EFFECT OF ENZYMATIC CROSS-LINKING OF MILK PROTEINS ON PROPERTIES OF ICE CREAM WITH DIFFERENT COMPOSITION

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ABSTRACT

Ice-cream mix was treated with transglutaminase enzyme. The enzyme forms covalent bonds between protein fractions enhancing polymerization. Enzyme treated ice cream mix greatly enhanced its viscosity and reduced their rate of shear thinning effect. The treated mix flow behavior changed form time thinning flow into a time thickening flow.

Regular fat control (10% fat and 0.7% stabilizer), regular fat enzyme treated (10% fat and 0.7% stabilizer), regular fat - low stabilizer enzyme treated (10% fat and 0.2% stabilizer), low fat control (5% fat and 0.7% stabilizer) and low fat enzyme

treated (5% fat and 0.7% stabilizer) ice cream samples were prepared.

The mix was treated with the enzyme at 45°C/2.5h, inactivated at 80°C/1min. and homogenized at 13.8/3.4 Mpa with a low stage homogenizer. Regular fat enzyme treated ice cream was superior in melting resistance, smoothness, creaminess, coarseness, overrun and dryness during extrusion and moldability over its control. Low fat treated ice cream showed good improvement in physical and sensory properties over its control that can be easily produced without the need of using fat substitutes. Also, the enzyme treated compensated low stabilizer content in the product. Physical properties of ice cream could be controlled with manipulating the enzyme reaction conditions.

INTRODUCTION

Structural elements of ice cream are composed of fat destabilization, ice-crystallization, air bubble formation and macromolecule structure in the unfrozen phase (Cottrell et al, 1980). Optimal fat destabilization, which is responsible for structure integrity is composed of partial coalescence of fat within the serum phase and enhanced adsorption of fat globules to air After homogenization, interface. proteins adsorption impedes destabilization (Goff, 2000). However, the presence of a powerful surfactant, which is a destabilizing agent, reduces the amount of protein adsorbed on fat by replacing the protein (Goff, 2000 and Goff and Jordan, 1989). Fat also affects mix viscosity, and creaminess, flavor release, smoothness and mouth coaling properties of finished product (Hyvonen, et al., 2003). Low fat (2.5%) and fat free (0.4%) ice cream showed lower mix viscosity and reduced the flavor, smoothness and mouth cooling properties for finished product, therefore in low fat ice cream, the use of fat replacer was recommended (Aime et al. 2001).

Stabilizer is another ice cream constitute that partially increase mix viscosity, improves the aeration and body, restricts ice-crystal growth during storage, controls rate of melt down and prevents shrinkage of product volume during storage. The increased concentration of stabilizers produced a non-

linear increase in the apparent viscosity of mix. This increase in viscosity is decreased by the increase in the amount of solute present in the mix and by increasing the temperature. Actually, there is an optimum viscosity for the original ice mix to give the proper final properties of ice cream (Cottrell et al, 1980 and Bolliger et al, 2000). Double homogenization was found to be effective in increasing the viscosity only in the presences of stabilizer (Ruger et al, 2002). Beside fat and stabilizer which effectively affect mix viscosity, mix proteins also have effect on the physical properties such as solubility, gelation, viscosity, emulsionfication and foaming (Cottrell et al, 1980).

Recently, proteins are polymerized to high molecular weight polymers by the formation of covalent bonds with transglutaminase enzyme (TGase). Transglutaminase (Protein-glutamine γ-glutamyl transferase) is an enzyme naturally present in most animal tissues and body fluid and it plays an important role in the blood clot formation. The enzyme catalyzes an acyl transfer reaction between y-carboxyamide groups of peptide bound glutamine residues (acyl donor) and the primary amino groups in a Varity of amine compounds (acyl acceptor) such as peptide bound ε-amino groups of lysine. The enzyme catalyzes protein cross-linking, deamination or amin incorporation. However, cross-linking dominates the first 30 min. of reaction. Caseins are good substrates for the enzyme action, particularly after heating due to their low degree of tertiary structure and porous nature. k-casein and β-casein are most susceptible for the reaction. β-lactoglobulin requires heating before reaction (Sharma et al, 2001). It was found that milk treated with TGase modified proteins functional properties like solubility, hydration ability, rheological and emulsifying properties, improves heat stability and reduces syneresis (Lorenzen et al, 2002). Acidified milk gel with the TGase gave a strong gel with lower permeability and finer microstructure, more creamy and less syneresis. Therefore, it was suggested that various dairy products e.g. yogurt with reduced fat and proteins could be produced which have similar texture to the full fat and high total solids products (Faergemand et al, 1999). In other words, the action of TGase on proteins of the product could replace fat substitutes in low fat products.

