

FUNCTIONAL PROPERTIES OF ENZYMATICALLY MODIFIED BUFFALO MILK PROTEIN PRODUCTS

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ABSTRACT

Buffalo milk protein products (Total milk proteinate (TMP), casein-co-precipitate, acid casein) were enzymatically modified using Maxrien enzyme. The chemical composition was determined and some properties (solubility, emulsion activity, foam expansion, buffer capacity, viscosity and freezing point) of these products were investigated at different rates of hydrolysis. The TMP possessed the lowest ash & moisture with highest lactose and protein contents compared to the other protein products. The enzymatic hydrolysis up to eight h markedly improved the protein solubility while the changes were slight after that. The pattern of electrophoresis showed that casein-co-precipitate was more resistant to hydrolysis than total milk proteinate or acid casein. Foam expansion of milk protein products was enhanced by the enzymatic modification being increased with increasing the time of hydrolysis. At zero time the acid casein showed the lowest foam expansion compared to the other two products. There were no differences in foam expansion among enzyme treated protein products. Different protein hydrolyzates showed poor foam stability with extending the time of hydrolysis being the lowest in acid casein. The emulsion activity index of protein hydrolyzates decreased with advancing time of hydrolysis showing the highest value with TMP. Stability of emulsion decreased with longer storage period showing the lowest stability in acid casein. Enzymatically modified protein products exhibited lower viscosity values and more reduction in freezing point with advancing time of hydrolysis. Total milk proteinate showed the highest buffer capacity followed by casein co-precipitate and lastly acid casein. The buffer capacity increased in the protein product with prolonging time of hydrolysis. It could be concluded that enzymatically modified protein products may be successfully used in some food and dairy products depending on the enhancement in their functional properties.

Key words: Buffalo milk protein products, Functional properties, Enzymatic hydrolysis

INTRODUCTION

Protein modification can be carried out either chemically, physically, enzy-

matically, or genetically to alter the functionality of protein. Enzymatic modification was reported as an important mean of converting food protein into

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products with very different and desirable properties (Chan and Ma 1999). Most of protein properties are affected by altering the structure, configuration and molecular weight of proteins. Enzymatic hydrolysis of food proteins such as soy protein has been extensively applied to improve the solubility and other functional properties. Since rennin and rennin substitutes belong to the same class of aspartic protease, it is not easy to understand the differences in their proteolytic activity at the same clotting activity. In Europe, the classic microbial rennin substitutes will retain their importance up to the time in which chymosin from transgenic microorganisms; can be generally used (Krause *et al* 1998). Viscosity is one of the most important functional properties of food proteins. It is important for providing physical stability to emulsions. The concentrations, molecular weight, polydispersity, hydrophobicity, and conformation of each protein species affect the viscosity of protein solution. All of these factors tend to confound the underlying inverse relationship of protein solubility and viscosity (Schenz and Morr, 1996). Processing-induced changes in proteins such as polymerization, aggregation and hydrolysis affect the viscosity of food products. The freezing point is dependent on the soluble constituents and varies with variation in composition. It is also highly affected by the molecular weight of solutes in the media. The available information about enzymatic modification of buffalo milk proteins or protein products is very limited. Thus, the target of this work was to examine the functional properties of enzymatically modified milk protein products, (total milk proteinate, casein co-precipitate or acid casein) using Maxrien enzyme aiming to

enhance the role of these modified protein products in food and dairy industry.

MATERIAL AND METHODS

Materials

Buffalo milk was obtained from the herd of Faculty of Agriculture, Ain Shams University and used for preparing the protein products. Total milk proteinate, casein co-precipitate, and acid casein were isolated from buffalo skim milk following the method of Morr (1985). Protein samples were lyophilized at -40°C using freeze-drying system LYPH-LOCK-4.5. Maxiren enzyme obtained from Gist-brocades, France, was used for enzymatic hydrolysis.

