Research Article

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Impact of probiotic and synbiotic supplementation on the physiochemical, texture and sensory characteristics of wheyless domiati-like cheese

Abstract

The effect of probiotic cultures of lactic acid bacteria in the absence and presence of Inulin on the quality of wheyless Domiati-like cheese made from dry dairy ingredients was assessed during 28days of storage at 7ºC. The treatments design was as follows: C, thermophilic starter of *Streptococcus thermophillus* and *Lactobacillus delbreckuii* subsp *bulgaricus* (standard control cheese); LR, Lactobacillusrhamnosus19070; LR+IN, *Lbrhamnosus* with 2%inulin; BL, *Bifidobacterium animalis* subsp *lactis* ATCC SD5219; and BL+IN, B. *lactis* with 2%inulin. Differences in physicochemical characteristics, proteolysis indexes, rheological texture and sensory attributes were found among the assessed cheeses. Results showed that addition of both probiotic strains improved cheese quality, whereas incorporation of Inulin in synbiotic cheeses enhanced the texture and sensory attributes. Relative degrees of proteolysis were found to be significantly higher in synbiotic cheese than the control and probiotic cheeses. The sensory evaluation showed that the total perception of Domiati-like cheeses was improved by the addition of the probiotics alone or in combination with Inulin. It is suggested that wheyless Domiati-like cheese fortified with probiotics and prebiotics well orientated functional food had accepted composition and sensory properties.

Keywords: probiotics, synbiotics, wheylee domiati-like cheese, proteolysis

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Introduction

The consumers' interest in food products which promote health positively has produced an increase in search of food functions, stimulating innovation and development of new products. Modern approach in nutrition illustrates that diet might modulate various functions in the body and able to play detrimental and beneficial beyond meeting nutrition needs, roles in some diseases in which defined as "Functional Foods.[1]"

One of the highly recommended approach in which foods can be modified to become functional is the probiotic supplementation. Probiotics are live microorganisms which support the health of consumers by maintaining/improving their intestinal microbial balance.[2] Probiotics are found to be capable in treatment of gastrointestinal disorders, respiratory infections and allergic symptoms[3] and also reduce blood cholesterol and improve immunity.[4] The most commonly strains used as probiotic belong to the genera of *Bifidobacterium*, *Lactobacillus* and *Enterococcus* sp.[5] Aprobiotic food is produced to contain viable probiotics in a convenient matrix and in effective concentration.[6] The most promising food delivery systems for probiotics are fermented dairy products such as cheeses, yoghurts and fermented milks. However, their survival and viability may be adversely affected byoperational processes as well as by the product environmental and storage conditions.[7] Cheese has been considered as a better probiotic carrier compare toother fermented milk products. Cheese characteristics like pH, higher content of fat and solid consistency matrix offer greater protection to these cultures in the gastrointestinal tract (GIT).[8] Moreover, probiotic viability might be enhanced by combination with prebiotic ingredients during food processing, as results of their protective effect while in food matrix and nutritive role in the GIT.[9] Prebiotics are short chain carbohydrates that are non-digestible by digestive enzymes in humans and selectively enhance the activity of some groups of beneficial bacteria.[10] Inulin and Fructooligosaccharides (FOS) are among the most famous prebiotic compounds.[11] Inulin is a storage carbohydrate in a large number of plants built up from β(2,1)-linked fructosylresidues ending with a glucose.[12] Inulin finds use in food and non-food applications as results of its nutritional and technological properties. On the side of nutritional effects, Inulin as a fiber has a positive effect on bowel habit[13] further, it can cause a specific shift in the composition of the colonic microbiota that has beneficial effects for the human host.[9] Inulin is known to reduce the risk of colon cancer, diabetes, obesity, and cardiovascular diseases in human beings.[14] The technological benefits of inulin are dependent on its properties as sugar and fat replacers and texture modifier.[15]

The concept of "Synbiotics" was firstly introduced by Gibson who, speculated to gain additional benefits if prebiotics were combined with probiotics.[16] Synbiotic products are produced in Europe and Japan asa recent concept; they can enhance health promotion in a synergistic manner, over either probiotics or prebiotics alone.[17]

The main crucial *challenges* of functional food *development are* focusing on the technological, nutritional and sensory factors determining the acceptance of developed product. Domiati cheese is an Egyptian trademark cheese belongs to soft pickled cheese varieties. Originally, it is produced from Buffalo's milk with direct addition of high salt concentration (up to 12%) to raw cheese-milk.[18]

Researchers have paid a lot of attention to develop Domiaticheese as one of the main ingredients in Egyptian diet. The traditional method of manufacturing Domiati cheese has been scaled-up over the years with some modifications. Technological modifications have been studied including the use of different milk types, heat treatment, ultrafiltration technique and adding of starter/adjunct cultures or flavoring agents.[18,19] IDF[20] has been recommended to produce recombined Feta cheese from certified powder milk, whey protein concentrate and other healthy ingredients to overcome the problem of lack in milk production in developing countries. In view of the shortage of fresh milk in Egypt, dairy factories have been encouraged to utilize reconstituted full/skimmed milk powder, casein concentrate and/or recombined milk in Domiati cheese milk.[21]

In order to reduce whey loss during cheese process in addition, to inability to reuse salted whey produced from Domiati cheese, wheyless soft cheese containing more than about 65 percent moisture with a high whey protein/casein ratio (e.g., about 60/40 or higher) and with desirable firmness which does not involve whey separation has been invented.[22]

In this context, this research aimed to develop wheyless recombined Domiati bio-cheese type with probiotic bacteria (*Lactobacillus rhamnosus* 19070 and *Bifidoacterium animalis subsp. lactis* ATCCSD5219) and a prebiotic ingredient (Inulin) and evaluate the effects of supplementation on the Physiochemical, texture and sensory characteristics of the cheese during 28days refrigerated storage.

Material and methods

Bacterial strains

Probiotic cultures used in the present study were *Lactobacillus rhamnosus* 19070 (DSM 26357); and *Bifidoacterium animalis subsp.lactis* ATCCSD5219were kindly provided by Prof. M. Tvede, University Hospital of Copenhagen, Copenhagen. Cheese starter culture was Express 0.2, DVS, containing *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (Chr. Hansen Laboratories, Copenhagen, Denmark).