Recently, the enzyme was prepared from microbial source and became commercially available. It is approved by the American FDA as a food additive; therefore its use would be advantageous for its effect in

changing protein function without having adverse effects.

Nowadays, the demand for low fat dairy products is increasing. Ice cream is a high fat dairy product (~10% fat) and as mentioned above reduction its fat contents would adversely affect the product physical and sensory properties. Also, the role of stabilizers in establishing processing parameter of ice cream is well documented. The objective of this research is to produce low fat and low stabilizer ice cream having the same properties of regular ice cream with the use of TGase.

The effect of treating low fat and low stabilizer ice cream mix with the enzyme on the mix physical properties and the final product physical and

sensory properties were studied.

MATERIALS AND METHODS

Materials:

Buffaloes' milk fat from the herd of faculty of Agriculture, Cairo University was separated in to 50% fat cream and 9% S.N.F. skim milk Skim milk powder (96% T.S.), Sugar Cane (100% T.S.) and gelatin stabilizer, were obtained from local markets Microbial (streptoverticillium spp.) Transglutaminase was a gift from Activa, Ajinomoto Europe Sales (Hamburh, Germany); the declared activity of the preparation was approximately 1000 units/g.

Ice cream mix preparation:

Five experimental mixes were prepared and their compositions are listed in Table 1. All mixes were heated at 80°C for 15 min., then rapidly cooled to 50°C and homogenized at 13.8/3.4 Mpa with a tow stage APV Gaulin homogenizer. Control mixes that were not treated with the enzyme were cooled to 5°C for ageing for 48 h. The enzyme-treated samples after homogenization were processed according to the flow sheet of Figure 1. Enzyme concentration of 0.6g enzyme powder/liter of mix was used in all experiments. Before addition to the mix batch, the calculated amount of enzyme was stirred into 50 ml of mix. The enzyme reaction was carried out at 45°C for 2.5 h and the deactivation was at 80°C for 1 min.

Table (1): Composition of the experimental mixes.

Ingredients	Composition of the mix(%)						
		Regula	Low fat				
	Control-	Enzyme- treated	Low-stabilizer enzyme-treated	Control- B	Enzyme- treated		
Milk fat	10	10	10	5	5		
MSNF	10	10	10	11	11		
Sugar	14	14	14	14	14		
Gelatine	0.7	0.7	0.2	0.7	0.7		

A batch of 3.5 L of mix was prepared for each treatment. Mix was frozen in a batch ice cream freezer, (Starmatic V 500, Italy) Packed in plastic cups (120 Cm³) and hardened at -18°C.

Methods of analysis:

The apparent viscosity of aged ice-cream mixes was carried out in triplicates after tempering for 10min, at 15°C and measured at the same temperature using a concentric cylinder Brookfield Programmable viscometer (Model DV -II+; Brookfield Engineering Laboratories, USA) with UL adaptor and ULA spindle over a shear rate range of 1.22 to 97.8 s⁻¹. WinGather version 1.1 (Brookfield Engineering Laboratories, Inc., Copyright® 1995) software was used to collect, store and plot the data on a personal computer connected to the viscometer. The power law model was used to calculate the flow behavior index (n) and consistency index (K) of the different ice-cream mixes (Innocente et al., 2002).

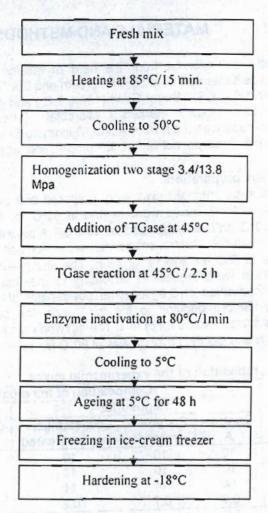


Fig. (1): Flow chart for the production of ice cream treated with TGase.

