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Research Article
Production of Healthy Fermented Milk Supplemented with Natural Sources of Antioxidants

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Abstract

Background: The current study was designed to raise the antioxidant activity of low fat stirred yoghurt by supplementing with natural sources of antioxidant (pomegranate peel and milk thistle seeds) and to raise its nutritive value by addition Whey Protein Concentrate (WPC) to milk. **Material and Methods:** The whole Pomegranate Peels (PPs) were dried by using oven (40°C/48 h.) and aqueous extract was prepared from the dried peels. Low fat stirred yoghurt supplemented with 25% Pomegranate Peel Extract (PPE), 0.5 (g/100 mL) WPC and MTSE (1, 1.5, 2, 2.5, 3, 3.5 and 4%) was manufactured. Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activities (DPPH and ABTS) were evaluated in extract and product. Total counts of *S. thermophilus*, *L. delbrueckii* subsp., *bulgaricus*, mold and yeast, chemical composition and physical properties were evaluated of the products. **Results:** Increasing the concentration of MTSE from 1-4% increased TPC from 17.241-20.225 (equivalent mg gallic acid g^{-1}), TFC from 3.545-6.996 (mg rutin g^{-1}), RSA from 94.085-94.887% and ABTS from 83.694-98.905%. During cold storage the TPC, TFC and antioxidant activity showed a gradually decrease for all yoghurt samples. Increasing the concentration of MTSE in yoghurt samples led to slight increase in total solids, lactose, fat and protein percentage, while these contents were slightly decreased during storage and there wasn't significantly difference during storage in fat percentage. Acidity percentage was decreased with increasing MSTE percentage and increased during storage. With increasing the MTSE concentration, the counts of *S. thermophilus*, *L. delbrueckii* subsp., *bulgaricus*, molds and yeast were decreased and this also observed during storage at 5±1°C for 15 days. **Conclusion:** Yoghurt samples with the different concentration of MTSE were accepted when fresh. While along the cold storage at 5°C for 15 days the sensory scores were decreased. The product was contained 25% PPE, 0.5% WPC and 4% MTSE can be recommended of meet health continues consumer demanded and has multihealthy benefits.

Key words: Pomegranate peel extract, total phenolic content, total flavonoids content, antioxidant activity, whey protein concentrate, milk thistle seeds extract

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yoghurt has gained a positive perception by consumers as a functional dairy product with health promoting ingredients[1]. Yoghurt which supplemented with antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yoghurt nutrients and health benefits[2]. To increase the functionality and antioxidant capacity of yoghurt it's important to add healthy ingredients and natural source rich in antioxidants. There are increasing interests in applying fruit processing wastes as functional food ingredients and most of the beneficial bioactive compounds are remained in those by-products[3]. Waste products (e.g., fruit peels) from processing of agricultural commodities could offer practical and economic sources of natural source of antioxidants which could replace the synthetic ones[3-5]. Pomegranate peels are exploited in traditional medicine because of their strong astringency, making them a popular remedy throughout the world and it was used for dysentery and diarrhea and also for stomatitis[6,7]. Peels of pomegranate contain a wide variety of phytochemical compounds like gallotannins, ellagic acid, gallic acid, punicalins, punicalagins, as it was previously stated by some researchers. These phenolic compounds have antioxidant phytochemicals with interesting properties and have deeply value to their biological and free radical scavenging activities[8]. In addition, milk whey has antioxidant activity would include chelation of transition metals by serum albumin and lactoferrin, an iron-binding glycoprotein, as well as free radical scavenging activity by amino acids, such as tyrosine and cysteine[9]. Milk thistle (*Silybum marianum* (L.) Gaertn) is a member of Asteraceae family. Beyond the hepatoprotective activity and this flavonoid complex also has antioxidant properties which seem to be related to liver damage prevention. Silybin, the major active constituent of silymarin acts as inhibitor of tumor growth in hepatocarcinoma cell[10,11]. The target of this study was preparing functional product (healthy fermented product) that low fat stirred yoghurt supplemented with rich active ingredients included PPE (by-product) rich in antioxidant activity, WPC (as partition replacement of casein) and milk thistle extract (rich in silymarin) for healthier ones and some patients. Total phenolic content, total flavonoid content and antioxidant activity were determined for the resultant stirred yoghurt. Moreover, the effect of these additives (PPE, WPC and MTSE) on the *S. thermophilus* and *L. delbrueckii* subsp., *bulgaricus* counts was evaluated. Evaluation the quality characteristics of healthy product: This evaluation included pH value, titratable acidity, sensory evaluation, chemical composition and apparent viscosity (cP sec) which measures the resistance to arotating spindle (Brookfield Engineering Laboratories, Inc., Middleboro, Mass., USA) depending on time of shearing.

MATERIALS AND METHODS

Materials: Low heat skim milk powder (USA), 34% protein, 51% lactose, 1.2% fat, 8.2% ash and 4% moisture was used. Whey Protein Concentrate (WPC) from Davisco Foods International, Inc. (Eden Prairie, MN, USA), 62% protein, 29% lactose, 5% fat and 4% ash. Pomegranate (*Punica granatum* L.) and Milk Thistle Seeds (MTS) were obtained from a local market. Commercial freeze-dried culture DVS mixed bacterial starter of *L. delbrueckii* subsp., *bulgaricus* and *S. thermophilus* as yoghurt starter was obtained from Chr., Hansens Laboratories, Denmark.