Manufacture of cheese

Recombined wheyless Domiati-like cheese treatments were prepared by using skim milk powder(SMP), milk protein concentrate(MPC), and butter (80% fat) according to Tamime[23] with some modifications. Table 1 presents the required ingredients for processing 100Kg of "designed" recombined cheese. Preparation of cheese milk was carried out in vats by blending SMP in warm water at 45±2°C, and then the MPC was added to the reconstituted milk. After the complete blending, the butter was added and the temperature was raised to 50±2°C to melt the butter, then the temperature was raised to 65±2°C where the stabilizer was added. The stirring velocity gradually increased to reach 1400 rpm with continuous mixing for 30min then the mixture was cooled to 40°C and then $CaCl_2$ (0.02%) was added.

The mixture was divided into five equal portions in duplicates as the following:

Control cheese (C): Standardized cheese-milk mixture was inoculated (1%) of Express 0.2, DVS commercial culture (*St.thermophillus* and *Lb.delbrueckii* subsp. *bulgaricus*.

Probiotic cheese treatments: Standardized cheese-milk mixture was inoculated with freeze-dried *Lb.rahmnosus* 19070 (LR) or *B.animalis subsp. Lactis* SD5219 (BL) at concentration of $\geq$11log_{10}cfu/g.

Synbiotic cheese treatments: Standardized cheese-milk mixture was fortified with 2% inulin then inoculated with *Lb.rahmnosus* 19070(LR+IN) or *B.animalis subsp. lactis* SD5219 (BL+IN) at concentration of$\geq$11 log_{10}cfu/g.

In all treatments, inoculated cheese-milk was incubated for an hour at 40°C before, 2% salt and diluted rennet were added. Thereafter, mixtures were poured into 0.5Kg plastic containers (final package) and left for complete coagulation at 40°C while whey was not separated. Produced cheeses stored in the refrigerator at 7°C for 28days. Cheese samples were analysed intervals of 1, 14 and 28days.

Table 1 Ingredients used in wheyless Domiati-like cheese manufacture

Ingredients	Quantity (Kg)
Skim milk powder(Fonterra, New Zealand)	8.72
Milk protein concentrate(Fonterra, New Zealand)	11.63
Butter (80% fat, NZMP, Fonterra, New Zealand)	4.72
Stabilizer (Mefad company, Egypt)	0.25
Salt (EL Nasr Saline's Company, Egypt)	1.5
Calcium chloride (EL Nasr Saline's Company, Egypt)	0.02
Rennet(Purchased from Alexandria local market)	0.08
Inulin (FabrulineÒ Instant, Cosucra Group Warcoing, Belgium)	2
Water	To complete to 100Kg (@71.1Kg)

Chemical analysis

Cheese samples of each treatment were withdrawn in duplicate from two sealed packs at everyspecific analysis time. Samples were analyzed for titratable acidity, pH, water soluble nitrogen (WSN) and total nitrogen (TN) as described by Ling.[24] Moisture content, fat percent and fat extraction for gas chromatographic analysis (GC) were done according to AOAC.[25] Salt content was determined according to the modified Volhard's method as described by Kosikowski.[26] The free amino acids (FAA) were determined in the water-soluble nitrogen extract (WSN) of cheese according to Ninhydrin method as described by Folkertsma et al.[27]

Sodium dodecyl sulphate polyacrylamide gel electrophoresis: Milk proteins were diluted 1:3 (v/v) with buffer 0.05 M Tris-HCl, pH 6.8, then diluted samples were mixed in the ratio 1:1 (v/v) with sample buffer 0.5M Tris-HCl, pH 6.8, containing glycerol (7.5%), sodium dodecyl sulphate (SDS) (2%), β-mercaptoethanol (5%) and bromophenol blue (0.5%) and subjected to heat in a boiling water bath at 100°C for 10min. Samples were cooled at room temperature, centrifuged at 10,000×g for 10min to remove any insoluble material, and then loaded onto the gel using the discontinuous buffer system.[28] Electrophoresis was performed using Min-Protean system (Bio-Rad, USA).The running buffer consisted of 0.192M glycine, 0.025M Tris

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and SDS (0.1%). Runs were carried out at 80V in stacking gel then increased to 180v until the end of electrophoresis. Protein bands were stained in the gels using Coomassie blue R-250 (0.1%).

Determination of instrumental Texture Profile Analysis: Texture profile analysis (TPA) in cheese treatments was conducted using a TA.XT*plus* Texture analyzer (Stable Micro System, London, England). Hardness (H), adhesiveness (A), springiness (S), cohesiveness (ratio), gumminess (G) and Chewiness (J) were calculated by the software instrument called Texture Exponent (Stable Micro System, London, England) as described by Bourne.[29]

Sensory evaluation: Sensory evaluation was carried out at the Department of Dairy Science and Technology, Alexandria University, by a panel consisting of 15 cheese experts, including staff members and assistants. Each individual was given 3 blocks (6×2×2cm) of cheese per sample. Samples were presented in identical plastic sample cups sealed with plastic lids and identified by a random 3-digit number. The coded samples were randomly presented. The score card of soft cheese was designed in the light of score suggested by Bodyfelt et al.,[30] as follows: 50points for flavor, 40points for body/texture and 10points for appearance with total perception of 100points. The cheese considered to be accepted at total perception of 65points.

Statistical analysis

All experimental data of were analyzed for the effect of main factors and their interactions on chemical, proteolytic and sensory characteristics by the method of Steel et al.[31] All data were also analyzed by analysis of variance and Duncan's multiple mean comparisons between main factors using SPSS statistical software (version 16.0; SPSS Inc., Chicago, IL, USA).

Result and discussion

Cheese composition

Table 2 illustrates the development of chemical composition of probiotic and synbiotic wheyless Domiati-like cheeses in compared with control treatment during storage at 7°C for 28days. The moisture content in all cheeses significantly (P>0.05) decreased as storage period proceeded which could be related to an increase in the percentage of protein. This decrease could be attributed to the contraction of curd as a result of developed acidity during storage period, which helps to expel the whey from the curd.

