Impact of Probiotic and Synbiotic Supplementation on the Physicochemical, 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 28 days of storage at 7°C. The treatments design was as follows: C, thermophilic starter of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (standard control cheese); LR, lactobacillus rhamnosus 19070; LR+IN, Lb.rhamnosus with 2% inulin; BL, Bifidobacterium animalis subsp. lactis ATCC S5219; 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; wheyless domiati-like Cheese; Proteolysis

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]. A probiotic 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 by operational processes as well as by the product environmental and storage conditions [7]. Cheese has been considered as a better probiotic carrier compared to other 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 colon 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 as a 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 Domiati cheese 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 Bifidobacterium animalis subsp. lactis ATCCSD5219) and a prebiotic ingredient (Inulin) and evaluate the effects of supplementation on the physicochemical, texture and sensory characteristics of the cheese during 28 days refrigerated storage.

**Material and Methods**

**Bacterial strains**

Probiotic cultures used in the present study were Lactobacillus rhamnosus 19070 (DSM 26357); and Bifidobacterium animalis subsp. lactis ATCCSD5219 and a prebiotic ingredient (Inulin) and evaluate the effects of supplementation on the physicochemical, texture and sensory characteristics of the cheese during 28 days refrigerated storage.

**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 100 Kg 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 30 min then the mixture was cooled to 40°C and then CaCl₂ (0.02%) was added. **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 (Fabruine® Instant, Cosuera Group Warcoing, Belgium)   | 2             |
| Water                                                         | To complete to 100 Kg (≥71.1Kg) |

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. thermophilus and Lb. delbrueckii subsp. bulgaricus).

**Probiotic cheese treatments:** Standardized cheese-milk mixture was inoculated with freeze-dried Lb. rhamnosus 19070 (LR) or B. animalis subsp. Lactis SD5219 (BL) at concentration of ≥11 log₆ cfu/g.

**Synbiotic cheese treatments:** Standardized cheese-milk mixture was fortified with 2% inulin then inoculated with Lb. rhamnosus 19070(LR+IN) or B. animalis subsp. lactis SD5219 (BL+IN) at concentration of ≥11 log₆ 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 0.5 Kg 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 28 days. Cheese samples were analysed intervals of 1, 14 and 28 days.

**Chemical analysis**

Cheese samples of each treatment were withdrawn in duplicate from two sealed packs at every specific 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
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Hydration 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 and Fox [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 0.5 M 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 10 min. Samples were cooled at room temperature, centrifuged at 10,000 × g for 10 min to remove any insoluble material, and then loaded onto the gel using the discontinuous buffer system [28]. Electrophoresis was performed using Mini-Protean system (Bio-Rad, USA). The running buffer consisted of 0.192 M glycine, 0.025 M Tris and SDS (0.1%). Runs were carried out at 80 V 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 T.A.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 × 2 cm) 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: 50 points for flavor, 40 points for body/texture and 10 points for appearance with total perception of 100 points. The cheese considered to be accepted at total perception of 65 points.

