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EFFECT OF ADDITION OF TRANSGLUTAMINASE ENZYME AND WHEY PROTEINS ON THE PHYSIOCHEMICAL AND SENSORY PROPERTIES OF YOGURT

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ABSTRACT

This study was done to investigate the influence of addition of Transglutaminase enzyme and whey proteins on physiochemical and sensory properties of yogurt that produced from whole cow milk where the TGase enzyme was added at a concentration of 0.15 and the whey proteins at concentrations of T1 = 1% and T2 = 2%, T3 = 3% and T4 = 4% with the manufacture of control sample C without addition. Physiochemical tests for yogurt samples included total acidity and PH while rheological tests were viscosity, syneresis and water holding capacity. The results showed a decrease in the pH values and an increase in the total acidity of the samples with storage. The results also showed an increase in the viscosity value with an increase in the concentration of whey proteins after manufacturing. The values of water holding capacity and syneresis varied by increasing the concentration of whey proteins immediately after manufacture. Also, Results showed an increase in the viscosity and syneresis values with cold storage while water holding capacity was decreased with cold storage. Treatment T2 was superior in terms of sensory evaluation, where it was given the highest marks.

Keywords: Transgiutamenase, whey protein, yogurt, physiochemical

Introduction

Yogurt is the most popular fermented milk product (walstra *et al.*, 1999). It is made from a variety of formulas (fats and dry matter), either plain or with additives. It is a milk product traditionally fermented in the Middle East (Tamime and Robinson, 2007) and fermentation has been used for thousands of years to produce milk products. Yogurt is produced by fermenting milk with thermophilic bacteria that are composed of a mixture of *Streptococcus salivarius* thermophiles and *Lactobacillus delbrurckii* bulgaricus. (Lee and Lucey, 2010). The texture of yogurt is as important as its taste and flavor in terms of consumer preferences, however the characteristics of the milk used in the production of yogurt and the conditions of storage or transportation to remote points of sale can lead to defects in the texture such as changes in viscosity and seepage of whey (Hematyar *et al.*, 2012). Various stabilizers are used to prevent these problems and create desirable textual properties (Athar *et al.*, 2000).

Transglutaminase is an enzyme that has a role in food protein-modulating reactions (Dickinson and Yamamoto, 1996) and has been shown in the United States of Safe Use Substance (GRAS) (Ohtsuka *et al.*, 2001). Research on the applications of this enzyme began with the beginnings of separating the enzyme from the liver tissue of the guinea pig and the blood of mammary animals, but the guinea pig liver enzyme was the only commercially available until the late eighties of the last century, and its use as an additive to strengthen the texture of food was the subject of discussion in that era. Due to its limited source and due to difficult, long and costly separation and purification methods, and its low extraction rate, it was difficult to produce in large quantities and its very high price in the market made its widespread use in food processing impossible (Motoki and Kumazamwa, 2000). Proteins can be modified in several ways, including chemical, physical and enzymatic. Enzymatic modification of food proteins is preferred because of its superiority over other methods in terms of efficiency, high specialization, working in moderate conditions, as well as safety and avoiding the risks resulting from the formation of toxic products.

Whey is a greenish yellowish liquid produced from cheese making by rennet or an organic acid such as lactic acid, citric acid or acetic acid and it contains a high percentage of organic ingredients such as the main whey proteins in cow's milk which are alpha-lactoclopolulin 54%, alpha-albumin 21%, albumin Serum 10% and immunoglobulin 10%. In addition to lactose sugar, salts, water soluble vitamins and organic ions, the whey has its own flavor. The whey protein is produced from cutting the cheese curd during the cheese manufacturing process and it is rapidly oxidized (Jandal, 1997). It is known that whey proteins are of high nutritional value and have good functional characteristics in food products. The nutritional and functional properties of whey proteins are related to the structural function vital to those proteins (De wit, 1998).

The objective of this study is to investigate the effect of adding TGase enzyme and milk proteins (whey proteins) as a base material on the physiochemical and sensory properties of yogurt immediately after manufacture and during cold storage at a temperature of (5 ± 1) °C for 14 days.

Materials and Methods

Materials

Whole cow milk was used in the manufacture of yogurt, obtained from fields near the College of Food Sciences-Al-Qasim Green University/ Iraq. The TGase enzyme was obtained from Activa Ajino-moto Europe Sales GmbH, Hamburg, Germany. The added whey proteins were obtained from Alinda velco S.A. Greece. The yogurt starter was produced by Danisco, France

Manufacture of yogurt

Making yogurt according to the method used by (Tamime and Robinson, 1999). Milk was treated at a temperature of 90 °C for 10 minutes, then it was cooled to a temperature of 43 °C, and TGase enzyme and whey proteins were added and incubated for two hours at a temperature of 43, then inoculated with the yogurt starter consisting of Streptococcus Salivarius thermophilus and Lactobacillus delbrueckii bulgaricus by direct addition by the percentage (0.002%) as indicated by the produced company (Danisco – France). Then it was packed in plastic containers of 150 ml and incubated at 42 ± 2 °C until curd is complete, then removed from the incubator and transferred to the refrigerator for cooling and keeping at a temperature of (5 ± 1) C until the necessary tests are carried out after 1, 7 and 14 days.