Freezing point was determined as it was described in FAO laboratory manual annual (1977). The whipping ability was carried out using a food mixer as given by El-Neshway et al., (1988). Specific gravity and weight per gallon for the mixes and their products were determined according to Burke, (1947). Ice cream overrun and melting resistance were determined according to Sommer, (1951) and Arbuckle, (1977), respectively. Scoring card was used for the sensory evaluation of the produced ice cream by experienced panelists from the Dairy Dept. Staff.

Fat destabilization was measured by spectroturbidity method reported by Innocente et al., (2002).

Statistical analysis:

Numerical results were reported or plotted as the arithmetic mean. Analysis of variance (one way ANOVA) was used for multiple comparisons over the different treatments. The statistical significance of the data was

determined using Fisher's L.S.D. post hoc test. *P* value was equal to or less than 0.05 was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SPSS 12.0 (SPSS Inc., Copyright© 2003, Chicago, IL, USA) package on a personal computer.

RESULTS AND DISCUSSION

A- Preliminary experiments on Ice-cream mix

The use of TGase enzyme on ice cream was not reported before, therefore preliminary experiments were run to select the proper time and processing steps to carry out the reaction. The effect of the following points on rheological properties of the mix was studied:

- Reaction time before or during the ageing step.
- b- The importance of homogenization.
- The effect of keeping the enzyme active or not in the mix. Table (2) shows the treatments tested.

Table (2); preliminary trials for selecting proper condition of the

Treatments Sample number		Reaction time Enzyme activity		Mix homogenization	
1	Control	-	-	Not homogenized	
2	Control	1.	-	Homogenized	
3	Enzyme- treated	During ageing	Active	Not homogenized	
4	Enzyme- treated	During ageing ^b	Active	Homogenized	
5	Enzyme- treated	After mix pasteurization	Deactiva- ted	Not homogenized	
6 Enzyme- treated		After mix pasteurization	Deactiva- ted	Homogenized	

a Mix composition was 10% fat %10 MSNF, 14% sugar and 0.7% gelatin and was not frozen.

b The enzymatic reaction was carried out during mix ageing at 5°C for 48h.

The enzyme manufacture literature pointed out that the reaction could be carried out at either 40°C/2h or 6-8°C/12-20h and the enzyme could be left active in yogurt production (Lorenzen et al., 2002). Both points, the reaction temperatures and enzyme activity were tested. Table (3) shows the effect of the tested points on the ice cream mix physical properties.

Table (3): The effect of transglutaminase reaction temperature, state of enzyme and homogenization on the rheological properties of ice cream mix.

Parameter	Treatments*							
Parameter	1	2	3	4	5	6		
Viscosity (mPa.s)	24.2	142,1	35.6	315.2	28.8	171.7		
K (mPa.s)	59 2	250 1	107.5	745.9	120.6	357.8		
n (dimensionless)	0 88	0.86	0.78	0.73	0.66	0.79		
Whipping ability ^b (%)	11	12	15	15	11	11_		

a See Table (2).

b Percent of increase from the original volume after 5 min. of whipping.

The enzyme reaction improved mix viscosity and the improvement greatly increased when the reaction was accompanied with homogenization. The viscosity increased from 24.2 to 35.6 mPa.s and from 142.1 to 315.2 mPa.s with the enzyme reactions and with the enzyme and homogenization, respectively. Running the reaction during mix preparation and before ageing resulted also in viscosity improvement particularly when accompanied with homogenization. The consistency index (k) followed the above trends and increased greatly by the enzyme reaction at ageing accompanied with homogenization, the change, which was highly significant (p < 0.001), was from 250 in the control into 745.9 mPa.s in the treated samples. Again, there was less improvement with carrying out the reaction during mix preparation due to the short period of reaction. The flow behavior index (n) of the mix samples were between 0.66 and 0.88. This was a non-Newtonian or pseudoplastic behavior. The pseudoplastic behavior of ice cream was well established (Goff et al. 1994 and Innocente et al. 2002), however this peseudoplasticity increased with the enzyme-homogenization treatment, which lowered n from 0.86 in control to 0.73. Figure (2) illustrates the effect of shear rate on apparent mix viscosity. All samples showed shear thinning behavior and the rate of thinning was greater with the enzyme treated homogenized samples. Figure (3) points out the effect of viscosity-time relationship on mix viscosity at constant shear rate. The behavior of the enzyme-treated samples changed from time thinning at fixed shear rate in the control that described as thixotropic flow into a different behavior in which the viscosity increased by time at a fixed shear rate. This carried some of the rheopectic flow behavior in which the sample viscosity increased with the time. Time-thickening effect was the highest with enzyme treated homogenized sample. hese results showed that the enzyme treatment greatly increased mix viscosity, which is an important property for production of ice cream with good rheological properties. The effect of the enzyme was enhanced when accompanied with homogenization. Homogenization causes casein particles to be dispersed and adsorbed on fat globules, a practice that makes various protein-sites easily accessible to the enzyme. During ageing the enzyme had more time to react than carrying the reaction at processing as shown by the effect on mix processing.

