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Comparative Study on the Use of Transglutaminase Enzyme in Making Labneh from Different Kinds of Milk

Reham k. Elmenawy[1*]; Y. I. Abd-Elkader[1]; M. M. Elmetwally[1] and Ola M. A. K. Shalabi[2]

[1]Animal production research institute, Agricultural Research Center, Dokki, Egypt
[2]Dairy Department, Faculty of Agriculture, Mansoura University

ABSTRACT

Present research aims to make labneh from different kinds of milk (buffalo , cow and goat)(BM,CM, and GM, respectively), and to compare the effect of the addition of transglutaminase (TG) on the examined treatments. Results show that the addition of TG did not considerably affect the development of acidity during the fermentation process, while the increase of acidity in B and C was faster than in G. On the other hand, an increase in the yield of labneh made with added enzyme, compared with samples without TG from was 43.16 to 50.55% ,38.78 to 48.16 % and 22.50 to 37.20%, in the same order. In addition, an improvement in the gel strength and decrease in the synersis were observed. The chemical composition and sensory properties was determined during 21 days showed an increase in the total solid (TS),total protein(TP) and total volatile fatty acid (TVFA) in the samples treated with TG, while there was no noticeable effect on acidity and PH. The addition of TG in all of the examined samples led to improvement in the sensory value and accessed to creamy body, particularly in the labneh made from cow milk with added TG.

Keywords: Transglutaminase, Gel strength, Synersis,Total volatile fatty acid .

INTRODUCTION

Labneh or concentrated yogurt is fermented milk in the Middle East having an acidic flavor and milky white color. Labneh usually characterized with softness, smoothness, and spreadability. It made by using strains of *lactobacillus delbrueckii subsp bulgaricus and streptococcus thermophilus*(Shamsia 2012).

Transglutaminases(EC2.3.2.123) (TG)are enzymes that stimulate forming an isopeptide bond between γ-carboxamide groups (-(C=O)NH2) of glutamine residue side chains and the ε-amino groups (-NH2) of lysine residue side chains with subsequent release of ammonia (NH3) naturally. Lysine and glutamine residues must be bound to a peptide or a protein to happen this cross-linking (between separate molecules) or intramolecular (within the same molecule) reaction. Bonds formed by transglutaminase show high resistance to proteolytic degradation (proteolysis).(Dejong and Koppelman 2002 and Griffin *et al* .,Truong *et al* ., 2004). Recently, The interest with improving the protein properties of food products has received great attention . Transglutaminase is one of the most important methods to modify the properties of protein in food and is one of the enzymes that stimulates an acyl transfer reaction in the existence of Ca +2(Folk1983).This reaction between Ɛ-carboxaminde group of peptide - bound glutamine (acyl donors) and primary amino groups in avariety of amino compunds (acyl acceptor)as lysine which results in curse (Ɛ-(Y-glutamyl)lysine(Aeschlimann and Paulsson1994; Soawes*et al*.,2004; Truong *et al* ., 2004: El nawawy *etal*.,2009).

Casein is an excellent substrate formicrobial transglutaminases(MTG) than whey protein , although the denaturation why protein makes their amino acid available for TGM (Abou mahmouund andSavello1990; Traore and Meunier1992).

Treatment of dairy products with microbial transglutaminases(MTG) led to improving the functional properties , flavour , viscosity, solubility, serum holding capacity , gel firmness and less allergic proteins (Ozrenk,2006;Lee andChin 2010;Fernando and Susan 2012).

The best result was obtained from addition microbial transglutaminases (MTG) on yoghurt milk was 0.04% for 120 min setting at 40^{O}C resulting in enhancing the functional properties of yogurt (Aprodu*etal*.,2012).

MATERIALS AND METHODS

Fresh raw goat, cow, and buffalo milk was obtained from El-Serw Animal Production Research station, Animal Production Research Institute, Agriculture Research center,Egypt. Starter of commercial classic yogurt containing *streptococcus thermophiles* and *lactobacillus delbruckii subsp bulgricus* (1:1) was obtained from Chr. Hansen,s lab A/S Copenhangen, Denmark). Transglutaminase (MTGase) Activa TG-1 was bought from Ajinomoto (Incteanec, Nj,U.S.A). the enzymatic product is consisted of 99% maltodextrin and MTGase with adeclared enzymatic activity of about 100 UE /g.

