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EFFECT OF TRANSGLUTAMINASE ON THE QUALITY OF YOGHURT MADE WITH DIFFERENT MILK PROTEIN SOURCES

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ABSTRACT

Cow's milk was fortified with 0.5, 1.0 or 1.5% milk protein supplied either from skim milk powder (SMP), sodium caseinate (NaCn), dried whey protein concentrate (WPC), or by concentrating the milk through the ultrafiltration process and treated with TG at a level of 0.5 g/l. All samples of yoghurt were stored for 21 days and changes in lactic acid bacterial count, acidity, acetaldehyde, firmness and consistency coefficient as well as the organoleptic properties were investigated. The obtained results reveal that, yoghurt treated with TG developed less lactic acid bacterial count, less acidity and acetaldehyde. On contrary, it developed higher firmness and consistency coefficient comparing with untreated samples. Organoleptic scoring recorded better scores for body and texture, and appearance. However, flavour and even the total sensory scores were lower than those of the enzymatic untreated ones. Yoghurt treated with TG could be considered as alternative of protein elevation with a level of 0.5% and improve the keeping quality as well as the shelf life of yoghurt.

Keywords: Skim milk powder, Sodium caseinate, Dried whey protein concentrate, Retentate, transglutaminase, Rheological parameters, Protein quality

INTRODUCTION

Milk proteins are known to exert a wide range of nutritional, functional and biological activities that make them potential ingredients of health-promoting foods. There are many benefits resulting from the addition of milk proteins in yoghurt formulations. These benefits include: improved flavor and texture improvement, nutritional enrichment, reduced syneresis, extended shelf-life, prebiotic effect and nutraceutical benefits. The appearance and texture of yoghurt is dependent upon numerous factors: total solids, protein content, type of protein, fat content and the type and concentration of any thickeners or stabilizer that are added (**Kuehn *et al* 2006**). The casein micelles in yoghurt form different matrices depending upon the concentration of the other proteins. When milk is fortified with WPC and heat treated, fine

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protein floccules are observed. When casein, skim milk powder, or milk protein concentrates are added, no floccules are observed. When milk is heated, ß-lactoglobulin is denatured and reacts with a-casein to form an insoluble complex. When milk is fortified with WPC, the concentration of ß-lactoglobulin greatly exceeds the concentration of α-casein. As a result other protein complexes, such as ß-lactoglobulin and α-lactalbumin complexes will form. In yoghurts fortified with WPC, it is the ß-lactoglobulin and α-lactalbumin complex, are formed rather than the casein complex, that probably stabilizes the yoghurt, resulting in different consistency. Fortification of milk for yoghurt with WPC results in yoghurt with better texture and consistency. Yoghurts fortified with casein or skim milk protein often have a firmer gel, but yoghurts fortified with WPC tend to be smoother and have a better appearance.

On the other hand, one of the most important criteria for consumer acceptance of foods is flavor. Food matrix components, such as proteins (**Gianelli *et al* 2005**) known to interact with flavor compounds. Proteins are added to foods primarily because of their functional properties, such as emulsifying and stabilizing capacities, and because of their nutritional value. However, interactions between proteins and flavors are known to influence the perceived flavor of a food product (**Land 1996**). Protein ingredients not only reduce the perceived impact of desirable flavors but also may transmit undesirable off-flavors to foods (**Semenova *et al* 2002**). In addition, proteins may change the texture of a food that is gelling, and thus decrease the flavor perception due to inhibition of mass transfer (**Wilson and Brown 1997**).

Transglutaminase (protein glutaminey-glutamyltransferase, EC 2.3.2.13) catalyzes an acyl-transfer reaction between the y-carboxamide group of peptide-bound glutamine residues and a variety of amino acids. Milk proteins (casein, α-lactalbumin, β-lactoglobulin) are good substrates for TG-catalyzed cross-linking, and among these, the caseins are excellent substrates for TG. Gels formed by TG-treated casein micelles have some interesting features: they are much stronger and they form more quickly than gels obtained by more traditional routes (acidification or renneting); they are temperature-dependent on heating; and they exhibit no syneresis even after long storage time. For this reason, it has been suggested that TG could be used for production of gelled products, less allergic proteins and food additives with improved properties in dairy products. Moreover, TG increases the water-holding capacity and emulsifying properties of milk proteins (**Faergemand *et al* 1999 and Oezrenk, 2006**).