B- Ice-cream-treated experiments:

Considering the above results, the experimental ice-cream were carried out by running the reaction after mix pasteurization but allowing more time for the reaction (2.5h) and deactivating the enzyme to prevent excessive reaction on ageing and the mix was homogenized.

In the above section the enzyme effectively increased mix viscosity over the control. Fat and stabilizers are the two ingredients that help developing mix viscosity can then be replaced by the enzyme action. Five mixes were prepared according to Table (1) under the experimental section. Rheological and other properties of the experimental mixes were recorded in Table (4). The viscosity of regular fat mix composition was greatly increased with the enzyme reaction; it changed significantly (p < 0.001) from 2700 in the control into 5000 mPa.s in the treated. Low stabilizer mix viscosity was

significantly higher (p < 0.01) than the control but the increase was less in the regular fat treated mix.

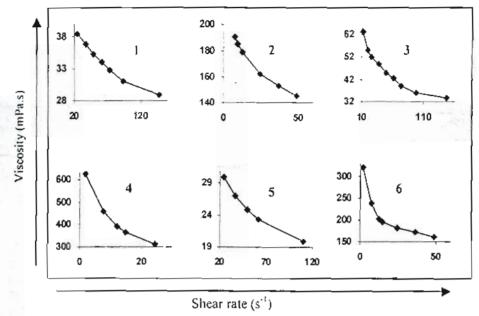


Fig. (2): The effect of treating ice cream mix with transglutaminase on mix viscosity, preliminary trails

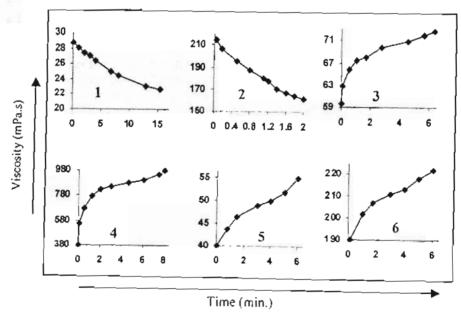


Fig. (3): The effect of treating ice cream mix with transglutaminase on mix viscosity time-dependence, preliminary trails.

Table (4): The effect of transglutaminase enzyme on rheological and other properties of ice cream mixes with low fat and low stabilizer.

	Mix treatments					
	LCC	Regular fa	Low fat			
Parameter	Control- Enzyme- treated		Low- stabilizer enzyme- treated	Control- B	Enzyme- treated	
Weight/gallon (Kg)	3.85	4.26	4.11	4.07	4.82	
Whipping ability(%)	12	22	14.2	19	21.9	
Specific gravity (g/cm ³)	1.016	1.127	1.087	1.076	1.272	
Viscosity (mPa.s)	2700	5000	2900	78	180	
K (mPa.s)	1797	3801	1900	150	234.4	
n	0.9	1.26	1.18	0.78	0.67	
Yield stress (mPa.s)	3.34	9.67	5	3	6.83	
Freezing point (°C)	3.2-	3.9-	3.5-	2.2-	2.6-	

The enzyme also enhanced the viscosity of low fat-mix over low fat control, (180 and 78 mPa.s, respectively). The enzyme treatment also significantly (ρ < 0.01) enhanced the whipping ability of regular fat, low stabilizer and low fat treated mixes. The highest whipping ability was the regular fat (22%) followed by the low fat (21.9%) treated samples. Low fat ice —cream showed higher (but not significant, ρ = 0.05) freezing point than regular fat ice cream. The lowest freezing point was of the regular fat treated sample (-3.9°C).

