes for casein types and then gradually increased until the end of incubation time (300 minutes). Moreover, the hydrolysis extent of cow and buffalo caseins was comparable (not significant), but it was significantly higher than that of camel casein. These results do not agree with that reported by Salami *et al.* (2008) who found that camel caseins were more resistance to hydrolysis by chymotrypsin than bovine caseins. According to the maximal absorbencies measured at 280 nm or at 340 nm, the extent of chymotryptic hydrolysis of the three different types of caseins was significantly higher than those obtained in the case of tryptic hydrolysis. These findings are well agreed with those obtained by Salami *et al.* (2008). They attributed the higher chemotryptic activity into the greater number of available cleavage sites by chymotrypsin than trypsin. Total nitrogen liberated in 4% TCA after the treatment of different caseins with chymotrypsin was determined. The results revealed that cow casein was more susceptible to hydrolysis by chymotrypsin enzyme than buffalo or camel caseins. The proteolysis of camel casein showed lower values in comparison to cow and buffalo caseins (data not shown).

When the degree of casein hydrolysis was measured at 340 nm (OPA), cow casein showed significant higher proteolysis than buffalo or camel caseins. However, there was no significant difference between camel and buffalo caseins (Fig. 2). Electrophoretic examination of the chymotrypsin-treated samples showed that considerable proteolysis had occurred in both camel and cow casein (Fig. 5). The α_s-casein was completely degraded after 1-minute incubation at 37°C of whole cow casein. However, a minor portion of α_s-casein of camel milk remained intact after 10 minutes hydrolysis. Moreover, after 10 minutes hydrolysis by chymotrypsin, a minor portion of β-casein of camel and cow milk were resistant to proteolysis. But, the nature of the proteolysis products was almost the same, where about four main low molecular protein bands were observed in both camel and cow treated-caseins (Fig. 5).

It can be assumed that chymotrypsin enzyme attack the same sites in cow and camel casein. These results confirmed the above findings that camel casein revealed lower susceptibility to proteolysis than cow casein.

Proteolytic activity of plasmin

Plasmin is an alkaline serine proteinase with trypsin-like properties (cleaves Lys-X and Arg-X bonds) and most active around pH 8.0 (Kaminogawa *et al.*, 1972). Plasmin is able to readily hydrolyzes β-casein, α_{s2}-casein, and more slowly α_{s1}-casein (Fox and McSweeney, 1996). SDS-PAGE revealed gradual disappearance of major caseins (α_s- and β-casein) accompanied by appearance and increase in the intensity of numerous electrophoretic bands when caseins were incubated with plasmin (Fig. 6). The electrophoretic pattern of α_s-casein of camel milk completely hydrolyzed at the end of incubation time (60 minutes). Contrary to α_s casein of camel milk, α_s-casein of buffalo milk showed resistance to the hydrolysis by plasmin. Although the α_s-casein of cow milk exhibited medium extend of hydrolysis, β-casein of different types of milk showed a different extent of hydrolysis by plasmin.

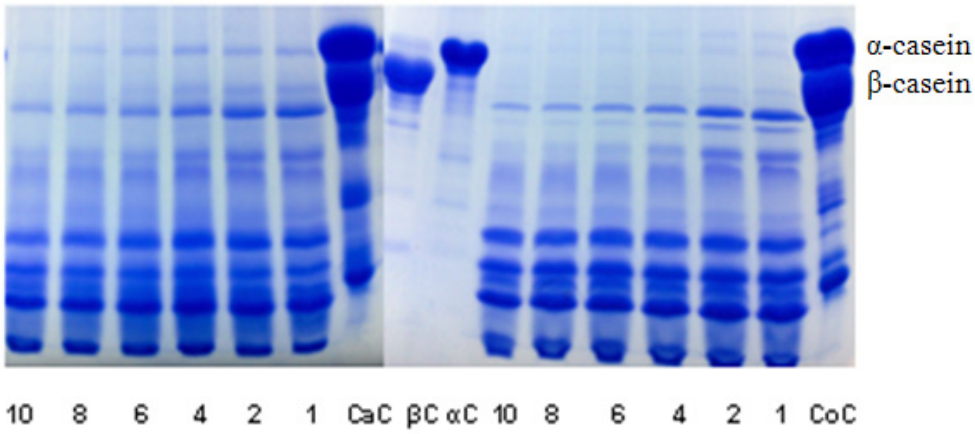

Fig. 5. SDS-PAGE profiles of caseins and of their hydrolysis with chymotrypsin enzyme. CaC and CoC are camel and cow caseins respectively. Lanes 1, 2, 4, 6, 8 and 10 are incubation times in minutes at 37°C.

