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Effect of Transeglutaminase enzyme on drinking yogurt properties

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Abstract

This study was carried out to examine the addition effect of microbial Transglutaminase (T. G) on the chemical and physical properties of drinking yogurt. Transglutaminase Enzyme was added to drinking yogurt in different concentration (0.050, 0.075, 0.100 and 0.125) g/kg with activity 100 unit /g and at different milk dilution (1:1,1:2,1:3and1:4) milk:water after pasteurization. The fermentation was carried with (*Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus of ratio* (1:1). The results reveled to that TG addition did not cause significant changes on chemical properties of drinking yogurt, while the physical properties were improved by T.G compared with the control treatment, pretreatment of milk with enzyme increased the viscosity and prevented whey separation. The best results in terms of whey separation and rheological properties were obtained when treated milk with 0.125 g./Kg in dilution 1: 2 milk to water. The results indicated that T. G may be successfully used for improving the functional properties of drinking yogurt. The results show that the addition of T.G in all concentration did not appeared any un favourable effect on the sensory properties of the final products.

Key words: Transglutaminase, drinking yogurt, whey separation, rheological properties.

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تأثير أضافة أنزيم الـ Transglutaminase في خصائص لبن الشنينة

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير أنزيم Transglutaminase (TG) المايكروبي في الصفات الكيميائية والفيزيائية للبن الشنينة، أذ أضيف الأنزيم للبن بتراكيز مختلفة (0.050 و0.075 و0.100 و 0.125) غم/ كغم وبفعالية أنزيمية مقدارها 100 وحدة/ غم لتخافيف مختلفة من الحليب (1:1 و 1:2 و 1:3 و 1:4) ماء: حليب بعد البسترة، وأجري التخمر بأستعمال بكتريا *Streptococcus theromophilus* و *Lactobacillus* subsp. *bulgaricus* *delbrueckii* (1:1)، وأشارت النتائج أن أضافة الأنزيم لم تسبب اي تأثير في الصفات الكيميائية للبن الشنينة بينما حسنت الصفات الفيزيائية للمنتج مقارنة مع معاملة السيطرة، أذ أزادت اللزوجة ومنع أنفصال الشرش بالمعاملة المسبقة للحليب بالأنزيم وحصل على أفضل النتائج من حيث أنفصال الشرش والصفات الريولوجية عند معاملة الحليب ب0.125 غم/ كغم بتخفيف 2:1 ماء: حليب، أشارت النتائج أمكانية أستخدام الأنزيم بنجاح لتحسين الصفات الوظيفية للبن الشنينة. كما لوحظ أن أضافة الأنزيم وبكل تراكيزه لم يظهر أي تأثير غير مرغوب في الصفات الحسية للمنتج النهائي.

الكلمات المفتاحية: ترانس كلوتاميناز، لبن الشنينة، انفصال الشرش، الصفات الريولوجية.

Introduction

Transglutaminase (TGase; EC 2. 3. 2. 13) catalyses covalent intermolecular protein cross-linking through an acyl-transfer reaction, between the γ- carboxyamide group of a peptide- bound glutamine residue (acyl donor) and the primary amino group of an amine (acyl acceptor). The application of TGase in various types of dairy products has been reviewed (9; 15). This enzymatic modification of protein structure appears a suitable tool for improving the functional properties of food proteins. Both caseins and heat denaturated whey protein are good substrates for transglutaminase (12; 15; 18).

Set yogurt is formed in retail pots as lactic acid bacteria ferment lactose into lactic acid givin continuous gel structure in the consumer container. In stirred yogurt, the acid gel formed during incubation in large fermentation tanks is disrupted by agitation (stirring),and the stirred product is usually pumped through a screen which gives the product a smooth and viscous texture(19). The total solids content of milk can be increased by concentration processes, such as, evaporation under vacuum, and membrane processing (i. e., reverse osmosis and ultrafiltration).Stabilizers, such as, pectin or gelatin, are often added to the milk base to enhance or maintain the appropriate yogurt properties including texture, mouthfeel, appearance,viscosity/consistency and to the prevention of whey separation (wheying- off) (19). Pretreatment of milk with enzyme increased the viscosity and prevented serum separation(17). The dietary intake is one of the important factors contributing significantly to the obesity epidemic. The best way to obtain a calorie balance is to monitor dietary intake and replace the high calories foods with nutrient-dense foods and beverages relatively low in calories(20).The drinking yogurt physical attributes of yogurt such as the lack of visual whey separation and perceived viscosity are effect of sensory consumer acceptance of drinking yogurt thus the aim of this study was curried out to conduct the influence of transglutaminase concentration on viscosity & and visual whey separation during storge for 14 days at (6± 1c) with low calories on final products.

