The Use of Transglutaminase Catalysed Cross-Linking in the Manufacture of Low Fat Ice Cream

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Abstract: *Low fat (2%) ice cream was prepared from mixes in which milk ingredients were subjected to enzymatic modification with Transglutaminase (TGase). The milk ingredients of the mix were treated with 0.0, 0.4, 0.6 and 0.8 g TGase/kg of mix for 2 h at 40°C. Sugar and gelatin were added and TGase was inactivated by holding the mix at 80°C for 1 min. Treated mixes were aged overnight at 4°C and ice cream (60-74% overrun) was made using the conventional techniques. The mix viscosity was found to increase after TGase treatment. Organoleptic scoring revealed that, ice cream made from the TGase treated mix as were ranked higher score for body and texture. TGase treated ice cream showed a slower rate of melting than the control. These characteristics were pronounced for all TGase treatments than the control.*

Key words: *Transglutaminase, low fat ice cream, rheological properties*

INTRODUCTION

The structure of ice cream is complicated and highly dependent on the amount, functionality, and interaction between its components. Researchers have developed several ingredients for ice cream that mimic the function of fat. However some of these ingredients contribute undesirable flavours or mask desirable flavour. Instead of using additives to mimic the functions of fat some research modified casein by controlled treatment with Chymosin (Chang *et al.*, 1995). Protease enzymes with activities resulting in protein modification had been made in frozen desserts as alternatives stabilizer (Dalgleish, 1979, Arbuckle, 1986). Curdy appearance of the melted product often occurred, high overrun and melting rate was decreased.

New enzymes including TGase which cross-links proteins process the potential for increasing water immobilization properties by forming a gel from milk protein. Enzymatic modification by cross-linking is a method that has received increasing attention during the last 10 years. TGase is a group of enzymes that catalyzes the formation of covalent cross-links between peptide-bound glutamine and various primary amines (Nio *et al.*, 1985). Particles smaller than 0.1 μ, such as casein micelles are perceived on tongue as slippery (Singer and Dunn, 1990); particles that rang in size from 0.1 to 3.0 μ are perceived as creamy (Kalab, 1990, Singer and Dunn, 1990). Among milk proteins, caseins are excellent substrate for TGase, probably due to their low degree of tertiary structure, leaving the reactive groups exposed to the enzymes (Nio *et al.* 1985, 1986; Traore and Meunier, 1991). Cross-linking reactions result in aggregates of protein polymers, this modification of milk protein results generally in products with higher strength and lower syneresis.

Because casein micelles that are cross-linked by TGase, have larger particles size than the original casein micelles in milk, these particles may roll on the tongue, producing a creamy feeling. Changes in volume and shape of casein particles were reported to be responsible for changes in the viscosity of milk (Guthy and Novak, 1977, Prentice, 1972).

Consequently TGase can be used to change the cross-linking of the target proteins which in turn can modify their hydration, gelation, rheological, emulsifying, rennetability and heat stability (Dickison, 1997; Lorenzen and Schlimme, 1998; Lorenzen, 2000a,b). There are several studies on the effect of TGase on casein (Ikura, *et al.*, 1980; Sakamoto *et al.*, 1994). Due to its high reactivity with casein, its application in dairy products has been widely investigated (Dickinson and Yamamoto, 1996; Lorenzen and Schlimme, 1998; Faergemand *et al.*, 1999; Lorenzen 2000a, b and .Abou El Nour *et al.*, 2004).

The research reported here was designed to modify milk protein by controlled treatment with TGase for achieving desirable texture for ice cream particularly that of low fat ice cream.

MATERIALS AND METHODS

Materials:

Cow's milk was obtained from herds of faculty of Agriculture, Suez Canal University and standardized to about 3.0% fat and 9% SNF. Skim milk powder 95% TS was imported from Holland, cane sugar and vanillin were obtained from local market, gelatin solution (10%) was prepared from gelatin powder (ADWIC, El-Nasr Pharmaceutics Chemicals Co., Egypt). TGase was gift from Sjimomoto Europe sales (Stubbenhuk 3, Hamburg, Germany), the declared activity of the preparation was approximately 100 units/g and shelf life of 18 months at room temperature.