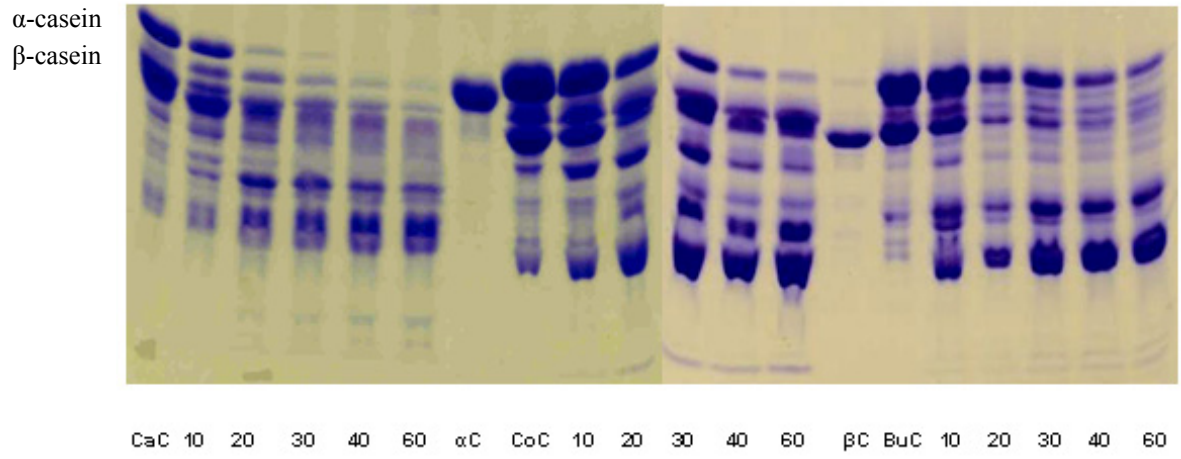

Fig. 6. SDS-PAGE profiles of caseins and of their hydrolysis with plasmin enzyme. CaC, CoC and BuC are camel cow and buffalo caseins respectively. Lanes 10, 20, 30, 40, and 60 are incubation times in minutes at 37°C, αC and βC are, α_s and ß casein respectively.

The electrophoretic patterns of different caseins revealed that the degree of β-casein hydrolysis for buffalo casein was higher than those for camel and cow caseins. Moreover, the α_s-casein exhibited higher extent of hydrolysis in different types of casein than β-casein. This may be attributed to the number of available cleavage sites in α_s casein (7 sites) is higher than those in ß-casein (5 sites). Plasmin hydrolysis of β-casein results in the formation of 3 γ-caseins [γ 1-(β-CN f29-209), γ 2- (β-CN f106-209), and γ 3- (β-CN f108-209) caseins], presenting C-terminal region, and 5 proteose-peptones [β-CN f1-28, β-CN 1-105/107, and β-CN f29-105/107] representing the corresponding N-terminal region (Bastian and Brown, 1996). The same fragments were obtained by the action of plasmin on β-casein of caprine milk, suggesting that plasmin probably attacks the same regions in different types of milk. Srinivasan and Lucey (2002) found that the amounts of intact α_s and β-casein of cow milk were about 24 and 14% respectively after the incubation of skim milk with plasmin for 8 hours.

Proteolytic activity of plasmin on different types of caseins was also measured with monitoring optical densities at 280 and 340 nm (Figs. 7 and 8). Results showed that there is a significant difference between the rate of hydrolysis of the three types of caseins. Buffalo casein exhibited the higher susceptibility of proteolysis than cow and camel caseins. Non-protein nitrogen in the final concentration of 4% TCA was also measured after incubation with plasmin. The results showed that buffalo casein was significantly susceptible to hydrolysis by plasmin than cow and camel caseins, while there was no significant difference between the rate of hydrolysis of cow and camel caseins (data not shown).