Methods of manufacturing and analysis

Preparation aqueous extract of whole pomegranate peel: According to Shiban *et al.*[12], PPs powder (5 g) were dissolved by blending powder into 300 mL of distilled water for 10 min then filtrated by Whatman No. 1 and centrifuged at 3500 rpm for 10 min. Extract was kept at -3°C prior to analysis.

Chemical composition of milk thistle seeds (MTS): Table 1 showed that moisture, ash, fat and carbohydrate content by standard methods[13]. Mineral compositions were determined by using atomic absorption spectrophotometer (air-acetylene flame in combination with single element hollow cathode lamps.

Preparation of Milk Thistle Seeds Extract (MTSE): The ground sample (20 g) was infused in 100 mL distilled water at different degree of temperature (40, 60, 80 and 100°C) then stirred for 2 h and allowed to stand in the refrigerator overnight, then filtered. The extract stored in dark bottles at -3°C until further use and the effect of difference temperatures of extract was investigated.

Table 1: Chemical composition of Milk Thistle Seeds (MTS)

Contents	Percentage
Moisture	8.07
Fat	23.50
Ash	5.20
Total carbohydrates	24.20
Silymarin (mg g^{-1})	499.75
Minerals content (mg/100 g dry weight)	
K	527.924
Ca	424.586
Na	94.352
Fe	7.099

Determination of silymarin from MTS extract by HPLC chromatography: The HPLC separation was performed on a Pursuit C18 column at room temperature. The mobile phase was MeOH-0.1% glacial acetic acid (pH 2.8) (46:54, v/v). The elution has been made in an isocratic mode at a flow-rate 1 mL min^{-1} and the detection at 288 nm Wen *et al.*[14].

Manufacture of low fat stirred yoghurt supplemented with PPE, WPC and MTSE: Skim milk (12 g) and WPC (0.5 g) powders were reconstituted by gradually dispersing the required amount of powder in 25% PPE and different concentration of MTSE 1, 1.5, 2, 2.5, 3, 3.5 and 4% with continual stirring. Solutions were left for at least 1 h for hydration before further processing. Reconstituted skim milk powder was heated to 90°C for 5 min and cooled to (45°C). Yoghurt starter culture (3%) was added and the mixtures were incubated at 45°C until the gel structure is formed. The gel was stirred and stored at refrigerator (5±1°C) for 15 days until analyzed.

Evaluation of antioxidant activity

Determination of Total Phenolic Content (TPC): Total Phenolic Content (TPC) was determined according to Jayaprakasha *et al.*[15] by using Folin-Ciocalteu reagent. The 0.5 mL of sample was mixed with 0.5 mL of 10-fold-diluted Folin-Ciocalteu reagent. After 3 min, 4 mL of 7.5% sodium carbonate was added. The mixture was allowed to stand for 30 min in the dark at room temperature before the absorbance was measured at 765 nm using a spectrophotometer (model 2010, Cecil Instr., Ltd., Cambridge, UK). The final results were expressed as milligrams gallic acid of equivalent per gram of Dry Weight (DW).

Determination of Total Flavonoids Content (TFC): The amount of TFC in the extracts was measured spectrophotometrically according to Djeridane *et al.*[16]. This method depends on the formation of flavonoids aluminium complex, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. The TFC was expressed as milligrams rutin equivalents per gram of dry weight.

DPPH radical scavenging activity: Free Radical Scavenging Activity (RSA) of the samples were measured using the method of Brand-Williams *et al.*[17]. An aliquot 100 µL of the sample solution was mixed with 2.9 mL of 1,1-diphenyl-2-pycrylhydrazyl (DPPH) in methanol. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV-visible spectrophotometer. The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = 1-\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\times 100$$

$ABTS^{\bullet+}$ radical cation scavenging activity: The 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) radical scavenging assay was done according to the method of Re *et al.*[18]. The ABTS radical cation solution was produced by reacting 7 mM ABTS and 2.45 mM ammonium persulphate at a ratio 2:1 (v/v). The mixture was kept in the dark at 25°C for 12-16 h and then diluted with phosphate buffer (pH 7.2). Aliquot (100 µL) of each sample was mixed with 3 mL of the prepared ABTS working solution and the change in absorbance was observed at 734 nm. The percentage inhibition of the samples was calculated as:

$$ABTS^{\bullet+}\text{ radical scavenging activity} = \frac{A_{\text{blank}}-A_{\text{sample}}}{A_{\text{blank}}}\times 100$$

Microbiological analysis: The *S. thermophilus* count M17 agar medium (Oxoid) was used for viable counts of strpetococci. Plates were incubated for 24 h at 37°C under aerobic incubation. The *L. delbrueckii* ssp., *bulgaricus* count De Man Rogosa Sharpe (MRS) agar (Oxoid) was used for viable count of lactobacilli from the examined samples. Plates were incubated for 72 h at 45°C under anaerobic incubation. Yeasts and moulds count were carried out using the potato dextrose agar medium M096 (Oxoid). Plates supplemented with 10% acid then incubated at 22-25°C for 3-5 days, colonies of yeasts and moulds were counted and calculated per mL of sample. All microbiological results expressed as colony forming unit (log_{10} CFU mL^{-1}) of sample FDA[19].

Chemical composition of yoghurt samples: Dry matter, protein, fat and ash of yoghurt samples were determined according to AOAC[13]. Titratable acidity as lactic acid (TA) was determined as given by Ling[20]. The lactose content was determined as described by Nickerson *et al.*[21]. The pH determined by using pH meter (Jenway, 3505 pH meter).