All treated samples developed significantly (p < 0.01) higher yield stress than their controls. Regular fat mix needed 9.67 mPa.s compared to 6.33 and 3.34 mPa.s for the low fat treated and control, respectively. The consistency index (k) increased significantly (p < 0.01) with enzyme treatment, to reach values of 3801 mPa.s with regular fat treated mix compared to 1797 mPa.s with control. Even with low fat treated mix, the k value was significantly (p < 0.01) higher than the low fat control. The big change was in the flow index (n), it was changed from non-Newtonian (0.9) shear thinning into non-Newtonian shear thickening (or Dilatant) behavior with n = 1.26. The great change in n with the enzyme occurred with the high fat mixes. The increase in fat globule numbers and their adsorption to polymerized casein led to change in the type of flow into a heavy solids flow which was clearly demonstrated in figure (4). The flow turned to be shear thickening and there was a break in the slope of their curves, tending to form a stable viscosity at a particular shear rate. The figure showed that low fat mixes and regular fat control were of shear thinning behavior. However, the low fat treated mix showed a break in the slope of thinning curve. Figure (5) demonstrates the time-viscosity curve at constant shear rate. Regular fat control showed thixotropic (time thinning) flow while the treated regular fat with normal and low stabilizer showed rheopectic (time thickening) flow behavior, ending with a constant viscosity. Low fat control showed no change in viscosity (time independent), but the treated low fat ice cream mix showed rheopectic flow behavior with different slopes. The effect of the enzyme treatment on controlling mix viscosity was established whether the reaction

was with high or low fat ice cream or with high or low stabilizer ice cream. These flow properties of treated ice cream were unfamiliar to the product literature (Goff et al, 1994 and Innocente et al, 2002).

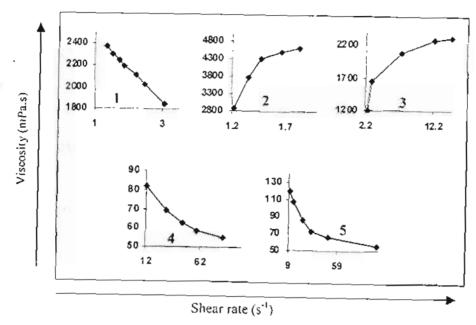


Fig. (4): The effect of treating ice cream mix with transglutaminase on mix viscosity. (1) regular-fat control, (2) regular-fat treated, (3) regular-fat low-stabilizer treated, (4) low-fat control, (5) low-fat treated.

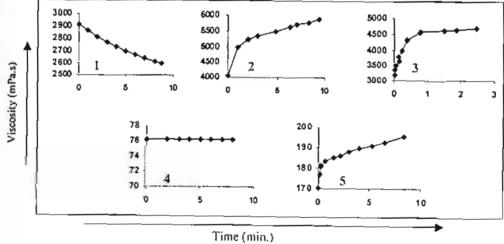


Fig. (5): The effect of treating ice cream mix with transglutaminase on mix viscosity time-dependence. (1) regular-fat control, (2) regular-fat treated, (3) regular-fat low-stabilizer treated, (4) low-fat control, (5) low-fat treated.

Table (5) shows the properties of ice cream treated with the enzyme. Regular-fat ice cream treated samples gave almost the maximum overrun of the used ice-cream freezer (48%); this was followed by regular-fat low-stabilizer ice cream (44%). Even, the low-fat treated sample gave significantly (p < 0.05) higher overrun than its control. The improvement in overrun was clearly demonstrated. Fat destabilization which is an important property for controlling ice-cream physical structure (Tirumalesha and Jayaprakasha, 1998 and Lee and White, 1991), significantly (p < 0.05) increased with all treated samples. Even, with the treated low-fat ice cream, fat destabilization was better than its control. Melting resistance is one of the important properties of ice cream. Figure (6) illustrates the effect of enzyme treatment on ice cream melting resistance.

Table 5: Properties of ice cream treated with transglutaminase enzyme.