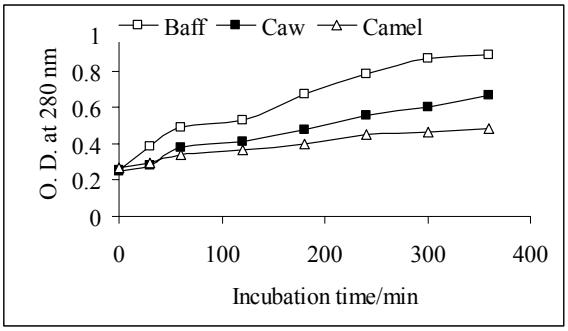

Fig. 7. Proteolytic activity of plasmin on different types of caseins measured at 280 nm.

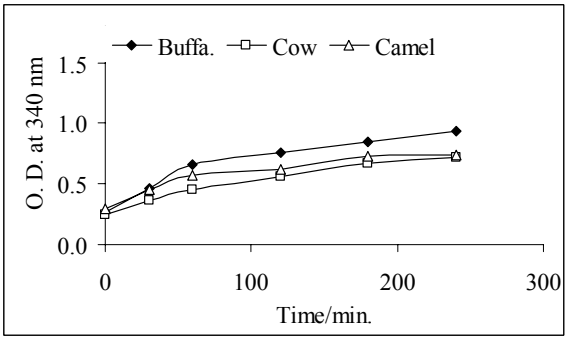

Fig. 8. Proteolytic activity of plasmin on different types of caseins measured at 340 nm (OPA).

Effect of rennet (microbial rennet from *Mucor miehei*) on casein

It is known that Jennes and Koops buffer contains salts as in milk serum. Therefore, caseins in this experiment were suspended in water to extend incubation time without casein coagulation. The degree of hydrolysis of different caseins by microbial rennet (*Mucor miehei*) measured at 280 nm is shown in Fig. 9. Buffalo casein was generally hydrolyzed significantly higher than cow and camel caseins. The degree of hydrolysis of camel and cow caseins was nearly the same through the incubation period.

When the degree of proteolytic of caseins was measured at 340 nm, the buffalo casein also exhibited higher degree than cow casein, and the latter was higher than that of camel (Fig. 10). These results were in agreement with those obtained by El-Shibiny and Abd El-Salam (1977) who observed that α_s-casein of buffalo milk exhibited more hydrolysis than α_s-casein of cow milk when treated with *Mucor miehie* protease.

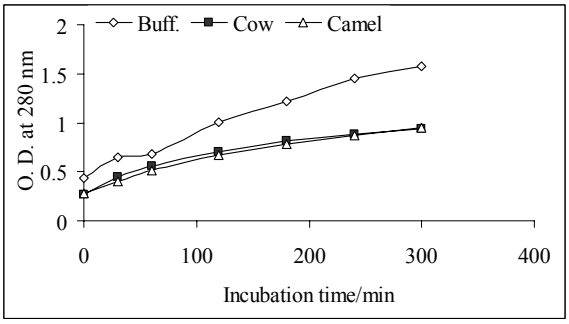

Fig. 9. Proteolytic activity of rennet (microbial rennet from *Mucor miehei*) on different types of caseins measured at 280 nm.

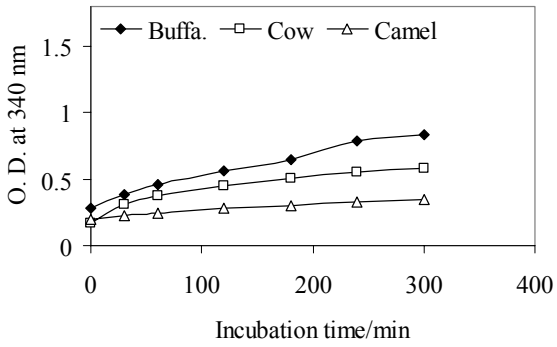

Fig. 10. Proteolytic activity of microbial rennet from *Mucor miehei* on different types of caseins measured at 340 nm (OPA).