Physical properties

Viscosity measurement: Apparent viscosity measurements of yoghurt samples were carried out at room temperature (22±2°C) using Brookfield programmable viscometer DV-II+(Brookfield Engineering Laboratories, Inc., Middleboro, Mass., USA) equipped with spindle No. 4 at 20 rpm.

Sensory evaluation: Yoghurt samples were organoleptically evaluated, by dairy science department staff members, National Research Center, Egypt. For evaluation a maximum number of flavour (50 points) body and texture (40 points) and appearance and color (10 points) to each sample characteristic identified according to Bodyfelt *et al.*[22].

Statistical analyses: The data obtained in this study were expressed as the mean of triplicate determinations. Statistical comparisons were made with Duncan's test which was analyzed with SPSS[23]. The $p<0.05$ values were considered to be significant.

RESULTS AND DISCUSSION

Effect of extraction temperature of MTSE on TPC, TFC and antioxidant activity (RSA and ABTS%): The effect of using different temperatures of the extraction of MTS on TPC, TFC and the antioxidant activity (RSA and ABTS%) was illustrated in Fig. 1. It is obvious that, increasing temperature of extraction of MTS increased TPC, TFC and antioxidant activity (RSA and ABTS%) and 100°C was the best temperature for extraction of Milk Thistle Seeds (MTS). This result in accordance with Duan *et al.*[24]. They reported that water at 85-100°C was effective in extracting flavanolignans and the dihydroquercitintaxifolin from milk thistle and the non polarsilybinin compounds were preferentially extracted at increasing temperature. Moreover, using high temperature insilymarin extraction from MTS explained by Kim *et al.*[25] and Wianowska and Wisniewski[26].

Total Phenolic Content (TPC): The TPC of stirred yoghurt supplemented with 25% PPE, 0.5% WPC and different concentration of MTSE either in fresh or stored samples were determined and recorded in Fig. 2. It was obvious that, there was marked increase in TPC in yoghurt samples supplemented with MTSE, whereas TPC gradually increased by increasing the concentration of MTSE added. This ascribed to the high level of phenolic compounds in MTSE as reported by Taha *et al.*[27]. They noticed that milk thistle had high amount of TPC, TFC and antioxidant activity. During cold storage, TPC gradually decreased for all yoghurt samples, whereas the supplemented samples with MTSE still retained more TPC along the storage time than control.

Total Flavonoid Content (TFC): The total flavonoid content of stirred yoghurt supplemented with PPE, WPC and MTSE for fresh and during cold storage periods was presented in Fig. 3. It was notable that with increasing the concentration of MTSE, TFC of yoghurt samples gradually increased while during storage periods, TFC decreased, though the supplemented samples still retained more TFC along the storage time than control. Comelli *et al.*[28] reported that silymarin containing

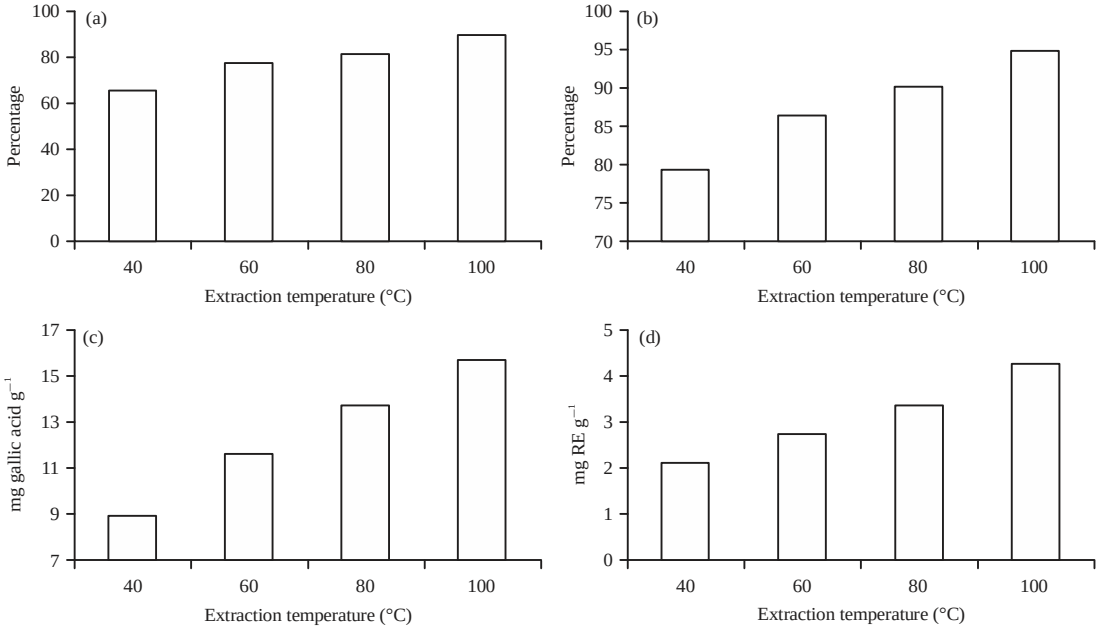

Fig. 1(a-d): Effect of extraction temperature of MTSE on (a) RSA%, (b) ABTS% antioxidant activity, (c) TPC and (d) TFC, TPC, TFC and antioxidant activity (RSA and ABTS%) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