Material and Methods

- Bulk milk: was obtained from the plant collage of Agriculture University of Baghdad.
- Enzyme: Microbial Transglutaminase was preparation from Ajinomoto food (EUROPE SAS, Hamburg, Branch, Germany.) The enzymatic product MTGase with a declared enzymatic activity of about 100 unit/g. (representative value).
- Starter: mixed culture of lactic acid bacteria(YO- mix 601,495 LYO) consisting of (*Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 1:1 provided by (Danisco, France).
- Salt (NaCl) for food used (application).
- Water: distill water pH7.0 - 7.5.

Preparation of drinking yogurt:

In the first step of preparation was the milk samples were diluted by distill water with ratio 1:1, 1:2, 1:3 and 1:4 milk: water treatments (A, B, C, D) then 2% salt was add and the mixture was homogenization at15-20mpa at 55- 65 °C and after that all samples were pasteurized at 80-85 °C for 30 min. and the final product cooled slowley to 45 °C (incubation temperature) later add the enzyme Transglutaminase with 4 concentration0.05,0.075,0.1 and 0.125g/kg with activity 100 unit/ g to all dilution samples with slow mixed period of 15-30min. then addition of starter culture 2- 3%,The samples were afterwards poured in to 500 ml plastic containers and incubated at 43°C for 4-6 hr.

The acidity decrease from 6.7≤ 4.6 during the entire incubation period and the fermentation was completed when the pH was reached to 4.4 for control samples, the all treatment were then cooled in the refrigerated cold store at 4°C for 14 days to reduce further acid development .

Control samples was produced without transglutaminase.

Note: A refered to treatment 1:1, B refered to treatment 1:2, C refered to treatment 1:3, D refered to treatmen1:4.

Determination of pH and acidity:

pH was measured according to (11) by immersing the sensor of digital pH- meter on the drinking yogurt and the total acidity was

determined by titration 10 ml of the samples with 0.1 *N* of NaOH, phenolphthalein was used as indicator.

Chemical test of milk:

1- Determination of total solid : follow with(1).
2- Determination of total fat : follow with method of Babkok as to be mentioned in (14).
3- Determination of total protein: follow with method as to be mentioned in (2).
4- Determination of wheying off : To determine wheying off for all the samples we used cylinder volume 100 ml after 24 hr storage for all the treatment at 4C, the syneresis was estimated as percentage of the released whey over the initial gel size and was average of three determination:

%Wheying off= volume of supernatant / volume of drinking yogurt sample*100

5- Determination of freeze point and Added water to milk:To determine freeze point and Added water to milk by means of MILKANA (milk analyzer) producted by mayasan instrument, Istanbul– Turkey.

Rheological Measurements:

Rheological properties of the yogurt samples after 24 h of storage at 4°C were determined in duplicate by means of Ostwald viscometer. All measurements were carried out on 50 g of sample which reviously prepared by gently stirring in identical conditions.
The apparent viscosity was calculated as:

Viscosity of known liquid/ Viscosity of unknown liquid= Density of known liquid* time of it‘s dropped/ Density of unknown liquid* time of it‘s dropped.

The determination of density achieved by density vial (volume 50 ml) using duplicate samples in all treatmeant. (1).

Statistical Analysis:

The Statistical Analysis System- (16) was used to effect of different factors in study parameters. Least significant difference -LSD test was used to significant compare between means in this study.

Results and discussion:

Chemical composition of milk which is used in all treatment

Before incubation and fermentation with lactic acid bacteria, the samples treated with T. G different ratio ranging from0.0 50 to 0.125 g./Kg. The influence of T. G treatment on the quality of the end product was determination the acidification and water holding capacity and rheological capacity and rheological properties of the end products. Composition of fresh cow's and all treatment are presented in (Table, 1).

Table (1): The composition of fresh cow's milk and all treatment .

Composition	Treatment					LSD value
	Row milk	A	B	C	D	
Fat	2.80	1.45	0.95	0.65	0.51	0.783 *
Solid non-fat	7.29	4.01	2.74	2.11	1.78	1.794 *
Protein	2.76	1.56	1.10	0.88	0.76	0.844 *
Total solid	10.09	5.46	3.69	2.76	2.29	2.091 *
Add water to milk	13.80	57.70	76.40	86.30	91.50	11.39 *
Freeze point	-0.47	-0.23	-0.13	-0.07	-0.04	-0.08*
* (P<0.05).						

From the resulted in(table, 1) different value could saw of fat, Solid non fat, Protein, Total solid, Added water to milk and freezing point in composition of all treatment.

Evolution of yogurt acidity and pH:

In order to study the effect of the milk treatment with T.G on the lactic acid bacteria metabolism, the development of pH was continuous during the entire fermentation period for all tested samples. The lactic acid fermentation started at once after the addition of starter culture the pH of the T. G treated samples was slightly higher in comparison with control samples(Table, 2).

Milk samples was fermented at 40- 45°C, the fermentation temperature is very important for obtaining high quality final products higher temperatures would favor the growth of lactobacilli, and the

development of higher acidity and sour yogurts with poor flavor, whereas lower temperatures would favor the growth of streptococci and the achievement of flavored yogurt well with low acidity and no specific taste(5).