Shammet *et al.* (1992) reported that *Mucor miehei* protease was not as specific for κ-casein as chymosin. One peak only was appeared by RP-HPLC as a result of chymosin action on κ-casein after incubation for 60 minutes, while seven peaks were appeared when κ-casein treated with *Mucor miehei* protease under the same condition. Emmons and Binns (1990) reported a higher loss of proteins with whey (0.44%) during the manufacturing of cheddar cheese with *Mucor miehei* protease compared with 0.14% when a mixture of calf rennet and bovine pepsin was used.

To study the degree of hydrolysis of camel, cow and buffalo caseins, the untreated and enzyme-treated samples were analyzed by SDS-PAGE after incubation time for 60 minutes. The results in Fig. 11 showed that α_s-casein was extensively degraded by *Mucor miehei* protease after 60-minute incubation, whereas hydrolysis of β-casein was less than of α_s-casein. Fox (1969) and Ledford *et al.* (1968) found that α_s-casein was more susceptible to proteolysis by calf rennet than β-casein at 32°C. However, β-casein of buffalo milk exhibited higher hydrolysis than those of cow and camel milk. When different types of caseins were subjected to proteolytic action of *Mucor miehei* protease, several faint and intense bands were appeared and moved ahead of the β-casein band (Fig. 11).

Conclusion

In conclusion, this study indicated that the extent of proteolysis of camel, cow and buffalo milk caseins by trypsin, chymotrypsin, pepsin, plasmin and *Mucor miehei* protease was significantly different. In general, the camel casein revealed resistance to digestibility than cow or buffalo milk caseins (with all proteolytic enzymes under studies). Moreover, SDS-PAGE revealed that the rate of enzymes activities on different caseins was in the following order: trypsin > chymotrypsin > pepsin > plasmin > *Mucor miehie* protease. It can be also concluded that the susceptibility of specific casein fraction to proteolysis by the above enzymes was different according to the type of milk casein.

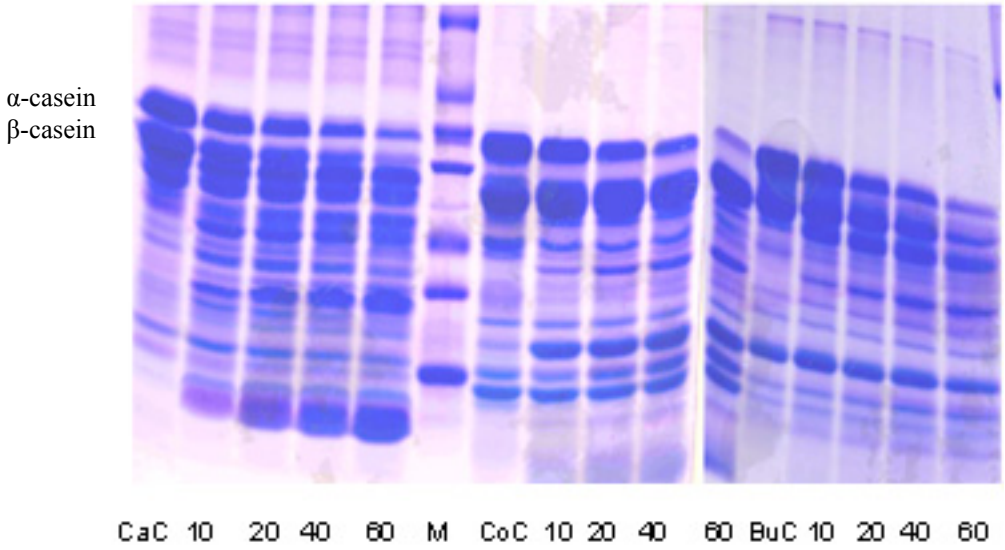

Fig. 11. SDS-PAGE profiles of caseins and of their hydrolysis with microbial rennet from *Mucor miehei*. CaC, CoC and BuC are camel, cow and buffalo caseins respectively. Lanes 10, 20, 40, and 60 are incubation times in minutes and M is protein marker (66-14.2 KDa).

References

Al-Saleh, A. A.; Metwalli, A. A. M. and Abu-Tarboush, H. M. "Partial Characterization of Bacteriocin-like Peptide Produced by Lactobacillus Acidophilus DSM 20079." *Saudi Society Journal for Food and Nutrition*, Vol. 4, (2009), 1-14.