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 & Torrie [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 28 days. 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. This decrease could be attributed to the contraction of curd and a 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 & Metin [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 increased 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 probiotic 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 6 g/100 g 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 metabolisation as an additional carbon and energy source.
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) | C | LR | BL | LR+IN | BL+IN |
|-----------|---------------------|---|----|----|-------|-------|
| Moisture (g/100g) | 1 | 72.68±0.03<sup>a</sup> | 71.73±0.23<sup>b</sup> | 72.70±0.30<sup>a</sup> | 73.05±0.05<sup>b</sup> | 74.84±0.17<sup>b</sup> |
| | 14 | 71.46±0.07<sup>b</sup> | 69.82±0.07<sup>a</sup> | 71.43±0.08<sup>b</sup> | 70.95±0.07<sup>b</sup> | 72.98±0.02<sup>b</sup> |
| | 28 | 65.60±0.10<sup>c</sup> | 64.90±0.10<sup>c</sup> | 64.75±0.25<sup>c</sup> | 68.90±0.10<sup>b</sup> | 70.90±0.10<sup>b</sup> |
| Titratable acidity (g/100g) | 1 | 0.26±0.01<sup>a</sup> | 0.30±0.03<sup>b</sup> | 0.23±0.04<sup>b</sup> | 0.52±0.03<sup>b</sup> | 0.45±0.04<sup>b</sup> |
| | 14 | 0.32±0.02<sup>b</sup> | 0.37±0.04<sup>ab</sup> | 0.35±0.02<sup>ab</sup> | 0.59±0.04<sup>b</sup> | 0.55±0.00<sup>b</sup> |
| | 28 | 0.40±0.05<sup>c</sup> | 0.39±0.03<sup>c</sup> | 0.45±0.04<sup>c</sup> | 0.85±0.03<sup>c</sup> | 0.67±0.01<sup>c</sup> |
| Fat (g/100g) | 1 | 3.00±0.00<sup>c</sup> | 3.00±0.00<sup>c</sup> | 3.00±0.00<sup>c</sup> | 3.00±0.00<sup>c</sup> | 3.00±0.00<sup>c</sup> |
| | 14 | 4.00±0.00<sup>c</sup> | 5.00±0.00<sup>c</sup> | 5.00±0.00<sup>c</sup> | 4.55±0.05<sup>c</sup> | 3.90±0.10<sup>c</sup> |
| | 28 | 5.00±0.00<sup>c</sup> | 5.95±0.05<sup>c</sup> | 7.00±0.00<sup>c</sup> | 5.55±0.05<sup>c</sup> | 6.00±0.00<sup>c</sup> |
| Salt (g/100g) | 1 | 2.06±0.05<sup>a</sup> | 2.04±0.03<sup>a</sup> | 2.03±0.03<sup>a</sup> | 2.01±0.05<sup>a</sup> | 1.98±0.03<sup>a</sup> |
| | 14 | 2.33±0.05<sup>c</sup> | 2.20±0.03<sup>c</sup> | 2.19±0.03<sup>c</sup> | 1.99±0.03<sup>c</sup> | 2.14±0.03<sup>c</sup> |
| | 28 | 2.38±0.05<sup>c</sup> | 2.33±0.05<sup>c</sup> | 2.24±0.03<sup>ab</sup> | 2.15±0.03<sup>ab</sup> | 2.27±0.06<sup>ab</sup> |
| Protein (g/100g) | 1 | 17.06±0.03<sup>a</sup> | 16.97±0.00<sup>b</sup> | 16.99±0.09<sup>b</sup> | 16.78±0.00<sup>b</sup> | 16.75±0.16<sup>b</sup> |
| | 14 | 17.93±0.03<sup>a</sup> | 19.57±0.03<sup>a</sup> | 17.33±0.03<sup>a</sup> | 18.58±0.25<sup>b</sup> | 18.25±0.19<sup>b</sup> |
| | 28 | 18.42±0.04<sup>c</sup> | 21.13±0.01<sup>c</sup> | 17.68±0.13<sup>c</sup> | 20.54±0.00<sup>c</sup> | 21.50±0.06<sup>c</sup> |
| pH | 1 | 6.47±0.04<sup>c</sup> | 6.15±0.01<sup>c</sup> | 6.54±0.15<sup>c</sup> | 4.88±0.01<sup>c</sup> | 5.47±0.01<sup>c</sup> |
| | 14 | 5.32±0.01<sup>c</sup> | 6.10±0.01<sup>c</sup> | 6.23±0.01<sup>c</sup> | 4.54±0.01<sup>c</sup> | 5.32±0.02<sup>c</sup> |
| | 28 | 5.35±0.01<sup>c</sup> | 6.08±0.01<sup>c</sup> | 6.11±0.01<sup>c</sup> | 4.84±0.01<sup>c</sup> | 5.29±0.02<sup>c</sup> |

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, DWS, St. thermophilus and Lb. delbrueckii subs. 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.

<sup>a</sup>Different letters for each column and for each type of cheese indicate significant differences (p < 0.05) among treatments at each interval ripening time.