Physiochemical tests of yogurt:

Total acidity:

The total acidity was measured according to (A.O.A.C, 2008) by weighing 9 g of the sample in a beaker and a few drops of phenophthaline reagent were added to it, then was titrated with (0.1 N) NaoH until the pink color appeared. The percentage of total acidity estimated on the basis of lactic acid was calculated according to the following equation:-

Total acidity% = (0.09 x 0.1 NaoH of consumer volume) / (Sample weight) x 100

pH

pH of yogurt samples was measured by placing the sensor of a pH meter (Model 211 HANNA Instruments Microprocessor - Romanian origin) directly into the yogurt sample after diluting it with a little distilled water before measurement.

Viscosity determination

The apparent viscosity of the yogurt samples was determined at a temperature of 10 ° C after 1, 7 and 14 days of refrigerated storage using the Brookfield DVII + viscometer produced by (Brookfield Engineering Lab Inc., Stoughton, Mass) according to the method mentioned by (Donkor *et al.*, 2007) with some modifications, by using the axial spindle No. 4, with a number of 10 revolutions / minute, with a volume of 150 ml for the sample, and the spindle was left to rotate inside the sample for 60 seconds after mixing the sample well by moving it ten times clockwise and ten times in the opposite direction, viscosity was measured in units of centipoise.

Water holding capacity (WHC)

Water holding capacity was measured according to the method mentioned by (Parnell-Clunies *et al.*, 1986), by subjecting 10 grams of the yogurt sample to a centrifugal force at 3000 revolutions/minute for 60 minutes at a temperature of 10 C. Then the filtrate was removed and the weight of the remaining wet precipitate. The water holding capacity was calculated as the ratio between the weight of the remaining precipitate and the weight of the original sample.

$$\text{Water holding capacity} = \frac{\text{The weight of the deposit}}{\text{The original weight of the sample}} \times 100$$

Syneresis

Syneresis of yogurt samples was determined according to the method mentioned by (Amatayakul *et al.*, 2006) by placing the cooled cup of yogurt diagonally at an angle of 45 ° for two hours at a temperature of 5 °C and withdrawing the exuded whey from the surface using the syringe, then weighing the cup again, and the process was performed within a period of 10 seconds to avoid excessive perfusion.

Sensory evaluation

Sensory tests of yogurt were conducted in the Department of Dairy Science and Technology - College of Food Science, Al-Qasim Green University, by a number of specialized professors according to the sensory evaluation form that placed by (Nelson and Trout, 1964) which included the characteristics of flavor, texture, color, appearance and exudation of whey.

Results and Discussion

pH

The results shown in Table (1) show the pH values of the yogurt for the control treatment (C) and the different treatments of the yogurt to which the TGase enzyme and the whey proteins were added. pH values were immediately after the manufacture where was 4.63 for the control treatment (c), and this is in agreement with results obtained by Shaghaghi *et al*. (2013) who found that pH was 4.63. The result also is near to findings of Ibrahim (2015) and Sadiq (2019) where they found that pH value were 4.59, 4.6 respectively and less than Ibrahim *et al*. (2016) who found that PH for yogurt was 4.5. The pH values for T1, T2, T3, T4 treatments were 4.71, 4.74, 4.77, 4.84 respectively. The results also showed decrease in the PH values of all treatments during storage for 14 days where was 4.48 for control treatment C while for the enzyme-added treatments and whey proteins they were 4.62, 4.61, 4.67, 4.73 respectively.

Results of the statistical analysis showed that there are no significant differences ($P \leq 0.05$) in the pH values immediately after manufacture and after 14 days of cold storage. Kailasapathy (2006) stated that acidity during the storage period is due to the activity of the B- galactosidase enzyme which remains effective at 0-5 ° C thereby the pH may drop to less than 4.2.

Total Titratable Acidity

The results shown in Table (1) show the values of the titratable acidity (calculated on the basis of lactic acid) for the control treatment (c) and the different treatments of the yogurt to which the TGase enzyme and whey proteins were added. Titratable acidity of control treatment (c) immediately after manufacture was 0.89%, this is in agreement with results obtained by Kawni (2011) who found that acidity was 0.89%. The result also is near to findings of Al-Bedrani *et al.* (2019). who found that acidity value was 0.90%. While the total titratable acidity of T1, T2, T3, T4 treatments were 0.84,

0.83, 0.87, 0.83% respectively, these results showed that there is a significant effect of the enzyme added with whey proteins on titratable acidity.