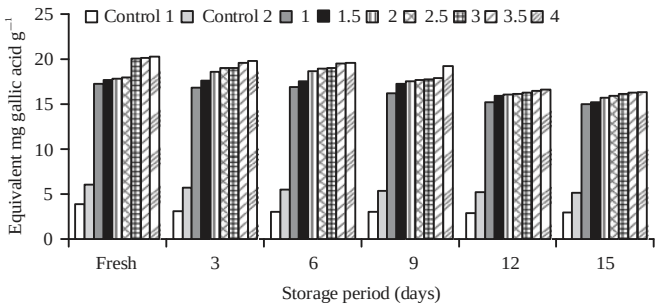

Fig. 2: Total phenolic content (equivalent mg gallic acid g^{-1}) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

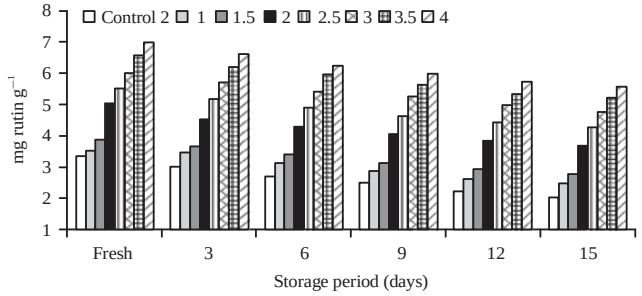

Fig. 3: Total flavonoid content (mg RE g^{-1}) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

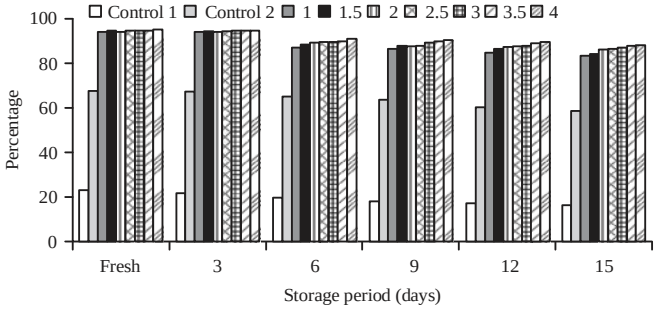

Fig. 4: DPPH Radical Scavenging Activity (RSA%) of low fat stirred yoghurt supplemented with PPE, WPC and different conc. of MTSE during storage at 5±1°C for 15 days

flavonoid mixture which contains approximately 65-80% flavonolignans silybin A and silybin B, iso-silybin A, iso-silybin B, silychristin and silydianin.

DPPH Radical Scavenging Activity (RSA%): The DPPH radical scavenging activity of yoghurt samples supplemented with PPE, WPC and MTSE had higher RSA than the two control samples and this was directly proportional to the percentage of the added MTS extract. During cold storage, the percentage of RSA decreased for all yoghurt samples with MTSE but the supplemented yoghurt samples with PPE and WPC still had higher RSA% than the control because the decline in the control values was more than the supplemented with PPE and WPC as RSA was reached to 23.002 and 16.504% for the control while it was 83.286, 86.435, 87.371 and 88.029% for yoghurt supplemented with 1, 2, 3 and 4% MTSE after 15 days of storage Fig. 4. The previous study was confirmed by Kiruthiga *et al.*[29] and Gaso-Sokae *et al.*[30] found that silymarin was a significantly stronger quencher of DPPH radical and this was in a concentrated dependent manner. Moreover, Asghar and Masood[31] evaluated the free radical scavenging activity and antioxidant properties of silymarin. High antioxidant

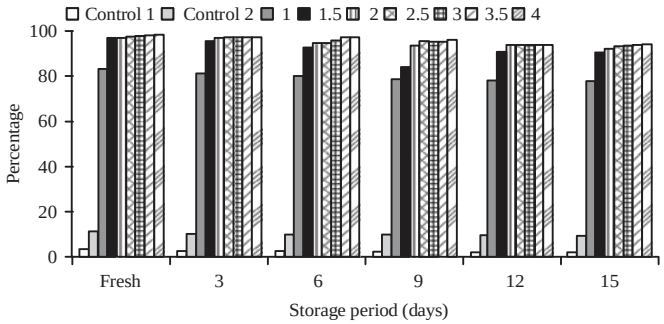

Fig. 5: ABTS radical scavenging activity (%) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

Table 2: Quantity of silymarin (mg/100 mL) in fresh low fat stirred yoghurt supplemented with different concentration of MTSE* by HPLC chromatography

	Concentration of flavonolignins (mg/100 mL yoghurt sample)						
MTSE (%)	Taxifolin	Silybin A	Silybin B	Silydianin	Silychristin	Iso-silybin	Total silymarin (mg/100 mL)
1	-	69.483	-	-	28.654	-	98.137
1.5	-	73.841	-	40.216	30.005	-	144.062
2	-	79.125	-	50.814	37.361	28.207	195.507
2.5	-	85.512	-	61.058	46.149	39.911	232.63
3	-	93.152	32.204	70.358	53.421	47.219	296.354
3.5	21.023	99.750	36.86	75.911	57.150	50.701	341.395
4	29.126	112.241	45.014	84.127	65.141	59.029	394.678

*MTSE: Milk thistle seeds extract

properties and significant protective effect of silymarin were in a concentration dependent manner in all methods. It may be used in preventing free radical-related diseases as a dietary natural antioxidant supplement.

ABTS radical scavenging activity: Data presented in Fig. 5 showed that supplemented yoghurt with MTSE besides PPE and WPC had highly increased ABTS% than control 1 (yoghurt without additives) and control 2 (yoghurt with PPE and WPC only) for fresh and stored yoghurt samples and this increase was proportional to the percentage of the added MTSE extract. During storage period, ABTS values decreased for all yoghurt samples to reach 1.880, 78.075, 92.702, 94.102 and 94.381% after 15 days for control 1 (without MTSE), 1, 2, 3 and 4% MTSE, respectively.