Bastian, E. D. and Brown, R. J. "Plasmin in Milk and Dairy Products: An Update." *International Dairy Journal*, Vol. 6, (1996), 435-457.

Beg, O. U.; Von Bahr-Lindstrom, H.; Zaidi, Z. H. and Jornvall, H. "A Camel Milk Whey Protein Rich in Half-cysine: Primary Structure, Assessment of Variations, Internal Repeat Patterns and Relationships with Neurophysin and Other Active Polypeptides." *European Journal of Biochemistry*, Vol. 159, (1986), 195-201.

Church, F. C.; Swaisgood, H. E.; Porter, D. H. and Catignani, G. L. "Spectrophotometric Assay Using O-phthaldialdehyde for Determination of Proteolysis in Milk and Isolated Milk Proteins." *Journal of Dairy Science*, Vol. 66, (1983), 1219-1227.

El-Shibiny, S. and Abd El-Salam, M. H. "Action of Milk Cloting Enzymes on α_s-caseins from Buffalo's and Cow's Milk." *Journal of Dairy Science*, Vol. 60, (1977), 1519-1521.

Emmons, D. B. and Binns, M. "Cheese Yield Experiments and Proteolysis by Milk-clotting Enzymes." *Journal of Dairy Science*, Vol. 73, (1990), 2028-2043.

Ferranti, P.; Scaloni, A.; Caira, S.; Chianese, L.; Malorni, A. and Addeo, F. "The Primary Structure of Water Buffalo α_{s1} and β-casein: Identification of Phosphorylation Sites and Characterization of a Novel β-casein Variant." *Journal of Protein Chemistry*, Vol. 17, (1998), 835-844.

FitzGerald, R. J.; Murray, B. A. and Walsh, D. J. "Hypotensive Peptides from Milk Proteins." *Journal of Nutrition*, Vol. 134, (2004), 980-988.

Fox, P. F. and McSweeney, P. L. H. "Proteolysis in Cheese." *Food Reviews International*, Vol. 12, (1996), 457-509.

Ganguli, N. C.; Prabhakaran, B. J. V. and Iya, K. K. "Composition of the Caseins of Buffalo and Cow Milk." *Journal of Dairy Science*, Vol. 47, (1963), 13-18.

Gaucheron, E.; Molle, D. and Pannetier, R. "Influence of pH on the Heat-induced Proteolysis of Casein Molecules." *Journal of Dairy Research*, Vol. 68, (2001), 71-80.

Gomez-Ruiz, J. A.; López-Expósito, I.; Pihlanto, A.; Ramos, M. and Recio, I. "Antioxidant Activity of Ovine Casein Hydrolysates: Identification of Active Peptides by HPLC-MS/MS." *European Food Research and Technology*, Vol. 227, (2008), 1061-1067.

Jenness, R. and Koops, J. "Preparation and Properties of a Salt Solution Which Simulates Milkultraf'dtrate." *Netherlands Milk Dairy Journal*, Vol. 16, (1962), 153.

Kaminogawa, S.; Mizobuchi, H. and Yamauchi, K. "Comparison of Bovine Milk Protease with Plasmin." *Agriculture Biological Chemistry Journal*, Vol. 36, (1972), 2163.

Kappeler, S. R.; Faraah, Z. and Puhan, Z. "5 Flanking Regions of Camel Genes Are Highly Similar to Homologous Regions of Other Species and Can Be Divided into Two Groups." *Journal of Dairy Science*, Vol. 86, (2003), 498-508.

Kennedy, J. G.; Spence, E. R. and Bernhart, W. "Comparison of the Rates of *in vitro* Proteolysis in Human Milk, Cow's Milk and Cow's Milk Preparation by Human Gastric and Duodenal Juices." *Journal of Dairy Science*, Vol. 38, (1955), 1307-1311.

Korhonen, H. and Pihlanto, A. "Milk Protein-derived Bioactive Peptides: Novel Opportunities for Health Promotion." *International Dairy Federation (IDF) Bulletin*, Vol. 363, (2001), 17-26.