Preparation of hydrolyzates

Protein hydrolysis was carried out using diluted protein products solutions (10g/L) at pH 6.5 and 30°C with adding Maxiren enzyme (30ug/ml solution). The hydrolyzates were taken after 0.5, 1, 2, 4, 6, 8, 12, 24 h and the enzyme activity was inhibited by heat treating the protein solutions in boiling water for two min and then cooled in ice.

Chemical analysis

Moisture and ash contents were determined according to AOAC (1984). Total nitrogen content was determined by micro-kjeldahl method as described by Ling (1963). Lactose content was measured according to Barnett and Tawab (1957). Polyacrylamide-gel electrophoresis was carried out according to Hillier (1976).

Functional properties

Protein solubility was determined by the method of Morr (1985). Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by the turbidimetric method of Pearce and Kinsella (1978). Foam expansion and foam stability was estimated by the procedure of Patel *et al* (1988). Buffer capacity was determined by the method of Morr *et al* (1973). Viscosity in centipoise was estimated on 30-40 ml of protein solution using Hoppler viscometer type BHN^o 0.9367. The freezing point was determined according to the method described in FAO Laboratory Manual (1977). Three replicates of each sample were produced and analyzed for each character assessed for 3 times.

RESULTS AND DISCUSSION

Chemical composition of total milk proteinate, casein co-precipitates and acid casein from buffalo milk is shown in Table (1). Among examined protein products, total milk proteinate exhibited the lowest ash & moisture with highest

lactose & protein contents, compared with those of other protein products. On the other hand, acid casein possessed the lowest values of protein and lactose. The results are in agreement with those reported by Morr (1985).

Electrophoretic characterization of hydrolyzes

Patterns of electrophoresis were performed to monitor hydrolysis of total milk proteinate, casein-co-precipitate and acid casein by Maxiren (Fig. 1 A, B and C) at different periods ranged from 0.5 to 24.0 h. The electrophoretic patterns for untreated total milk proteinate (column 1) seemed to be divided into three major regions including κ -casein, β -casein and α_1 -casein. The electrophoresis patterns (column 2,3,4) were characterized by appearance of some new bands in α_1 -casein region. This could be attributed to the degradation of α_1 -casein. After 8 h of hydrolysis, it could be observed that the intensity of β -casein band decreased compared to that of untreated pattern (column 1). Although the intensity of casein bands decreased, no bands could

Table 1. Gross chemical composition % of buffalo milk protein products

Protein product	Moisture	Protein (N x 6.38)	Lactose	Ash
Total milk proteinate	4.21	90.89	1.04	3.21
Casein co-precipitate	5.20	88.82	0.82	4.45
Acid casein	4.96	87.18	0.69	5.14