These results are in an agreement with these reported by Awad et al.[32] and Badawiand Kabary.[33] As a wheyless soft cheese, the average moisture content at the beginning of storage was higher than those reported by Awad et al.[32] & Hamad[34] However, the produced probiotic and synobiotic cheeses with high moisture levels did not show any manifestations of spoilage or significant increase in the yeast and fungal numbers until the fifth week of storage compared to control treatment (data not shown). Inulin in synbiotic cheeses had significantly increased (P>0.05) moisture content which it might be explained as inulin increases of water binding capacity of the cheese matrix. Koca et al.[35] stated that addition of fat replacer, i.e. inulin in general to low-fat cheese increased moisture content and yield of produced cheese. The same results were found by Zalazar et al.[36] & Alnemr et al.[37] in low-fat soft cheese and Karish cheese, respectively. The bio-cheese in the present study was designed to have low fat and salt (□3% and 2% respectively) and high protein (□17%) contents (Table 2). The increase of fat and protein contents in cheeses during storage were found to be concomitant with an increase in dry matter, which related to the decrease of moisture content. Similar behavior trends of protein and fat in Domiati cheese had been reported.[38–41] There was a gradual increase in salt during the storage period (Table 2); similar results were reported by Awad et al.[32]

Development of titratable acidity during cheese manufacture and storage was dependent on strain used and addition of inulin (Table 2). Addition of inulin (2%) with probiotics in synbiotic cheese led to increase acid production. Cardarelli et al.[42] reported that combination of probiotics and probiotics resulted in a rather promising functional *petit-suisse* cheese, where as presence of inulin had no implications upon growth and viability of *Lb. paracasei* in a synbiotic fresh cream cheese.[11] In yoghurt, addition of inulin (1%) exhibited the lowest pH values in compare with control, and yoghurt made with FOS or resistance starch at the same concentration of inulin.[43] According to Su et al.,[44] probiotics (*Lb. casei* and *B. lactis*) are able to grow in basal medium supplemented with FOS or inulin. The presence of prebiotic compounds contributes to a higher acid production; a similar accelerating effect of Inulin i.e. a reduction by □10% of the fermentation time of different binary co-cultures, was reported by Oliveira et al.[45]

Effect of strain type and inulin on acidification rate was investigated by Oliveira et al.[46] They found that *Lb. rhamnosus* (Lr) grown in skim milk was able to metabolize 6g/100g more galactose than *Str. Thermophilus* (Str) and Str+Lr. Inulin stimulated both biomass growth and levels of all end-products, as the likely result of fructose release from its partial hydrolysis and subsequent metabolization as an additional carbon and energy source.

Domiati cheese may be a potential synbiotic vector, in particular for the *Lb. rhamnosus* 19070 and *B. lactis* DS5219 strains under consideration here. Similar trend is observed in probiotic UF-Domiati cheese produced with *Lb. rhamnosus*ATCC7460where the inoculating rate was the determining factor for the rate of acidity.[41]

Proteolytic behavior

Proteolysis is indispensable biochemical changes for the development of proper flavor in cheeses during storage, mainly due to the production of peptides and free amino acids.[47] The mean values of water-soluble nitrogen (WSN) and free amino acids composition (FAA) of pro- and- synbiotic Domiati cheeses during 28days of storage at 7°C are listed in Table 3.

The proteolytic patterns of cheeses were also analyzed by assessing the hydrolysis of αs-casein and β-casein using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) as shown in Figure 1. The results showed that the rate of accumulation of WSN and FAA marked a constant increase during cheese ripening (p<0.05) in all cheese treatments as the storage period proceeded. Addition of inulin with *Lb.rhamnosus* significantly enhanced (p<0.05) WSN content while, with *B. lactis* it is only noticed at the end of storage period. The FAA composition followed the same trend of increasing during storage. Meanwhile probiotic cultures showed highest potential activities. It is stated that *Bifidobacterium* sp. was a weak proteolytic bacterium, when compared with strains of *Lactobacillus*.[48] Kabary et al.[41] noted that the proteolytic effect of *Lb. rhamnosus* ATCC7460 in UF-Domiati cheese was dependent on inoculating level. Our findings are consistent with previous results of Ong et al.[8,48] who were investigated the impact of different probiotics on the properties of Cheddar cheese during 6 months of ripening.

These results were expected due to fact that primary proteolysis of most cheeses takes place under the action of residual rennet and starter proteinases. Hence, starter cultures, including probiotics, usually have different peptidase systems which do not assume a significant role during primary proteolysis, but influence the secondary proteolytic changes.[47] The higher rates of secondary proteolysis due to the addition of probiotics were also found during studies of different kind of cheeses.[8,49,50]

The stimulation influence of inulin on proteolytic activities of *Lb. acidophilus* and *Bifidobacterium* BB12 in UF-soft cheese was reported by El-Baz.[51] In yoghurt, inulin did not increase the degree of proteolysis[52] however; a higher level of proteolysis has demonstrated in some probiotic yogurt and was attributed to the higher viability of *Lb. bulgaricus*.[53]

Electrophoretograms of the experimental cheeses at various stages of ripening are presented in Figure 1. The hydrolysis of α_{s1}-casein was more intensive than that of β-casein. The α_{s1} -casein was hydrolyzed initially and other peptides with electrophoretic motilities faster than α_{s1}-I. The differences in the accumulation of α_{s1}-I and α_{s1}-II were seen in all cheese treatments. However, the degradation of α_{s1}-casein in symbiotic cheeses (LR+IN and BL+IN) was more pronounced than in probiotic cheeses (Figure 1). The obtained results showed that the addition of inulin to cheese promoted the proteolysis throughout the storage period as there were significant different among treatments. However, the degradation of α_{s1}-casein in symbiotic cheeses (LR+IN and BL+IN) was more pronounced than in probiotic cheeses (Figure 1). The obtained results showed that the addition of inulin to cheese promoted the proteolysis throughout the storage period as there were significant differences among treatments.

The proteolysis of cheese is mainly related to moisture content as the most critical factor; there was a direct relationship between the residual clotting activity in cheese and its moisture content. As well as, the high moisture content led to reduce the cheese pH by activation the microorganisms and continuous fermentation of lactose to lactic acid. At the low cheese pH, the casein degradation increased by activation the residual clotting enzymes. This phenomenon is clearly evident in the case of cheese made with *Lb rhamnosus* 19070 and inulin which gave lowest pH (Table 2) and highest proteolytic rate (Table 3) (Figure 1).

Intracellular endopeptidase (PepO) was identified and characterized in *B. animals* subsp. *lactis*. The predominant peptide bond cleaved by *B. lactis* PepO was on the N-terminal side of phenylalanine residues.[54] Proteolytic system in *Lb. rhamnosus* BGT10, probiotic human isolate, has an efficient proteinase (PrtR) at pH 6.5 able to cleavage α_{S1}- and β-casein and is distributed throughout all *Lb. rhamnosus* strains tested.[54,55]