Determination the amount of total silymarin (mg/100 mL) in fresh low fat stirred yoghurt supplemented with different concentration of MTSE by HPLC chromatography: The chromatographic data of methanolic solutions of low fat stirred yoghurt supplemented with 1, 1.5, 2, 2.5, 3, 3.5 and 4% MSTE as compared to that of standard silymarin presented in Table 2. The quantitative data obtained from the samples analysis by HPLC showed that the amount of total silymarin varied from 98.137-394.678 (mg/100 mL) for yoghurt samples fortified with 1 and 4% MSTE, respectively. The highest amounts silybin and other components such as silychristin, silydianin and iso-silybinin were obtained from 4% MSTE which with increasing of MSTE concentration increase silymarin amounts.

It clears from Table 2 a little decrease in the amounts of silymarin in yoghurt samples that may be attributed to the interaction between phenolic compounds of silymarin with casein[32]. The interaction of plant phenolics and proteins are covalently or non-covalently. The non-covalent interactions between phenolic compounds and proteins suggested to be created by hydrophobic association, which may subsequently be stabilized by hydrogen bonding[33,34]. The types of interaction depend on the molar ratio of phenolic compounds to proteins Haslam[33] so, the total polyphenol content affected by the addition to milk. In general, both ways may lead to precipitation of proteins, via either multisite interactions (several phenolics bound to one protein molecule) or multi dentate interactions (one phenolic bound to several protein sites or protein molecules). This could lead to the masking of polyphenols i.e., catechins and the flavonoids on the milk proteins.

Chemical composition of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days: Data given in Table 3 revealed that, control 1 had a highest titratable acidity

followed by control 2 (PPE and WPC) and yoghurt samples supplemented with 1, 1.5, 2, 2.5, 3, 3.5 and 4% MSTE, respectively. The titratable acidity values increased as storage period progressed for all yoghurt samples. The pH values increased by increasing MTSE% in yoghurt samples, while the pH values decreased during storage period in all yoghurt samples as shown in Table 4. Data also, showed a slight effect of additive (MTSE%), on titratable acidity, pH values of fresh, 3, 6, 9, 12 and 15 days.

The obtained results given in Table 5 indicated that increasing the concentration of MTSE in stirred yoghurt led to slight increasing the total solids, this ascribed to the total solids of MTSE, while it was slightly decreased during storage.

Supplementation yoghurt with milk thistle extract caused a slight increase in fat percentage as a result of its content in milk thistle extract, whereas, there wasn't any effect on the recovery of fat percentage during the cold storage at the late of storage period Table 6.

Lactose content of the yoghurt supplemented with MTSE was shown in Table 7. It was evident that there was insignificant difference between yoghurt samples supplemented with different conc. of MTSE, while during the

Table 3: Titratable acidity percentage of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	Fresh	3	6	9	12	15
Control 1	0.81^{h}	0.83^{h}	0.85^{d}	0.86^{i}	0.86^{h}	0.87^{i}
Control 2	0.79^{g}	0.81^{g}	0.82^{c}	0.83^{h}	0.85^{g}	0.85^{h}
1	0.77^{f}	0.79^{f}	0.81^{c}	0.84^{g}	0.85^{g}	0.86^{g}
1.5	0.75^{e}	0.78^{e}	0.79^{c}	0.81^{f}	0.83^{f}	0.83^{f}
2	0.74^{d}	0.76^{d}	0.79^{b}	0.80^{e}	0.81^{e}	0.82^{e}
2.5	0.73^{c}	0.75^{c}	0.76^{b}	0.79^{d}	0.80^{d}	0.81^{d}
3	0.71^{b}	0.73^{b}	0.74^{a}	0.77^{c}	0.78^{c}	0.80^{c}
3.5	0.70^{a}	0.72^{a}	0.73^{a}	0.75^{b}	0.76^{b}	0.78^{b}
4	0.70^{a}	0.72^{a}	0.72^{a}	0.74^{a}	0.75^{a}	0.77^{a}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

Table 4: pH of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	Fresh	3	6	9	12	15
Control 1	4.64^{f}	4.62^{f}	4.61^{f}	4.60^{f}	4.60^{f}	4.59^{h}
Control 2	4.65^{e}	4.64^{e}	4.62^{e}	4.60^{f}	4.60^{f}	4.58^{f}
1	4.66^{d}	4.65^{d}	4.64^{d}	4.62^{e}	4.62^{e}	4.61^{e}
1.5	4.66^{d}	4.65^{d}	4.64^{d}	4.63^{d}	4.62^{e}	4.62^{d}
2	4.67^{c}	4.66^{c}	4.65^{c}	4.63^{d}	4.63^{d}	4.62^{d}
2.5	4.68^{b}	4.67^{b}	4.66^{b}	4.65^{c}	4.64^{c}	4.63^{c}
3	4.69^{a}	4.68^{a}	4.68^{a}	4.66^{b}	4.65^{b}	4.64^{b}
3.5	4.69^{a}	4.68^{a}	4.68^{a}	4.67^{a}	4.65^{b}	4.64^{b}
4	4.69^{a}	4.68^{a}	4.68^{a}	4.67^{a}	4.66^{a}	4.65^{a}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