Thus our objectives were to test the effect of TG on the yoghurt characteristics when manufactured from cow milk fortified with different protein source and levels.

MATERIAL AND METHODS

Materials

Fresh cow's milk (3.60% fat and 3.37% protein) was obtained from Higher Institute of Agric. Co-operation, Shoubra El-Kheima at Faculty of Agriculture, Ain Shams University. Skim milk powder (SMP, 36% protein) and Dried whey protein concentrate (WPC, 82% protein) made in Denmark were obtained from the local market at Cairo. Sodium caseinate (NaCn, Lactovit Co., Germany, 84% protein) was obtained from Arab Dairy Co., Cairo, Egypt. Lyophilized mixed yoghurt starter culture containing *Streptococcus thermphilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Cagilificio Clerici, Cadorag, Italy (strain 3.63). Microbial transglutaminase enzyme derived from *Streptovertierllium* sp. was obtained from Gewuerzmueller GmbH, Salzburg, Bergheim, Germany (100 units / g protein).

Preparation of milk protein fortified yoghurt in the presence of transglutaminase

Thirteen treatments including the control were designed, where cow's milk was firstly fortified either with 0.5, 1.0 or 1.5% protein whether directly by adding SMP, Na caseniate, WPC or by the milk concentration (after its previously heat treatment at (72°C/2 min.) by ultrafiltration technique at 50°C (as recommended by **Maubois *et al* 1971**) using CARBOSEP UF-unit (type 2S 37, France) with zirconium oxide membrane area 1.68 m^2 at Agric. Secondary school, Giza. The yoghurt bases were procedure as described by **Tamime and Robinson (1999)** with adopting the manufacture conditions enacted by **EOSQC (2005)**, where they were heat treated at 85°C for 5 min. then cooled to 42°C. Thereafter, TG was added at a level of 0.5 g/l yoghurt milk. After 2 h incubation at 42°C, yoghurt mixes were heat treated again but at 80°C for 1 min. for enzyme inactivation then cooled to 42°C, inoculated with 2% of activate starter culture as aforementioned, filled into 100 ml polystyrene containers, covered, and incubated until complete coagulation (through about 3 h.). Thereafter, the containers were transferred to the refrigerator (5±1°C), where they were kept for the periodical analyses.

Analytical methods

Dry matter (DM), fat, protein (total nitrogen x 6.38), soluble nitrogen and titratable acidity (TA) contents were determined according to **AOAC (2000)**. Acetaldehyde was estimated as described by **Lees and Jago (1969)**. pH value was measured using a pH meter (HANNA Instruments, USA). The electrophoretic determinations, SDS poly acrylamide gel electrophoresis (PAGE) technique was applied according to the method of **Laemmli (1970)** as modified by **Studier (1973)**.

The firmness of set-style yoghurt was measured using penetrometer model SUR, BERLIN, PNR as described by **Bourne (1982)**. The depth to which a loaded perforated disc penetrates into the yoghurt curd in a given time is measured (using cone weight 35g). The depth of penetration (0.1 mm, penetrometer unit, PE) is a function of the firmness of yoghurt curd. The measurements are always carried out at about 10°C and the depth of penetration was measured after 5 sec.

The count of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were carried out using in order M17 and MRS agar media as described by **Gueimonde *et al* (2003)**.

Sensory evaluation of yoghurt samples was applied for storage period by regular score panels including the staff members of Food Science Department, Faculty of Agriculture, Ain Shams University according to **Tamime and Robin-**

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son (1999) using the yoghurt evaluation scheme III approved by the American Dairy Science Association

The data obtained were exposed to proper statistical ANOVA analysis according to statistical analyses system user's guide (SPSS, 1998).