Texture assessment

Textural parameters of probiotic and synbiotic wheyless Domiati-like cheeses are listed in Table 4. After 4weeks of storage, an increase in hardness was observed for probiotic cheeses (LR and BL) while it is dropped in synbiotic cheeses. At the end of storage, the lowest values of springiness was found in C cheese whereas; LR+IN cheeses presented the lowest values for cohesiveness. The gumminess and chewiness increased in probiotic cheeses during storage period. At day one in synbiotic cheeses, inulin addition led to increase hardness and decrease the rest of texture parameters. During storage, hardness decreased but springiness, cohesiveness, and gumminess have increased. These trends are among the most difficult to explain in the present study as they seem to result from a complex interaction of a number of variables. Meanwhile, in yoghurt at high whey protein concentration and in condition similar to wheyless cheese as no whey separation inulin resulted in increasing of hardness.[43] Glibowski et al.[56] reported higher hardness of inulin-whey protein gels probably due to interaction inulin-whey proteins. Others have shown that the effect of fat replacement on cheese texture is dependent on the nature of the fat being replaced.[57] Hennelly et al.[58] compared the use of shear-induced inulin gels and heated inulin solutions to replace 63% of the fat in imitation cheese. They observed that at equivalent moisture levels, the inulin cheeses had significantly higher hardness values than the control sample with fat. Our results are in agreement with the previous studies.[37,59,60] Effect of inulin could be attributed to the corresponding increase the capacity for holding water. Awad et al.[61] demonstrated importance of fat and moisture as the filler with the network of cheese, whilst water acts as a lubricant or plasticizer between proteins. Softening the protein matrix is greatly affected by moisture in non-fat cheese.[62]

According to Koca et al.,[35] the softening effect observed in the cheese made with inulin might be attributed to the higher ratio of moisture to protein and to the increase in filler volume that results in a decrease in the amount of protein matrix. Springiness is the rate at which a deformed material returns to its original shape on removal of the deforming force.[29,60] In spite of cheeses with BL+IN had the highest springiness among all cheeses, the differences in the rest of treatments were insignificant.

Inulin decreased gumminess of synbiotic cheeses; the presence of inulin in the cheese matrix may also be a mitigating factor in this respect. Whereas adhesiveness is the tendency of cheese material to adhere with other material or surface and cohesiveness is the strength of internal bonds making up the body of the product.[29,63] The lower values of chewiness in synbiotic cheeses compared to the other treatments may be due to the addition of inulin which changes the protein structure by entrapping in the matrix serving to weak the elastic cheese matrix.[50] Advancing storage period until 14days showed an increase followed by decreasing in following storage period. Overall at the end of storage, hardness was significantly lower than control and probiotic cheeses. These results are confirmed the results of Juan et al.[60] who found that cheeses produced with inulin were less hard than reduced-fat cheeses, and more similar to cheeses made from whole milk.

Sensory evaluation

The sensory attributes of probiotic and synbiotic wheyless Domiati-like cheeses are presented in Table 5. The resultant cheeses had a good body, texture (soft, smooth and lubricity texture) and pleasant creamy flavor. The sensory attributes such as general appearance, body and texture, flavor and total perception were found to be significantly different between samples with increased storage period of 4week ($p<0.05$). Changes occurred during storage period were improved sensory attributes in probiotic and synbiotic cheeses. The lowest ($p<0.05$) values for appearance, flavor, body and texture and total perception were found for cheese C (control treatment) at all evaluated storage periods (Table 5). Differences in sensory attributes between synbiotic cheeses (LR+IN and BL+IN) and probiotic cheeses (LR and LB) were noticed at the end of storage time, indicates the enhancement effect of Inulin on the cheese sensory profile. The highest sensory scores were gained by LR+IN cheeses followed by BL+IN cheeses ($p<0.05$).

In agreement with the present results, Cardarelli et al.[64] reported that the addition of medium chain Inulin plus oligofructose (50/50) in probiotic *petit-suisse* gave the highest sensory acceptance after 28 days of refrigerated storage. Along the same line, Araujo et al.[65] developed a synbiotic cottage cheese with *Lactobacillus delbrueckii* UFV H2b20 and 8% medium chain Inulin. Probiotic bacteria and inulin did not change taste or texture of the cottage cheese after 15days of storage at 5°C in comparison with the control non-probiotic cheese. In the present study, the lower acidity values were observed in synbiotic cheeses however, it had high acceptability scores. This is might be explained by the ability of inulin to mask the taste. Prebiotic fiber such as Trehalose, Inulin and Oligosaccharides impart a touch of sweetness to food and beverage products while it is not actually be classified as sweeteners.[66]

Table 2 Mean values of the chemical composition and pH of probiotic and synbiotic cheese treatments during 28 days of storage at 7°C

Variables	Storage time (Days)	Cheese treatments				
		C	LR	BL	LR+IN	BL+IN
Moisture (g/100g)	1	72.68±0.03^{Ac}	71.73±0.23^{Ac}	72.70±0.30^{Ac}	73.05±0.05^{Ab}	74.84±0.17^{Aa}
	14	71.46±0.07^{Bb}	69.82±0.07^{Bd}	71.43±0.08^{Bb}	70.95 ±0.07^{Bc}	72.98±0.02^{Ba}
	28	65.60±0.10^{Cc}	64.90±0.10^{Cc}	64.75±0.25^{Cc}	68.90±0.10^{Cb}	70.90±0.10^{Ca}
Titratable acidity (g/100g)	1	0.26±0.01^{Bb}	0.30±0.03^{Ab}	0.23±0.04^{Bb}	0.52 ±0.03^{Ba}	0.45±0.04^{Ba}
	14	0.32±0.02^{Bb}	0.37±0.04^{Ab}	0.35 ±0.02^{ABb}	0.59±0.04^{Ba}	0.55±0.00^{Ba}
	28	0.40±0.05^{Ac}	0.39±0.03^{Ac}	0.45±0.04^{Ac}	0.85±0.03^{Aa}	0.67±0.01^{Ab}
Fat (g/100g)	1	3.00±0.00^{Ca}	3.00±0.00^{Ca}	3.00±0.00^{Ca}	3.00±0.00^{Ca}	3.00±0.00^{Ca}
	14	4.00±0.00^{Bc}	5.00±0.00^{Ba}	5.00±0.00^{Ba}	4.55±0.05^{Bb}	3.90±0.10^{Bc}
	28	5.00±0.00^{Ad}	5.95±0.05^{Ab}	7.00±0.00^{Aa}	5.55±0.05^{Ac}	6.00±0.00^{Ab}
Salt (g/100g)	1	2.06±0.05^{Ba}	2.04±0.03^{Ba}	2.03±0.03^{Ba}	2.01±0.05^{Ba}	1.98±0.03^{Ba}
	14	2.33±0.05^{Aa}	2.20±0.03^{Ab}	2.19±0.03^{Ab}	1.99±0.03^{ABc}	2.14±0.03^{Ab}
	28	2.38±0.05^{Aa}	2.33±0.05^{Aa}	2.24±0.03^{Aab}	2.15±0.03^{Ab}	2.27±0.06^{Aab}
Protein (g/100g)	1	17.06±0.03^{Ca}	16.97±0.00^{Ca}	16.99±0.09^{Ba}	16.78±0.00^{Ca}	16.75±0.16^{Ca}
	14	17.93±0.03^{Bc}	19.57±0.03^{Ba}	17.33±0.00^{ABd}	18.58±0.25^{Bb}	18.25±0.19^{Bbc}
	28	18.42±0.04^{Ad}	21.13±0.01^{Ab}	17.68±0.13^{Ae}	20.54±0.00^{Ac}	21.50±0.06^{Aa}
pH	1	6.47±0.0^{Aa}	6.15±0.01^{Ab}	6.54±0.15^{Aa}	4.88±0.01^{Ad}	5.47^{Ac}±0.01
	14	5.32±0.01^{Bc}	6.10±0.01^{Bb}	6.23±0.01^{ABa}	4.54±0.01^{Cd}	5.32^{Bc}±0.02
	28	5.35±0.01^{Bc}	6.08±0.01^{Bb}	6.11±0.01^{Ba}	4.84±0.01^{Be}	5.29^{Bd}±0.02