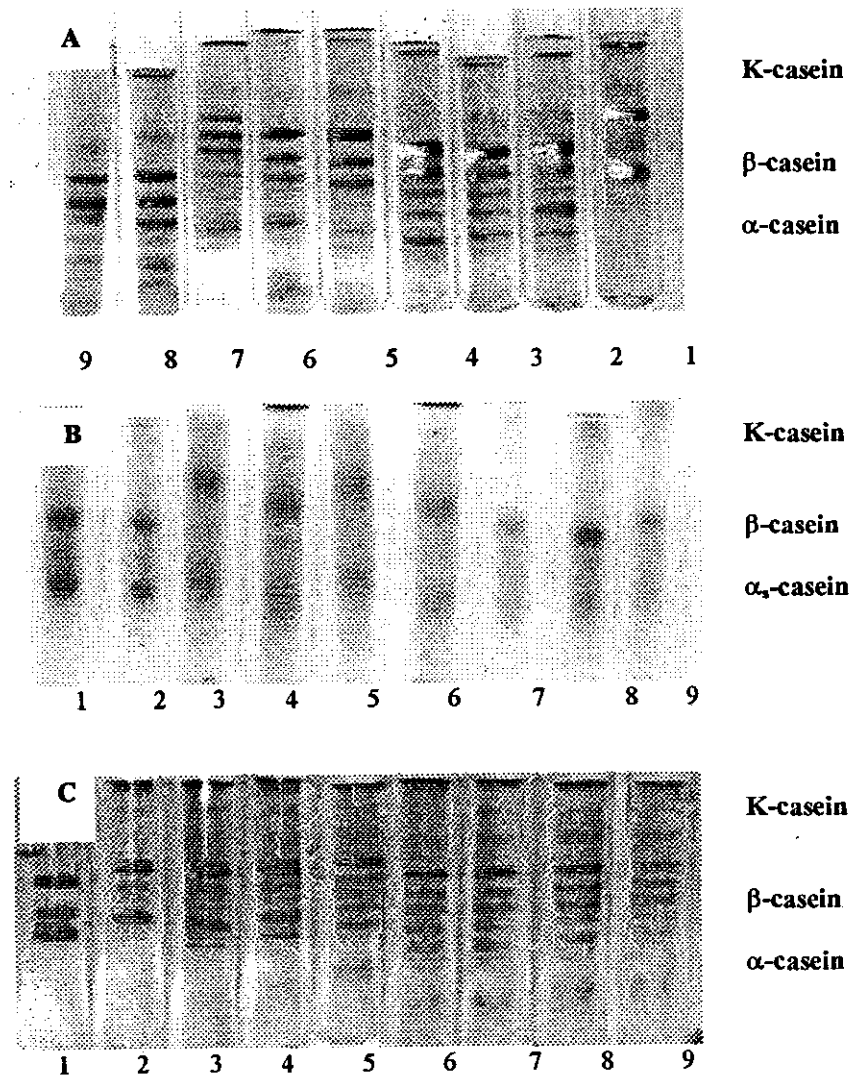


Fig. 1. Polyacrylamide gel electrophoresis patterns of (A) total milk proteinate, (B) casein co-precipitates and (C) acid casein treated with Maxrien enzyme. The hydrolyzates 1 to 9 were taken after 0.0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h respectively.

be detected in the α_2 -casein region. This could be attributed to loss of casein fragments (column 5,6,7,8,9). The degradation in both β -casein and α_2 -casein of casein co-precipitate by Maxrien enzyme (Fig. 1 B, column 7,8,9) was very slight compared to other protein products. It could be concluded that milk protein breakdown was more pronounced in total milk proteinate. Moreover, the rate of hydrolysis was clearly proportional after 2 h of hydrolysis. The rate of degradation increased with extending the time of hydrolyses. Among tested buffalo milk protein products, the casein-co-precipitate was the most resistant against Maxrien hydrolysis.

Protein Solubility

Effect of hydrolysis time of Maxrien at pH 6.5 on solubility of buffalo milk protein products is shown in (Table 2).

The results indicated that the enzymatic hydrolysis improved the protein solubility of milk protein products. The increase in solubility was more noticeable up to 8 h while after that it was very slight. The casein-co-precipitate showed the highest resistance against hydrolysis by Maxrien compared to total milk proteinate or acid casein. The solubility degree of casein-co-precipitate was 75% after 8 h whereas it was 98.11% and 95.64% for total milk proteinate and acid casein respectively. The poor solubility of casein-co-precipitate could be attributed to its high content of denatured whey protein. The solubility of milk protein products was improved by Maxrien hydrolysis over a wide range of time. This can be attributed to the reduction occurred in molecular size of protein, and the increase of net charge on hydrolyzed milk protein micelles. The cleavage of peptide bonds adjacent to basic amino acids could

Table 2. Solubility (%) of buffalo total milk proteinate, casein co-precipitate and acid-casein hydrolyzed by Maxrien enzyme during 24 h incubation at pH 6.5 and 30 °C.

Protein product	Protein solubility after (h)							
	0.0	0.5	0.1	4.0	6.0	8.0	12.0	24.0
Total milk proteinate	6.55	72.81	76.85	81.23	85.90	98.11	98.10	98.21
Casein co-precipitate	5.40	50.61	53.89	58.05	61.22	75.78	76.45	77.35
Acid-casein	5.46	65.14	70.54	76.63	83.96	95.64	96.32	96.75

dissociate the protein complex to expose more charged groups to surrounding water (Jones and Tung, 1983). This could promote protein-water interaction and enhance protein solubility (Kinsella and Shetty, 1979).