After 14 days of storage, the values of titratable acidity for the control treatment C was 1.07%, while for enzyme and whey proteins treatments were 0.92, 0.91, 0.92, 0.93% respectively. These results showed that the treatments with the addition of the TGase enzyme and the whey proteins obtained the lowest acidity development compared to the control treatment, these results is in agreement with the findings of Sebo (2008) who indicated that enzyme treatment slowed acidity development during the three weeks of storage. The results of the statistical analysis indicated that there is no significant difference (P≤0.05) in the titratable acidity between the control treatment C and the treatments of the TGase enzyme and whey proteins immediately after manufacture and during cold storage for 14 days.

Table 1: Physiochemical properties of the control treatment and the treatments with the addition of TGase enzyme and the whey proteins were measured at different concentrations immediately after manufacture and during the storage period for (5 ± 1) for a period of 14 days.

Physiochemical properties of yogurt						Treatments
Water holding capacity	Syneresis	Viscosity (centipoises)	Acidity%	Ph	Age of yogurt (day)	
47.97	9.80	2740	0.89	4.63	1	Control
49.00	7.00	3010	0.97	4.55	7	
52.00	6.00	3514	1.07	4.48	14	
50.65	3.10	4540	0.84	4.71	1	T1=1%
57.24	2.10	6070	0.87	4.67	7	
64.00	1.51	6247	0.92	4.62	14	
67.66	1.50	5290	0.83	4.74	1	T2=2%
70.53	1.11	5788	0.85	4.67	7	
77.12	0.70	6470	0.91	4.61	14	
60.40	2.77	6704	0.87	4.77	1	T3=3%
64.00	2.14	7989	0.85	4.71	7	
70.55	1.10	8137	0.92	4.67	14	
56.41	2.94	6950	0.83	4.84	1	T4=4%
59.76	2.10	7115	0.87	4.78	7	
67.00	1.30	7578	0.93	4.73	14	
7.844*	2.251 *	612.46*	0.267 NS	0.398 NS	---	L.S.D

*(P≤0.05), Each number in the table represents the average of three replications

Rheological characteristics of yogurt with added TGase and whey proteins

Viscosity

The results shown in Table (1) show that the viscosity value of the control treatment immediately after manufacturing was 2740 centipoise while the viscosity values of the yogurt treatments with the addition of TGase enzyme and whey proteins were increased and reached 4540, 5290, 6704, 6950 centipoise respectively. Results showed that increased of viscosity values with storage where reached after 14 days 3514 centipoise for control treatment C and for treatments T1,T2,T3,T4 they reached 6247, 6470, 8137, 7578 centipoise respectively. Our results indicated that the viscosity values of the TGase enzyme and whey proteins treatments were much higher than the control treatment, this may be due to the enzyme's role in forming the cross-linkages more in the presence of the added whey proteins, these results is in agreement with the findings of Ibrahim (2017) who indicated that the apparent viscosity of yogurt supported by whey proteins and treated with TGase enzyme is higher than the control treatment that does not contain TGase enzyme, where the reaction catalyzed by TGase leads to the formation of high molecular weight polymers. It could be due to each of the internal molecules forming bonds between protein molecules. The results of the statistical analysis indicated that there are significant differences ((P≤0.05) in the viscosity values between the control treatment (c) and all the treatments with the addition of TG enzyme and whey proteins immediately after manufacture and during storage for 14 days.

Syneresis

It is a measure of the amount of whey proteins separated from the yogurt, which is one of the most important factors affecting the acceptability of yogurt for the consumer Gursoy *et al*. (2010). The results shown in Table (1) show the quantities of exuded whey for the control treatment C and the treatments with added TGase enzyme and the whey proteins at different concentrations mentioned above, where syneresis value immediately after manufacture for control treatment C was 9.80 ml/50 gm, and for the yogurt treatments T1, T2, T3, T4 were 3.10, 1.50, 2.77 and 2.94 mL/50 g, respectively. Results showed that there is a clear difference between the control treatment C and the treatments with added TGase enzyme and the whey proteins, this is may be due to the role of the enzyme where Kuraishi *et al*. (1996), Motoki and Seguro (1998) indicated that the problem of whey separation in milk product as a result of changing degrees Temperature or physical condition can be avoided by adding TGase, which improves the holding capacity of the gel for water. Also, results indicated that the exudative whey was reduced with storage for all treatment, where it was reached 6.00 ml/50 g after 14 days for control treatment C whereas for yogurt treatments with added TGase enzyme and whey proteins were 1.51, 0.70, 1.10, 1.30 ml/50

g, respectively. The results of the statistical analysis showed that there were significant differences (P≤0.05) in the values of whey exudation between control treatment C and TGase enzyme and whey proteins treatments immediately after manufacture and during storage for 14 days.