Results are means of two replicates±standard error, the following abbreviations are used: C, control cheese made with commercially available lyophilized culture Chr. Hansen® (Express 0.2, DVS, St. thermopilus and Lb. delbrueckii subsp. bulgaricus); LR, cheese made with Lb. rhamnosus 19070; LR+IN, cheese made with Lb. rhamnosus 19070 and 2% inulin (Cosucra Group Warcoing, Belgium.); BL: cheese made with B. animalis subsp.lactis SD5219; BL+IN, cheese made with B. lactis SD5219and 2% inulin.

A-CDifferent letters for each column and for each type of cheese indicate significant differences (p<0.05) among treatments at each interval ripening time.

a-bDifferent letters for each row and for each type of cheese indicate significant differences (p<0.05) throughout ripening time.

Table 3 Mean values for proteolysis parameters of wheyless Domiati-like cheese with probiotic and synbiotic supplementation during 28days of storage at 7°C

Variables	Storage time (Days)	Cheese treatments				
		C	LR	BL	LR+IN	BL+IN
Soluble Nitrogen (g/100g)	1	0.22±0.01^{Ca}	0.22±0.01^{Ba}	0.20±0.01^{Ca}	0.25±0.01^{Ca}	0.23±0.01^{Ba}
	14	0.25±0.02^{Bb}	0.27±0.01^{Ab}	0.25±0.00^{Bb}	0.31±0.01^{Ba}	0.26±0.01^{Bb}
	28	0.30±0.01^{Ab}	0.30±0.01^{Ab}	0.29±0.00^{Ac}	0.49±0.01^{Aa}	0.34±0.02^{Ab}
Free Amino Acids (mg/100g)	1	2.80±0.20^{Cab}	2.45 ±0.25^{Cb}	2.93±0.08^{Cab}	2.93±0.08^{Cab}	3.50±0.50^{Ca}
	14	4.95±0.05^{Bb}	5.60±0.40^{Bb}	4.80±0.20^{Bb}	8.00±0.50^{Ba}	8.75±0.25^{Ba}
	28	6.50±0.50^{Ad}	7.50±0.50^{Acd}	9.00±0.00^{Ac}	11.50±0.50^{Ab}	14.50±0.50^{Aa}

Results are means of two replicates± standard error, the following abbreviations are used: C, control cheese made with commercially available lyophilized culture Chr. Hansen® (Express 0.2, DVS, St. thermopilus and Lb. delbrueckii subsp.bulgaricus); LR, cheese made with Lb. rhamnosus 19070; LR+IN, cheese made with Lb. rhamnosus 19070 and 2% inulin (Cosucra Group Warcoing, Belgium.); BL: cheese made with B. lactis SD5219; BL+IN, cheese made with B. lactis SD5219and 2% inulin.

A-CDifferent letters for each column and for each type of cheese indicate significant differences (p<0.05) among treatments at each interval ripening time.

a-bDifferent letters for each row and for each type of cheese indicate significant differences (p<0.05) throughout ripening time.

Table 4 Texture analysis of probiotic and synbiotic cheeses during cold storage

Storage Time (day)	Cheese treatments				
	C	LR	BL	LR+IN	BL+IN
Hardness(N)					
0	724.45±11.36^{Cd}	804.81±8.35^{Bc}	730.86±5.44^{Cd}	1150.05±24.56^{Ab}	1238.34^{Aa}±35.71
14	815.31±11.58^{Bc}	1035.99±35.65^{Aa}	929.37±6.08^{Bb}	772.27±16.94^{Bd}	806.56^{Bc}±53.03
28	920.40±18.34^{Ac}	1050.30±39.34^{Ab}	1274.50±29.67^{Aa}	546.58±13.16^{Ce}	785.80±38.49^{Cd}
Adhesiveness (J)					
0	4.15±0.50^{Cc}	11.02±1.14^{Ba}	6.85±0.34^{Cb}	5.17±0.10^{Cb}	12.04±0.19^{Ca}
14	61.28±4.76^{Aa}	64.11±3.48^{Aa}	41.80±3.82^{Bb}	35.63±0.66^{Ab}	37.84±2.72^{Ab}
28	42.78±0.80^{Bb}	52.94±5.43^{Aa}	51.75±1.55^{Aa}	13.68±0.60^{Bd}	23.25±0.68^{Bc}
Springiness(mm)					
0	1.45±0.16^{Aa}	0.95±0.06^{Ab}	0.96±0.04^{Ab}	0.73±0.02^{Bb}	0.74±0.02^{Cb}
14	0.96±0.07^{Ba}	0.96±0.01^{Aa}	0.98±0.02^{Aa}	0.96±0.02^{Aa}	0.95±0.02^{Ba}
28	0.88±0.05^{Bc}	0.92±0.02^{Bb}	0.99±0.02^{Ab}	0.94±0.01^{Ab}	1.20±0.03^{Aa}
Cohesiveness(ratio)					
0	0.84±0.07^{Ba}	0.83±0.04^{Aa}	0.86±0.02^{Aa}	0.38±0.02^{Cb}	0.13±0.01^{Cc}
14	0.90±0.05^{Aa}	0.89±0.07^{Aa}	0.90±0.05^{Aa}	0.85±0.01^{Aa}	0.78±0.07^{Bb}
28	0.90±0.07^{Aa}	0.89±0.04^{Aa}	0.84±0.04^{Aa}	0.67±0.01^{Bb}	0.95±0.04^{Aa}
Gumminess(N)					
0	607.91±46.49^{Ba}	668.19±37.18^{Ba}	628.69±18.53^{Ca}	437.09±20.68^{Bb}	161.36±16.51^{Cc}
14	734.80±47.44^{Babc}	924.82±92.99^{Aa}	835.89±36.18^{Bb}	656.53±17.14^{Abc}	630.27±69.81^{Bc}
28	830.70±23.97^{Ac}	931.86±21.13^{Ab}	1076.57±68.43^{Aa}	366.06±5.66^{Ce}	744.38±25.55^{Ad}
Chewiness(J)					
0	895.45±168.53^{Aa}	630.24±3.67^{Cb}	602.34±18.7^{Cb}	318.44±8.22^{Bc}	119.62±14.02^{Cd}
14	709.08±87.24^{Bab}	886.83±84.73^{Aa}	820.55±53.24^{Bab}	629.96±13.18^{Ab}	601.08±76.34^{Bb}
28	872.23±26.07^{Ab}	859.98±11.67^{Bb}	1063.74±52.20^{Aa}	344.03±3.21^{Bc}	893.25±53.84^{Ab}

