



Properties of Low Fat Bio-frozen Yoghurt Fortified with Extract and Powder of Pomegranate Peel (*Punica ganatum L.*)



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The present study aimed at using Pomegranate peel powder and extract (PPPE) (*Punica ganatum L.*) as a source of dietary fiber, natural colorant, prebiotic, polyphenols and antioxidant activity in developing low fat frozen yoghurt. Pomegranate peel has been added in form of powder (added to yoghurt at a rate of 1.5%) and extract (added at a rate of 0, 25, 50, 75, and 100% as a substitute of water used in preparing the frozen yoghurts that given codes of T1, T2, T3, T4 and T5). Also, control frozen yoghurt sample was prepared without fat replacer (simplesse) as compared to other treatments. The results of frozen yoghurt revealed that the conversion of yoghurt into frozen mix caused increments in acidity, viscosity, total phenolic content, total flavonoids content, and antioxidant activity values. Moreover, the viable count of lactic acid bacteria strains increased in frozen yoghurt mix. While the pH value, specific gravity (sp.gr.) and freezing point have been decreased by adding PPPE. Also, adding PPPE along with the fat replacer (simplesse) led to calorie reduction. Additionally, adding PPPE into traditional frozen yoghurt or bio-frozen yoghurt increased mineral content (Fe, Zn, P, and K). The freezing and hardening processes decreased the viable counts of LAB strains keeping its therapeutic level (10^6 cfu/g). Thus, T5 sample had the highest viable counts over storage time. The final frozen yoghurt was characterized with higher overrun, stronger melting resistance and the highest value of hardness as the PPPE were replaced and when it was made using ordinary yoghurt *versus* the bio-yoghurt. The quality of all sensory criteria of body and texture, flavour as well as overall quality of the PPPE-replaced frozen yoghurt was evidently better than that of the control, which was made with fat replacement. Scores of sensory attributes increased with adding PPPE up to 100%.

Keywords: Frozen yoghurt and quality attributes, Antioxidants and polyphenols, Pomegranate peel powder and extract

Introduction

Healthy foods have met with marked success in the last two decade which they termed as functional foods. Despite the growing popularity of functional foods, scientists have identified few specific substances that proved to reduce the risk of disease. Out of these substances, photochemical exhibited several healthy effects including anti-cancer effects, lowering blood cholesterol, reducing risk of cardiovascular diseases, anti-hypertension, improvement of renal function and diabetes (Arvantoyamis & Houwelingen, 2005).

Dietary fat has been shown to be associated with an increased risk of obesity, atherosclerosis, elevated blood pressure and cardiovascular disease (Steijns, 2008). This has created a high demand for low-fat/fat free foods with similar sensory properties with conventional full fat products. Decreasing the fat content in yoghurt resulted in a weak body and texture product, increased the syneresis and decreased viscosity (Houzé et al., 2005). Therefore, production of low fat yoghurt requires careful control of textural attributes.

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Received: 9/2/2021; accepted: 8/8/2021

DOI: 10.21608/ejfs.2021.62253.1095

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Iron deficiency anemia is still the most prevalent nutritional problem, which affects 30% of the world's population (Juneja et al., 2004). It is estimated that up to half of all anemias are caused by dietary iron deficiency. Dairy products are widely consumed, providing high quality proteins, vitamins, and minerals except iron. Lack of iron in dairy products decreases the iron density of diets when the proportion of dairy products in the diets increases. So, it would be beneficial to fortify dairy products with iron to increase the iron density of the people who consume large amounts of dairy products. During manufacture of frozen yoghurt the variation and quality of milk solid not fat source have an important effect on the physico-chemicals properties of frozen yoghurt mixes and resultant product.

Pomegranate peel (PP) can be considered as a very rich natural material of antioxidants, minerals especially (iron and zinc) as well as natural colour. Milk and milk products are very poor sources of zinc and iron and therefore its fortification with pomegranate peel would be very useful. Salama (2012) studied the production and properties of pomegranate flavored ice milk can be produced as functional food by adding pomegranate juice in the base blend of ice milk. Pomegranate peels have attracted a great attention for their nutritional and health benefits in the last year (Mehder, 2013). It is considered as a nutritive antioxidant rich by-product, easily available after production of pomegranate products (Sayed-Ahmed, 2014). The pomegranate peel makes up to 50% of the fruit weight (Viuda-Martos et al., 2010b), whereas it can be easily converted in to powder form (PPP) to be used as nutritive supplementation of some foods and dairy products. Arjmand (2011) and Khalil (2013) found that addition of concentrated pomegranate peel to yoghurt after fermentation increased its total phenolic compounds, antioxidant activity, decreased the fracture stress and firmness as compared with control one, but still considered acceptable. The pomegranate peel powder (PPP) contain proteins much higher leucine, lysine, aromatic amino acid (phenylalanine and tyrosine), valine and threonine contents and slightly lower sulphur containing amino acids (methionine and cysteine) and isoleucine than the reference protein pattern (Rowashed et al., 2013). Therefore, the incorporation of PPP into the foodstuff can offer great economic and nutritive values in food technology and human nutrition (Mehder, 2013). Also, pomegranate peels is a

good source of bioactive compounds such as polyphenols, carotenoids, vitamins, enzymes and dietary fibers (Ashoush et al., 2013) which can play an important role in prevention of diseases. Ashoush et al. (2013) mentioned that PPP is a good source of total phenolic compounds and has a great free radical scavenging activity. They concluded that, the mixture of PPP and whey powder could be used as natural antioxidants to enhance the antioxidant properties of functional food. Pomegranate peel powder (PPP) can be used also in the food industry as a potential ingredient to develop functional foods and to promote health benefits (Gullon et al., 2015). Moreover, PPP can exhibit antibacterial activity (Khan & Haneef, 2011) against *E. coli*, *P. aeruginosa* and *S. aureus*. Sakr et al. (2018) stated that, improving the quality of stirred yoghurt by using pomegranate peel powder (PPP) 1.5%, with good of 1.5% PPP to yoghurt milk led to significant increase in the flavour score. It could be concluded, that PPP can be used as ingredient in making functional yoghurt with improved health benefits. Also, using of Pomegranate peel powdered and the extract gave the product expect improvement physicochemical properties and sensory properties special flavour and natural colour.

Frozen yoghurt was introduced in New England in the late 1970s as a soft serve dessert by Hoodunder the name *Frogurt*. In 1978, Brigham's, a Boston-based ice cream, candy and sandwich chain, developed and introduced the first packaged frozen yoghurt under the name *Humphreez Yogurt*. Frozen yoghurt is a frozen dessert containing yoghurt or other dairy products. It is slightly tarter than ice cream, as well as lower in fat due to the use of milk instead of cream (Chandan & Shahani, 1993). Frozen yoghurt has become a delicious alternative to ice cream which contains both the nutritional value of yoghurt and the refreshing taste of ice cream. Moreover, the digestibility of yoghurt is better than the ice cream. Further the acceptability of the frozen yoghurt will be good when compared with the yoghurt. Conversion of plain yoghurt to frozen product and hardening at -20°C could improve its keeping quality and acceptability (Mashayekh & Brown, 1992). The growing interest of consumers in therapeutic products has led to the incorporation of probiotic cultures into ice cream to result in dietetic ice cream. Some studies have demonstrated that it is possible to produce ice cream type frozen yoghurt using different ratios of fermented mixes (Davidson et al., 2000;

Alamprese et al., 2005). Fermented ice cream products are considered a healthy challenge to the ice cream industry which emphasizes the ways of avoiding or masking too strong a yoghurt flavour, and the use of other cultured milk products as a base for healthy ice cream products. Considering that, probiotic *Bifidobacterium* strains in fermented milk have anti-inflammatory effects against ulcerative colitis (Imaoka et al., 2008). Many microorganisms have been utilized in the manufacture of fermented milk products. Probiotic products are those containing single or mixed probiotic microorganisms. Fuller (1989) described these microorganisms as "living microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition". The strains that currently used in probiotic include: *Lactobacillus spp.*, *Bifidobacterium spp.* and other species (O'Sullivan et al., 1992).

For that in view, the present study was aimed to study the manufacture possibility of reduced fat yoghurt and hence frozen yoghurt *via* proportional fat and pomegranate peel powdered and extract (PPPE) in relation to dietetical, bacterial, biochemical, physical, rheological and organoleptical attributes of yoghurt and frozen yoghurt made using milk inoculated whether with ordinary or probiotic bacterial starter culture (BSC).