RESULTS AND DISCUSSION

The gross composition of yoghurt fortified with different milk protein sources further stabilized *via* TG displayed in **Table (1)**. The obtained results reveal that, the DM content raised as well as the fat content decreased, opposite to that protein enriched *via* the UF concentration, as the protein fortification level increased in yoghurt. Concerning the effect of enzymatic treatment in relation to those two properties, although the DM content was not influenced, the fat content decreased significantly (P<0.001) due to treating with TG.

Figs. (1 and 2) show the lactic acid bacterial counts in yoghurt during storage period. The counts of *Lb. delbrueckii* subsp. *bulgaricus* were gradually icreased as the protein level increased, the count of *Str. thermophilus* raised significantly only when the protein level heightened to 1.5%. With regard to the protein source in relation to the yoghurt bacterial count, SMP offered the best condition for growth of both strains enumerated, followed by WPC, UF and NaCn respectively. Duration of storage of yoghurt was associated with gradual decline in the count of both strains.

Similar findings were described by **Neve *et al* (2001)**. The effect of TG was significantly depended on the level and source of fortification protein (P<0.05) as well as the storage period (P<0.001) as statistically ANOVA declared.

Fig. (3) reveal that the titratable acidity raised significantly as the protein level increased in the presence of TG. SMP imparted the resultant yoghurt the highest titratable acidity followed by WPC, retentate and NaCn. Inversely, the protein source that caused the highest titratable acidity imparted the lowest pH value and *visa versa*.

Table 1. Gross composition of yoghurt as affected by the level and source of milk protein fortification in the presence of transglutaminase.

Component %	control	Level and source of protein fortification*											
		0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
Dry matter	12.62	13.90	13.85	13.08	13.21	15.45	15	13.75	13.82	16.75	16.10	14.31	14.38
Fat	3.80	3.70	4.28	3.74	3.72	3.66	4.72	3.71	3.70	3.62	5.27	3.70	3.68
Protein	3.51	4.13	4.20	4.11	4.10	4.60	4.55	4.61	4.48	5.11	5.19	5.11	5.05

* SMP: Skim milk powder , NaCn: Sodium caseinate, UF:Milk Retentate,
WPC: Whey protein concentrate

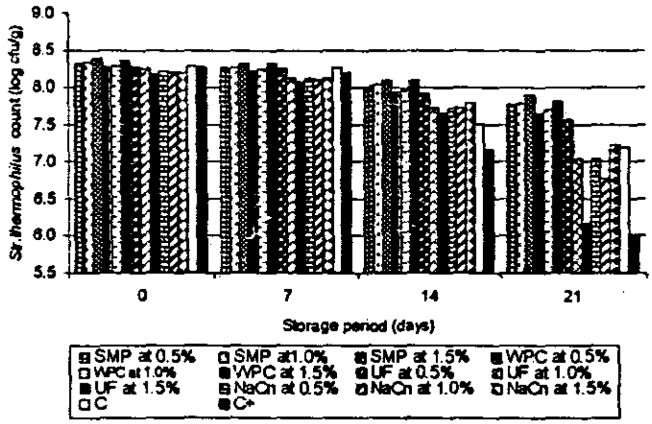

Fig. 1. *Streptococus.thermophilus* count as affected by different protein levels and source* in the presence of transglutaminase along storage period.
* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

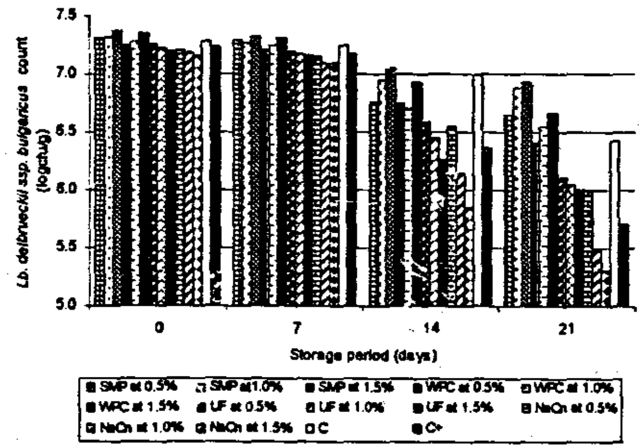

Fig. 2. *Lactobacillus.delbrueckii subsp. bulgaricus* count as affected by different protein levels and source* in the presence of transglutaminase along storage period.
* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

