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ORIGINAL PAPER

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DEVELOPMENT OF FUNCTIONAL LOW-FAT YOGHURT FORTIFIED WITH PSYLLIUM HUSK MUCILAGE: QUALITY ATTRIBUTES, MICROSTRUCTURE, AND ANTIOXIDANT CHARACTERISTICS

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ABSTRACT

Background. Psyllium has a long history as a dietary supplement, since it is a rich source of both soluble and insoluble fibre that is reported as a traditional medicine in areas of India and China. Its consumption has been shown to provide nutritional benefits, Thus, interest in the incorporation of psyllium into food products is growing. This study was designed to assess how adding psyllium husk mucilage (PHM) at different concentrations impacts on the physical and quality characteristics of yoghurt during storage for 15 days at 5 ±1°C.
Materials and methods. Three different concentrations [33.3 (low), 66.6 (medium), and 100 mg/100 g (high)] were used in low-fat yoghurt fortifications. Water-holding capacity, syneresis, pH, titratable acidity, total phenolic content (TPC), DPPH scavenging activity, flavour compounds, texture profile, and sensory properties of resultant yoghurt were analyzed.
Results. The addition of PHM to low-fat yoghurts modified the acidification rate, fermentation time and significantly ($p < 0.05$) increased the viscosity, water-holding capacity, further improved the diacetyl concentration during storage. Total phenolic compounds and DPPH scavenging activity were significantly increased by the addition of PHM at a high dose (100 mg/100 g). Furthermore, low-fat yoghurt enriched with PHM had a compact and thick gel structure, together with a homogenous systematic protein aggregation network. Sensorially, low-fat yoghurt with 66.6 mg/100 g PHM revealed the highest sensory attributes score.
Conclusion. Overall, PHM extract offers a promising choice as a natural stabilizer for a unique fibre-fortified yoghurt with nutritional value.

Keywords: low-fat yoghurt, psyllium husk, physiochemical, antioxidant characteristics, microstructure, and sensory attribute

INTRODUCTION

The market for functional products is seeing significant growth due to the increasing demand for foods that have additional health-promoting ingredients beyond regular nutrition (Baker et al., 2022). In this respect, the introduction of dietary fibre-mixed products has been a significant advancement in this area. This is because the addition of dietary fibre to foods is a successful method for improving functionality,

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physiological and nutritional feature (Petruzziello et al., 2006). Numerous health advantages of consuming dietary fibre have been reported, including reducing the chance of contracting the following illnesses: heart disease, hypertension, diabetes, obesity, and some gastrointestinal disorders. Also, they may be used as functional additives to enhance the physical and structural features of hydration, oil-holding capacity, viscosity, and texture (Yadav et al., 2016).

Psyllium, scientifically known as *Plantago ovata*, is one of the most important and known forms of fibre, with a wide variety of uses (Amini et al., 2018). Consumption of psyllium has been demonstrated to have nutritional advantages including the ability to lower cholesterol, constipation and diarrhea problems as well as glycemia, and the risk of heart disease. Psyllium is produced mainly for its mucilage content, and is a highly branched arabinoxylan polysaccharide that has a high water-holding and gelling capacity (Fischer et al., 2004). Psyllium mucilage has a long history as a dietary supplement, since it is a rich source of both soluble and insoluble fibre that is also reported to be a medicinally active gel-forming natural polysaccharide (Fischer et al., 2004).

The use of dietary fibre to improve antioxidant activity and sensory acceptance of yoghurts has expanded significantly in recent decades, as customers prefer foods supplemented with natural ingredients rather than synthetic chemical molecules. As a result, significant investments have been made in the health food sector, and many milk-based formulations with various fibres have emerged. In creating formulations for yoghurt enrichment; the enrichment agent added to yoghurt has an effect on the acidification and reduction capacity (Bulut et al., 2019; Alwazeer et al., 2022), so physicochemical, rheological, textural and sensory properties (Bulut et al., 2021) and sensory properties (Bulut et al., 2022) should be taken into consideration of the product during storage. Psyllium mucilage has been widely used in the food, cosmetics, and pharmaceutical industries (Belorio and Gómez, 2020). Considering fibre's benefits, the possibility of developing fibre-fortified yoghurt using psyllium husk as a source of dietary fibre was attempted. However, a wide gap exists in psyllium research as a dietary fibre in dairy food fortification. As a consequence, this study was designed to investigate the effect of using psyllium husk mucilage (PHM) on the chemical, physical, rheological, and organoleptic properties of low-fat yoghurt.

MATERIALS AND METHODS

Materials and chemicals

Fresh whole and skimmed buffalo milk was obtained from the Dairy Technology Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Spray-dried low heat skimmed milk powder (96.2% TS, 0.8% Fat, and pH 6.5 ±0.02) was purchased from Mifad (Misr Food Additives), Egypt. Psyllium husk (PH) was also obtained from the local market, Giza, Egypt. Freeze–dried lactic culture (*Lb. delbrueckii* ssp. *bulgaricus* and *St. thermophilus*) was obtained from Chr. Hansen laboratories (YC-X11, Hoersholm, Denmark).1,1-diphenyl-2-picrylhydrazyl (DPPH), and Gallic acid was purchased from Sigma-Aldrich (Egyptian International Centre for Import, Cairo, Egypt).