Table(2): PH of all samples after fermentation.

Concentration (g./kg)	Treatment				LSD
	A	B	C	D	
Control	4.10	4.40	4.40	4.40	NS
0.050	4.30	4.55	4.45	4.30	NS
0.075	4.40	4.50	4.50	4.40	NS
0.100	4.50	4.60	4.60	4.50	NS
0.125	4.55	4.65	4.60	4.70	NS
LSD	NS	NS	NS	NS	---
NS: Non-significant.					

The pH before addition culture for all the samples is between 6.5-6.7In agreement with (13), our results propose that T.G interferes with the growth of starter culture yogurt ,leading to longer fermentation .After 24 hr of storage at 4°C the high pH was available in control cases for all treatment (Table. 2), and the low pH is appearance in treatment B that once treated with 0.125g/ Kg. The acidification procedure continues at a lower development during drinking yogurt samples storage. The pH evolution at 4C depended on T.G concentration. We can see differences of pH units in case of the milk samples treated with enzyme for 0.050- 0.125 g./Kg .

The best treatment is appearance in treatment B it was treated with o.125g of T.G/Kg, treatment C and D it has high pH unit but it's fail in sensory evalution, the results agree with (3). The addition of T.G concentration induced a lower acidification of the yogurt sample resulting in the final products with high pH.

Whey separation:

The samples syneresis is a measure of the quantity of whey separated from the the most important factors effecting on consumers' acceptance (8). The syneresis of drinking yogurt samples produced from fresh cow's milk treated with different concentration of transglutaminase at 45 °C was determined by the volume of separated whey by using cylinders and it was a present in (Table, 3).The best treatment is appearance in treatment AandB which it was treated with 0.125g enzyme of T.G per kg .

Table (3): Whey separation for drinking yogurt treatment after fermentation at 4 °C for 24hr.

Concentration (g./kg)	Treatment				LSD value
	A	B	C	D	
Control	25	40	66	77	8.34 *
0.050	10	23	58	54	6.91 *
0.075	5	10	47	40	6.38 *
0.100	3	6	28	34	7.64 *
0.125	0	0	20	29	8.03 *
LSD value	6.24 *	8.63 *	8.96 *	10.75 *	---
* ($P<0.05$).					

The intensity of whey separation is higher in the cases of drinking yogurt which deals with low concentration of enzyme composed with cases that deals with high concentration of enzyme. The ratio of whey separation influenced with ratio of dilution of milk and concentration of addition of enzyme, the best treatment is B which is deals with 0.125 g/kg it has 0% syneresis and it was succeed in sensory test, However treatment A which is deals with 0.125 g/kg it has 0% wheying off but it was refuse in sensory test because of higher

viscosity. We can see also decrease of ratio of wheying off in all concentration of enzyme composed to control Samples.

Resulted agree with (17) suggested that pretreatment of milk with TGase was prevented whey separation and TGase treatment did not show any unfavorable effect on the sensory properties of the final product ,In agreement with (10) and (13), our results indicate that the MTGase treatment of milk allowed improving the water holding capacity of the yogurt. The acidification process of the MTGase treated milk or casein micelles allows obtaining gels with increased firmness, lower permeability, finer protein networks and improved whey drainage (6; 7; 15; 18).

Rheological Measurements:

The viscosity is an important parameter that can be successfully used for comparing the quality of the yogurt samples prepared in different conditions(3; 4) (Table, 4) are presented the apparent viscosity for the drinking yogurt prepared using different enzyme concentrations, the TGase treated samples have improved rheological properties compared to the control samples(3).

properties of yogurt with low fat content.also resulted agree with(17) our result that treatment of milk with enzyme increased the viscosity dramatically.

The viscosity improvement for all treatment was not only directly related to the enzyme because of concentration of enzyme addition but also for the ratio of dilution of samples, we can saw the influence of concentration of enzyme addition in(Table, 4).

Table (4): Viscosity for all treatments drinking yogurt.

Concentration (g./kg)	Treatment				LSD value
	A	B	C	D	
Control	199.63	110.96	108.26	90.47	23.56 *
0.050	354.13	130.52	112.44	110.26	38.14 *
0.075	377.96	143.14	121.12	123.64	31.77 *
0.100	436.73	168.89	130.37	129.87	48.65 *
0.125	611.43	171.68	137.22	131.39	61.49 *
LSD value	39.44 *	21.35 *	19.34 *	26.82 *	---
* ($P<0.05$).					

Analysing the results in (Table, 4), one can see that for all treatment viscosity, the apparent viscosity of the drinking yogurt increases with increased enzyme concentration .Higher concentration of enzyme allowed obtaining yogurts with slightly increased rheological properties. There are not significant differences with in samples C and D because the active side of enzyme was busy with substrate.

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