Results are means of two replicates± standard error, the following abbreviations are used: C, control cheese made with commercially available lyophilized culture Chr. Hansen® (Express 0.2, DVS, St. thermopilus and Lb. delbrueckii subsp.bulgaricus); LR, cheese made with Lb. rhamnosus 19070; LR+IN, cheese made with Lb. rhamnosus 19070 and 2% inulin (Cosucra Group Warcoing, Belgium.); BL: cheese made with B. animalis subsp.lactis SD5219; BL+IN, cheese made with B. lactis SD5219 and 2% inulin.

A-CDifferent letters for each column and for each type of cheese indicate significant differences (p<0.05) among treatments at each interval ripening time.

a-bDifferent letters for each row and for each type of cheese indicate significant differences (p<0.05) throughout ripening time.

Table 5 Sensory evaluation of probiotic and synbiotic cheeses during cold storage time

Storage Time	Cheese Treatment	Appearance	Body & Texture	Flavor	Total Perception
1	C	8.10±0.43^{a}	31.80±0.88^{a}	34.50±3.43^{a}	74.40±4.07^{a}
	LR	8.50±0.22^{a}	33.70±1.27^{a}	41.80±2.23^{a}	84.00±2.61^{a}
	BL	8.00±0.52^{a}	31.20±1.38^{a}	37.10±2.65^{a}	76.30±2.91^{a}
	LR+IN	8.40±0.45^{a}	33.10±0.71^{a}	40.70±1.89^{a}	82.20±2.13^{a}
	BL+IN	8.60±0.22^{a}	33.90±1.49^{a}	40.60±2.46^{a}	83.10±3.18^{a}
14	C	7.40±0.54^{c}	34.10±1.47^{a}	35.70±2.16^{bc}	77.20±3.44^{bc}
	LR	7.90±0.46^{bc}	29.40±1.54^{b}	34.60±3.16^{c}	71.90±4.62^{c}
	BL	8.20±0.29^{abc}	33.40±1.59^{a}	35.80±1.94^{bc}	77.40±3.38^{bc}
	LR+IN	9.10±0.23^{a}	36.70±0.97^{a}	44.50±1.02^{a}	90.30±1.70^{a}
	BL+IN	8.60±0.31^{ab}	35.50±1.80^{a}	42.10±2.14^{ab}	86.20±3.94^{ab}
28	C	5.80±0.50^{d}	25.40±1.16^{d}	26.20±2.50^{c}	57.7±1.75^{d}
	LR	6.70±0.50^{c}	26.90±1.53^{c}	30.90±2.07^{b}	64.50±3.66^{c}
	BL	8.00±0.26^{b}	30.90±1.54^{b}	31.50±0.43^{b}	70.40±1.73^{b}
	LR+IN	9.20±0.20^{a}	38.40±0.31^{a}	46.90±0.74^{a}	94.50±1.10^{a}
	BL+IN	9.40±0.16^{a}	38.70±0.40^{a}	45.20±0.51^{a}	93.30±0.67^{a}

Results are means of two replicates± standard error, the following abbreviations are used: C, control cheese made with commercially available lyophilized culture Chr. Hansen® (Express 0.2, DVS, St. thermopilus and Lb. delbrueckii subsp.bulgaricus); LR, cheese made with Lb. rhamnosus 19070; LR+IN, cheese made with Lb. rhamnosus and 2% inulin (Cosucra Group Warcoing, Belgium.); BL: cheese made with B. animalis subsp.lactis SD5219; BL+IN, cheese made with B. lactis and 2% inulin.

a-dDifferent letters for each column of each sensory attribute indicate significant differences ($p<0.05$) among treatments throughout ripening time.

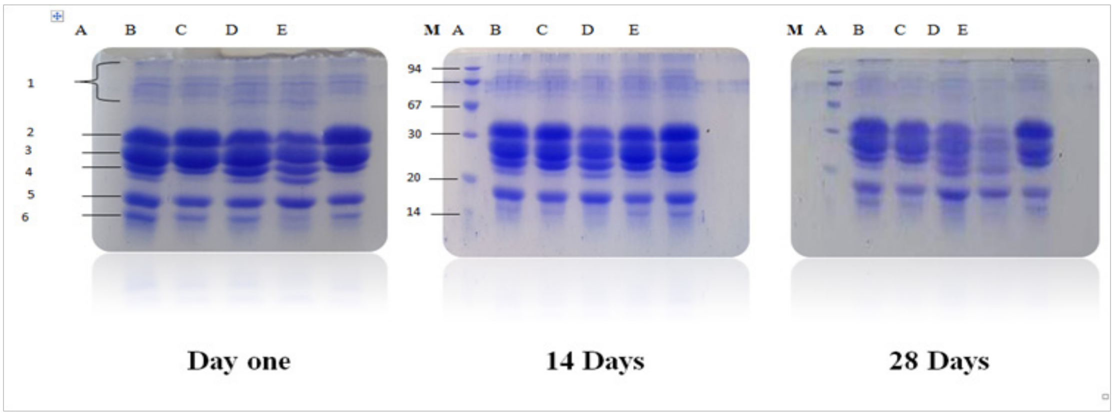

Figure 1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of probiotic and synbiotic cheese proteins during cold storage. Lane M, molecular weight standard (Phosphorylase 94kd, Bovine serum albumin 67kd, Ovalbumin 43kd, Carbonic anhydrous 30kd, Soya trypsin inhibitor 20kd and □- lactalbumin 14kd); lane A, control cheese made with commercially available lyophilized culture Chr. Hansen® (Express 0.2, DVS, St. thermopilus and Lb. delbrueckii subsp.bulgaricus); lane B, cheese made with Lb. rhamnosus19070; lane C, cheese made with B. lactis SD5219 and 2% inulin; lane D, cheese made with Lb. rhamnosus and 2% inulin; lane E: cheese made with B. lactis SD5219. 1, minor whey proteins (Lf, BSA, and Ig); 2, □S-CN; 3, □-CN; 4, k-CN; 5, □-Lg; 6, □-La.