_	Mix treatments						
	Regular fa	at	Low fat				
Parameter	Control-	Enzyme- treated	Low- stabilizer Control enzyme- treated		- Enzyme- treated		
Specific gravity (g/cm3)	0.78	0.69	0.71	0.86	0.81		
Weight/gallon (Kg)	2.95	2.61	2.68	3.25	3.06		
Overrun(%)	38	48	44	40	46		
Fat destabilization(%)	72	80	56	2.5	3.5		

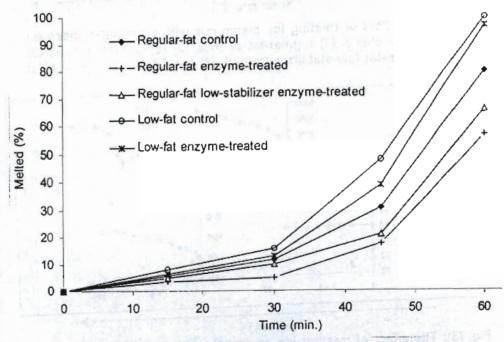


Fig. (6): Effect of milk proteins cross-linking on the ice cream melting resistance.

The treatment improved melting resistance of product. Regular-fat treated samples showed the highest resistance followed by low-stabilizer treated samples. The low-fat ice cream was better than its control.

Figure (7) illustrates the sensory evaluation of Low-fat ice cream samples. Treated low-fat ice cream was significantly (p < 0.05) better in melting quality and creaminess but equal in the coarseness and flavor with its control

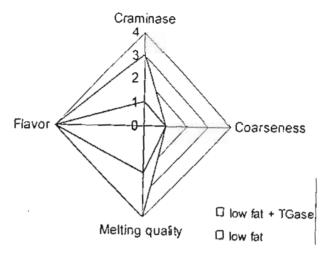


Fig. (7): Sensory evaluation of Enzyme-treated and non treated low fat ice cream.

Figure (8) demonstrates the sensory evaluation of regular-fat ice cream samples. Regular-fat treated samples were superb in creaminess and melting quality followed by regular-fat low-stabilizer ice cream compared to their control (the differences were significant at p < 0.05).

The enzyme forms covalent bonds between casein fractions and β -lactoglobulin forming high molecular weight polymers. This increased mix viscosity and greatly changed the behavior of the mixes into shear thickening flow. This was manifested in the improvement in ice cream properties. Treated ice cream was better in the following properties: aeration, body, controlled ice-crystalles, melting resistance, creaminess, mouth coating and flavor perception than their control. Regular-fat samples showed better dryness at extrusion, better moldability and low rate melt down after scooping. These properties helped greatly in giving the product a good heat shock resistance (unreported data). Low stabilizer was greatly compensated with the enzyme treatment. A useful use of the enzyme was the production of low fat ice cream which was preferred with the panelists without knowing its fat content.

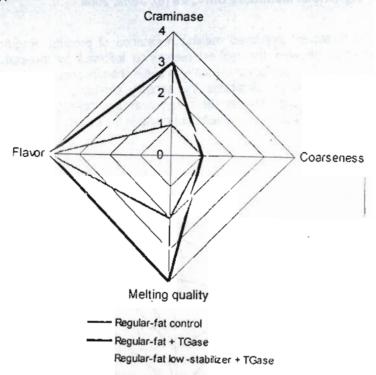


Fig. (8): Sensory evaluation of Enzyme-treated and non treated regular fat ice cream.

In conclusion, the enzyme treatment provided a good method to control ice cream mix viscosity, flow properties and the finished product physical and sensory properties. Actually, with manipulating the enzyme concentration and reaction time to fit the required mix composition, the required ice cream properties, could be tailored.

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تأثير عمل روابط تساهمية تبادلية بين بروتينات اللبن انزيميا على خواص المتلجات القشدية المصنعة من مخاليط مختلفة التركيب. أحمد محمد السيد متولي كلية الزراعة – جامعة القاهرة – الجيزة – مصر.