Materials and Methods

Materials

Skimmed milk powder (SMP) (96.2%TS, 0.8% fat and 33, 4% protein) was obtained from Fonterra Company (Auckland, New Zealand). Cream (50% fat) was obtained from the Faculty of Agriculture, Cairo University. Pomegranate fruits (*Punica granatum* L.) were obtained from local market. Commercial grade granulated cane sugar produced by Sugar (sucrose) and Integrated Industries Co. at Hawamdia was obtained from the local market. Folin-Ciocalteu's phenol reagent and gallic acid monohydrate were purchased from Fluka (Madrid, Spain), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, (Germany). Simplese 100® (microparticulated whey protein concentrate) made by CPKelco, Penrhyn Road, Knowsley Business Park, Denmark, LOT No: 4G4197F, was obtained from the local market at Cairo. Stabilizer, sodium carboxy methylcellulose (CMC) and all-other analytical grade chemicals used were purchased from El-Gomhouria Co., Cairo, Egypt. Vanillin (chem. rein 100%) made

by Boehringer Mannheim GMB, Germany was obtained from the local market. Probiotic starter culture (FD-DVS ABT-2-Probio-Tec) containing [*Streptococcus thermophilus* (*Str. thermophilus*) ST-20Y, *Lactobacillus acidophilus* (*Lb. acidophilus*) LA-5 and *Bifidobacterium bifidum* (*Bifidobacterium* sp.) BB-12] and freeze dried conventional yoghurt starter culture (FD-DVS YC-X11-YO-Flex) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. delbrueckii* subsp. *bulgaricus*) strains (1:1) were obtained from Chr. Hansen A/S, DK-2970 Horsholm, laboratories, (Denmark). Starter cultures were in freeze-dried direct-to-vat set (DVS) form and stored at -18 °C until used. ABT-2 was added to the milk after preliminary incubation at 37±1 °C for 18hr.

Experimental procedures

Cream separation

Cream was mechanically separated from cow's milk at 45 °C. The fat content of the resultant cream was adjusted to 30%.

Preparation of pomegranate peel powdered and extract (PPPE)

Pomegranate fruits were washed by tap water and then air dried. The outer edible portion of the fruit was removed from the seed and then crushed. The peel was powdered by milling in a laboratory mill type (Kenwood major titanium, Japan), than freezing precipitated pomegranate peel powdered at -20°C for 24hr, and dried for 24 hr in freeze dryer (Martin Christ, 8891, type317, Germany) at -30 °C. The dried pomegranate peel powdered was dry milled, and then sieved through a 60 mesh sieve (250 microns). The resultant powdered was packed in polyethylene bags and stored at 5±1°C in a refrigerator until used. Preparing pomegranate peel extract taking 1 kilogram of ground pomegranate peel with 4 kilogram of tap water, then put it on the temperature to a boiling point, after which the filtration is taken, and the filtrate is taken and used instead of water. Replace the water with pomegranate peel powder extract (PPPE) with 0% (T₁), 25% (T₂), 50% (T₃), 75% (T₄) and 100% (T₅) of water. The composition of pomegranate peel powder (PPP) and skim milk powder (SMP) used in frozen yoghurt are presented in Table 1.

Preparation of bacterial starter culture

Lyophilized bacterial cultures were separately inoculated in previously autoclaved (121°C/15 min.) skimmed milk and incubated at 42°C for the YC-X11 or at 37°C for the ABT-2 type. The complete curdling occurred within 8h. Starter cultures were freshly used.

TABLE 1. Composition of pomegranate peel powder (PPP) and skim milk powder (SMP) used in preparation of frozen yoghurt.

Property	Skim milk powder (SMP)	Cream	Pomegranate peel powder (PPP)
Total solids (TS) %	96.2±0.14	54.00±0.21	87.76±0.17
Fat %	0.80±0.06	50.00±0.16	1.30±0.11
Protein %	33.4±0.24	1.90±0.12	5.20±0.14
Ash %	7.90±0.28	0.60±0.06	1.90±0.06
Crude fiber %	Free	Free	3.30±0.16
pH values	6.60±0.25*	6.62±0.23*	7.10±0.20
carbohydrate**	54.10±0.20	1.5±0.20	76.06±0.33
Total phenols mg GAE/100g dw.	ND	ND	695.4
Total flavonoids mg quartine/100g dw	ND	ND	112.6
Antioxidant activity (DPPH) %	ND	ND	76.85
Fe (mg/100g)	ND	ND	3.99
Zn (mg/100g)	ND	ND	2.50
P (mg/100g)	ND	ND	320
K (mg/100g)	ND	ND	368
Ca (mg/100g)	ND	ND	18.80

*: In 20% solution

**: Carbohydrate content of pomegranate peel powder (PPP) was determined by difference.

DPPH: 2,2 diphenyl-1-picrichydrazyl.

Values are means of three replicates ± SD

ND: Not determined

Preparation of fermented milk

Skimmed cow's milk powdered (SMP) (96.2% TS) was diluted to 12%TS either by tap water, pomegranate peel powder (PPP) (1.5% for all samples), pomegranate peel extract (PPPE) or by their mixture (1:1) to reduce the tap water content by 0%, 25,50,75 or 100% respectively. Fat content was adjusted to 3% (the control) using the resultant fresh cream (30% fat). Conversely, Simplese 100® was added to full fat mimic at the level of 3%, respectively. That was expressed on the base of each part of fat would be replaced with one part of Simplese 100® as recommended by Summerkamp & Hesser (1990) and Kirby (1994). Then milk preparations were heat treated at 85°C for 5 min. followed by temperature adjustment to 42°C. Milk preparations were converted into yoghurt according to the protocol proposed by Tamime & Robinson (1999) with adopting the manufacture conditions enacted by EOSQ (2010), where they were inoculated with 2% of freshly activated YC-X11 or ABT-2, filled into one kg polystyrene containers, covered, and incubated at the same temperature degree (42°C) until complete coagulation (through about 3 hr).

Thereafter, the small containers were transferred to the refrigerator (5±1°C), where they were kept to the next day for frozen yoghurt making.

Preparation of frozen yoghurt

The main manufacturing procedure was carried out as described by Bradley & Winder (1977) and Arbuckle (1986). Frozen-yoghurt base mix 1.5% pomegranate peel powdered (PPP) was added to each, (the control) was prepared to contain 3% fat, 12% milk solids not fat (MSNF), 15% sucrose, and 2.5% CMC using the same dairy ingredients mentioned before, namely, Skimmed cow's milk powdered (SMP) (96.2% TS), and fresh cream (30% fat). Defatted milk preparations, those were made by diluting Skimmed cow's milk powdered (SMP) (96.2% TS) to 12% TS either using tap water for T₁, pomegranate peel powdered extract (PPPE) for T₅ or by their mixture (1:1) for T₃, to reduce the tap water with pomegranate peel powdered extract (PPPE), in order, by T₁0%, T₂25%, T₃50%, T₄75% or T₅100%. Then 3% of simplese 100® was added to each one for full fat mimicking. Every milk preparation was divided into six portions. Mixes of frozen yoghurt

portions from the aforementioned ingredients with quantities calculated as shown in Table 2. The 1st and 2nd portions were exposed, in order, for ordinary- and bio-yoghurts as before mentioned. Pomegranate peel powdered extract (PPPE) was used to replace 0, 25, 50, 75 and 100% of tap water added in the mix as shown in Table 2. All complementary mixes were stabilized with 2.5% CMC, heat treated at 85±1°C for 5 min. then rapidly cooled to 5±1°C and aged at this temperature for the 1st day. Thereafter, each aged complementary mix was mixed with an equal part of the analogous cold ordinary- or bio-yoghurt. All frozen yoghurt mixes were flavoured with suitable amounts of 0.01% vanilla powder was directly to the mixes before frozen in horizontal batch freezer and frozen in an experimental ice cream batch freezer at -18°C. This machine (Taylor Co.USA Model, 103) is automatically controlled to stop whipping when ice cream was frozen. The resultant product was filled into PVC cups (cap, 120 mL) covered and hardened in a deep freezer at -26°C for 24h before analyses. All

Analytical methods

Dry matter, fat, total nitrogen and ash contents as well as titratable acidity (TA) % were determined according to the methods outlined in AOAC (2007). Total carbohydrates of pomegranate peel powdered extract (PPPE), the mix and the final frozen yoghurt were calculated by differences as described by Ceirwyn (1995) using the following formula: Total carbohydrates

% =100- (% fat+ % protein + % fiber + % ash + % moisture). Acetaldehyde and diacetyl contents (µ mol/g) were determined according to Lees & Jago (1970) respectively. Total phenols were estimated by the Folin-Ciocalteu method reported by Elfalleh et al. (2009). The amount of total flavonoids was according to the method of Nasri et al. (2011). The Antioxidants activity (DPPH) (2,2 diphenyl-1- picrichydrazyl) radical scavenging activity of methanolic extracts was determined according to the method reported by Olivera et al. (2006) and Okonogi et al. (2007).

The pH value was measured electrometrically using LabpH meter with a glass electrode,(HI 93 1400, Hanna instruments) pH meter. The specific gravity of the mix and the final frozen yoghurt was determined according to Winton (1958)at 20°C. Freezing point of frozen yoghurt was measured as described in FAO Report (1977) using a Digital Probe Thermometer (-40 to +200°C) with probe 125 mm (acc. to HACCP), TFA Dostmann, GmbH & Co. KG, Wertheim, Germany. Mineral contents were determined as described by Hankinson (1975) using Atomic absorption spectrophotometer NO.3300 (PerkinElmer, US instrument Division Norwalk, CT, USA.). The overrun percent was calculated as mentioned by Marshall et al. (2003)from the figures obtained for the weight of the mix and the resultant frozen yoghurt. The melting resistance of the frozen yoghurt was determined as described by Segall & Goff (2002).