<sup>b</sup>Different letters for each row and for each type of cheese indicate significant differences (p < 0.05) throughout ripening time.

Domiati cheese may be a potential synbiotic vector, in particular for the Lb. rhamnosus 19070 and B. lactis DDS219 strains under consideration here. Similar trend is observed in probiotic UF-Domiati cheese produced with Lb. rhamnosus ATCC7460 where 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 28 days 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,-casein was more intensive than that of β-casein. The α,-casein was hydrolyzed initially and other peptides with electrophoretic mobilities faster than α,-l casein. The differences in the accumulation of α,-l and α,-II were seen in all cheese treatments. However, the degradation of α,-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,-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.

### Table 3: Mean values for proteolysis parameters of wheyless Domiati-like cheese with probiotic and synbiotic supplementation during 28 days 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±0.01⁸        | 0.22±0.01⁸ | 0.20±0.01⁸ | 0.25±0.01⁸ | 0.23±0.01⁸ |
|               | 14                  | 0.25±0.02<sup>ns</sup> | 0.27±0.01<sup>ns</sup> | 0.25±0.00<sup>ns</sup> | 0.31±0.01<sup>ns</sup> | 0.26±0.01<sup>ns</sup> |
|               | 28                  | 0.30±0.01<sup>ns</sup> | 0.30±0.01<sup>ns</sup> | 0.29±0.00<sup>ns</sup> | 0.49±0.01<sup>ns</sup> | 0.34±0.02<sup>ns</sup> |
| Free Amino Acids (mg/g) | 1                   | 2.80±0.20<sup>ns</sup> | 2.45±0.25<sup>ns</sup> | 2.93±0.08<sup>ns</sup> | 2.93±0.08<sup>ns</sup> | 3.50±0.50<sup>ns</sup> |
|               | 14                  | 4.95±0.05<sup>ns</sup> | 5.60±0.40<sup>ns</sup> | 4.80±0.20<sup>ns</sup> | 8.00±0.50<sup>ns</sup> | 8.75±0.25<sup>ns</sup> |
|               | 28                  | 6.50±0.50<sup>ns</sup> | 7.50±0.50<sup>ns</sup> | 9.00±0.00<sup>ns</sup> | 11.50±0.50<sup>ns</sup> | 14.50±0.50<sup>ns</sup> |

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. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*); LR, cheese made with *Lb. rhamnosus* 19070; LR+IN, cheese made with *Lb. rhamnosus* 19070 and 2% inulin; BL, cheese made with *B. lactis* SDS219; BL+IN, cheese made with *B. lactis* SDS219 and 2% inulin.

<sup>ns</sup> Different letters for each column and for each type of cheese indicate significant differences (p < 0.05) among treatments at each interval ripening time.

<sup>**</sup> Different letters for each row and for each type of cheese indicate significant differences (p < 0.05) throughout ripening time.

![Electrophoretograms of the experimental cheeses at various stages of ripening are presented in Figure 1.](image-url)
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 (PrR) at pH 6.5 able to cleavage α-, β- and γ-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 4 weeks 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 probiotic 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 and Bochynska [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]. Henneelly 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 & Metin [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 weaken the elastic cheese matrix [50]. Advancing storage period until 14 days 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 4 week (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* UVF H2b20 and 8% medium chain Inulin. Probiotic bacteria and inulin did not change taste or texture of the cottage cheese after 15 days 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].

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Citation: Abd-Rabou HS, El-Ziney MG, Awad SM, El Sohaimy SA, Dabour NA (2016) Impact of Probiotic and Synbiotic Supplementation on the Physicochemical, Texture and Sensory Characteristics of Wheyless Domiati-Like Cheese. MOJ Food process Technol 3(3): 00074. DOI: 10.15406/mojfpt.2016.03.00074
### Table 4: Texture analysis of probiotic and synbiotic cheeses during cold storage.