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Conclusion

Wheyless Domiati-like cheese made with low concentration of fat and salt fortified with functional effective dosage of *Lb. rahmnosus* 19070 or *B. lactis* SD 5219 alone or in synbiotic form with Inulin represents a good model of functional food. Probiotic and synbiotic Domiati-like cheeses, produced according to the established production procedure, are distinguished by acceptable composition, sensory quality and satisfactory dietetic.

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Conflict of interest

The author declares no conflict of interest.

References

1. Roberfroid MB. Concepts and strategy of functional food science: the European perspective. *A J ClinNutr*. 2000;71(6 suppl):S1660–S1664.
2. Mattila-Sandholm T, Myllärinen P, Crittenden R, et al. Technological challenges for future probiotic foods. *Int Dairy J*. 2002;12(2-3):173–182.
3. Wohlgemuth S, Loh G, Blaut M. Recent developments and perspectives in the investigation of probiotic effects. *Int J Med Microbiol*. 2010;300(1):3–10.
4. Shah NP. Functional cultures and health benefits. *Int Dairy J*. 2007;17(11):1262–1277.
5. Fooks LJ, Fuller R, Gibson BR. Prebiotics, Probiotics and human gut Microbiology. *Int Dairy J*. 1999;9(1):53–61.
6. Saxelin M, Korpela R, Maÿrä-Mäkinen A. Introduction: classifying functional dairy products. In: Mattila-Sandholm T, et al. editors. *Functional Dairy Products*. Boca Raton, La, USA: CRC Press; 2003. p. 1–16.
7. Castro JM, Tornadijo ME, Fresno JM, et al. Biocheese: a food probiotic carrier. *Bio Med Resh Int*. 2015;2015:1–11.
8. Ong L, Henriksson A, NP Shah. Development of probiotic Cheddar cheese containing *Lactobacillus acidophilus*, *Lb. casei*, *Lb. paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. *Int Dairy J*. 2006;16(5):446–456.
9. Gibson GR, Probert HM, van Loo J, et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev*. 2004;17(2):259–275.
10. YasminA, Butt MS, Afzaal M, Marleen van Baak M, et al. Prebiotics, gut microbiota and metabolic risks: Unveiling the relationship. *J Funct Foods*. 2013;17(2):189–201.
11. Buriti FC, Cardarelli HR, Filisetti TM, et al. Synbiotic potential of fresh cream cheese supplemented with inulin and *Lactobacillus paracasei* in co-culture with *Streptococcus thermophillus*. *Food Chem*. 2007;104(4):1605–1610.
12. Ritsema T, Smeekens S. Fructans: beneficial for plants and humans. *Curr Opin Plant Biol*. 2003;6(3):223–230.
13. Tungland BC, Meyer D. Non-digestible oligosaccharides (dietary fibre): their physiology and role in human health and food. *Compr Rev Food Sci Food Saf*. 2002;3:73–92.
14. Miremadi F, Shah NP. Applications of inulin and probiotics in health and nutrition. *Int Food Res J*. 2012;19:1337–1350.
15. Meyer D, Bayarri S, Tárrega A, et al. Inulin as texture modifier in dairy products. *Food Hydrocolloids*. 2011;25(8):1881–1890.
16. DeVrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. In: Stahl U, et al. editors. *Food Biotechnology*. USA: Springer Berlin Heidelber; 2008. p. 1–66.
17. Maukonen J, Matto J, Kajander K, et al. Diversity and temporal stability of fecal bacterial populations in elderly subjects consuming galacto-oligosaccharide probiotic yoghurt. *Int Dairy J*. 2008;18(4):386–395.
18. Abou-Donia SA. Recent development in Egyptian Domiati research: An overview. *Egypt J Dairy Sci*. 2007;35:1–14.
19. Awad S, Ahmed N, El Soda M. Influence of microfiltration and adjunct culture on quality of Domiati cheese. *J Dairy Sci*. 2010;93(5):1807–1814.
20. IDF. *Monograph on recombination of milk and milk products -Technology and engineering aspects, Doc. 116*. Brussels, Belgium: International Dairy Federation; 1979.
21. Abd-El Salam M, Benkerroum N. North African brined cheeses In: Adnan Tamime, editor. *Brained Cheese*. 1st ed. USA: Blackwell Publishing Ltd; 2006. p. 142–151.
22. Han XQ. *Process for making cream cheese products without whey separation*. Patent US. 6406736 B1; 2002.
23. Tamime AY. *Modern Dairy technology*. Vol 2 – advances, Milk products, London UK: Elsevier Applied Science; 1986.
24. Ling ER. *A text book of Dairy Chemistry*. Vol. II. 3rd ed. London, UK: Chapman & Hall Ltd; 1963.
25. AOAC. *Official methods of analysisof the Association of Official Analytical Chemists*. 17th ed. Washington DC, USA; 2000.
26. Koskikowski FV. *Cheese and fermented milk foods*. 2nd ed. USA: New York; 1966.
27. Folkertsma B, Fox PF. Use of Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. *J Dairy Res*. 1992;59:217–224.
28. Laemmli UK. Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature*. 1970;277(5259):680–685.
29. Bourne M. Texture Profile Analysis. *Food Technol*. 1978;32:62–66.
30. Bodyfelt FW, Tobias J,Trout GM. *The sensory evaluation of dairy products*. Pub Van Nostrand Reinhold, New York, USA, Library of Congress Catalog card no.67-28078; 1988.
31. Steel RG, Torrie JH. *Principles and Procedures of Statistics*. New York, USA: McGraw-Hill; 1960. 190 p.
32. Awad SA, Abdel-Kader YI, Nawar MA. The quality of white pickled cheese as affected with the types of calcium salt. *Mansoura Univ J Agric Sci*. 2001;26:2183–2203.
33. Badawi, RM, Kebary KMK. Accelerated ripening of cheese by partial lyzedLactococci. *Menofiya J Agric Res*. 1996;21:63–81.
34. Hamad MNF. Comparative study between traditional Domiati cheese and recombined Feta cheese. *Indian J Dairy Sci*. 2015;68:442–451.
35. Koca N, Metin M. Textural, melting and sensory properties of low-fat fresh Kashar cheeses produced by using fat replacers. *Int Dairy J*. 2004;14(4):365–373.
36. Zalazar CA, Zalazar CS, Bernal S, et al. Effect of moisture level and fat replacer on physicochemical, rheological and sensory properties of low fat soft cheeses. *Int Dairy J*. 2002;12(1):45–50.
37. Alnemr TM, Abd El-RazeK AM, Hasan HM, et al. Improving of Karish cheese by using enhanced technological texturizing inulin. *Alex J Agric Res*. 2013;58(2):173–181.