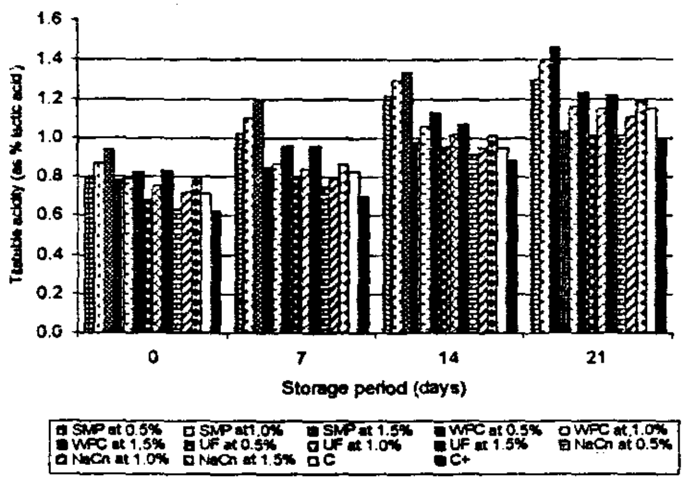

Fig. 3. Titratable acidity as affected by different protein levels and sources* in the presence of transglutaminase along storage period.

* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

As the storage of yoghurt prolonged, the acidity increased. Similar observations were reported by Neve *et al* (2001); **Lorenzen** ***et al*** **(2002) and Abou El-Nour** ***et al*** **(2004).** The effect of TG treatment on TA% was not related to the protein level (P>0.05), but its effect thereon was correlated to the kind of protein source as well as the duration of storage periods as indicated.

Fig. (4) shows the acetaldehyde content of yoghurt during storage period as affected by the level and source of fortifying protein in the presence of TG. Data confirmed that, gradual increase in acetaldehyde content was associated with the proportional increase in the protein level of the yoghurt (P<0.001). Moreover, SMP as protein source gave the yoghurt the highest acetaldehyde value followed by WPC, UF and NaCn respectively. During storage period of yoghurt, the acetaldehyde increased until the 7th day gradually. Then they trended thereafter to decrease. The TG treatment of yoghurt milk led to delay the formation of acetaldehyde. These trends agree with those reported by **Abo-El Nour** ***et al*** **(2004).** Moreover, the statistical interactions between the TG treatment and the level as well as the kind of protein source were significant (P<0.001), while that between it and the storage period was not significant (P>0.05).

Fig. (5) shows the soluble nitrogen / total nitrogen of yoghurt during storage period as affected by the level and source of fortifying protein in the presence of TG. Data confirmed that, gradual increase in soluble nitrogen was associated with the increase of storage period. Moreover, WPC as protein source gave the yoghurt

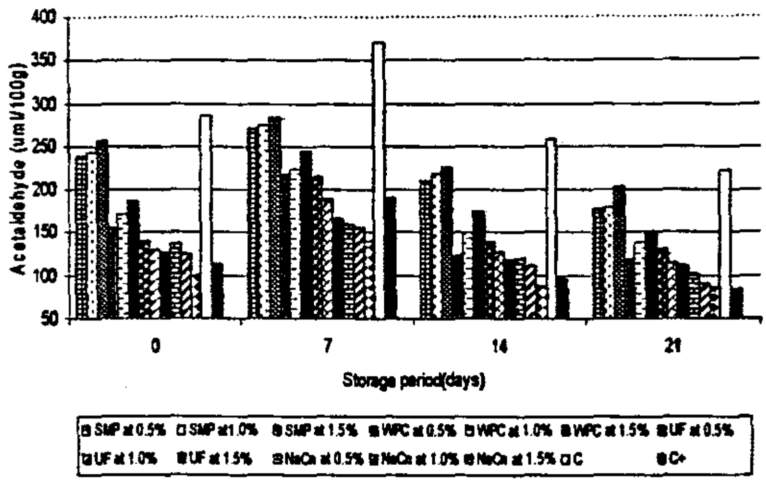

Figure 4. Acetaldehyde content as affected by different protein levels and source* in the presence of transglutaminase along storage period.

* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

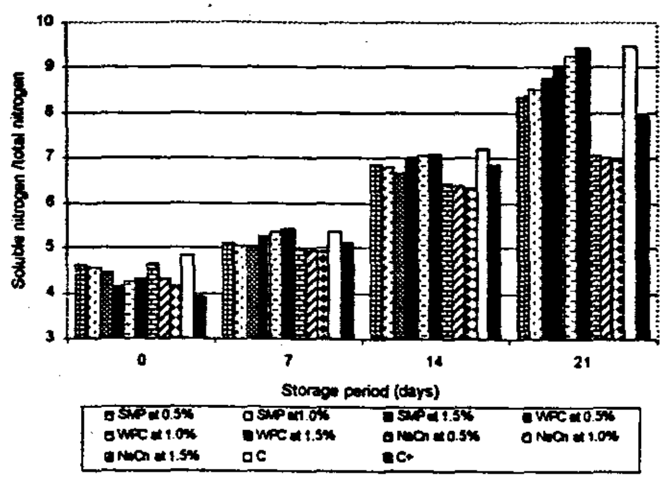

Fig. 5. Soluble nitrogen /total nitrogen of the yoghurt as affected by different protein levels and source* in the presence of transglutaminase along storage period.

* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

the highest soluble nitrogen value followed by SMP and NaCn respectively. The TG treatment of yoghurt milk led to more protein lysis.

Rheological parameters (the penetration values, those inversely indicating the firmness of set yoghurt as well as the consistency coefficient) during storage period of yoghurt treated samples are presented in **Table (2)**

The results demonstrate that, There are gradual strengthening in the set yoghurt firmness as the protein level raised as inversely indicated from the penetration values (P<0.001). With respect to the protein source, NaCn caused the lowest penetration value, i.e. the highest firmness, followed by retentate, WPC and SMP. By duration of storage the penetration values of yoghurt gradually decreased. The effect of TG treatment on the yoghurt firmness was significantly correlated only to the kind of protein source. That could be depending on its casein content. Where casein is the main substrate for TG in milk, while the globular whey proteins are poor substrates (**Nonaka *et al* 1989** and **Miwa *et al* 2001**). These results are in agreement with those reported by **Faergemand *et al* (1999)**; **Lorenzen & Neve (2002)** and **Abo-El Nour *et al* (2004)**. The reason for the enzyme-induced increase of the gel strength is due to a reduction of mish sizes of the protein network and to a more regular distribution of the protein chains in the product (**Lorenzen and Neve, 2002**).

Table 2. Penetration value of set yoghurt as well as consistency coefficient during storage period as affected by the level and sources of milk protein fortification in the presence of transglutaminase.

Storage period (day)	Level and source of protein fortification*												
	control	0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Penetration (mm)												
0	27.0	26.4	24.0	22.8	24.3	25.5	24.0	22.5	24.1	23.8	23.0	21.5	23.5
7	26.0	25.0	23.5	22.3	24.0	24.2	23.3	22.0	23.5	23.4	22.5	21.0	23.0
14	25.5	24.2	23.2	22.0	23.6	23.8	23.0	21.4	23.0	23.0	22.0	20.2	22.7
21	24.9	23.7	22.8	21.4	23.2	23.3	22.3	20.9	22.7	22.5	21.4	19.8	22.1
	Consistency coefficient (dyne.sec./cm^2)												
0	15.5	17.65	19.71	19.88	18.32	18.28	20.13	21.31	19.59	20.11	21.96	22.5	20.43
7	17.51	18.55	20.52	21.62	19.43	19.2	22.34	22.94	20.2	21.69	23.43	24.9	21.8
14	19.9	19.52	21.6	23.04	20.3	20.02	23.64	24.47	21.39	22.18	24.2	25.85	22.95
21	21.99	20.37	22.02	25.15	21.33	22.87	24.56	25.38	22.57	23.98	25.00	26.64	23.19