Foam expansion and Stability

Foam expansion (FE) and Foam volume stability (FVS) of total milk proteinate, casein-co-precipitate, and acid casein before and after enzymatic hydrolysis were presented in Table (3). The enzymatic modification improved the foam expansion of milk protein products. Therefore, FE increased with increasing the time of hydrolysis up to 6 h. At zero time, the acid casein showed the lowest foam expansion, while casein co-precipitate exhibited the highest one. Mean while, after 6 h there was no difference in FE among all tested protein products. Enzymatic modification of milk protein products resulted in poor stability of foam volume. The foam height decreased after 20 min by about 50, 25 and 27% of untreated total milk proteinate, casein-co-precipitate and acid casein respectively. The foam disappeared after 25 min for modified TMP and acid casein while it disappeared after 30 min in modified casein-co-precipitate even at the lowest rate of hydrolysis. The data indicated that casein co-precipitate either treated or untreated had the highest ability to produce and maintain the foam. The highest FVS of casein co-precipitate could be due to its low rate of hydrolysis compared to the other protein products. The increase in solubility of protein by hydrolysis would enhance foam ability (Kinsella, 1976), but the excessive increase of net charge may reduce the protein-protein interaction and prevent

the formation of elastic film at the air-liquid interface, hence decrease the foam stability (Chan and Ma, 1999).

Emulsifying properties

The emulsion activity index of emulsions prepared by homogenizing a solution of protein hydrolyzed at different rates with corn oil is shown in Table (4). The results clearly demonstrated that the emulsion activity of milk protein products progressively decreased with increasing the time of hydrolysis. The emulsion activity index of hydrolyzed protein was lower than that of untreated protein (at 0.0 time). Untreated and treated TMP showed the best emulsion activity index against the other protein products. The effect of storage on stability of emulsion indicated that the emulsions made from hydrolyzed milk protein products were generally less stable than the emulsions of non-hydrolyzed (untreated) proteins. This may be due to that the hydrolysis of protein increased the number of polar group and thus the hydrophilicity decreased the molecular weight, altered the globular structure of protein, and exposed previously buried hydrophobic regions. All these changes would affect the emulsifying properties (Nielsen, 1997; Schwenke, 1997). These results are in agreement with those of Slattery and Fitzgerald (1998). Also, the decrease of emulsion activity by increasing the time of hydrolysis may be due to that the small formed peptides have lost the capacity to interact with both aqueous and nonaqueous phases. A peptide with very long chain length has a greater probability of having both hydrophilic and hydrophobic moieties on the same unit (Agboola and Dalgleish, 1996).

Table 3. Foam expansion (FE) and foam volume stability (FVS) of buffalo total milk proteinate, casein co-precipitate and acid-casein hydrolyzed by Maxrien enzyme during 24 h incubation at pH 6.5 and 30°C