| Storage Time (day) | Cheese Treatments | C       | LR      | BL      | LR+IN   | BL+IN   |
|--------------------|-------------------|---------|---------|---------|---------|---------|
|                    |                    | C       | LR      | BL      | LR+IN   | BL+IN   |
| Hardness (N)       |                   | 0       | 724.45±11.36<sup>b</sup> | 804.81±8.35<sup>c</sup> | 730.86±5.44<sup>cd</sup> | 1150.05±24.56<sup>ab</sup> | 1238.34±35.71<sup>ac</sup> |
|                    |                   | 14      | 815.3±11.58<sup>bc</sup> | 1035.99±35.65<sup>bc</sup> | 929.37±6.08<sup>b</sup> | 772.27±16.94<sup>bc</sup> | 806.56±53.03<sup>bc</sup> |
|                    |                   | 28      | 920.4±18.34<sup>ac</sup> | 1050.30±39.34<sup>ab</sup> | 1274.50±29.67<sup>aa</sup> | 546.58±13.16<sup>ca</sup> | 785.80±38.49<sup>cd</sup> |
| Adhesiveness (J)   |                   | 0       | 4.15±0.50<sup>bc</sup> | 11.02±1.14<sup>bc</sup> | 6.85±0.34<sup>ab</sup> | 5.17±0.10<sup>ab</sup> | 12.04±0.19<sup>ac</sup> |
|                    |                   | 14      | 61.28±4.76<sup>AB</sup> | 64.11±3.48<sup>AB</sup> | 41.80±3.82<sup>AB</sup> | 35.63±0.66<sup>AB</sup> | 37.84±2.72<sup>AB</sup> |
|                    |                   | 28      | 42.78±0.80<sup>BC</sup> | 52.94±5.43<sup>BC</sup> | 51.75±1.55<sup>BC</sup> | 13.68±0.60<sup>BC</sup> | 23.25±0.68<sup>BC</sup> |
| Springiness (mm)   |                   | 0       | 1.45±0.16<sup>AB</sup> | 0.95±0.06<sup>AB</sup> | 0.96±0.04<sup>AB</sup> | 0.73±0.02<sup>AB</sup> | 0.74±0.02<sup>AB</sup> |
|                    |                   | 14      | 0.96±0.07<sup>AB</sup> | 0.96±0.01<sup>AB</sup> | 0.98±0.02<sup>AB</sup> | 0.96±0.02<sup>AB</sup> | 0.95±0.02<sup>AB</sup> |
|                    |                   | 28      | 0.88±0.05<sup>AB</sup> | 0.92±0.02<sup>AB</sup> | 0.99±0.02<sup>AB</sup> | 0.94±0.01<sup>AB</sup> | 1.20±0.03<sup>AB</sup> |
| Cohesiveness (ratio) |                 | 0       | 0.84±0.07<sup>BC</sup> | 0.83±0.04<sup>BC</sup> | 0.86±0.02<sup>BC</sup> | 0.38±0.02<sup>BC</sup> | 0.13±0.01<sup>BC</sup> |
|                    |                   | 14      | 0.90±0.05<sup>BC</sup> | 0.89±0.07<sup>BC</sup> | 0.90±0.05<sup>BC</sup> | 0.85±0.01<sup>BC</sup> | 0.78±0.07<sup>BC</sup> |
|                    |                   | 28      | 0.90±0.07<sup>BC</sup> | 0.89±0.04<sup>BC</sup> | 0.84±0.04<sup>BC</sup> | 0.67±0.01<sup>BC</sup> | 0.95±0.04<sup>BC</sup> |
| Gumminess (N)      |                   | 0       | 607.91±46.49<sup>ab</sup> | 668.19±37.18<sup>ab</sup> | 628.69±18.53<sup>ab</sup> | 437.09±20.68<sup>ab</sup> | 161.36±16.51<sup>ab</sup> |
|                    |                   | 14      | 734.80±47.44<sup>abc</sup> | 924.82±92.99<sup>ab</sup> | 835.89±36.18<sup>ab</sup> | 656.53±17.14<sup>ab</sup> | 630.27±69.81<sup>ab</sup> |
|                    |                   | 28      | 830.70±23.97<sup>bc</sup> | 931.86±21.13<sup>bc</sup> | 1076.57±68.43<sup>bc</sup> | 366.06±5.66<sup>bc</sup> | 744.38±25.55<sup>bc</sup> |
| Chewiness (J)      |                   | 0       | 895.45±168.53<sup>ab</sup> | 630.24±3.67<sup>ab</sup> | 602.34±18.7<sup>ab</sup> | 318.44±8.22<sup>ab</sup> | 119.62±14.02<sup>ab</sup> |
|                    |                   | 14      | 709.08±87.24<sup>ab</sup> | 886.83±94.73<sup>ab</sup> | 820.55±53.24<sup>ab</sup> | 629.96±13.18<sup>ab</sup> | 601.08±76.34<sup>ab</sup> |
|                    |                   | 28      | 872.23±26.07<sup>ab</sup> | 859.98±11.67<sup>ab</sup> | 1063.74±52.20<sup>ab</sup> | 344.03±3.21<sup>ab</sup> | 893.25±53.84<sup>ab</sup> |