The chemical composition of different used milk was presented in Table(1)

Table 1. Chemical analyses of different milk

Typeof milk	Ph	Acidity	Fat	Protein	Total solid
Goat	6.26	0.19	4.0	4.2	13.11
Cow	6.44	0.18	3.1	3.8	11.43
Buffalo	6.50	0.17	8.0	5.0	15.33

* **Corresponding author.**
E-mail address: gananabil2013@yahoo.com
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For the preparation of Labenh, milk samples was heated to 90 ^{O}C /10min, followed by cooling to 40^{O}C. TG enzyme used in experiment was 0.04g MTGase/100g milk. Inoculation was carried out for 120 min., and the cross-linking reaction was stopped by thermally treating at 90^{o}c/2 min followed by cooling to 40^{o}c. Inoculation with 2% yogurt starter was carried out until complete coagulation. Then it was put into cheese bags which was hanged in the refrigerator room at 4^{o}c over night (12h) to allow why drainage.

Total solids (T.S %), titratable acidity (T.A %) and fat contents of the different labneh samples were determined following the methods described in Ling (1963). Protein content was determined by the kjeldhl (AOAC 2000). Total volatile free fatty acids (TVFFA) was estimated as ml. 0.1N NaOH/10gm, and labneh samples was measured by using the method of Kosikowski (1982).The determination of pH in ten grams of the labenh water and was mixed to measure pH and was diluted with 70 ml distilled. The pH meter (Mettles, Toledo MP220, Switzerland) was calibrated with standard buffers at PH 4 and 7 (BDHL laboratory, England) prior to measuring the pH of the mixture.

The gel strength was measured at 4-6 ^{O}C. by penetration measurements(Stevens-L.F.R.A Texture Analysiser, CNS Farnell, Borehamwood ,UK) the instrument was adjusted to the following conditions :cylindrical probe area 5.07 cm^2 ,penetration speed 1.0 mm/ s; penetration distance , 20 mm into surface .The determination of Gel strength was done in triplicate and was showed as N/cm^2 of probe area.

Synersis was estimated by the centrifugation procedure. Approximately 20 g of yogurt was transmitted to a 50 mL glass tube and was centrifuged at 3500 rpm for 15 min at 20 °C. It was measured as the released percentage whey over the initial gel weight and as an average of three determinations:

$$\textbf{Syneresis \%} = \frac{\textbf{weight of supernatant}}{\textbf{weight of yogurt}} \times \mathbf{100.}$$

Yield was calculated as follows:

$$\textbf{Yield\%} = \frac{\textbf{weight } \boldsymbol{of labenh}}{\textbf{weight of milk used to make labenh}} \times \mathbf{100}$$

Statistical analysis of the obtained data were carried out as mean ± standard deviation of three replicates. Except for the data of texture and sensory evaluation that were analyzed using one-way ANOVA and the other data were statistically analyzed by SPSS statistics 22.0 using two-way ANOVA to evaluate the significant differences between the means of samples and storage period. The means of results were compared by the Tukey test at a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Results indicated in Figure (1) show the effect addition of MTG on the coagulation time of labneh. It is clear that the use of milk with MTG resulted in reduction in the fermentation time in all samples, which came in agreement with Abdulqadr*et al*,(2014). MTGase with different doses significantly increase yogurt pH value, compared to the untreated yogurt. The use of MTG accelerates the gel-forming product, especially in goat milk that has long fermentation with fragile gel, which agrees with Aprodu*etal.*,*(*2012), while disagrees with the results in samples in the absence of the enzyme obtained by Lorenzen *etal.*, 2002; and Neve *etal.*, (2001).