TABLE 2. Formulation of one kg frozen yoghurt containing different ratios of pomegranate peel powdered extract (PPPE)

Ingredients	Level of pomegranate peel powdered (PPP) (1.5%)					
	(control)	Level of fat replacement with simplese100® 100% Level of tap water replacement with pomegranate peel powdered extract (PPPE)				
		T ₁ (0%)	T ₂ (25%)	T ₃ (50%)	T ₄ (75%)	T ₅ (100%)
Skim milk powder (SMP)(g)	125.8	125.8	125.8	125.8	125.8	125.8
Cream (g) (30% fat)	66.6	-	-	-	-	-
Simplese100® (g)	-	30	30	30	30	30
sugar (g)	150	150	150	150	150	150
CMC (g)	2.5	2.5	2.5	2.5	2.5	2.5
pomegranate peel powdered extract (PPPE)(g)	-	-	172.925	345.85	518.775	691.7
Water	655.1	691.7	518.775	345.85	172.925	-
Total	1000	1000	1000	1000	1000	1000

Vanilla was added at 0.1g/kg.

Determination of viscosity was determined using a coaxial rotational viscometer (Rheotest II, Medingen, Germany) at shear rates ranging from 3.0 to 1312 s⁻¹. The measuring device (S1) was used with a sample volume of 30 ml per run. All samples were adjusted to 20 ±1°C before loading in the viscometer device. Apparent viscosity was calculated at shear rate of 48.6 s⁻¹. (RPM=20, Spindle=21). Hardness of frozen yoghurt samples were examined by adapting the method recommended by Bourne & Comstock (1986).

The count of *Lb. delbrueckii* ssp. *bulgaricus* was enumerated using MRS agar medium as described by Gueimonde et al. (2003). Culture was incubated anaerobically for 2 days at 37°C. Whilst, *Str. thermophilus*, *Lb. acidophilus* and *Bifidobacterium* sp. were enumerated using M17 agar, MRS-salicin agar and MRS agar media, respectively after the incubation at 37°C for 72 hr as in Dave & Shah (1996). The count was expressed as colony forming units (10⁶cfu/g) of product.

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 & Robinson (1999) using the yoghurt evaluation scheme III approved by the American Dairy Science Association.

The calorie value was calculated using the modified factors of 4.27, 8.79 and 3.86 kcal/g of milk protein, fat and carbohydrate (lactose), respectively. These figures have been reported by the Food and Agriculture Organization (FAO) of the United Nation on May 1947 (Lambert, 1975). The modified factor for simplese was 1¹/₃ kcal/g (Summerkamp & Hesser, 1990).

Statistical analysis all the data obtained were exposed to proper statistical analysis according to statistical analyses system user's guide (SAS, 1996).

Results and Discussion

Gross composition of yoghurt

The results of Table 3 indicated that, neither full fat nor water replacement with pomegranate peel powdered extract (PPPE) led to any significant influence on the dry matter (DM) content of yoghurt. It is worthy to mention that, all obtained DM contents in this study are, in general, in coincidence with the Egyptian Organization for Standards and Quality (EOSQ, 2010). Similar observations were reported by Fayed et al. (2011a, b). Logically, the fat content was not significantly affected by the water replacement

with pomegranate peel powdered extract (PPPE). While, the fat mimic with simplese caused a significant increase in the protein content. This phenomenon is; indeed, because of simplese is protein origin. Moreover, the content was not significantly affected by the fat replacement. That could be attributed to the fat substitution with simplese was executed part to part. Needless to add that, the partial replacement of water with pomegranate peel powdered extract (PPPE) resulted logically in significant reduction in the water content of the final products. Further, neither full fat and/nor partial water replacement with pomegranate peel powdered extract (PPPE) led to increase significant changes in the ash content of yoghurt. The kind of bacterial starter culture (BSC) used did not cause any significant differences in all foregoing criteria.

Lactic acid bacteria (LAB) population of yoghurt

As a result of the fat elimination of yoghurt via its replacement, the bacterial growth of all strains studied was significantly promoted as indicated evidently from their count (Table 3). There is a reverse relationship between the fat content of milk and the population as well as activity of lactic acid bacteria (LAB). Similar trends were reported by Hefny et al. (1995) and Farahat (1999). Another reason for this phenomenon could be explained with regard to the kind of fat mimetic used viz. simplese which is, indeed, hydrated whey and egg proteins, i.e. simplese is distinguished with sulphur amino acids (Benz, 1992; Kirby, 1994). That may be a considerable reason for explain any the bacterial stimulating observed in the simplese containing yoghurts.

Radke-Mitchell & Sandine (1984) reported that, both species exhibit a symbiotic relationship during the processing of yoghurt, with the ratio between the species changing constantly. During fermentation *Str. thermophilus* grows quickly at first, utilizing essential amino acids produced by *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*, in return, produces lactic acid, which reduces the pH to an optimal level for growth of *Lb. delbrueckii* ssp. *bulgaricus* the lactic acid produced, and lesser amounts of formic acid stimulate the growth of *Lb. delbrueckii* ssp. *bulgaricus* (Gurr, 1987). Similarly, Dave & Shah (1998) found that, the fortification of yoghurt with 2% whey protein concentrate supported the growth of *Str. thermophilus* and multiplication of this organism was faster, which could have been the reason for the shorter incubation time needed to reach pH of 4.5 for samples.

Moreover, the obtained results demonstrated also that, the partial substitution of water content with pomegranate peel powdered extract (PPPE) was

associated with significant increase in the count of all strains of bacterial starter culture (BSC) applied whether for ordinary or bio-yoghurt production. Suskovic et al. (2001) elucidated that, besides its bacterial protective effect, pomegranate peel powdered extract (PPPE) acts as prebiotic agent and converts bio-yoghurt to be synbiotic product.

Moreover, the count of *Str. thermophilus* in the ordinary yoghurt was always significantly higher than those counted in the bio-yoghurt. Similar findings were reported by Gab-Alla (2011).

It could be noticed also that, the *Str. thermophilus* count is always higher than that of *Lb. delbrueckii* ssp. *bulgaricus* or *Lb. acidophilus* and *Bifidobacterium* sp. regardless the kind of BSC used. Rasic & Kurmann (1978) reviewed that *Str. thermophilus* grows faster at the beginning of lactic acid fermentation, outnumbering *Lb. delbrueckii* ssp. *bulgaricus* by 3 or 4 times often the 1st hr. The obtained results are in complete agreement with those found by Fayed et al. (2011a,b).

Generally, it is worthy to mention that, each bacterial count of all yoghurt treatments, in relation either to the full fat and/or partial water replacement with pomegranate peel powdered extract (PPPE), stilled conforming the figures provided by Codex Alimentarius Commission of FAO/WHO (2002) which approved an international standard as established a minimum of 10⁷cfu/g for the starter cultures of fermented milks and a minimum of 10⁶cfu/g for specific starter bacteria for which a claim is made for specific microorganism that has been added as supplement.

The letters before comma possess the factor of fat replacement. While those after comma possess the factors of water replacement with pomegranate peel powdered extract (PPPE), kind of bacterial starter culture and conversion into frozen mix, respectively. The means with the same letter at any position did not significantly differ ($P > 0.05$).

Biochemical properties of yoghurt

Titrateable acidity and pH value of yoghurt

The results of Table 3 showed also that, the yoghurt possessed significantly higher titrateable acidity (TA) % and hence lower pH value when the fat was replaced. These trends are totally harmonious with the bacterial growth previously recorded in Table 3. Moreover, there was positive relationship between the partial water replacement with pomegranate peel powdered extract (PPPE) and the acidity in yoghurt when pomegranate peel powdered extract (PPPE) was used as water substitute. Whereas, besides to the relatively acidic effect of pomegranate peel powdered extract (PPPE) added itself, it

had a bacterial stimulating action as previously mentioned with regard to the LAB population in yoghurt and as reported by Suskovic et al. (2001). On the other hand, the pH value lowering was reversely dependent on the quantity of acid produced. Furthermore, the ordinary yoghurt was distinguished with TA% higher and hence pH value lower than there found in bio-yoghurt. Similar findings were reported by Hussein et al. (2008) and Gab-Alla (2011).

Acetaldehyde and diacetyl contents of yoghurt

The obtained results of Table 3 confirmed also that, both acetaldehyde (AC) and diacetyl (DA) contents significantly, increased when the fat replacement with simplese. These trends became logic with regard to the population of LAB counted in yoghurt regardless their species.

At the parallel time the water replacement with pomegranate peel powdered extract (PPPE) was associated with significant increment in the level of AC or DA of yoghurt. These trends are completely harmonious with the acid production in yoghurt. Rasic & Kurmann (1978) described that, the rate of AC production is highly positively dependent on acidity level. Similar observations were reported by El-Nawawy et al. (2011).

Concerning the production of AC and DC in relation to the kind of BSC used for yoghurt making data reveal that, the ordinary yoghurt was significantly distinguished with AC and DA contents higher than those of bio-yoghurt. These results are in agreement with those found by Hussein et al. (2008) and Fayed et al. (2011a,b).