Incubation time (h)	FE %	FVS % after (min.)						
		5	10	15	20	25	30	35
Total milk proteinate								
0.0	600	100	100	85.7	50.0	42.9	25.0	0.0
1	600	100	99.3	71.4	39.3	0.0	0.0	0.0
4	650	100	88.9	70.3	31.6	0.0	0.0	0.0
6	750	88.9	88.0	57.8	30.1	0.0	0.0	0.0
8	750	88.0	88.0	57.0	30.0	0.0	0.0	0.0
12	750	81.0	76.0	39.0	0.0	0.0	0.0	0.0
24	750	80.0	72.0	34.0	0.0	0.0	0.0	0.0
Casein co-precipitate								
0.0	650	100	100	93.3	75.3	66.7	60.0	50.0
1	700	100	94.1	70.7	46.9	21.9	0.0	0.0
4	700	100	85.9	70.4	46.9	0.0	0.0	0.0
6	750	97.1	85.3	69.4	40.0	0.0	0.0	0.0
8	750	96.9	76.5	65.6	40.0	0.0	0.0	0.0
12	750	93.8	75.0	64.4	40.0	0.0	0.0	0.0
24	750	81.3	71.8	60.0	35.4	0.0	0.0	0.0
Acid-casein								
0.0	500	100	100	83.3	73.0	62.5	20.8	20.0
1	700	94.1	70.7	44.2	25.3	0.0	0.0	0.0
4	700	94.1	63.9	38.2	11.0	0.0	0.0	0.0
6	750	87.5	52.3	16.3	0.0	0.0	0.0	0.0
8	750	85.4	43.0	8.0	0.0	0.0	0.0	0.0
12	750	80.7	33.8	0.0	0.0	0.0	0.0	0.0
24	750	65.7	28.7	0.0	0.0	0.0	0.0	0.0

Table 4. Emulsion activity (EAI) and emulsion stability (ESI) indexes of buffalo total milk proteinate, casein co-precipitate and acid-casein hydrolyzed by Maxrien enzyme during 24 h incubation at pH 6.5 and 30°C

Incubation time	EAI (m ² g ⁻¹)	ESI after*		
		1 day	2 days	3 days
Total milk proteinate				
0.0	147	100	100	90
1	136	100	97	86
4	136	97	97	83
6	132	97	97	83
8	125	94	88	82
12	120	92	86	80
24	120	91	83	76
Casein co-precipitate				
0.0	121	100	96	87
1	99	100	92	81
4	99	100	88	74
6	92	100	72	60
8	84	91	82	43
12	76	90	81	42
24	68	90	79	38
Acid-Casein				
0.0	95	96	76	61
1	84	95	86	52
4	77	94	80	52
6	66	94	55	44
8	62	82	58	41
12	61	82	58	40
24	61	82	56	40

* Samples stored at 25°C

Buffer capacity

Buffer index (BI) versus pH values of buffalo milk protein products, total milk proteinate, casein co-precipitate and acid casein, as titrated with 0.1N HCl from pH 10.0 to 3.0 are illustrated in Fig. 2 (A, B and C). Buffer intensity curves of all protein products either non-hydrolyzed or at different rates of hydrolysis followed the same trend in the peak. From the data it could be generally concluded that the buffer index of buffalo milk protein products showed a broad BI peak at pH range from 5.0 to 6.0. At acidic side the BI decreased progressively and reached a first minimum value at pH about 4.0. As pH was further lowered, an increase in BI was observed at pH 3.0. At alkaline side the second minimum value of BI was observed at pH range from 7.0 to 8.0, then an increase was noticed again at pH about 10.0. From the results presented it could be observed that the buffer index values of buffalo milk acid casein protein was slightly lower than that of TMP and casein co-precipitate. The different behaviour of protein products in buffer index could be due to the form and nature of protein fractions, which previously confirmed by electrophoresis (Metwally and Awad, 2001). The buffer index at pH 3 of untreated TMP was about 0.3 while it was about 0.25 for untreated casein co-precipitate or acid casein. On the other hand, BI of previous hydrolyzed proteins for 8 hrs was about 0.7, 0.65 and 0.5 for TMP, casein co-precipitate and acid casein respectively. Such differences among protein samples could be due to the variations in protein composition and structure. The results are in the same trend given by Mahran *et al* (1994), who reported that maximum buffering capac-

ity of acid casein was in the range of pH 5-6. Morr *et al* (1973) found that the greater BI at pH around 4.5 was related to whey proteins than those of casein.