منذ ان سمحت الـ American FDA باستخدام إنزيم الـ American FDA كمادة يمكن إضافتها الغذاء و بدات الأبحاث العلمية في مجال الألبان و الصناعات الغذائية في محاولة الاستفادة من التغيرات التي يحدثها هذا الأتزيم البروتينات. يقوم هذا الإنزيم بإجراء عملية على البروتينات تسمى Cross-linking حيث يقوم بعمل روابط من النوع التساهمي بين جزيئات البروتينات تسمى البعض مما يودي هذا الحدوث تغير في الخصاص الطبيعية و الوظيفية لهذة البروتينات. و من أهم الخصائص التي تتغير بعد إجراء هذة العملية هي الخواص الريولوجية مثل المزوجة و غيرها. هناك العديد من الأبحاث في مجال الصناعات الغذائية الأن على استخدام هذا الإنزيم و تطبيقاتة فقد استخدم في تغير خواص العديد من البروتينات الغذائية مثل بروتين السمك و البيض و غيرها. البتت الأبحاث أن الكازين من اكثر البروتينات المنامية لنشاط اللإنزيم، إلا انته البيض و غيرها. الأتزيم في مجال الصناعات اللبنية محدودا لقلة الأبحاث في هذا الصدد. أغلب الأبحاث في محاولة استخدامة في التي اجريت على محاولة استخدامة في صناعة الزبادي متعالى الغثرة و قد اثبت نجاحة في انتاج زبادي معتاز في القوام بدون استخدام مثبتات القوام.

أجري هذا البحث بغرض محاولة الأستفادة من التغيرات التي يحدثها هذا الإنزيم -Cross على بروتينات الكازين و بروتينات الشرش في تحسين خواص المتلجات القشدية و المصنعة من مخاليط بها النسبة العادية من الدهن و المثبت القوام و كذلك المصنعة من مخاليط منخفضة المحتوى من الدهن و من مثبت القوام أيضا. نظرا الأنة لم يستخدم هذا الأنزيم في أي من الأبحاث السابقة في هذا الغرض فكان لابد من إجراء تجارب مبدنية لتحديد الظروف المثلى لتفاعل الإنزيم مع بروتينات اللبن فدرس تأثير المعاملة الحرارية و التجنيس و درجة حرارة التفاعل و كذلك صورة الانزيم (سواء بقائة نشط الثاء خطوة التعتيق أو إتلاقة قبلها). و بعد ان إختيرت الظروف المثلى التنفاعل تم دراسة تأثيرة على خواص المخاليط ذات التركيب المختلف السابق ذكرها.

أثبتت النتائج ان الإنزيم قام بتغيرات كبيرة جدا في المتلجات التشدية المعاملة به خصوصا في الخواص الريولوجية فمثلا زائت اللزوجة نتيجة للمعاملة بالانزيم من 2700 mPa.s الى shear thinning الى shear thinning الى shear thinning و تغير نوع التنفق تماما كنتيجة لهذة المعاملة من 5000 و تغير نوع التنفق تماما كنتيجة لهذة المعاملة من time thickening و زاد الربع من 38% إلى thickening و كذلك من هذة التغيرات fat destabilization من 20% إلى 80% و كانت هذة التغيرات معنوية بدرجة كبيرة و كانت المتلجات الناتجة من مخاليط معاملة بالإنزيم لها أكبر درجة مقاومة للإنصهار وغيرها من الصفات المرغوبة.

كما اثبت النتائج نجاح الإنزيم في تغير صفات المتلجات القشدية منخفة المحتوى من الدهن لجعلة مشابية تماما للمثلجات المصنعة من مخاليط بها ضعف الكمية من الدهن و كان هذا التغير شديد لدرجة ان المحكمين الذين قاموا بالتحكيم الحسي لم يستطيعوا تميز الفرق بينيما. و نفس النتائج مع المنخفض في نسبة مثبتات القوام فقد حصلت على درجات التحكيم الحسي أكبر من الكنترول الخاص بيا.

تشير النتائج إلى أهمية أستخدام هذا الإنزيم في صناعة المثلجات القشدية لما لمة من تأثير واضح و قوي في تغير صفاتة إلى صفات مرغوبة بل انة يمكن بالتحكم في ظروف التفاعل و نسبة البروتين في المخاليط التوصل للزوجة و التركيب و المقاومة للإنصيار المرادة بالضبط للمنتج الفشدي الناتج.