Antioxidant activity of yoghurt

The obtained results of Table 3 incorporation of pomegranate peel powdered extract (PPPE) showed significant higher values of antioxidant activity all simples. Increasing the ratio of substitution with pomegranate peel powdered extract (PPPE) of yoghurt increased the values of antioxidant activity in treated simples. These results due to higher antioxidant of pomegranate peel powdered extract (PPPE). Similar observations were reported by Sakr et al. (2018).

Rheological properties of yoghurt

Apparent viscosity (AV) of yoghurt was significantly increased due to the fat replacement (Table 3). The relatively increased level of protein and TA contents associated with fat replacement with simplese may cause such significant increments. Prentice (1992) confirmed that whichever method used, the increase of protein content is the principle factor influencing the texture of yoghurt. Similar findings were reported by El-Nawawy et al. (2011).

TABLE 3. General properties of yoghurt made using ordinary (YC-X11) or bio-yoghurt (ABT-2) as affected by fat and/or water replacement with of pomegranate peel powdered extract (PPPE).

Property	Level of pomegranate peel powdered (PPP) (1.5%)											
	(control)		Level of fat replacement with simplese 100 [®] 100%		Level of tap water replacement with pomegranate peel powdered extract (PPPE)		T ₃ (50%)		T ₄ (75%)		T ₅ (100%)	
	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2
Dry matter (%)	15.87 ^{aaa}	15.82 ^{aaa}	15.91 ^{aaa}	15.86 ^{aaa}	15.92 ^{aaa}	15.92 ^{aaa}	15.96 ^{aaa}	15.92 ^{aaa}	15.97 ^{aaa}	15.97 ^{aaa}	15.99 ^{aaa}	15.97 ^{aaa}
Fat (%)	3.5	3.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Protein (%) (T.NX6.38)	5.37 ^{baa}	5.35 ^{baa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}	8.000 ^{aaa}
Simplese added (%)	0.0	0.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
carbohydrate* (%)	5.922 ^{cba}	5.892 ^{bcb}	6.831 ^{bba}	6.781 ^{cab}	6.831 ^{bba}	6.791 ^{bab}	6.864 ^{aba}	6.824 ^{abb}	6.874 ^{aaa}	6.850 ^{aab}	6.840 ^{aca}	6.820 ^{acb}
Ash (%)	1.078 ^{baa}	1.078 ^{baa}	1.079 ^{baa}	1.079 ^{baa}	1.089 ^{aaa}	1.089 ^{aaa}	1.096 ^{aaa}	1.096 ^{aaa}	1.100 ^{aaa}	1.100 ^{aaa}	1.150 ^{aaa}	1.150 ^{aaa}
Titratable acidity (%) (as lactic acid)	0.86 ^{bca}	0.77 ^{bcb}	0.89 ^{aca}	0.79 ^{acb}	0.92 ^{aba}	0.81 ^{abb}	0.95 ^{aba}	0.85 ^{abb}	0.97 ^{aaa}	0.89 ^{aab}	0.99 ^{aaa}	0.91 ^{aab}
PH value	4.60 ^{aab}	4.91 ^{aaa}	4.41 ^{bab}	4.83 ^{baa}	4.35 ^{bbb}	4.81 ^{bba}	4.26 ^{bcb}	4.80 ^{bca}	4.20 ^{bcb}	4.74 ^{bca}	4.15 ^{ccb}	4.69 ^{cba}
Acetaldehyde (µmol/g)	350.7 ^{bca}	255.0 ^{bcb}	412.1 ^{cba}	321.2 ^{cab}	415.6 ^{bba}	330.3 ^{bab}	418.9 ^{aca}	342.6 ^{acb}	422.6 ^{aba}	365.5 ^{abb}	426.2 ^{aaa}	382.1 ^{aab}
Diacyl (µmol/g)	18.62 ^{cba}	17.20 ^{cab}	19.82 ^{bba}	17.36 ^{bab}	20.08 ^{aca}	17.38 ^{acb}	20.20 ^{aba}	17.40 ^{abb}	20.35 ^{aaba}	17.45 ^{aabb}	20.52 ^{aaa}	17.47 ^{aab}
Antioxidant activity (DPPH) (%)	3.11 ^{bca}	3.10 ^{bcb}	3.12 ^{cba}	3.10 ^{cab}	5.28 ^{bba}	5.26 ^{bab}	7.88 ^{aca}	7.87 ^{acb}	11.10 ^{aba}	11.09 ^{abb}	13.85 ^{aaa}	13.84 ^{aab}
Apparent viscosity (centipoises)	43.60 ^{bcaa}	15.96 ^{bcbcb}	68.15 ^{acaaa}	35.29 ^{acbbb}	78.30 ^{abaaa}	38.58 ^{abbbb}	89.41 ^{aaaca}	41.31 ^{aaacb}	98.50 ^{aabaa}	45.44 ^{aabb}	115.09 ^{aaaa}	50.50 ^{aaab}
<i>Streptococcus thermophilus</i>	53 ^{bca}	39 ^{bcb}	57 ^{caa}	44 ^{cab}	59 ^{baa}	48 ^{bab}	61 ^{aca}	50 ^{acb}	64 ^{aba}	55 ^{abb}	66 ^{aaa}	59 ^{aab}
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	55 ^{cc}	ND	59 ^{cb}	ND	62 ^{bcb}	ND	64 ^{ca}	ND	66 ^{ba}	ND	68 ^{aa}	ND
<i>Lactobacillus acidophilus</i>	ND	32 ^{cc}	ND	33 ^{cb}	ND	34 ^{bbb}	ND	35 ^{ca}	ND	37 ^{ba}	ND	39 ^{aa}
<i>Bifidobacterium</i> sp.	ND	28 ^{cc}	ND	28 ^{cb}	ND	29 ^{bb}	ND	30 ^{ca}	ND	32 ^{ba}	ND	34 ^{aa}

*Calculated by the difference. TN: Total nitrogen. NA Not added.

ND: Not determined. DPPH: 2,2 diphenyl-1-picrylhydrazyl.

All bacterial counts are colony forming unit x 10⁷cfu/g.

Beside the relatively acidic effect of pomegranate peel powdered extract (PPPE) itself its bacterial stimulating action for growth and acid production was reported by Suskovic et al. (2001). Moreover, data showed also that, the ordinary yoghurt possessed AV higher than those appeared in the bio-yoghurt. That could be positively depending on the difference in TA%. Similar observations were reported by Hussein et al. (2008) and Fayed et al. (2011a,b).

Gross composition of frozen yoghurt mix

The DM % of frozen yoghurt mix was not significantly influenced either by the fat and/or water replacement with pomegranate peel powdered extract (PPPE) (Table 4). Moreover, the use of simplese as fat replacer caused a significant in cement in the protein content of fat-replaced frozen yoghurt mix, while the same effect did not occurred when the water was also replaced at any level with pomegranate peel powdered extract (PPPE) in combination. Neither the fat nor water replacement with pomegranate peel powdered extract (PPPE) resulted in any significant differences in the ash content of frozen yoghurt mix. The type of bacterial starter culture (BSC) applied did not cause any significant differences in all compositional criteria of frozen yoghurt mix. These phenomena were previously observed in yoghurt.

Dietetic aspects of frozen yoghurt mix

The calorific data indicated the achievement of among the aim planned in this study toward fat reduction, which carried out either by fat (8.79 Kcal/g) replacement with simplese (1 $\frac{1}{3}$ Kcal/g) respectively. As seemed from the caloric value of resultant frozen yoghurt mix, considerable reduction was recorded to be significantly associated with fat replacement (Table 4). These results are in accordance to those reported by Farahat (1999). The reduction in caloric value was positively proportionally correlated to the level of fat replacement. In this respect the kind of BSC was ineffective on the criterion. Tharp & Gottemoller (1990) reported that, to qualify as reduced caloric a product must provide at least one-third fewer calories than a reference product. *Viz.* in this own study such stipulation could not be accomplished unless by full fat elimination. But recently, definitions for nutrient content claims on food labels published by Insel et al. (2011) appeared that, the reduced-calorie food is that nutritionally altered product containing at least 25% less of a nutrient or of calories than the regular or reference product.

Lactic acid bacteria population of frozen yoghurt mix

As beginning, significant increments in the counts of all strains were enumerated in frozen yoghurt mix in correspondence with the fat replacement (Table 4). Moreover, the presence of pomegranate peel powdered extract (PPPE) in combination with water replacer at any level acted significantly as growth promoter of all strains. For *Str. thermophilus*, its count was raised as the portion of pomegranate peel powdered extract (PPPE) added was increased. While the increase in the water replacement from 25 to 50 and 75 to 100% with pomegranate peel powdered extract (PPPE) did not lead to any further growth promotion either for *La. delbrueckii* ssp. *bulgaricus*, *Lb. acidophilus* or *Bifidobacterium* sp. Moreover, *Str. thermophilus* count of frozen ordinary yoghurt mix was significantly higher than counted in bio-yoghurt one.