38. Hussien SA. *Studies on the use of starter cultures in the manufacture of cheese*. M.Sc Thesis, Egypt: Minufiya Univ; 1985.

39. Farag SI, El-Saify NA, Hamed AI. Microbiological and chemical studies on pickled Domiati cheese treated with some ripening factors. *Minofia J Agric Res*. 1988;13:1845–1851.

40. Abd El-Aziz M, Kholif AM, Sayed AF. Evaluation of Domiati cheese quality during storage as affected by live salinized yeast supplementation to rations of lactating Buffaloes. *Int J Dairy Sci*. 2007;2:152–158.

41. Kabary EK, El-Shazly HA, Youssef IT. Quality of Probiotic UF Domiati Cheese made by *Lactobacillus rhamnosus*. *Int J CurrMicrobiol App Sci*. 2015;4(7):647–656.

42. Cardarelli HR, Saad SM, Gibson GR, et al. Functional *petit-suisse* cheese: measure of the prebiotic effect. *Anaerobe*. 2007;13(5-6):200–207.

43. Gustaw W, Kordowska-Wiater M, Koziol J. The influence of selected prebiotics on the growth of lactic acid bacteria for bio-yoghurt production. *Acta Sci Pol Technol Aliment*. 2011;10(4):455–466.

44. Su P, Henriksson A, Mitchell H. Selected prebiotics support the growth of probiotic mono-cultures in vitro. *Anaerobe*. 2007;13(3-4):134–139.

45. Oliveira RP, Perego P, Oliveira MN, et al. Growth, organic acids profile and sugar metabolism of *Bifidobacterium lactis* in co-culture with *Streptococcus thermophilus:* The inulin effect. *Food Res Int*. 2012;48(1):21–27.

46. Oliveira RP, Perego P, Oliveira MN, et al. Effect of inulin on the growth and metabolism of a probiotic strain of *Lactobacillus rhamnosus* in co-culture with *Streptococcus thermophiles*. *LWT - Food SciTechnol*. 2012;47(1):358–363.

47. Sousa MJ, Ardo Y, McSweeney PL. Advances in the study of proteolysis during cheese ripening. *Int Dairy J*. 2001;11(4-7):327–345.

48. Ong L, Henriksson A, Shah NP. Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei*, *Lb. casei* or *Bifidobacterium* sp. *Int Dairy J*. 2007;17(1):67–78.

49. Bergamini CV, Hynes ER, Zalazar CA. Influence of probiotic bacteria on the proteolysis profile of a semi hard cheese. *Int Dairy J*. 2006;16(8):856–866.

50. Bergamini CV, Hynes ER, Palma SB, et al. Proteolytic activity of three probiotic strains in semi-hard cheese as single and mixed cultures: *Lactobacillus acidophilus, Lactobacillus paracasei* and Bifidobacterium lactis. *Int Dairy J*. 2009;19(8):467–475.

51. El-Baz AM. The use of Inulin as a dietary fiber in the production of symbiotic UF-soft cheese. *J Food Dairy Sci Mansoura Univ*. 2013;4:663–677.

52. Shakerian M, Razavi SH, Khodaiyan F, et al. Effect of different levels of fat and inulin on the microbial growth and metabolites in probiotic yogurt containing nonviable bacteria. *Int J Food Sci Technol*. 2014;49:261–268.

53. Cruz A, Castro W, Faria J, et al. Stability of probiotic yogurt added with glucose oxidase in plastic materials with different permeability oxygen rates during the refrigerated storage. *Food Res Int*. 2013;51(2):723–728.

54. Janer C, Arigoni FBH, Lee B, et al. Enzymatic Ability of *Bifidobacterium animalis* subsp.*lactis* to hydrolyze milk proteins: Identification and characterization of endopeptidase O. *Appl Environ Microbiol*. 2005;71(12):8460–8465.

55. Paŝtar I, Tonic I, Golic N, et al. Identification and genetic Characterization of a novel proteinase, PrtR, from the human isolate *Lactobacillus rhamnosus* BGT10. *Appl Environ Microbiol*. 2003;69(10):5802–5811.

56. Glibowski P, Bochyńska R. Effect of inulin on rheological properties of whey protein solutions. *Acta Agrophysica*. 2006;8(2):337–345.

57. Lobato-Calleros C, Vernon-Carter E J, Hornelus-Uribe Y. Microstructure and texture of cheese analogs containing different types of fat. *J Texture Stud*. 1998;29:569–586.

58. Hennelly PJ, Dunne PG, O'Sullivan M, et al. Textural, rheological and microstructural properties of imitation cheese containing inulin. *J Food Engin*. 2006;75(3):388–395.

59. Dabour N, Kheadr E, Benhamou N, et al. Improvement of texture and structure of reduced-fat Cheddar cheese by exopolysaccharide-producing Lactococci. *J Dairy Sci*. 2006;89(1):95–110.

60. Juan B, Zamora A, Quintana F, et al. Effect of inulin additon on the sensorial properties of reduced-fat fresh cheese. *Int J Dairy Technol*. 2013;66(4):478–483.

61. Awad S, Hassan AN, Muthukumarappan K. Application of exopolysaccharide- producing cultures in reduced fat Cheddar cheese: Texture and melting properties. *J Dairy Sci*. 2005;88(12):4204–4213.

62. Lucey JM, Johnson ME, Horn DS. Perspectives on the basis of the rheology and texture properties of cheese. *J Dairy Sci*. 2003;86:2725–2743.

63. Szczesniak AS, Brandt MA, Friedman HH. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. *J Food Sci*. 1963;28(4):397–403.

64. Cardarelli HR, Buriti FC, de Castro IA, et al. Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially symbiotic petit-suisse cheese. *LWT- Food Sci Technol*. 2008;41:1037–1046.

65. Araujo EA, de Carvalho AF, Leandro ES, et al. Development of a symbiotic cottage cheese added with *Lactobacillus delbrueckii* UFV H2b20 and inulin. *J Funct Foods*. 2010 2(1):85-89.

66. http://www.naturalproductsinsider.com/articles/2003/03/how-sweet-it-is-functional-sweeteners.aspx