Viscosity and freezing point

The viscosity values and freezing points of 1.0% solutions of buffalo milk protein products, TMP, casein co-precipitate and acid casein, either hydrolyzed or non-hydrolyzed are presented in Table (5). Among all tested samples,

Table 5. Viscosity and Freezing point of buffalo total milk proteinate, casein co-precipitate and acid-casein hydrolyzed by Maxrien enzyme during 24 h incubation at pH 6.5 and 30°C

Incubation time (h)	Viscosity (cp)	Freezing point °C
Total milk proteinate		
0.0	1.38	-1.00
1	1.35	-1.00
4	1.29	-1.11
6	1.24	-1.19
8	1.19	-1.28
12	1.16	-1.36
24	1.13	-1.43
Casein co-precipitate		
0.0	1.46	-1.20
1	1.46	-1.20
4	1.43	-1.22
6	1.39	-1.24
8	1.35	-1.29
12	1.33	-1.34
24	1.29	-1.37
Acid-Casein		
0.0	1.29	-1.05
1	1.24	-1.10
4	1.21	-1.20
6	1.19	-1.21
8	1.16	-1.23
12	1.13	-1.26
24	1.11	-1.30

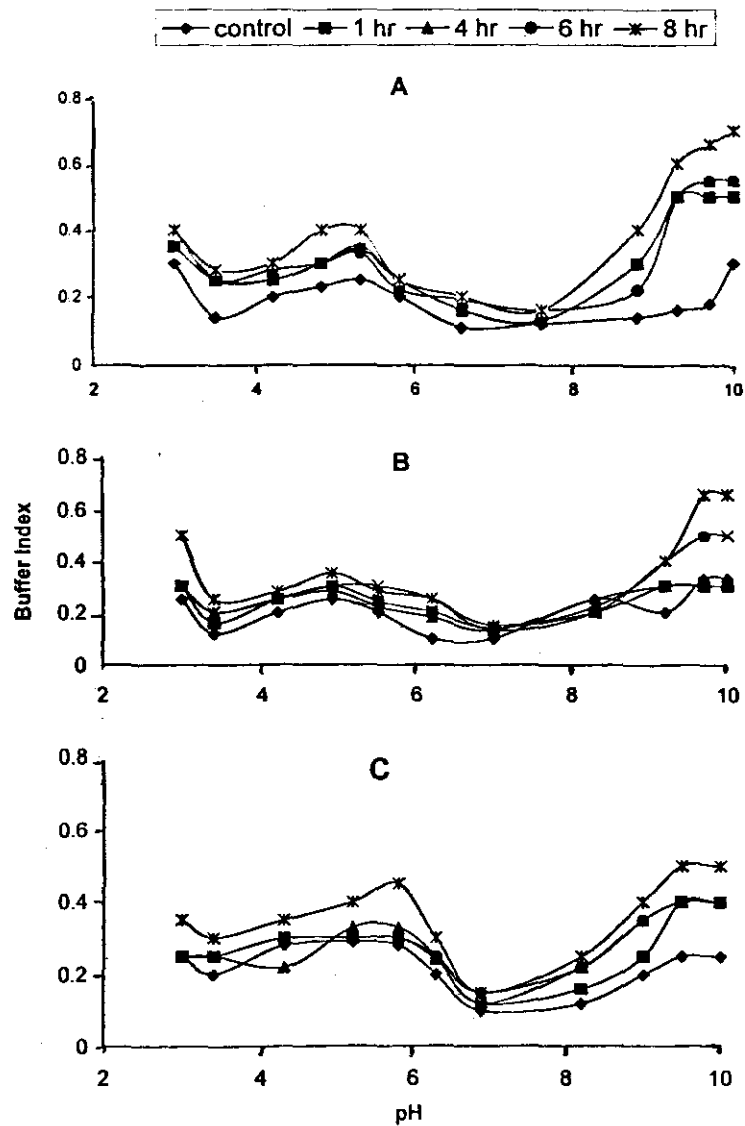


Fig. 2. Buffer intensity curves of hydrolyzed total milk proteinate (A), casein coprecipitate (B) and acid casein (C)

casein co-precipitate exhibited the highest viscosity values followed by TMP then acid casein. This could be due to casein co-precipitate contains high amount of whey proteins in denatured form which may cause higher viscosity (Awad and Metwally, 2000). The viscosity values of all protein products solutions decreased with increasing the hydrolysis time. Therefore, samples hydrolyzed by Maxrien enzyme for 24 hrs had the lowest viscosity values. The viscosity of protein solutions is affected by several factors such as molecular weight, polydispersity, hydrophobicity, and protein configuration (Schenz and Morr, 1996).