The letters before comma possess the factor of fat replacement. While those after comma possess the factors of water replacement with pomegranate peel powdered extract (PPPE), kind of bacterial starter culture and conversion into frozen mix or freezing process, respectively. The means with the same letter at any position did not significantly differ ($P>0.05$).

Biochemical properties of frozen yoghurt mix

Concerning the titratable acidity (TA) % and pH value of mixes date did not indicate that, the fat nor water replacement exhibited any significant differences in both criteria (Table 4). Likewise the differences in TA% and PH value between ordinary and bio-yoghurts had disappeared when they converted into frozen yoghurt mixes. This phenomenon could be ascribed to the buffering capacity of native milk protein added through the complementary mix portion. Besides, some non-dairy ingredients used such as CMC many caused similar alkaline effect toward the yoghurt acidity. All those factors led to eliminate any significant differences in TA% and PH value between yoghurt portions wherefrom the mixes were prepared.

Regarding the Acetaldehyde (AC) and diacetyl (DA) contents, significant increments were occurred in their levels when the fat and/or water were replaced with pomegranate peel powdered extract (PPPE). The Duncan's letters showed that DA was relatively more stable component than AC along treatments (Table 4).

TABLE 4. General properties of frozen yoghurt mix made using ordinary (YC-X11) or bio-yoghurt (ABT-2) as affected by fat and/or water replacement with of pomegranate peel powdered extract (PPPE).

Property	Level of pomegranate peel powdered (PPP) (1.5%)											
	(control)		Level of fat replacement with simplese100* 100% Level of tap water replacement with pomegranate peel powdered extract (PPPE)									
			T ₁ (0%)		T ₂ (25%)		T ₃ (50%)		T ₄ (75%)		T ₅ (100%)	
	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	Y C - X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2
Dry matter (%)	32.60 ^{baaa}	32.56 ^{baaa}	32.62 ^{baa}	32.59 ^{aaa}	32.65 ^{aaa}	32.62 ^{aaa}	32.67 ^{aaa}	32.65 ^{aaa}	32.69 ^{aaa}	32.68 ^{aaa}	32.72 ^{aaa}	32.70 ^{aaa}
Fat (%)	3.5	3.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Protein (%) (TNX6.38)	5.36 ^{b,aaa}	5.33 ^{b,aaa}	8.44 ^{aaa}	8.43 ^{aaa}	8.42 ^{aaa}	8.40 ^{aaa}	8.36 ^{aaa}	8.34 ^{aaa}	8.30 ^{aaa}	8.28 ^{aaa}	8.25 ^{aaa}	8.23 ^{aaa}
Simplese added (%)	0.0	0.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
carbohydrate* (%)	22.767 ^{aba}	22.766 ^{ba}	23.194 ^{baaa}	23.192 ^{baaa}	23.153 ^{baaa}	23.171 ^{baaa}	23.204 ^{aaa}	23.206 ^{baaa}	23.24 ^{baaa}	23.256 ^{baaa}	23.28 ^{baaa}	23.32 ^{baaa}
Ash (%)	0.973 ^{b,ca}	0.964 ^{b,cb}	0.986 ^{ca}	0.968 ^{cab}	1.077 ^{b,aa}	1.049 ^{b,ab}	1.106 ^{ca}	1.104 ^{ac,cb}	1.150 ^{aba}	1.144 ^{ab,bb}	1.190 ^{aaa}	1.150 ^{aab}
Caloric value (kcal/100g)	141.53 ^{baaa}	141.40 ^{baaa}	109.50 ^{b,baa}	109.47 ^{b,baa}	109.29 ^{b,baa}	109.30 ^{b,baa}	109.31 ^{b,baa}	109.26 ^{b,baa}	109.27 ^{b,baa}	109.27 ^{b,baa}	109.28 ^{b,baa}	109.37 ^{b,baa}
Titrate acidity (%) (as lactic acid)	0.84 ^{b,ca,cb}	0.71 ^{c,cb,bb}	0.86 ^{ca,ab}	0.75 ^{b,cb,bb}	0.90 ^{ba,ab}	0.80 ^{cb,bb}	0.95 ^{ca,cb}	0.85 ^{ac,cb}	0.99 ^{ab,ab}	0.90 ^{ab,bb}	1.04 ^{aaa,ab}	0.95 ^{ab,bb}
PH value	5.69 ^{aa,ba}	5.71 ^{aaaa}	5.51 ^{b,aba}	5.54 ^{baaa}	5.42 ^{ca,ba}	5.45 ^{ca,aa}	5.32 ^{b,ba}	5.35 ^{b,baa}	5.21 ^{b,ca}	5.24 ^{b,ca}	5.10 ^{c,cb,ba}	5.14 ^{c,ca}
Acetaldehyde (µmol/g)	291.5 ^{b,ca,cb}	235.9 ^{b,cb,bb}	312.9 ^{b,ba,ab}	249.8 ^{ca,ab}	326.5 ^{ca,cb}	259.9 ^{b,ab,bb}	350.4 ^{ab,ab}	329.5 ^{ac,cb}	374.1 ^{aaa,ab}	353.6 ^{ab,bb}	399.5 ^{aaaa}	377.3 ^{ab,bb}
Diacetyl (µmol/g)	21.37 ^{ca,baa}	17.40 ^{ca,ba,ba}	21.90 ^{b,ba,ba}	17.54 ^{b,ab,ba}	22.74 ^{b,ba,ba}	17.73 ^{b,ab,ba}	23.46 ^{ab,ba}	17.76 ^{ab,ba}	24.20 ^{ab,ba}	17.82 ^{ab,ba}	24.98 ^{aaaa}	17.89 ^{ab,ba}
Total phenols GAE/100g dw.	30.59 ^{b,ca}	30.58 ^{b,cb}	30.60 ^{cb,ba}	30.58 ^{ca,ab}	69.93 ^{b,ba}	69.91 ^{b,ab}	75.87 ^{ca}	75.86 ^{ac,cb}	89.91 ^{ab,ba}	89.90 ^{ab,bb}	98.50 ^{aaa}	98.49 ^{aab}
Total flavonoids quartine/100g dw	9.76 ^{b,ca}	9.75 ^{b,cb}	9.77 ^{cb,ba}	9.75 ^{ca,ab}	19.88 ^{b,ba}	19.86 ^{b,ab}	21.50 ^{ca}	21.49 ^{ac,cb}	26.99 ^{ab,ba}	26.98 ^{ab,bb}	30.50 ^{aaa}	30.49 ^{aab}
Antioxidant (DPPH) (%)	4.83 ^{b,ca}	4.82 ^{b,cb}	4.84 ^{cb,ba}	4.82 ^{ca,ab}	8.68 ^{b,ba}	8.66 ^{b,ab}	10.12 ^{ca}	10.11 ^{ac,cb}	17.93 ^{ab,ba}	17.92 ^{ab,bb}	20.72 ^{aaa}	20.71 ^{aab}
Specific gravity	1.119 ^{b,ba}	1.120 ^{baaa}	1.143 ^{aa,ba}	1.138 ^{aaaa}	1.149 ^{ac,ba}	1.148 ^{ca,aa}	1.141 ^{ad,ba}	1.139 ^{ad,aa}	1.135 ^{b,cb,ba}	1.132 ^{b,ca,aa}	1.130 ^{bd,ab}	1.127 ^{bd,aa}
Freezing point (°C)	-2.49 ^{aab}	-2.45 ^{aaa}	-2.67 ^{b,ab}	-2.56 ^{b,aa}	-2.85 ^{ca,cb}	-2.80 ^{ca,aa}	-3.90 ^{b,bb}	-3.05 ^{aba}	-4.05 ^{ac,cb}	-3.35 ^{aca}	-4.95 ^{b,cb}	-3.85 ^{b,ca}
Apparent viscosity (centipoises)	310.2 ^{b,ca,aa}	306.9 ^{b,cb,ba}	380.7 ^{ca,aa}	376.2 ^{ca,ba}	446.2 ^{b,baa}	420.7 ^{b,ba,ba}	491.7 ^{ca,aa}	480.2 ^{ac,ba}	540.6 ^{ab,ba}	529.9 ^{ab,ba}	590.8 ^{aaaa}	575.6 ^{aa,ba}
<i>Streptococcus thermophilus</i>	55 ^{b,ca,aa}	45 ^{b,cb,ba}	58 ^{ca,aa}	46 ^{ca,ba}	60 ^{baaa}	50 ^{b,aba}	62 ^{ca,aa}	53 ^{ac,ba}	66 ^{ab,ba}	56 ^{ab,ba}	67 ^{aaaa}	60 ^{aa,ba}
<i>Lactobacillus delbrueckii ssp. bulgaricus</i>	57 ^{b,ba}	ND	60 ^{ca,aa}	ND	64 ^{aba}	ND	67 ^{aba}	ND	68 ^{aaa}	ND	69 ^{aaa}	ND
<i>Lactobacillus acidophilus</i>	ND	40 ^{ca,aa}	ND	42 ^{ca,aa}	ND	44 ^{baa}	ND	45 ^{baa}	ND	47 ^{aaa}	ND	49 ^{aaa}
<i>Bifidobacterium sp.</i>	ND	30 ^{b,ba}	ND	32 ^{aba}	ND	33 ^{aba}	ND	34 ^{aba}	ND	35 ^{ab,ba}	ND	37 ^{aaa}

*Calculated by the difference. TN: Total nitrogen. NA: Not added. ND: Not determined. DPPH: 2,2 diphenyl-1-picrylhydrazyl. All bacterial counts are colony forming unit x 10⁷/cfu/g.