Preparation of different mixes:

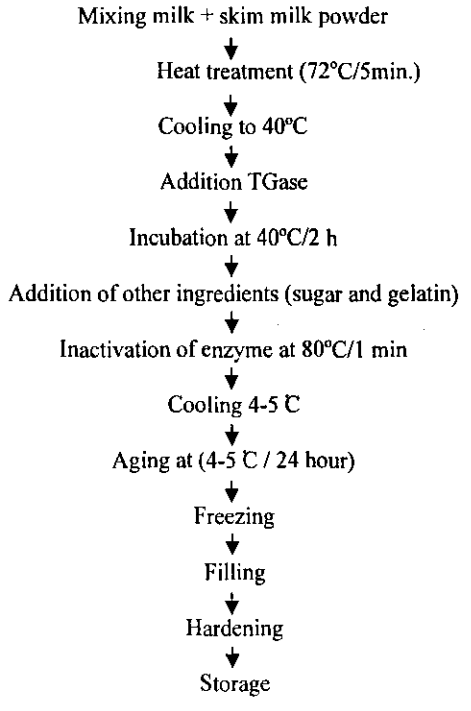
The ice cream mixes were prepared according to Arbuckle (1986). The mixes consisted of 2% fat, 15% SNF, 15% sugar, 0.5% gelatin and 0.03 % vanilla. The mixes were prepared using the formulation in (Table 1) and processed as shown in Figure (1).

Table 1: Composition of low fat ice cream mixes containing different level of TGase (g/100g mix)

Treatments	T_O	T_1	T_2	T_3
Ingredients				
Sugars	15	15	15	15
Gelatin solution	5	5	5	5
Standardized milk	68	68	68	68
Skim milk powder	9.5	9.5	9.5	9.5
TGase	0.00	0.04	0.06	0.08
water	2.5	2.5	2.5	2.5

T_O: low fat ice cream mix containing 0.00% TGase
T_1: low fat ice cream mix containing 0.04% TGase
T_2: low fat ice cream mix containing 0.06% TGase
T_3: low fat ice cream mix containing 0.08% TGase

Figure (1): Processing steps for making of ice cream – treated with TGase.



Control (0.00%TGase) and three levels of TGase were used 0.04, 0.06 and 0.08 % of the mix. The levels, incubation time and inactivation temperature of TGase were used according to Ajinomoto application data. The mixes were cooled to 4-5°C and aged at that temperature overnight and vanilla was added. Mixes were frozen in the following day in a continuous freezer to 60-74% overrun. The product was packed and stored at -18°C for hardening before evaluation. The whole experiment was repeated three times.

Methods of analyses:

The total solids, fat, acidity were determined according to (AOAC, 1990), pH by using digital pH meter. The specific gravity of the mix was determined using the method described by Winton (1958), weight per gallon according to (Burke, 1947). Freezing point (FAO, 1977). The viscosity, consistency and flow index of the mix were carried out as described by Petersen *et al.*, (2000) using a Brookfield viscometer (Brookfied Engineering Laboratories. Inc., MA, USA), equipped with a SC 4-21 spingle running at 25 rpm. Measurements were made at temperature of 5°C in shear rate ranging from 9.3 to 93.

Melting down percent and overrun were determined according to Arbuckle (1986). The sensory evaluations were assessed by the staff of the Dairy Department., Suez Canal University using the following scale for flavour, (50 points), body and texture (40 points) and appearance (10 points) .

Table 2: Rheological properties of different low fat ice cream mixes in preliminary experiment.

Treatments	T_O	T_1	T_2	T_3	T_4
Aging period (Hours)					
			Plastic viscosity (m Pa s)		
Zero time	16.7	15.5	16.1	15.8	20.3
3	112.0	20.2	166.0	19.9	177.9
6	201.9	20.2	206.4	33.2	299.7
24	275.2	50.0	311.2	38.0	803.2
			Consistency index (m Pa s^n)		
Zero time	1.90	2.3	3.15	2.6	3.2
3	99.4	23.8	122.7	23.9	120.0
6	308.6	21.3	336.4	41.7	345.8
24	312.0	61.3	422.2	61.2	826.8
			Flow index (n)		
Zero time	0.98	1.00	0.97	1.00	0.90
3	0.61	0.93	0.65	0.93	0.57
6	0.55	0.89	0.57	0.86	0.50
24	0.51	0.89	0.50	0.86	0.44

T_0 : control without TGase, T_1: 0.08% TGase add to the whole mix, T_2:0.08% TGase add to the whole mix without gelatin, T_3: 0.08% TGase add to the whole mix without sugar, T_4: 0.08% TGase add to the whole mix without sugar and gelatin.