Table 5: Total solids percentage of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	F	3	6	9	12	15
Control 1	10.264^{d}	10.263^{d}	10.258^{d}	10.251^{c}	10.247^{c}	10.243^{d}
Control 2	10.581^{c}	10.577^{c}	10.572^{d}	10.568^{c}	10.565^{c}	10.561^{d}
1	10.733^{c}	10.73^{c}	10.728^{c}	10.724^{b}	10.72^{b}	10.717^{c}
1.5	10.889^{c}	10.885^{c}	10.881^{c}	10.878^{b}	10.875^{b}	10.873^{c}
2	11.175^{b}	11.172^{b}	11.087^{c}	10.983^{b}	10.981^{b}	10.976^{b}
2.5	11.395^{b}	11.391^{b}	11.172^{b}	11.166^{b}	11.166^{b}	11.1658^{b}
3	11.456^{b}	11.452^{b}	11.448^{b}	11.445^{a}	11.441^{b}	11.438^{b}
3.5	11.705^{a}	11.702^{a}	11.70^{a}	11.695^{a}	11.692^{a}	11.69^{a}
4	11.954^{a}	11.951^{a}	11.947^{a}	11.945^{a}	11.941^{a}	11.937^{a}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

storage of yoghurt samples the lactose content markedly decreased; this may be due to by hydrolysis of lactose with increasing the activity of starter culture of yoghurt samples.

Concerning of the protein percentage of yoghurt samples as illustrated in Table 8, it was clear that protein percentage increased as a result of MTSE addition. No remarkable differences in percentage of protein were observed during storage periods of yoghurt samples until 15 days.

Total counts of *S. thermophilus*, *L. delbrueckii* subsp., *bulgaricus*, mold and yeast: Figure 6 and 7 showed that increasing the MTSE concentration in stirred yoghurt samples lead to decrease in the counts of *S. thermophilus* and *L. delbrueckii* subsp., *bulgaricus* and molds and this also observed during storage periods. It is noted that there wasn't any growth or colony of molds in all yoghurt samples with different concentration of MSTE in fresh samples and during storage.

Table 6: Fat percentage of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	Fresh	3	6	9	12	15
Control 1	0.1^{f}	0.1^{f}	0.1^{f}	0.1^{f}	0.1^{e}	0.1^{e}
Control 2	0.1^{f}	0.1^{f}	0.1^{f}	0.1^{f}	0.1^{e}	0.1^{e}
1	0.2^{e}	0.2^{e}	0.2^{e}	0.2^{e}	0.2^{d}	0.1^{e}
1.5	0.2^{e}	0.2^{e}	0.2^{e}	0.2^{e}	0.2^{d}	0.2^{d}
2	0.3^{d}	0.3^{d}	0.3^{d}	0.3^{d}	0.2^{d}	0.2^{d}
2.5	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}
3	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}	0.5^{c}
3.5	0.6^{b}	0.6^{b}	0.6^{b}	0.6^{b}	0.6^{b}	0.6^{b}
4	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

Table 7: Lactose percentage of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	Fresh	3	6	9	12	15
Control 1	5.041^{a}	4.875^{a}	4.680^{a}	4.575^{a}	4.268^{a}	4.135^{a}
Control 2	4.962^{b}	4.864^{ab}	4.668^{ab}	4.560^{ab}	4.262^{a}	4.134^{ab}
1	4.971^{b}	4.860^{ab}	4.660^{abc}	4.530^{bc}	4.259^{a}	4.129^{ab}
1.5	4.968^{b}	4.859^{ab}	4.630^{abc}	4.528^{bc}	4.254^{a}	4.124^{ab}
2	4.969^{b}	4.840^{abc}	4.628^{bc}	4.520^{bc}	4.249^{a}	4.122^{ab}
2.5	4.966^{b}	4.835^{abc}	4.625^{bc}	4.517^{bc}	4.247^{a}	4.120^{ab}
3	4.963^{b}	4.818^{c}	4.617^{bc}	4.512^{c}	4.242^{a}	4.116^{ab}
3.5	4.964^{b}	4.815^{c}	4.613^{c}	4.506^{c}	4.241^{a}	4.107^{ab}
4	4.965^{b}	4.826^{c}	4.621^{c}	4.514^{c}	4.244^{a}	4.118^{b}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

Table 8: Protein percentage of low fat stirred yoghurt supplemented with PPE, WPC and different conc. of MTSE during storage at 5±1°C for 15 days

	Storage period (days)					
Treatments	Fresh	3	6	9	12	15
Control 1	4.091^{b}	4.094^{c}	4.089^{c}	4.095^{d}	4.092^{d}	4.092^{c}
Control 2	4.186^{ab}	4.185^{bc}	4.187^{bc}	4.184^{cd}	4.188^{cd}	4.185^{ab}
1	4.216^{ab}	4.215^{abc}	4.217^{abc}	4.218^{bcd}	4.214^{bcd}	4.217^{ab}
1.5	4.368^{ab}	4.367^{abc}	4.369^{abc}	4.368^{abcd}	4.365^{abcd}	4.368^{ab}
2	4.547^{ab}	4.549^{abc}	4.548^{abc}	4.546^{abcd}	4.547^{abc}	4.546^{ab}
2.5	4.571^{ab}	4.570^{abc}	4.573^{abc}	4.570^{abcd}	4.572^{abc}	4.574^{ab}
3	4.629^{a}	4.628^{ab}	4.630^{ab}	4.632^{abc}	4.627^{ab}	4.626^{ab}
3.5	4.684^{a}	4.685^{ab}	4.683^{ab}	4.682^{ab}	4.685^{a}	4.686^{a}
4	4.715^{a}	4.717^{a}	4.715^{a}	4.716^{a}	4.715^{a}	4.716^{a}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