Antioxidant activity of frozen yoghurt mix

The results in Table 4 revealed that total phenols, total flavonoids and DPPH were slightly increased with pomegranate peel powdered extract (PPPE) ratios 75 and 100%. Total phenols, total flavonoids and DPPH recorded values 98.50, 30.50 and 20.72, respectively at the ratio 100% of pomegranate peel powdered extract (PPPE). Moreover, total phenols, total flavonoids and DPPH of frozen ordinary yoghurt mix did not lead to any further higher than frozen bio-yoghurt mix.

Physical and rheological properties of frozen yoghurt mix

Data of Table 4 demonstrated that, specific gravity (sp.gr.) of the mix was heightened by the fat replacement. That could be attributed to the relatively low density possessed the milk fat *versus* the simplese (which is proteinous origin). While, the sp.gr. of mix lowered as the level of water replacement with pomegranate peel powdered extract (PPPE) increased. Similar observations were reported by Abdallah (2003). Nevertheless, the statistical analysis seemed also that, the frozen bio-yoghurt mix exhibited sp.gr. significantly higher than that of ordinary one.

Concerning the Apparent viscosity (AV) of frozen yoghurt mix, data confirmed that, the product had higher values as the fat and pomegranate peel powdered extract (PPPE) were replaced. That could be attributed to the use of simplese (the proteinous origin) as fat mimetic (Table 4). As previously mentioned, Prentice (1992) confirmed that whichever method used, the increase of protein content is the principle factor influencing the texture of yoghurt. Likewise, this phenomenon may be ascribed also to the use of pomegranate peel powdered extract (PPPE) were replacement in a study on pomegranate peel powdered extract (PPPE) *versus* pectin gelatinize. The results an in same manner with Tawfek et al. (2017) who found an increase in viscosity of ice milk mixes with adding dehulled black rice flour. With respect to the freezing point (FP), data demonstrated that, the fat replacement led to lower the FP of the frozen yoghurt mix (Table 4). This phenomenon is mainly to the relatively high solidification temperature of milk fat, besides it might also be due to the consequence impact on the level and nature of some other directly effective components caused especially, in the presence of the increased acid developed by BSC in correspondence with the absence of the milk fat, which, also is characterized with relatively

higher solidifying point. Similar observations were found by Farahat (1999).

Moreover, the FP of frozen yoghurt mixes is highly dependent directly on, the soluble components like sweeteners and indirectly to, the ratio of fat and protein in the frozen yoghurt mix. FP of frozen yoghurt mixes was significantly affected with adding pomegranate peel powdered extract (PPPE). The Obtained results are in line with Tawfek et al. (2017). Furthermore, the results indicated also that, as the acid produced by ordinary *versus* bio BSC was high the FP of the related frozen yoghurt mix was low. Similar observations were reported by Abdallah (2003).

Minerals content of frozen yoghurt mix

The histograms illustrated in Fig 1 declared that, mineral contents of frozen yoghurt mix made using ordinary (YC-X11) or bio-yoghurt (ABT-2) as affected by fat and/or water replacement with of pomegranate peel powdered extract (PPPE).

Milk and milk products are considered as poor sources of Fe and therefore, fortification of milk products with natural Fe source would be a helpful tool. Results in Fig 1 showed that the iron content of treatments with pomegranate peel powdered extract (PPPE) replacement varied between 0.32-1.50 (mg/100) compared to 0.19 (mg/100) in nil (control) treatment. Adding pomegranate peel powdered extract (PPPE) to frozen yoghurt blends was also accompanied by high level of potassium content due to the high content of this element in pomegranate peel powdered extract (PPPE) (368 mg/100g). Adding of pomegranate peel powdered extract (PPPE) to frozen yoghurt mix slightly increased the zinc content of resultant frozen yoghurt. On the other hand, replacement with pomegranate peel powdered extract (PPPE) in frozen yoghurt recipes led to decrease the content of calcium in the resultant product which could be attributed to the low contents of these elements in pomegranate peel powdered extract (PPPE).

Lactic acid bacteria population of final frozen yoghurt

The freezing process followed by hardening step led to significant reductions in the counts of all bacterial starter culture (BSC) strains to about the tithe, without any exception (Table 5). Nevertheless, each bacterial count of all frozen yoghurt treatments, in relation either to the full fat and/or water replacement with pomegranate peel powdered extract (PPPE), stilled conforming the figures provided, which

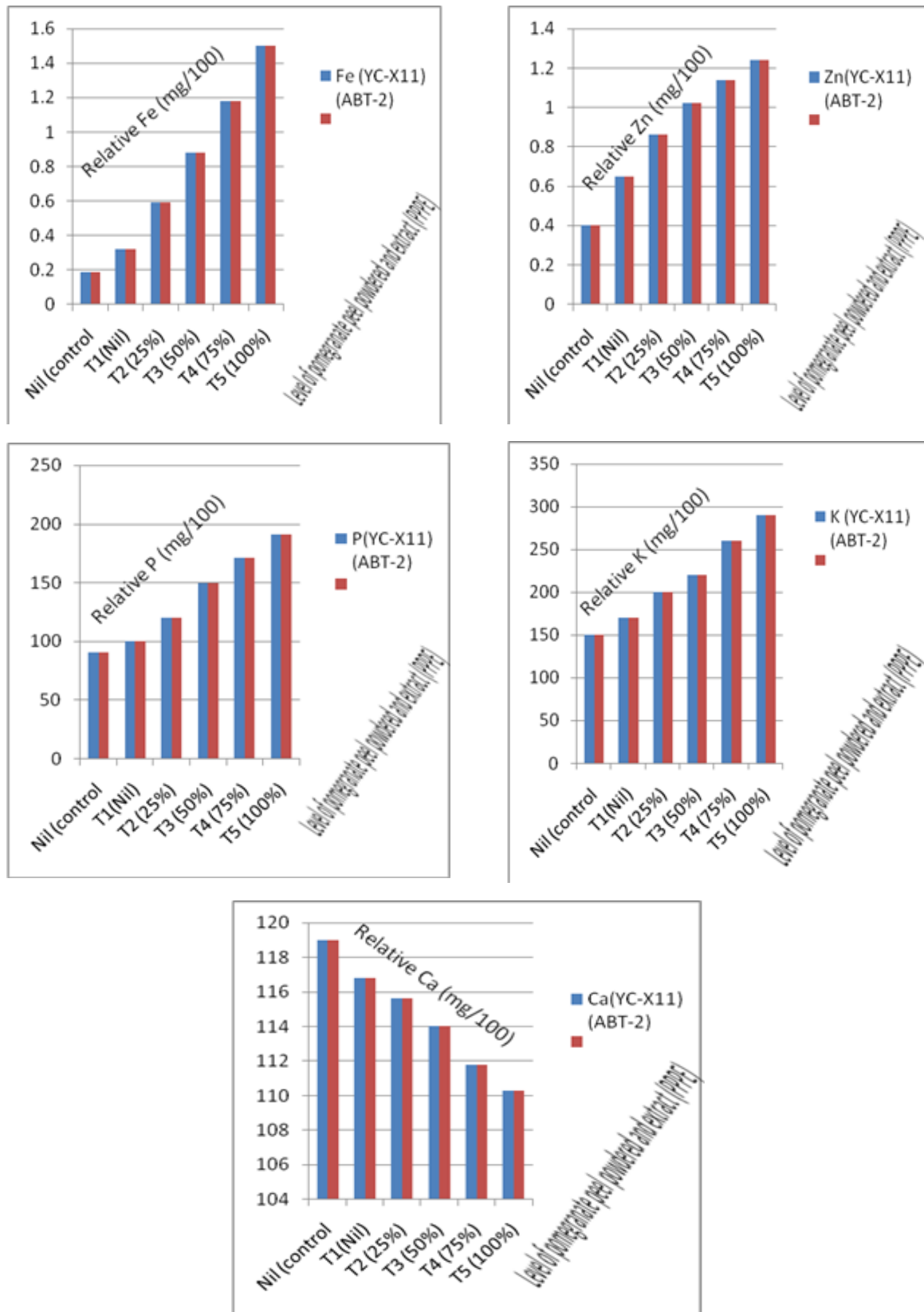


Fig. 1. Mineral contents (mg/100) of frozen yoghurt mix treatments as percentage of the control as function of fat and/or pomegranate peel powdered extract (PPPE) replacement.

approved an international standard as established a minimum of 10⁷cfu/g for the starter cultures of fermented milks and a minimum of 10⁶cfu/g for specific starter bacteria for which a claim is made for specific microorganism that has been added as supplement. Beside, Table 5 shows low changes in the bacterial counts of frozen products during storage. The reduction in viable bacterial counts, which occurred during freezing, may be associated with the freeze injury of cells. Furthermore, the mechanical stresses associated with the mixing and freezing processes, which incorporates oxygen into the mixture, may be responsible for the further bacterial count decline (Haynes & Playne 2002). The highest survival rate of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium* sp. occurred in T₅ (100%) than other treatments (Table 5).