RESULT AND DISCUSSION

Preliminary experiment:

In ice cream mix, there are some ingredients that can interfere or affect the mode of action of TGase. Therefore, preliminary experiment was carried out to explore the ample substrate conditions for TGase treatments in ice cream manufacture. TGase was add at level of 0.08% to the whole mix, mix without sugar, mix without gelatin and mix without sugar and gelatin (milk ingredients only), then incubated at 40°C /2h and the processing procedure was completed. During aging the rheological properties of the mixes were followed (Table 2).

From this experiment, enhancing the rheological properties of the mix can be obtained by the action of TGase on the low fat ice cream mix before adding sugar and gelatin. These results are in accordance with those of Ohr (2003) who reported that, one of the biggest effect of TGase in frozen desserts, its change of the mouth feel properties, especially on low calorie or sugar free products.

Mix properties

As shown in (Table 3) slight decrease was observed in the titratable acidity of ice cream mixes associated with the addition of TGase as a cross-linking enzyme. The trend of changes in pH of ice cream mixes of all treatments was opposite to that for the titratable acidity. These result are in accordance with those of Singh, (1991); Ikura *et al.*, (1980) and Lorenzen and Schlimme (1998), who reported that TGase is capable of catalyzing the deamidation of glutamine residues whereby water is used as nucleophil and ammonia liberated. Also, O'Sullivan *et al.*, (2001) and Abou El Nour *et al.*, (2004), reported that the development of pH in yoghurt made from TGase treated milk was slower than control treatment. There was no noticeable changes was found in the specific gravity and weight per gallon of low fat ice cream mixes treated with TGase.

Table 3: Effect of using different levels of TGase on some properties of low fat ice cream mixes.

property / Treatments	T_O	T_1	T_2	T_3
Specific gravity	1.175	1.175	1.177	1.180
Weight / gallon (kg)	5.340	5.345	5.350	5.363
Acidity%	0.27	0.26	0.26	0.25
pH value	6.42	6.44	6.45	6.45
Freezing point °C	-3.0	-3.1	-3.1	-3.1

Table 4: Effect of using different levels of TGase on rheological properties of low fat ice cream mixes.

Treatments / Aging period (Hours)	T_0	T_1	T_2	T_3
		Plastic viscosity (m Pa s)		
Zero time	16.7	19.9	20	20.3
3	112	155.4	173.1	177.9
6	201.9	233.3	289.3	299.7
24	275.2	628.1	784.4	803.2
		Consistency index(m Pa s^n)		
Zero time	1.90	2.57	2.61	3.28
3	99.4	112	119	120
6	308.6	312.1	335	345.8
24	312	512.8	796	826.8
		Flow index (n)		
Zero time	0.98	0.95	0.96	0.90
3	0.61	0.59	0.58	0.57
6	0.55	0.53	0.54	0.50
24	0.51	0.47	0.44	0.44

Addition of TGase showed no effect on the freezing point of ice cream mixes of all treatments. Rheological parameters (viscosity, consistency index and flow behavior index) of ice cream mix samples during aging at 5°C for 24h are presented in (Table 4).

Differences can be noticed between control and treatments. The viscosity and consistency index of ice cream mixes increased with increasing the level of TGase added. This could be attributed to the considerable increase in the water binding capacity of ice cream mixes containing TGase (Lorenzen and Schlimme. (1998). The trend of changes in flow behaviour index of all treatments was opposite to that found for viscosity.

Ice cream properties

As shown in (Table 5), addition of TGase, at levels of 0.04% showed noticeable increase in the overrun than the control treatment. These results are in accordance with those of Lorenzen and Schlimme (1998), they reported that TGase reduced time of whipping and increased overrun of the whipped cream. Increasing the levels of TGase more than 0.04% showed slight increase in the overrun. The increase in the overrun of ice cream as a result of adding TGase could be attributed to the increase in the amount of incorporated air.

The specific gravity of resulting ice cream was inversely proportional to changes occurring in the overrun (Mahran *et al.*, 1984). So it was clearly indicated from (Table 5) that as the specific gravity and weight per gallon decreased, the overrun increased.