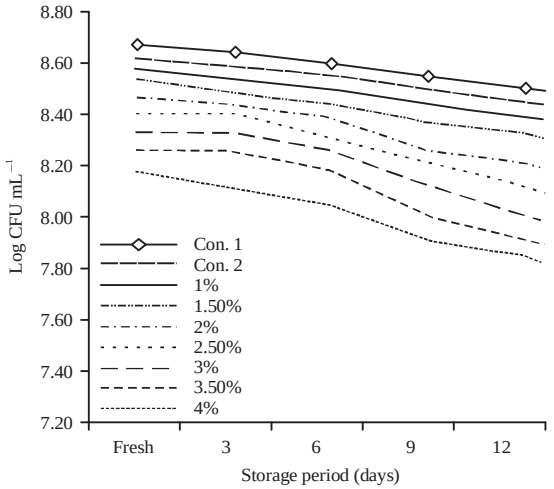

Fig. 6: *Lactobacillus delbrueckii* subsp., *bulgaricus* (log CFU mL^{-1}) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

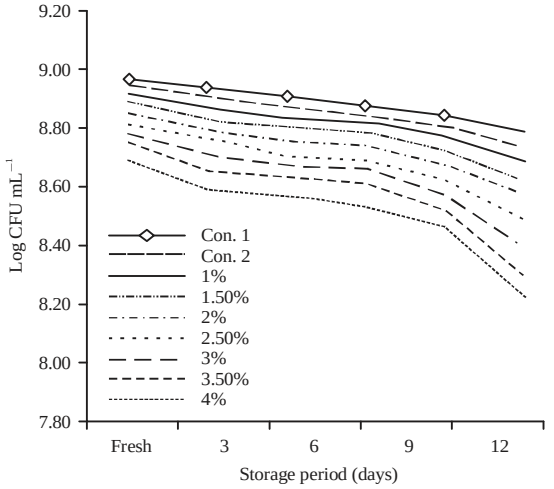

Fig. 7: *Streptococcus thermophilus* counts (log CFU mL^{-1}) of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

This finding is conformable with reported by Bessam and Mehdadi[35] who tested the effective of *Silybum marianum* L. seeds on three bacterial strains (*Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922 and *Bacillus cereus* ATCC 10876) and two fungal strains (*Candida albicans* ATCC 10231 and ATCC 16404 *Aspergillus brasiliensis*), they found *Silybum marianum* L., extract presented an antibacterial and antifungal power and revealed that the flavonoiques (sylibine, catechin and naringin) may be responsible for antimicrobial *Silybum marianum* power.

These results confirmed by Askun *et al.*[36] results which stated that flavonoids such as epigallocatechin, catechin, myricetin, quercetin and luteolin are important antibacterial substances. Moreover, the decreasing in the counts of *S. thermophilus*, *L. bulgaricus* and molds was explained by Cushnie and Lamb[37] who reported that extracts of plants rich in flavonoids and many other phytochemical possessed antimicrobial activities.

Karou *et al.*[38] explained the antibacterial activity of flavonoids is related to the mechanism of toxicity towards the microorganisms which is made the establishment of hydrogen bonds with proteins or enzymes of the cell walls or chelating of metal ions, inhibition of bacterial metabolism and sequestration of substances that necessary for the growth of bacteria.

Physical properties

Apparent viscosity of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days: Apparent viscosity is a primary factor in the prevention of settling and aggregation of solid suspended in yoghurt. Apparent viscosity of yoghurt supplemented with PPE, WPC and different ratios of MTSE measured as function of shearing rate used as a physical quality parameter to study the effect of the additives on the aggregation of protein during milk fermentation. Yoghurt samples were subjected to shearing period (0.0-5.0 min) and different values of shear rate. Results in Fig. 8 revealed that there were considerable decrease in the viscosity of yoghurt samples with increasing the concentration of MTSE that confirm products are pseudoplastic character. Moreover, there were no great differences in the viscosity between different ratios, even control 1 (yoghurt without any additives) and control 2 (yoghurt with PPE and WPC) had slightly higher viscosity than the corresponding yoghurt samples with PPE MWPC and MTSE.

Sensory evaluation of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days: Sensory evaluation of yoghurt supplemented with different ratios of MTSE was recorded in Table 9. Total scores which reflected the general quality of the product indicated that yoghurt with the different ratios of MTSE was accepted when fresh. While along the cold storage at 6°C for 15 days the sensory scores decreased not only for the MTSE yoghurt but also for the control.

An overall look to the total scores yoghurt supplemented with MTSE it could be recommended that yoghurt could

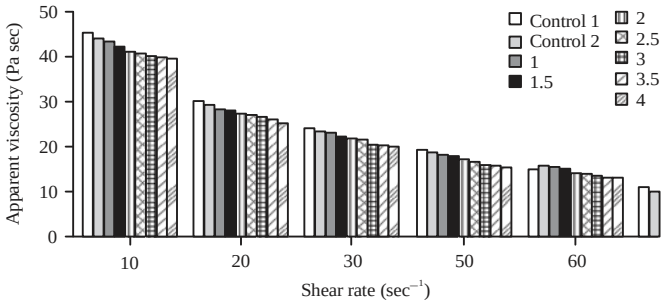

Fig. 8: Apparent viscosity of low fat stirred yoghurt supplemented with PPE, WPC and different concentration of MTSE during storage at 5±1°C for 15 days

Table 9: Sensory evaluation of low fat stirred yoghurt supplemented with PPE, WPC and MTSE percentage during storage (day) at 5±1°C