Similar results were reported by Akin et al. (2007). The survival rate of probiotic (ABT-2) increased with increasing pomegranate peel powdered extract (PPPE) content in samples in agreement with (FDA, 2008; El-Kholy, 2018) for the probiotic products, who found that probiotic can grow more extensively in pomegranate peel powdered extract (PPPE) than in control. This is probably because pomegranate peel powdered extract (PPPE) contains pectin, which may be utilized by probiotic. The produced products contained probiotic bacteria viable cells count above the required probiotic minimum (10⁶ cfu/g) for probiotic products (Raj & Prasad, 2014).

TABLE 5. Log counts (cfu/g) of final frozen yoghurt made using ordinary (YC-X11) or bio-yoghurt (ABT-2) as affected by fat and/or water replacement with of pomegranate peel powdered extract (PPPE) during storage period (fresh, after 30 days).

Property	Level of pomegranate peel powdered (PPP) (1.5%)											
	Level of fat replacement with simplesse100® 100%											
	(Level of tap water replacement with pomegranate peel powdered extract (PPPE) control)											
	T ₁ (0%)		T ₂ (25%)		T ₃ (50%)		T ₄ (75%)		T ₅ (100%)			
YC-X11	ABT-2	Y C - X11	ABT-2	YC-X11	ABT-2	Y C - X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	
<i>Streptococcus thermophilus</i>	Fresh											
	55 ^{b,c,a,b}	45 ^{b,c,b,b}	58 ^{a,a,a,b}	46 ^{a,c,b,b}	60 ^{a,b,a,b}	50 ^{a,c,b,b}	62 ^{a,b,a,b}	53 ^{a,b,b,b}	66 ^{a,a,a,b}	56 ^{a,a,b,b}	67 ^{a,a,a,b}	60 ^{a,a,b,b}
	After 30 days											
		42 ^{b,c,b}		43 ^{b,a,b}		47 ^{b,a,b}		51 ^{b,a,b}		54 ^{a,a,b}		59 ^{a,a,b}
Survival Rate (%)												
		93.33		93.47		94.00		96.22		96.42		98.33
<i>Lactobacillus delbrueckii ssp. bulgaricus</i>	Fresh											
	57 ^{b,b,b}	ND	60 ^{a,b,b}	ND	64 ^{a,b,b}	ND	67 ^{a,b,b}	ND	68 ^{a,a,b}	ND	69 ^{a,a,b}	ND
	After 30 days											
			38 ^{b,c,b}		40 ^{b,a,b}		42 ^{b,a,b}		43 ^{b,a,b}		46 ^{a,a,b}	
Survival Rate (%)												
		95.00		95.23		95.45		95.55		97.87		97.95
<i>Lactobacillus acidophilus</i>	Fresh											
	ND	40 ^{b,c,b}	ND	42 ^{b,a,b}	ND	44 ^{b,a,b}	ND	45 ^{b,a,b}	ND	47 ^{a,a,b}	ND	49 ^{a,a,b}
	After 30 days											
			38 ^{b,c,b}		40 ^{b,a,b}		42 ^{b,a,b}		43 ^{b,a,b}		46 ^{a,a,b}	
Survival Rate (%)												
		95.00		95.23		95.45		95.55		97.87		97.95
<i>Bifidobacterium</i> sp.	Fresh											
	ND	30 ^{b,c,b}	ND	32 ^{a,b,b}	ND	33 ^{a,a,b,b}	ND	34 ^{a,a,b,b}	ND	35 ^{a,a,b}	ND	37 ^{a,a,b}
	After 30 days											
			28 ^{b,c,b}		30 ^{a,b,b}		31 ^{a,a,b}		32 ^{a,a,b,b}		34 ^{a,a,b}	
Survival Rate (%)												
		93.33		93.75		93.93		94.11		97.14		97.29

ND: Not determined. All bacterial counts are colony forming unit x 10⁷cfu/g.

The letters before comma possess the factor of fat replacement. While those after comma possess the factors of water replacement with pomegranate peel powdered extract (PPPE), kind of bacterial starter culture and freezing process, respectively. The means with the same letter at any position did not significantly differ (P>0.05).

Biochemical properties of final frozen yoghurt

The freezing process followed by hardening step yielded in fixation the volatile carbonyl components so that, the Acetaldehyde (AC) content of frozen product was exhibited to be higher than that of the mix prior freezing. While, the diacetyl (DA) content did not exhibit any significant change (Table 6).

Physical properties of final frozen yoghurt

As beginning, the freezing and hardening processes led significantly to reduce the specific gravity (sp.gr.) of all treatments without any exception (Table 6). The calculated overrun % revealed that, the full fat and/or the water replacement with pomegranate peel powdered extract (PPPE) resulted in enhancement of the whipping ability of the mix, which was better when ordinary *versus* bio-yoghurt was used for frozen yoghurt making, (Table 6). This phenomenon emphasized the fact that previously reported

by some investigators, who mentioned that, the fat harmed, while pomegranate peel powdered extract (PPPE) improved the gained overrun of the dairy frozen desserts (Salama, 2012).

Concerning the melting resistance of the final frozen yoghurt, data given in Table 6 explain that, the melted quantities along the experimental time increased due to full fat replacement. Omer (1983) reported that, the weakest melting resistance for frozen yoghurt was the lowest level of fat content. On the other hand, the melting resistance of frozen yoghurt was gradually strengthened as the pomegranate peel powdered extract (PPPE) replaced with water. This is probably due to the decrease in the freezing point (FP) associated with pomegranate peel powdered extract (PPPE) replacement Arbuckle (1986) reviewed similar relationship between the FP and the melting reentrance. Moreover, the frozen ordinary yoghurt exhibited a melting resistance higher than that done by the bio-one.

TABLE 6. General properties of final frozen yoghurt made using ordinary (YC-X11) or bio-yoghurt (ABT-2) as affected by fat and/or water replacement with of pomegranate peel powdered extract (PPPE).

Property	Level of pomegranate peel powdered (PPP) (1.5%)											
	(control)		Level of fat replacement with <i>simplesse100</i> [®] 100%									
			Level of tap water replacement with pomegranate peel powdered extract (PPPE)									
	T ₁ (0%)		T ₂ (25%)		T ₃ (50%)		T ₄ (75%)		T ₅ (100%)			
	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2	YC-X11	ABT-2
Acetaldehyde (µmol/g)	370.4 ^{b,c,a,a}	280.6 ^{b,c,b,a}	425.3 ^{a,c,a,a}	331.2 ^{a,c,b,a}	436.2 ^{a,c,a,a}	350.3 ^{a,c,b,a}	440.9 ^{a,b,a,a}	354.2 ^{a,b,b,a}	445.4 ^{a,b,a,a}	372.9 ^{a,b,b,a}	450.5 ^{a,a,a,a}	392.3 ^{a,a,b,a}
Diacetyl (µmol/g)	20.1 ^{b,ab,a,a}	18.9 ^{b,ab,a}	20.91 ^{a,ab,a,a}	19.01 ^{a,ab,b,a}	21.67 ^{a,ab,a,a}	19.15 ^{a,ab,b,a}	22.07 ^{a,ab,a,a}	19.9 ^{a,ab,b,a}	22.83 ^{a,ab,a,a}	19.9 ^{a,ab,b,a}	23.55 ^{a,a,a,a}	20.30 ^{a,a,b,a}
Specific gravity	0.68 ^{b,a,b,b}	0.69 ^{b,a,b,b}	0.682 ^{a,a,b,b}	0.691 ^{a,a,a,b}	0.653 ^{a,c,b,b}	0.664 ^{a,c,a,b}	0.624 ^{a,c,b,b}	0.635 ^{a,c,a,b}	0.601 ^{a,d,b,b}	0.618 ^{a,d,a,b}	0.578 ^{a,d,b,b}	0.589 ^{a,d,a,b}
Overrun %	63.70 ^{b,c,a}	60.12 ^{b,c,b}	67.61 ^{a,c,a}	64.74 ^{a,c,b}	75.94 ^{a,b,a}	72.91 ^{a,b,b}	82.78 ^{a,b,a}	79.43 ^{a,b,b}	90.10 ^{a,b,a}	87.52 ^{a,a,b}	96.50 ^{a,a,a}	93.60 ^{a,a,b}
Melting loss % 15 min	2.57 ^{b,c,a}	2.94 ^{b,c,a}	9.72 ^{a,a,a}	9.88 ^{a,a,a}	3.91 ^{a,b,a}	4.40 ^{a,b,a}	2.70 ^{a,c,a}	2.94 ^{a,c,a}	2.50 ^{a,c,a}	2.69 ^{a,c,a}	2.29 ^{a,c,a}	2.45 ^{a,c,a}
30 min	18.92 ^{b,c,a}	19.30 ^{b,c,a}	41.50 ^{a,a,a}	42.50 ^{a,a,a}	24.12 ^{a,b,a}	24.50 ^{a,b,a}	19.19 ^{a,c,a}	20.32 ^{a,c,a}	15.15 ^{a,c,a}	16.30 ^{a,c,a}	12.92 ^{a,c,a}	14.01 ^{a,c,a}
45 min	61.99 ^{b,c,b}	65.34 ^{b,c,a}	77.70 ^{a,a,b}	78.80 ^{a,a,a}	64.93 ^{a,b,b}	70.34 ^{a,b,a}	61.98 ^{a,b,b}	69.94 ^{a,c,a}	58.10 ^{a,c,b}	66.80 ^{a,c,a}	55.91 ^{a,c,b}	63.50 ^{a,c,a}
60 min	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Hardness	9.21 ^{b,a,a}	8.11 ^{b,b,a}	9.20 ^{b,a,a}	8.10 ^{b,b,a}	9.25 ^{b,a,a}	8.15 ^{b,b,a}	9.30 ^{b,a,a}	8.20 ^{b,b,a}	9.71 ^{a,a,a}	8.60 ^{a,b,a}	9.95 ^{a,a,a}	8.85 ^{a,b,a}
Appearance (out of 5 points)	5	5	5	5	5	5	5	5	5	5	5	5
Body and texture (out of 5 points)	4.5 ^{b,ab,a}	4.5 ^{b,ab,a}	5 ^{a,ab,a}	5 ^{a,ab,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}	5 ^{a,a,a}
Flavour (out of 10 points)	8 ^{b,ab,a}	9 ^{b,ab,a}	9 ^{a,ab,a}	9 ^{a,ab,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}	10 ^{a,a,a}
Total (out of 20 points)	17.5 ^{b,ab,a}	18.5 ^{b,ab,a}	19 ^{a,ab,a}	1 ^{a,ab,a}	9 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}	20 ^{a,a,a}