Table (5) shows that melting down decreased with adding TGase which can be attributed to the increase in ice cream mix viscosity and improve hydration ability and water bending of protein (Abou El Nour *et al.*, 2004).

Organoleptic properties:

From (Table 6), it could noticed that adding TGase to low fat ice cream mixes had no effect on appearance or flavour of the resultant ice cream. Otherwise, the TGase treated samples gained a higher score points for body and texture.

Table 5: Effect of using different levels of TGase on some properties of the resultant low fat ice cream.

Treatments / property	T_0	T_1	T_2	T_3
Overrun (%)	60	72	72	74
Specific gravity	0.732	0.684	0.684	0.674
Weight / gallon (kg)	3.336	3.109	3.11	3.063
Melt- down				
% after 30 min	18	8	8	7
% after 60 min	53	29	31	29
% after 90 min	100	100	100	95

Table 6: Organoleptic properties of low fat ice cream containing different levels of TGase

property	Treatments	T_O	T_1	T_2	T_3
Flavour	**(40)**	36	38	38	38
Body & texture	**(50)**	44	48	48	48
Appearance	**(10)**	9	9	9	9
Total	**(100)**	89	95	95	95

Most of taste panels mentioned that ice cream containing TGase revealed softer body and smoother texture and higher melting resistance than the control. These results are in accordance with those of Ohr, (2003), who concluded TGase treatments changed the mouthfeel properties in frozen desserts. Increasing the level of added TGase more than 0.04% did not affect the score points of body and texture. From the forgoing, results indicate that TGase could be used successfully as cross-linking enzyme at the level of 0.04% for the production of a good quality low fat ice cream.

REFERENCE

Abou El-Nour, A. M.; El-Kholy, A. M. and Abd El-Salam, M. H. (2004). Rheological properties of cows milk yoghurt treated by transglutaminase (TGASE). Egyptian J. Dairy Sci., 32: 73-86.

AOAC (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists. Food Composition, Additive, Natural Contaminant. Vol. 2. Helrich, k. (Editor). ISBN: 0-935584-42-0.

Arbuckle, W. S. (1986). Ice Cream. 4th ed The AVI Publishing Company, Inc., Westport, Connecticut.

Burke, A. D. (1947). Practical Ice Cream Making. The Olsen Publishing Co., Milwauke, Wis., USA.

Chang, J., Mrshall. R. and Heymann, h. (1995). Casein micelles partially hydrolyzed by chymosin to modify texture of low fat ice cream. J. Dairy. Sci. 78:2617-2623.

Dalgleish, D. G.(1979). Proteolysis and aggregation of casein micelles treated with immobilized or soluble chymosin. J. Dairy Res. 46:653.

Dickinson, E. (1997). Enzymic crosslinking as a tool for food colloid rheology control and interfacial stabilization.Trends Food Sci. Technol. 8:334.

Dickinson, E., and Yamamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels crosslinked with transglutaminase. J. Agric. Food Chem. 44:1371.

Faergemand, M., Sorensen, M. V., Jorgensen, U., Budolfsen, G., and Qvist, K. B. (1999). Transglutaminase: Effect on Instrumental and sensory texture of set style yoghurt. Milchwissenschaft, 54:563.

FAO LAB (1977). Laboratory Mannual. FAO Regional Dairy Development and Training Center for the Near East.

Guthy, K. and Novak. G. (1977). Observation on the primary phase of milk coagulation by rennet under standardized conditions. J. Dairy Res. 44:363.

Ikura, K., Komitani, T., Yoshikawa, M., Sasaki, R., & Chiba, H. (1980). Crosslinking of casein components by transglutaminase. Agric. Biol. Chem. 44:1567.

Kalab, (1990). Micro particulate protein in foods.J. Am. Coll. Nutr. 9:374.

Lorenzen, P. Chr. (2000a). Renneting properties of transglutaminase treated milk. Milchwissenschaft,55:433.

Lorenzen, P. Chr. (2000b). Technofunctional properties of transglutaminase-treated milk proteins. Milchwissenschsft, 55:667.

Lorenzen, P. Chr., and Schlimme, E. (1998). Properties and potential fields of application of transglutaminase preparations in dairying . Bulletin of the IDF Bull. No. 332:47.

Mahran, G. A.; El-Ghandour, M.A.; El-Bagoury, E.H. and Sayed, A.F.(1984). Effect of skim milk powder storage on ice cream quality. Egyptian J. Dairy Sci., 12(2) : 267-273.