IDF. *Milk Determination of the Total Nitrogen Content*. Standard 20B, International Dairy Federation, Brussels, Belgium, (1993).

Lahov, E. and Regelson, W. "Antibacterial and Immunostimulating Casein-derived Substances from Milk: Casecidin, Isracidin Peptides." *Food Chemical Toxicology*, Vol. 34, (1996), 131-145.

Ledford, R. A.; Chen, J. H. and Nath, K. R. "Degradation of Casein Fractions by Rennet Extract." *Journal of Dairy Science*, Vol. 51, (1968), 792-794.

Lucas, A.; Sodini. I. C.; Monnet, C.; Jolivet, P. and Corrieu, G. "Probiotic Cell Counts and Acidification in Fermented Milks Supplemented with Milk Protein Hydrolysates." *International Dairy Journal*, Vol. 14, (2004), 46-53.

Ma, W.; Tang, C. and Lai, L. "Specificity of Trypsin and Chymotrypsin: Loop-motion-controlled Dynamic Correlation as a Determinant." *Biophys. Journal*, Vol. 89, (2005), 1183-1193.

Makoto, S. and Dong, O. S. "Food-derived Peptides and Intestinal Functions." *Current Pharmaceutical Design*, Vol. 13, (2007), 885-895.

Matar, C.; LeBlanc, J. G.; Martin, L. and Perdigón, G. "Biologically Active Peptides Released in Fermented Milk: Role and Functions." In: E. R. Farnworth (Ed.), *Handbook of Fermented Functional Foods: Functional Foods and Nutraceuticals Series*. Florida, USA: CRC Press, (2003), pp. 177-201.

Meisel, H. and FitzGerald, R. J. "Opioid Peptides Encrypted in Intact Milk Protein Sequences." *British Journal of Nutrition*, Vol. 84, (2000), 27-31.

Mickelsen and Fish. "Comparing Proteolytic Action of Milk Cloting Enzymes on Caseins and Cheese." *Journal of Dairy Science*, Vol. 53, (1970), 704-710.

Mizuno, S.; Nishimura, S.; Matsuura, K.; Gotou, T. and Yamamoto, N. "Release of Short and Proline-rich Antihypertensive Peptides from Casein Hydrolysate with an *Aspergillus oryzae* Protease." *Journal of Dairy Science*, Vol. 87, (2004), 3183-3188.

Moneret-Vautrin, D. A.; Hatahet, R. and Kanny, G. "Protein Hydrolysates: Hypoallergenic Milks and Extensively Hydrolyzed Formulas: Immuno-allergic Basis for Their Use in Prevention and Treatment of Milk Allergy." *Arch. Pediatr.*, Vol. 8, (2001), 1348-1357.

Nagaoka, S.; Futamura, Y.; Miwa, K.; Awano, T.; Yamauchi, K. and Kanamaru, Y. "Identification of Novel Hypocholesterolemic Peptides Derived from Bovine Milk *β*-lactoglobulin." *Biochemical and Biophysical Research Communications*, Vol. 218, (2001), 11-17.

Otte, J.; Shalaby, S. M.; Zakora, M.; Pripp, A. H. and El-Shabrawy, S. A. "Angiotensin-converting Enzyme Inhibitory Activity of Milk Protein Hydrolysates: Effect of Substrate, Enzyme and Time of Hydrolysis." *International Dairy Journal*, Vol. 17, (2007), 488-503.

Recio, I. and Visser, S. "Two Ion-exchange Methods for the Isolation of Antibacterial Peptides from Lactoferrin: *In situ* Enzymatic Hydrolysis on an Ion-exchange Membrane." *Journal of Chromatography*, Vol. 831, (2007), 191-201.

Rerranti, P.; Scaloni, A.; Caira, S.; Chianese, L.; Malorni, A. and Addeo, F. "The Primary Structure of Water Buffalo α_{S1} and β-casein: Identification of Phosphorylation Sites and Characterization of a Novel β-casein Variant." *Journal Protein Chemistry*, Vol. 17, (1998), 835-844.