The letters before comma possess the factor of fat replacement. While those after comma possess the factors of water replacement with pomegranate peel powdered extract (PPPE), kind of bacterial starter culture and freezing process, respectively. The means with the same letter at any position did not significantly differ (P>0.05).

That could be also attributed to the depressing in the FP correlated to the relatively increased acid production in the former. The results trends gained in this study agree with those previously found by Fayed et al. (1999) and Hassaan (2000). They reported that, there are reverse correlation between the FP of the mix and melting reentrance of its resultant frozen dairy desserts. Similar observations were reported by Fayed et al. (2012).

Hardness values of the final frozen yoghurt, data given in Table 6 reveal a significant increase associated with pomegranate peel powdered extract (PPPE) replacement. The highest value of hardness observed in 100% replacement with pomegranate peel powdered extracts (PPPE). The increase in hardness of frozen yoghurt samples with pomegranate peel powdered extract (PPPE) replacement could be attributed to the higher pectin contents which may act as stabilizing material and reduce the free water contents. Generally, the hardness values of (ordinary *versus* and bio-yoghurt) are related to several factors such. Dry matter (DM), the freezing point (FP), the amount and type of stabilizer, and thus the free water content, overrun percent and the consistency of the treatment Awad (2007) and Tawfek et al. (2017).

Organoleptic quality of final frozen yoghurt

Regarding the appearance score, data in Table 6 indicated that the appearance of all treatments was as good as that of the control without any evident change. Moreover, all full fat replaced treatments gained consistency score more than the control. That could be ascribed to the advanced preference role of simplese as protein *versus* the fat toward the consistency especially, the smooth texture gained for the simplese-containing frozen yoghurt. The small size and uniformly spherical shape of simplese particles are responsible for this product's resemblance to a creamy fluid. The texture of simplese is similar to that of fats, and its use in frozen desserts can inhibit the growth of ice crystals. Neither the pomegranate peel powdered extract (PPPE) replacement at any level nor the kind of bacterial starter culture (BSC) used for yoghurt making yielded in any significant differences in the consistency score of resultant frozen yoghurt.

Concerning the flavour criterion, which is a combination of mouthfeel, taste and aroma (Arbuckle, 1986), the judging scoring stated that, the palatability of all full fat replacement treatments was better than that of the control indicating that, simplese, which used as fat replacer, did not disappeared the taste lacking because of fat absence only but also improved the product flavour as confirmed from the panelist score listed in Table 6. Kirby (1994) reported that, during simplese preparation the precise blending or shearing in the microparticulation process shapes the protein gel into spheroidal particles so small (0.1 to 2.0 microns) that the tongue perceives them as fluid rather than individual particles. Because the protein is shaped into tiny particles below the perceptual threshold of the tongue, the fat mimetic has the richness and creaminess normally associated with fat. Moreover, the use of pomegranate peel powdered extract (PPPE) replacement led to complete recovery of the flavour and natural colour deficiency due to colour reduction. This is, indeed, indicating that pomegranate peel powdered extract (PPPE) performed various functions involving a) give of flavour and natural colour and thus palatability, b) balance of the fattiness of the added fat, c) providing bulk and body and hence improving mouthfeel, d) controlling ice crystallization and thereby helping to produce a smooth textured product, e) bringing about suitable depression in the freezing point of the mixture enabling the mix to be whipped and frozen simultaneously and thus affording the customer a delectable, refreshing food, f) enhancing flavour. Furthermore, the flavour of frozen bio-yoghurt did not vary from that of the ordinary one as stated from their scores recorded in Table 6.

As a result of the forgoing sensory criteria, the overall quality score of all frozen yoghurt treatments were significantly higher than that of the control (Table 6). Furthermore, the total organoleptic score of pomegranate peel powdered extract (PPPE) replaced samples were as high as those of the un-replaced one.

Conclusion

Finally, the foregoing results led satisfactory to conclude that, yoghurt and hence frozen yoghurt beyond their ability to be probiotic food *via* its culturing with the gut strains, it could further carry more healthy benefits when it was made in the reduced *via* proportional fat with pomegranate peel powdered extract (PPPE) replacement by certain advanced ingredients such as simplese and pomegranate peel powdered extract (PPPE), which acts as natural prebiotic converts the product to be, synbiotic. Besides, the product would become quite suitable for diabetes and gave the product expect improvement physicochemical properties and sensory properties special flavour. Also pomegranate peel powdered extract (PPPE) adding as natural source of colour, dietary fiber, phenolic compounds, flavonoids compounds and antioxidant activity could improve nutritive and health values of the resultant products.

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خواص اليوجهورت المجد الحيوي المنخفض الدهن المدعم بمستخلص ومسحوق قشر الرمان

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قسم بحوث تكنولوجيا تصنيع الالبان- معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية -الجيزة - مصر

هدفت الدراسة الحالية إلى استخدام مسحوق قشر الرمان ومستخلصه (PPPE) كمصدر للألياف الغذائية ، والملونات الطبيعية ، والبريبايوتيك ، والبوليفينول ، والنشاط المضاد للأكسدة في تطوير اليوجهورت المجد منخفض الدهن. تمت إضافة قشر الرمان على شكل مسحوق (يضاف إلى اللبن بمعدل ١,٥%) ومستخلصه (يضاف بمعدل ٠, ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠% كبديل للماء المستخدم في تحضير اليوجهورت المجد الذي أعطى رموز T1،T2،T3،T4،T5). كما تم تحضير عينة اليوجهورت المجد الكنترول بدون بديل للدهن (simplese) مقارنة بالمعاملات الأخرى. أظهرت النتائج أن تحويل اليوجهورت إلى خليط مجد تسبب في زيادة الحموضة واللزوجة والمحتوى الفينولي الكلي ومحتوى الفلافونويد الكلي وقيم النشاط المضاد للأكسدة. علاوة على ذلك، فقد زاد اعداد البكتريا الحية لسلاطات بكتيريا حمض اللاكتيك في مزيج اليوجهورت المجد. بينما انخفضت قيمة ال pH، الوزن النوعي (sp.gr) ونقطة التجمد بإضافة PPPE. أيضاً ، أدت إضافة PPPE مع بديل الدهن (simplese) إلى تقليل السرعات الحرارية. بالإضافة إلى ذلك ، فإن إضافة PPPE إلى الزبادي اليوجهورت التقليدي أو اليوجهورت المجد الحيوي أدى إلى زيادة المحتوى من المعادن (الحديد، الزنك، الفسفور والبوتاسيوم). وهكذا وجد ان اعلى معدل للمقاومة كان للمعاملة T5 خلال فترة التخزين. تميز اليوجهورت المجد النهائي بالريع المرتفع ، مقاومة عالية للإنبهار واعلى صلابة وذلك بإستبدال بمستخلص مسحوق قشر الرمان عندما يصنع بإستخدام اليوجهورت التقليدي بالمقارنة باليوجهورت الحيوي. وكانت درجات التحكيم وصفات الجودة الحسية للقوام، التركيب والتكهه لليوجهورت المجد المستبدل بمستخلص مسحوق قشر الرمان أفضل من الكنترول. وقد بينت نتائج التقييم الحسي زيادة مع مستخلص مسحوق قشر الرمان تصل الي معدل استبدال ١٠٠%.