* SMP: Skim milk powder, NaCn: Sodium caseinate, UF:Milk Retentate, WPC: Whey protein concentrate

As noticed from (**Table, 2**) there are forward relationship between the consistency coefficient of yoghurt and its protein level. Moreover, NaCn or retentate gave yoghurt the highest consistency coefficient followed by WPC and SMP respectively. The corresponding values increased as the storage period prolonged. The TG treated yoghurt exhibited consistency coefficient values significantly higher than that of the untreated one. Similar observations were reported by **Abo-El Nour *et al* (2004).**

Table (3) illustrate the organoleptic evaluation of the treated samples in terms of appearance, consistency and flavour.

Concerning the appearance criterion, that was significant affected among the protein fortification level ($P<0.001$). The yoghurt supplemented with 0.5% protein led to obtain the highest score, followed by 1.0, 1.5 without any significant differences between SMP, UF process and NaCn as milk protein source. While WPC caused some yellowness leading to significant reduce in the appearance score of its yoghurt.

The enzymatic treatment of yoghurt milk with TG improved significantly ($P<0.001$) the appearance of yoghurt.

The highest score for one was as revealed a dry, smooth and whiter shining surface.

Similar observations were reported by **Lorenzen *et al* (2002) and Abo-El Nour *et al* (2004).**

Table 3. **Appearance and Consistency scores of yoghurt during storage as affected by the level and source of milk protein fortification in the presence of transglutaminase.**

storage period (day)	control	Level and source of protein fortification*											
		0.5%				1.0%				1.5%			
		SMP*	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Appearance (out of 5 points)												
0	4.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
7	3.5	4.5	4.5	4.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
14	3.0	4.0	4.0	4.0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
21	3.0	3.5	3.5	3.5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
	Body and Texture (out of 5 points)												
0	4.5	5.0	5.0	4.0	4.5	5.0	5.0	3.5	4.5	5.0	5.0	3.0	4.0
7	4.5	5.0	5.0	4.5	4.5	5.0	5.0	3.5	4.5	5.0	5.0	3.0	4.0
14	4.5	5.0	5.0	4.5	4.5	5.0	5.0	3.5	4.5	5.0	5.0	3.0	4.0
21	5.0	5.0	5.0	4.5	4.5	5.0	5.0	3.0	4.5	5.0	5.0	2.5	4.0
	Flavor (out of 10 points)												
0	8	9	8	7	8	9	9	7	8	7	9	7	8
7	8	9	8	7	8	8	8	7	8	7	8	7	7
14	7	8	7	6	7	7	7	6	7	6	7	6	6
21	6	7	6	5	6	6	7	5	6	6	7	5	5
	Total (out of 20points)												
0	16.5	19.0	18.0	16.0	17.5	19.0	19.0	15.5	17.5	17.0	19.0	15.0	17.0
7	16.0	18.5	17.5	16.0	17.5	18.0	18.0	15.5	17.5	17.0	18.0	15.0	16.0
14	14.5	17.0	16.0	14.5	16.0	16.5	16.5	14.0	16.0	15.5	16.5	13.5	14.5
21	14.0	15.5	14.5	13.0	14.5	15.0	16.0	12.0	14.5	15.0	16.0	11.5	13.0

*SMP: Skim milk powder , NaCn: Sodium caseinate , UF:Milk Retentate ,WPC: Whey protein concentrate

The body and texture score of yoghurt increased by adding 0.5 or 1.0% protein more than that done when the level raised to 1.5% ($P<0.001$), especially when SMP or UF process was applied, followed by WPC and NaCn ($P<0.001$). The body and texture score of TG treated yoghurts varied slightly significantly ($P<0.05$) from those untreated, where some cheesy body was observed in the former especially when protein was added at the highest level (1.5%) and supplied from NaCn ($P<0.001$). There are no significant relationship ($P>0.05$) between the TG treatment and the storage period of yoghurt in body and texture score.