Nio, N.; Motoki, M. and Takinami, K. (1985). Gelation of casein and soybean globulins by T G ase. Agric.,Biol. Chem. 47: 2283-2286.

Nio, N.; Motoki, M. and Takinami, K. (1986). Gelation mechanisms of protein solutions by TGase. Agric.,Biol. Chem. 48: 851-855.

Ohr, L. M. (2003). Enzyme builds links to creative products. Prepared foods, new poducts conference. Four Seasons Resort Palm Beach, Florida.

O'Sulliven,M.; Lorenzen, P.; O'Connell,J.; Kelly,A.; Schlimme, E.; and Fox, P. (2001). Short communication : Influence of transglutaminase on the heat stability of milk. J. Dairy Sci. 84: 1331-1334.

Petersen, B.L.; Dave, R. I.; McMahon, D.j; Oberg, C.J. and Broadbent, J.R. (2000). Influence of capsular and ropy exopolysaccharide producing *Streptococcus thermophilus* on Mozzarella cheese and cheese whey. J. Dairy Sci. 83:1952.

Prentice, J. (1972). Rheology and texture of dairy products. J.texture stud. 3:415.

Sakamoto, H., Kumizawa, Y., and Motoki, M. (1994). Strength of protein gels prepared with microbial transglutaminase as related to reaction conditions. J. Food Sci. 59:866.

Singer, N. and Dunn, J.(1990). Protein micro particulation :The principle and the process. J. Am. Coll. Nutr. 9:39.

Singh, H. (1991). Modification of food proteins by covalent crosslinking. Trends Food Sci. Technol. 2: 196-200.

Traore, F. and Meunier, J. (1991). Cross-linking of caseins by human placental factor.J. of Agric.,and Food Chemistry. 39:1892-1896.

Winton, A. L. (1958). Analysis of Foods .3rd Printing pp. 6, John Wiley and Sons Inc., New York.

استخدام انزيم الترانس جلوتامينيز فى صناعة الآيس كريم المنخفض الدهن

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يوجد انزيم الترانس جلوتامينـاز Transglutaminase على نطـاق واسع فـى كثير مـن الكائنـات الحية مثل الانسـان والحيوان والنبات والكائنات الدقيقة . ولهذا الانزيم القدرة على ربط البروتينات ببعضها أو ربط البروتينات بالاحماض الامينية وأيضا عمل تحلل للاميد جلوتامين وكل هذا يؤدى الى تحسين الخواص الوظيفية للبروتين من ناحية القيمة الغذائية والقوام والطعم والمقدرة الحفظية . ويستخدم هذا الانزيم فى كثير من الصناعات الغذائية ومنها بعض منتجات الالبان مثل الجبن الطرية والالبان المتخمرة .

وقد تم استخدامه فى هذه الدراسة فى صناعة الأيس كريم بعد عمل تجربة مبدئية باضافة الانزيم بتركيز ٠,٨ جم/كجم مخلوط الى مخلوط أيس كريم (مخلوط بدون سكر ، مخلوط بدون جيلاتين ، ومخلوط بدون سكر وجيلاتين مع وجود كنترول بدون اضافة انزيم). وبتقدير الخواص الريولوجية لهذه المعاملات أثناء التعتيق وجد أن أنسب ظروف لعمل الانزيم هى اضافته الى المكونات اللبنية (مخلوط بدون سكر وجيلاتين) وبعد تمام عمله يضاف السكر والجيلاتين ثم تجرى البسترة لتثبيط الانزيم. تم عمل ٤ معاملات بعد ذلك استخدم فيها الانزيم بتركيزات صفر ، ٠,٠٤ ، ٠,٠٦،٠,٠٨ % من المخلوط وقد وجد أن المخـاليط المعاملـة بـالانزيم كانـت ذات حموضـة أقل وتحسنت اللزوجـة والصلابة عن الكنترول .

وبالنسبة للآيس كريم الناتج وجد أن المخاليط المعاملة بالانزيم كانت ذات ريع أعلى وأظهرت التقديرات الحسية حصولها على تقديرات أعلى بالنسبة للقوام والتركيب عن الكنترول الخالى من الانزيم ولم تكن لزيادة نسبة الانزيم عن ٠,٠٤% تأثير واضح على هذه الخواص .