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. thermophilus* 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.

<sup>a-c</sup>Different letters for each column and for each type of cheese indicate significant differences (*p* < 0.05) among treatments at each interval ripening time.

<sup>a-b</sup>Different 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 |
|--------------|-----------------|------------|----------------|--------|------------------|
|              | C               | 8.10±0.43* | 8.10±0.46*     | 8.20±0.29* | 8.10±0.54*      |
|              | LR              | 8.50±0.22* | 8.40±0.45*     | 8.40±0.45* | 8.60±0.22*      |
|              | BL              | 8.00±0.52* | 8.90±0.46*     | 8.90±0.46* | 8.90±0.46*      |
|              | LR+IN           | 8.40±0.45* | 8.40±0.45*     | 8.40±0.45* | 8.40±0.45*      |
|              | BL+IN           | 8.60±0.22* | 8.60±0.22*     | 8.60±0.22* | 8.60±0.22*      |
|              | C               | 7.40±0.54* | 7.40±0.54*     | 7.40±0.54* | 7.40±0.54*      |
|              | LR              | 7.90±0.46* | 7.90±0.46*     | 7.90±0.46* | 7.90±0.46*      |
|              | BL              | 8.20±0.29* | 8.20±0.29*     | 8.20±0.29* | 8.20±0.29*      |
|              | LR+IN           | 9.10±0.23* | 9.10±0.23*     | 9.10±0.23* | 9.10±0.23*      |
|              | BL+IN           | 8.60±0.31* | 8.60±0.31*     | 8.60±0.31* | 8.60±0.31*      |
|              | C               | 5.80±0.50* | 5.80±0.50*     | 5.80±0.50* | 5.80±0.50*      |
|              | LR              | 6.70±0.50* | 6.70±0.50*     | 6.70±0.50* | 6.70±0.50*      |
|              | BL              | 8.00±0.26* | 8.00±0.26*     | 8.00±0.26* | 8.00±0.26*      |
|              | LR+IN           | 9.20±0.20* | 9.20±0.20*     | 9.20±0.20* | 9.20±0.20*      |
|              | BL+IN           | 9.40±0.16* | 9.40±0.16*     | 9.40±0.16* | 9.40±0.16*      |

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. thermophilus 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.

**Different letters for each column of each sensory attribute indicate significant differences (p < 0.05) among treatments throughout ripening time.**

### Conclusion

Wheyless Domiati-like cheese made with low concentration of fat and salt fortified with functional effective dosage of Lb. rhamnosus 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.

### Acknowledgment

The authors greatly appreciate the financial support from the Science and Technological Development Fund of Egypt (STDF); RSTDG project ID #12676.

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Citation: Abd-Rabou HS, El-Ziney MG, Awad SM, El-Sohaimy SA, Dabour NA (2016) Impact of Probiotic and Symbiotic Supplementation on the Physicochemical, Texture and Sensory Characteristics of Wheyless Domiati-Like Cheese. MOJ Food process Technol 3(3): 00074. DOI: 10.15406/mojfpt.2016.03.00074