Furthermore, gradual increase in the flavor score was recorded, when the protein level of yoghurt raised until 1.0%. The highest level (1.5%) suffered from flavor score lower than even the control. the adding of WPC or UF process was the better protein enrichment procedure toward the yoghurt flavor, followed by SMP and NaCn. Nevertheless, the TG treated samples attained the lowest flavor score *vis a vis* the untreated ones. Yoghurt made from TG treated milk was considered to be flat and less intense in yoghurt specific flavor particularly for aroma attributes. Similar observations were reported by **Lorenzen *et al*** (2002) **and Abo El-Nour *et al*** (2004). This phenomenon was significantly correlated only to the kind of protein source ($P<0.01$) nor to the protein level or to the prolonging of storage period of yoghurt, which did not lead to any significant differences in the flavor score until the 14th day. Then significant reduction therein was occurred. Finally, it can conclude that yoghurt treated with TG could be considered as alternative of protein elevation with a level of 0.5% and improve the keeping quality as well as the shelf life of yoghurt.

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المؤتمر العاشر لبحوث التنمية الزراعية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر، ٢٠٠٦
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تأثير ترانس جلوتامينيز علي خواص اليوجهورت المصنع بإستخدام مصادر مختلفه من بروتينات اللبن

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تم في هذا البحث تدعيم الحليب البقري ببروتين اللبن بنسب مختلفة ومصادر مختلفة. حيث تم تصنيع ١٣ معاملة من الحليب البقري مدعمة بنسب بروتينية وهي ٠,٥%، ١,٠%، ١,٥% وذلك عن طريق الإضافة سواء باستخدام مساحيق: الحليب الفرز، كازينات الصوديوم، مركزبروتينات الشرش أو بتركيز الحليب نفسه باستخدام تقنية الترشيح الفائق لرفع نسبة البروتين بنفس المعدلات السابقة ثم معاملة العينات السابقة بانزيم الترانس جلوتامينيزبتركيز ٠,٥غرام/ ليتر. تم تخزين اليوجهورت لمدة ٢١ يوم وتقدير الحموضة واعداد بكتريا حمض اللاكتيك، المحتوى من الاسيتالدهيد، والتغير فى النيتروجين الذائب بالنسبه للنيتروجين الكلى، الصلابة، معامل القوام وقيم جهد القص الابتدائي، بالاضافة للصفات الحسية.

ولقد أوضحت النتائج انخفاض نسبة الحموضة والاسيتالدهيد وأعداد بكتريا حمض اللاكتيك اثناء التخزين في العينات المعاملة بالأنزيم مقارنة بالعينات غير المعاملة. كما أظهرت العينات المعاملة بالأنزيم ارتفاعاً في قيم النيتروجين الذائب والصفات الريولوجيه مقارنة بالعينات غير المعاملة، بينت التقديرات الحسية ان العينات المعاملة بالأنزيم كانت الأفضل من حيث المظهر والقوام مقارنة بالعينات غير المعاملة، بينما حصلت العينات غير المعاملة على الأفضلية من حيث النكهة ودرجات التقييم الحسية الكلية.وقد كانت العينات المدعمة بنسبة ٠,٥% بروتين هى الأفضل من حيث المظهر بينما حصلت العينات المدعمة بنسبة ١,٠% بروتين على الأفضلية من حيث القوام ومجموع درجات التقييم الحسي.

وبناء على ذلك فيمكن استخدام انزيم الترانس جلوتامينيز لإنتاج يوجهورت ذو جوده تركيبيه وحسيه جيده مع خفض نسبة الإضافات بمعدل ٠,٥%.

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