The freezing points of protein solutions (Table, 5) indicated that there was a slight difference among different protein solutions. The freezing point was lowered by 0.43°C from -1.0 for non-hydrolyzed to -1.43 for hydrolyzed to 24 h in TMP solution. On the other hand the values reduced by 0.17 and 0.25 for casein co-precipitate and acid casein respectively. The greatest reduction of freezing point in TMP solutions than that of other protein solutions may be due to the higher rate of proteolysis in TMP than the other proteins. The hydrolysis of proteins will produce peptides with lower molecular weights. Arbuckle (1986) mentioned that the freezing point is inversely proportional to the molecular weight.

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الخواص الوظيفية لمستحضرات بروتينات اللبن الجاموسى المعدلة انزيميا

[٣٥]

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يهدف هذا البحث الى دراسة الخواص الوظيفية للمستحضرات البروتينية للبن الجاموسى المعدلة انزيميا باستخدام انزيم المكسرين المخلوق وراثيا وذلك لتحسين معدل الاستفادة منها فى تصنيع المنتجات الغذائية واللبنيه. وقد تم فحص بعض المستحضرات البروتينية مثل بروتينات اللبن الكلية ، الكازين ومرافقاته ، والكازين الحامضى

التحلل وكان الكازين الحامضي هو أقل المستحضرات البروتينية تكويناً للرغوة وعلى العكس من ذلك أظهرت المستحضرات البروتينية المعدلة أنزيميا قدره ضعيفه على ثبات الرغوة في محاليلها وكانت أقل ما يمكن عند التحلل بالانزيم لمدته أطول من ثمان ساعات خصوصاً في الكازين الحامضي. وقد أظهرت بروتينات اللبن الكلية مقدره عاليه على تكوين المستحلب بينما انخفضت هذه الخاصية في كل من المستحضرات البروتينية الأخرى المعدلة أنزيميا وكانت أقل ما يمكن عند التحلل لمدته طويله أكثر من ست ساعات خصوصاً مع الكازين الحامضي. وكان اتجاه كل من قيم اللزوجة ونقطه التجمد نحو الانخفاض في المحاليل المعدله على عكس المحاليل التي لم يتم تعديلها أنزيميا. تشير نتائج هذه الدراسة الى أنه يمكن استخدام المستحضرات البروتينيه المعدله أنزيميا طبقاً للتحسن في خواصها الوظيفيه بما يلائم خواص المنتجات الغذائية المطلوبه.

من حيث بعض الخواص الكيمائيه والطبيعيه والوظيفية والتي أشتملت خاصيه الذوبان والقابلية لتكوين وثبات المستحلب القابلية لتكوين وثبات الرغوة والقدرة التنظيمية وكذلك اللزوجة ونقطة التجمد عند درجات مختلفة من التحلل. وقد اوضحت النتائج الكيمائيه أن بروتينات اللبن الكلية احتوت على أقل قيمة من الرطوبة و الرماد وأعلى قيمة من اللاكتوز والبروتين مقارنة با لمستحضرات البروتينية الأخرى. كما أظهرت النتائج أن التحلل الأنزيمي أدى الى تحسين في خاصية الذوبان حتى ثمانية ساعات من التحلل بينما أظهر تحسناً طفيفاً عند التحلل لمدة أطول. أظهر الفصل الكهربى على جل الاكريلاميد بأن الكازين ومرافقاته كان أكثر ثباتاً للتحلل الأنزيمي مقارنة ببروتينات اللبن الكلية والكازين الحامضي. كما تحسنت خواص تكوين الرغوة للمستحضرات البروتينية للبن الجاموسى بالتعديل الأنزيمي وزاد التحسن بزياده مدة

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