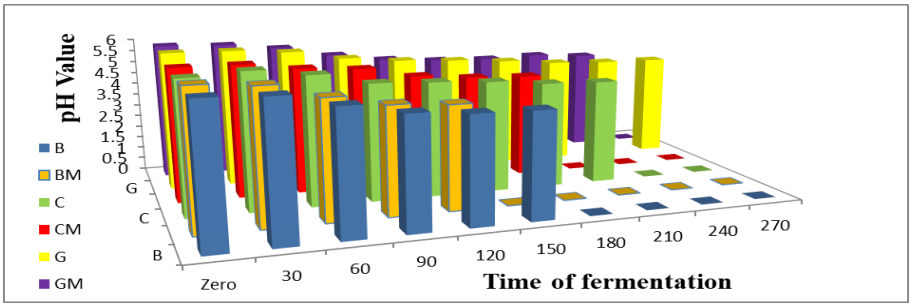

Figure1. pH value during fermentation time of labneh with and without transglutaminase.

Data illustrated in Figures (2 and 3) appear that yogurt before putting into cheese bags show that the use of TGM resulted in significant increase in gel strength, compared with yoghurt without enzyme were 56.16,45.72 and 47.76 %, respectively in buffalo, cow and goat, respectively, and on the contrary a significant decrease in why synersis was observed to14.89,18.18 and 24.24%, respectively, which came in agreement with Lorenzen *etal.*,(2002)..

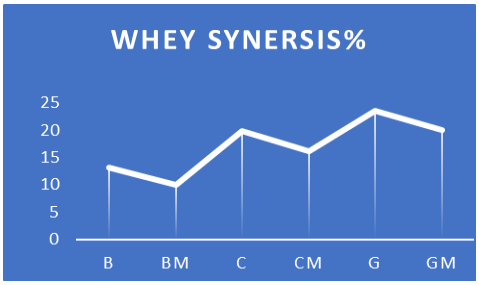

Figure (2)

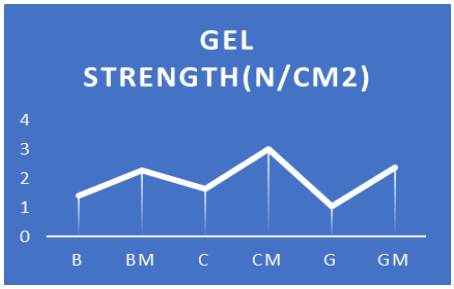

Figure (3)

Figure 2 and 3. Determination of gel strength and whey synersis of yoghurt before add in cheese cloth.

Data shown in Table (2) show that the yield of all samples with TMG increase in labeneh from buffalo, cow and goat were 14.62,16.06 and 25.50%, respectively, which might be probably attributed to what is known about MTG increasing the water hold capacity and less synersis (Motoki and Seguro 1998;Lorenzen *et al.*,2002).

Table 2. The yield of labneh.

Treatments	Yield%
B	43.16± 1.00 [c]
BM	50.55±1.00 [a]
C	38.78±1.00 [d]
CM	46.20±0.01 [b]
G	22.50±1.00 [f]
GM	30.20±0.10 [e]
Means	38.39 ±9.89

Duplicate labneh samples were analyzed in triplicate : means in the same row bearing a common superscript letter do not differ significantly (P>0.05) labneh were B,C and G labneh from milk without enzyme. BM, CM and GM labneh from milk with enzyme.

Total protein content in labenh and whey illustrated in Figure(4) show that the addition of the transglutaminase enzyme resulted in an increase in the concentration of protein in curd over the amount of protein in whey. Transglutaminase catalyses an acyl transmit reaction between γ-carboxyamide groups of peptide-bound glutamine residues (acyl donor) and the primary amino groups in many amine compounds (acyl acceptor) that includs peptide-bound ε-amino groups of lysine residues. Because of cross-linking of peptide-bound glutamine and lysine residues ε-(γ-glutamyl), lysine iso-peptide bonds and high-molecular weight polymers are composed. The nonexistence of amine substrates, transglutaminase is able to catalyze the deamidation and amine incorporation of glutamine residues (Soawes*et al*., 2004).