Storage period (days)	Control 1	Control 2	1%	1.50%	2%	2.50%	3%	3.50%	4%
Flavor (50)									
Fresh	46.5^{a}	45.40^{a}	44.30^{a}	43.60^{a}	43.30^{a}	43.10^{a}	42.90^{a}	42.50^{a}	42.20^{a}
3	45.00^{a}	45.60^{a}	44.30^{a}	43.80^{a}	44.00^{a}	43.80^{a}	43.70^{a}	43.70^{a}	42.11^{a}
6	44.50^{a}	44.10^{a}	43.60^{a}	43.30^{a}	43.00^{a}	42.80^{a}	42.80^{a}	42.50^{a}	41.23^{a}
9	42.80^{b}	42.00^{b}	41.90^{b}	41.80^{b}	41.33^{b}	40.78^{a}	40.44^{a}	40.33^{a}	40.11^{a}
12	41.60^{b}	41.30^{b}	41.00^{b}	40.80^{b}	40.400^{b}	40.10^{a}	39.60^{a}	39.30^{a}	39.20^{a}
15	41.52^{b}	41.20^{b}	40.69^{b}	40.42^{b}	40.00^{b}	39.71^{a}	39.54^{a}	39.26^{a}	39.15^{a}
Body and texture (40)									
Fresh	39.40^{a}	38.10^{a}	36.70^{a}	36.60^{a}	35.90^{a}	35.60^{a}	35.60^{a}	35.5^{a}	34.80^{a}
3	38.60^{a}	37.70^{a}	37.40^{a}	36.90^{a}	36.30^{a}	36.00^{a}	35.70^{a}	35.7^{a}	35.40^{a}
6	37.90^{b}	37.50^{a}	37.40^{a}	37.00^{a}	36.50^{a}	36.50^{a}	36.50^{a}	36.4^{a}	36.20^{a}
9	36.60^{b}	33.70^{b}	33.80^{b}	33.60^{b}	33.00^{b}	32.2^{b}	31.80^{b}	31.5^{b}	30.70^{b}
12	36.00^{b}	33.50^{b}	33.20^{b}	33.00^{b}	32.80^{b}	32.50^{b}	32.30^{b}	31.7^{b}	31.52^{b}
15	35.70^{b}	32.90^{b}	32.70^{b}	32.10^{b}	31.50^{b}	31.10^{b}	30.88^{b}	30.45^{b}	30.12^{b}
Appearance (10)									
Fresh	10.00^{a}	9.70^{a}	9.60^{a}	9.60^{a}	9.50^{a}	9.50^{a}	9.40^{a}	9.30^{a}	9.30^{a}
3	10.00^{a}	9.60^{a}	9.50^{a}	9.50^{a}	9.40^{a}	9.30^{a}	9.30^{a}	9.20^{a}	9.20^{a}
6	10.00^{a}	9.50^{a}	9.40^{a}	9.30^{a}	9.30^{a}	9.20^{a}	9.20^{a}	9.20^{a}	9.00^{a}
9	9.80^{a}	9.30^{a}	9.20^{a}	9.20^{a}	9.20^{a}	9.10^{a}	9.00^{a}	8.80^{b}	8.70^{b}
12	9.50^{a}	9.20^{a}	9.00^{a}	9.00^{a}	8.80^{b}	8.50^{b}	8.50^{b}	8.20^{b}	8.00^{b}
15	9.30^{a}	9.00^{a}	9.00^{a}	9.00^{a}	8.70^{b}	8.70^{b}	8.30^{b}	8.20^{b}	8.00^{b}
Overall acceptability (100)									
Fresh	95.90^{a}	93.20^{a}	90.60^{a}	89.80^{a}	88.70^{a}	88.20^{a}	87.90^{a}	87.30^{a}	86.30^{a}
3	93.60^{ab}	92.90^{a}	91.20^{a}	90.20^{a}	89.70^{a}	89.10^{a}	88.70^{a}	88.60^{a}	86.71^{a}
6	92.40^{ab}	91.10^{a}	90.40^{a}	89.60^{a}	88.80^{a}	88.50^{a}	88.50^{a}	88.10^{a}	86.43^{a}
9	89.20^{b}	85.00^{b}	84.90^{b}	85.60^{ab}	83.53^{b}	82.08^{b}	81.24^{b}	80.63^{b}	79.51^{b}
12	87.10^{b}	84.00^{b}	83.20^{b}	82.80^{b}	82.00^{b}	81.10^{b}	80.40^{b}	79.20^{b}	78.72^{b}
15	86.52^{b}	83.10^{b}	82.39^{b}	81.52^{b}	80.20^{b}	79.51^{b}	78.72^{b}	77.91^{b}	77.27^{b}

PPE: Pomegranate peel extract, WPC: Whey protein concentrate, MTSE: Milk thistle seeds extract, Control 1: Low fat yoghurt, Control 2: Low fat yoghurt supplemented with WPC and PPE, means within columns showing the same letter are not significantly

be supplemented with MTSE up to 4% and kept at 5°C for 15 days to get acceptable and high potential health product.

CONCLUSION

The current study revealed that PPE and WPC exhibited a potential antioxidant and radical scavenging properties whereas MTSE exhibited therapeutic potential. These combinations of ingredients gave combination of activity in the healthy fermented product, able to protect against the oxidative stress of free radicals and has heptaprotective activity. Product contain 25% PPE, 0.5% WPC and 4% MTSE was found to be more effective than the other ingredients concentrate and can be recommended to meet the health conscious consumer demanded. These achieve the multihealthy benefits especially for heptatoxicity. This product also has antifungal and antimicrobial power.

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