Salami, M.; Yousefi, R.; Ehsani, M. R.; Dalgalarrondo, M.; Chobert, J. M.; Haertle, T.; Razavi, S. H.; Saboury, A. A.; Naslaji, A. N. and Movahedi, A. A. M. "Kinetic Characterization of Hydrolysis of Camel and Bovine Milk Proteins by Pancreatic Enzymes." *International Dairy Journal*, Vol. 18, (2008), 1097-1102.

Shammet, K. M.; Brown, R. J. and McMahon, J. "Proteolytic Activity of Some Milk-cloting Enzymes on κ-casein." *Journal of Dairy Science*, Vol. 75, (1992), 1373-1379.

Srinivasan, M. and Lucey, J. A. "Effect of Added Plasmin on the Formation and Rheological Properties of Rennet-induced Skim Milk Gels." *Journal of Dairy Science*, Vol. 85, (2002), 1070-1078.

Tezcucano Molina, A. C.; Allia, I.; Konishi, Y. and Kermasha, S. "Effect of Dephosphorylation on Bovine Casein." *Food Chemistry*, Vol. 101, (2007), 1263-1271.

Voynick, I. M. and Fruton, J. S. "The Comparative Specificity of Acid Proteinases." *Proceedings of the National Academy of Sciences*, Vol. 68, (1971), 257-259.

Walstra, P.; Jenness, R. and Badings, H. T. *Dairy Chemistry and Physics*. New York: Wiley, (1984).

Zar, J. H. *Biostatisical Analysis*. 3rd ed., New Jersey: Prentice Hall, (1996).

Zhang, H.; Yao, J.; Zhao, D.; Liu, H.; Li, J. and Guo, M. "Changes in Chemical Composition of Alxa Bactrian camel Milk during Lactation." *Journal of Dairy Science*, Vol. 88, (2005), 3402-3410.

قابلية تحلل كازينات حليب النوق والجاموس والأبقار بالإنزيمات المحللة للبروتين

علي أحمد محمد متولي، وعبدالرحمن عبدالله الصالح

قسم علوم الأغذية والتغذية، كلية علوم الأغذية والزراعة، جامعة الملك سعود،
ص ب ٢٤٦٠، الرياض ١١٤٥١، المملكة العربية السعودية

(قدم للنشر في ١٤٣٠/٥/١٠هـ؛ وقبل للنشر في ١٤٣١/١/١١هـ)

الكلمات المفتاحية: كازين الحليب، الإنزيمات المحللة للبروتين، الهجرة في المجال الكهربي، النيتروجين الكلي.

ملخص البحث. تم في هذه الدراسة تقدير النشاط التحللي لإنزيمات التربسين والكيموتربسين والببسين والبلازمين والمنفحة الناتجة من عفن *Mucor miehei* على كازين حليب النوق والجاموس والأبقار وتم تقدير النشاط التحللي للإنزيمات المختلفة بقياس النيتروجين الذائب في محلول ٤٪ Trichloroacetic acid (ثالث كلوريد حمض الخل) وقياس الكثافة الضوئية على الطول الموجي ٢٨٠ و ٣٤٠ نانوميتراً، كما تم قياس هجرة البروتين في المجال الكهربي. وأوضحت الدراسة أن كازين حليب النوق أقل في تحلله بدرجة معنوية بالإنزيمات تحت الدراسة من كازين حليب الأبقار وكازين حليب الجاموس. كما اختلفت أيضاً درجة تحلل كازين الأبقار والجاموس بالإنزيمات المستخدمة فكان درجة تحلل كازين حليب الجاموس أعلى من تحلل كازين حليب الأبقار عند استخدام إنزيمات التربسين والبلازمين والمنفحة الناتجة من عفن *Mucor miehei*. من ناحية أخرى أظهر كازين حليب الأبقار تحللاً أعلى من كازين حليب الجاموس عند استخدام إنزيم الببسين والكيموتربسين، كما أوضحت الدراسة أيضاً أن إنزيمات الكيموتربسين والتربسين والببسين لها درجة تحلل أعلى من تلك التي للبلازمين والمنفحة الناتجة من عفن *Mucor miehei* على كل أنواع الكازين تحت الدراسة. كما أوضحت الدراسة أيضاً أن الهجرة في المجال الكهربي للكازينات المختلفة اختلفت اختلافاً واضحاً عند معاملتها بالإنزيمات تحت الدراسة.