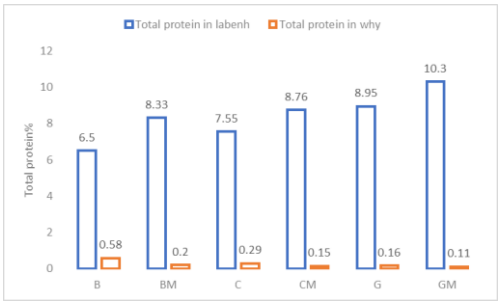

Figure 4. Comparison between the total protein in labenh and whey that put out during the manufacture of labneh

Regarding the chemical composition of labneh indicated in Table (3), it is clear that the labneh from goat milk gained the lowest score in the pH , on the contrary, it was the highest score in acidity. The addition of TGM resulted in slight increase in acidity and slight decrease in pH values due to the reduction of the proteolytic activity by usingTG ,(Dinkci 2012), while yogurt samples with TGM was increased as compared to without enzyme. The addition of enzyme led to increased (p<0.05)in total solid and TVFA with the continuous of increasing through storage time. Reaveled that addition of TGM led to increase dry matter of yoghurt. While no change in chemical composition in labneh with enzyme (Bucert *et al.*,2010; Lorenzen *et al.* ,2002; Aloglu and Oner 2013;Abdulqadr *et al.*,2014).

Table 3. Chemical analyses of labenh during 21 days.

	Treatment	zero	7	14	21	means
pH	B	3.82±0.01	3.69±0.01	3.66±0.06	3.63±0.10	3.70±0.90 [cd]
	BM	3.88±0.01	3.91±0.01	3.85±0.10	3.80±0.01	3.86±0.72 [a]
	C	3.87±0.10	3.85±0.01	3.80±0.01	3.73±0.01	3.81±0.51 [b]
	CM	3.85±0.01	3.61±0.01	3.58±0.10	3.50±0.01	3.64±0.15 [cd]
	G	3.68±0.01	3.66±0.10	3.63±0.01	3.55±0.10	3.63±0.90 [c]
	GM	3.75±0.10	3.82±0.01	3.78±0.10	3.60±0.10	3.71±0.12 [bc]
Means		3.80±0.12 [a]	3.75±0.12 [a]	3.72±0.12 [a]	3.64±0.12 [b]	
Acidity%	B	1.80±0.10	1.93±0.01	1.98±0.01	2.05±0.10	2.06±0.11 [b]
	BM	1.56±0.01	1.75±0.01	1.90±0.10	2.18±0.01	1.85±0.24 [c]
	C	1.55±0.01	1.60±0.10	1.75±0.05	1.88±0.01	1.70±0.14 [d]
	CM	1.60±0.01	1.68±0.10	1.70±0.01	1.80±0.10	1.69±0.20[d]
	G	1.88±0.10	2.02±0.01	2.10±0.10	2.22±0.10	2.06±0.15 [a]
	GM	1.79±0.01	1.85±0.10	2.00±0.01	2.08±0.01	1.93±0.29 [b]
Means		1.69±0.20 [d]	1.80±0.20 [c]	1.90±0.16 [b]	2.04±0.17 [a]	
Ts%	B	27.88±0.01	28.76±0.06	29.33±0.10	31.60±0.10	29.41±1.67 [c]
	BM	35.75±0.10	36.09±0.01	37.00±1.00	37.90±0.10	36.69±0.97 [a]
	C	23.80±1.00	24.50±0.10	25.06±0.01	26.12±0.01	24.87±0.98 [d]
	CM	30.56±0.10	31.80±0.10	31.96±0.01	32.32±0.57	31.58±0.67 [b]
	G	19.88±1.00	20.12±0.00	21.00±0.22	21.14±0.04	20.54±0.72 [e]
	GM	28.90±0.49	29.12±0.00	29.25±0.05	29.33±0.33	29.23±0.27 [c]
Means		27.85±0.20 [d]	28.39±0.23 [c]	28.93±0.20 [b]	29.69±0.36 [a]	
TVFA%	B	1.64±0.04	1.80±0.10	1.95±0.05	2.00±0.10	1.85±0.16 [d]
	BM	1.80±0.10	1.98±0.10	2.20±0.10	2.30±0.10	2.07±0.22 [c]
	C	1.50±0.10	1.66±0.10	1.74±0.04	2.00±0.10	1.73±0.20 [e]
	CM	1.60±0.10	1.95±0.05	2.12±0.02	2.35±0.05	2.01±0.29 [c]
	G	3.00±0.10	3.40±0.10	3.53±0.01	3.60±0.10	3.38±0.25 [b]
	GM	3.30±0.10	3.55±0.05	3.70±0.05	3.79±0.01	3.59±0.20 [a]
Means		2.14±0.75 [d]	2.39±0.80 [c]	2.54±0.86 [b]	2.67±0.76 [a]	