Water holding capacity

Table (1) shows the water holding capacity for the aforementioned yogurt treatments, where water holding capacity immediately after manufacturing for control treatment C was 47.97 while for treatments T1, T2, T3, T4 were 50.65, 67.66, 60.40 and 56.41% respectively. Our results indicated that the treatments with the addition of the TGase enzyme and the whey proteins had a higher water holding capacity than the control treatment where Sodini *et al*. (2005) indicated that the water holding capacity of yogurt fortified with whey proteins was higher than the control treatment. Also, Damin *et al*. (2009) indicated that both protein-protein interactions and protein bonds increase the elasticity characteristic of producing yogurt gel that is less prone to rupture. Results of statistical analysis showed that there is a significant difference (P≤0.05) between the traetments T1, T2, T3, T4 compared to the control treatment immediately after manufacturing. Our results is also noted that the ability to retain water with storage for all treatment, where water holding capacity after 14 days for control treatment C was 52.00% while for the treatments of yogurt with the addition of TGase enzyme and whey proteins were 64.00, 77.12, 70.55 and 67.00%, respectively, these results is in agreement with the findings of Al-Bedrani (2017) who indicated an increase in the water holding capacity for control treatment from 45% immediately after manufacturing to 77% after 14 days of refrigerated storage. This may be due to the effect of lower moisture content of yogurt treatments. The results of the statistical analysis showed that there is a significant difference ((P≤0.05) between the treatments T1, T2, T3, and T4 compared with the control treatment after 14 days of storage.

Sensory evaluation

Table (2) shows the results of the sensory evaluation of the control treatment C and the treatments T1, T2, T3, and T4. Results showed that the superiority of the grades given to the flavor and texture characteristic of the treatment T2 which the TGase enzyme and whey proteins were added where it obtained the highest score of 97.64 immediately after manufacturing Compared with the control treatment C, which obtained a total score of 91.06, this indicates that the concentration of the enzyme is 0.15 and the whey proteins 2% is the best concentration, which was more palatable by the Evaluation Committee, this result is in agreement with the findings of Al-Bedrani (2016) who indicated that adding 2% of whey protein concentrate to yogurt was the best on sensory evaluation. Hussein and Fadel (2017) also mentioned that the addition of whey proteins improved the organoleptic properties as a result of raising the total solids and giving the consumer a desired flavor and texture. The results of the statistical analysis of the total scores for sensory evaluation indicated that there are no significant differences ((P≤0.05) between the control treatment and the treatments with the addition of the TG enzyme and whey proteins immediately after manufacturing.. During storage that the degrees of the sensory evaluation for most of the characteristics were decreased, the cause of the high acidity. However, treatment T2 obtained the highest score for day 14 during storage, with an overall score of 85.0, while control treatment, it got the lowest total score of 72.20, wherease treatments T1, T3, and T4, were 80.47, 78.54 and 74.68. The results of the statistical analysis of the total scores indicate that there is a significant difference (P≤0.05) between the control treatment and the T2 treatment after 14 days of storage.

Table 2 : Results of the sensory evaluation of the control treatment and the treatments with the addition of the TGase enzyme and different concentrations of whey proteins after manufacturing and during storage at a temperature of (5 ± 1) C for a period of 14 days.

Total 100	The package 5	appearance 10	Acidity 10	Texture 30	Taste and flavor 45	Age of yogurt (day)	Treatment
91.06	5	9.66	9.20	25.50	41.70	1	Control
81.24	5	8.73	8.00	23.41	36.10	7	
72.20	5	7.10	7.33	20.78	31.99	14	
95.78	5	10.00	9.80	28.20	42.78	1	T1
87.74	5	9.10	8.50	26.90	38.24	7	
80.47	5	8.66	7.20	23.64	35.97	14	
97.64	5	10.0	10.0	29.10	43.54	1	T2
93.10	5	9.30	9.00	28.00	41.80	7	
85.07	5	8.00	8.33	24.80	38.94	14	
94.66	5	9.66	9.66	28.00	42.34	1	T3
88.24	5	8.80	8.20	26.99	39.25	7	
78.54	5	7.50	7.80	23.74	34.50	14	
92.85	5	9.00	9.20	28.10	41.55	1	T4
84.54	5	8.10	8.11	25.91	37.42	7	
74.68	5	7.40	7.73	22.55	32.00	14	
8.39*	---	2.18 *	1.66*	4.28*	6.64*	---	L.S.D

*(P≤0.05), Each number in the table represents the average of three replications

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