As with the organoleptic properties of the examined labneh, it is clear from the results in Table (4) that labneh from treatment milk with TGM gained high score of organoleptic properties which cross linking of milk protein by TGM improved the sensory properties as flavour, texture and color (El nawawy *etal.*, 2009;Aprodu*etal* ., 2012;Dinkci 2012) .Especially texture of labneh from goat milk (GM).The goats gel is weaker than cow's milk gel (Ardelean *etal.*,2013) but the use of TGM was improved of the total sensory properties.

Table 4. Effect of enzyme addition on the organoleptic properties on labneh from different milk .

Treatments	Flavour(50)	Texture(35)	Color(15)	Total(100)
B	46±1.0[b]	31±1.0[bc]	14±1.0[ab]	91±1.0[b]
BM	48±1.0[a]	33.5±0.5[ab]	14±1.0[a]	95.5±0.5[a]
C	42±1.0[c]	29±1.0[cd]	12±1.0[b]	83±1.0[c]
CM	47±1.0[ab]	34±1.0[a]	14±1.0[a]	95±1.0[a]
G	40±1.0[d]	28±1.0[d]	13±1.0[ab]	81±1.0[d]
GM	43±1.0[c]	32±0.5[ab]	14±1.0[a]	89±1.0[b]

Values are described in means ±Stander Division (SD) of three independent replicates. Means in the same columns with different superscripts are significantly different (P< 0.05). labneh were B , C and G labneh from milk without enzyme. BM , CM and GM labneh from milk with enzyme .

CONCLUSION

Enzymatic treatment of milk with TGase accelerated the gelling product especially goat's milk , and led to significant higher in yield of labneh inaddition of inceased gel strength and less synersis. The enzymatic cross- linking reaction led to improve the rheological properties.

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دراسة مقارنة لاستخدام إنزيم الترانسجلوتاميناز في تصنيع اللبنة من أنواع مختلفة من اللبن

ريهام كمال عبد الحميد المناوى[١]، يحيى ابراهيم عبد القادر[١] ، محمد محمد محمد المتولى الحديدى[١] وعلا محمد عادل كامل شلبى[٢]

[١]معهد الانتاج الحيوانى ، مركز البحوث الزراعية ،الدقى ، مصر

[٢]قسم الألبان – كلية الزراعة – جامعة المنصورة .

تهدف الدراسة الي انتاج اللبنة من أنواع مختلفة من اللبن(جاموسي- بقري ماعز) (B-C-G) ومقارنتها بنفس الالبان المعامله بانزيم الترانس جلوتاميناز (BM-CM GM)علي التوالى . يتضح من النتائج أن اضافة الانزيم ليس له تأثير كبير في تطور الحموضة أثناء عملية التخمر، بينما أدت الي زيادة الحموضة في المنتج النهائي B, C عن G وعلي الجانب الاخر ادي اضافة الانزيم الي زيادة نسبة التصافي للبنة كالاتي: من ٤٣,١٦ الي ٥٠,٥٥% و ٣٨,٧٨ الي ٤٨,١٦% و ٢٢,٥٠ الي ٣٧,٢٠% علي التوالى. كما ادي اضافة الانزيم الي تحسن قوة الخثره ونقص نسبة التشريش . وباجراء التحليل الكيميائي لعينات اللبنة خلال فتره التخزين حتى ٢١ يوم أدت الى زيادة الجوامد الصلبة الكلية و البروتين الكلي و الاحماض الدهنية الطياره في العينات المعامله بالانزيم عن الكونترول في حين لم يكن هناك اختلاف واضح في الحموضة وpH بين العينات . ايضا اضافة الانزيم ادي الي تحسين قيم الخواص الحسية وانتاج منتج ذو قوام كريمي وكانت اعلي القيم للعينة (CM) اى اللبنة الناتجة من اللبن البقري المعامل بالانزيم.