Extraction of psyllium husk mucilage (PHM)

PHM was extracted according to the procedure described by Antigo et al. (2017). Briefly, sieved powder was extracted as follows: 1.0 g of psyllium husk powder was dispersed and hydrated in water at different ratios namely 1:25, 1:50, 1:75 and 1:100 and was left for 1.2 h at 80ºC under constant stirring with a magnetic stirrer. It was very difficult to obtain a suitable extract to be applied from the first and the second ratios, while it was easy with the third and fourth ratios, therefore the 1:75 ratio was chosen, in which 1 ml of extract contained 13.3 mg psyillium husk, three concentrations were applied in this study (2.5 ml, 5 ml and 7.5 ml/100 gm, which was calculated mathematically as 33.3, 66.6 and 100 mg/100 g. Insoluble fractions of psyllium husk extract were removed by filtration using cloth fabric and organza fabric, and the filtrate (or hydrocolloid) was then kept at 5ºC until usage. The psyllium mucilage extract was chemically analyzed for its protein, fat, ash, fibre, and total solid contents, while the carbohydrate content was calculated by the differences.

Low-fat yoghurt preparation

Yoghurt was prepared following the procedures described by Hashim et al. (2009). Five different treatments of yoghurt were manufactured on the laboratory scale, as shown in (Fig. 1). The filling process was

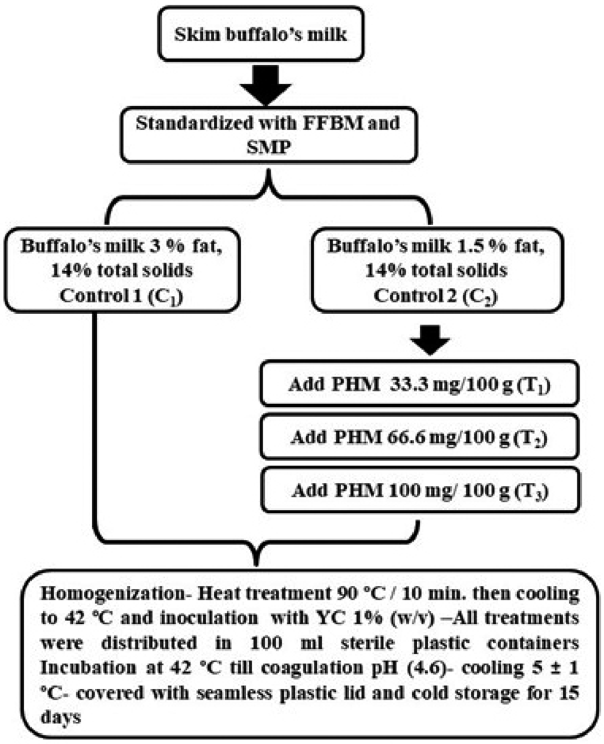

Fig. 1. Flow chart for the manufacturing of yoghurt treatments with different psyllium husk mucilage (PHM) amounts (SMP – skimmed milk powder, FFBM – full-fat buffalo milk, YC – yoghurt culture YC-X11)

performed in high-density polyethylene cups covered with seamless plastic lids. The resultant yoghurt cups with final pH 4.6 were then stored in the refrigerator at 5 ±1ºC for 15 days. All experiments were conducted in triplicate. All yoghurt samples were analyzed periodically when fresh and on 4, 8, 12 and 15 days of cold storage, except for the texture profile analysis, antioxidant activity and total phenolic content, which were performed when fresh and by the end of storage.

Physico-chemical properties of psyllium husk powder (PH) and yoghurt

Protein, fat, fibre, moisture, total solids of psyllium husk powder, titratable acidity, total solids, and fat of standardized buffalo milk and yoghurt samples were determined using AOAC methods (AOAC, 2005). The pH values of the yoghurt samples were measured using a digital pH-meter (WTW 720-inolab, D-82362 Weilheim, Germany).

Determination of total phenolic (TPC)

The TPC of the PHM and the yoghurt samples was measured using the Folin–Ciocalteu assay (Zhang et al., 2019), and the absorbance of the mixture was measured at a wavelength of 725 nm using a spectrophotometer. The results are expressed as the µg gallic acid equivalents (GAE) per milliliter.

Radical Scavenging Activity (RSA %) assay

The total free radical scavenging capacity of psyllium husk powder (PH) and yoghurt was evaluated using 2,2-diphenyl-1-picrylhydrazyl DPPH, as previously described by Shori and Baba (2013). A water-soluble extract (WSE) of yoghurts was prepared according to the procedure described by Shori et al., (2013). Briefly, the WSE (1 ml) was mixed with 0.1 mmol/l DPPH (1 ml) dissolved in 95% ethanol. The mixture was shaken and kept at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. Distilled water was used as a blank instead of the sample. Ascorbic acid (0.2 mmol/l) was used as a positive control. The scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_s)/A_0 \times 100$$

where
A_0 is the absorbance at 517 nm of blank
A_s is the absorbance at 517 nm of WSE.

It was used for to determine antioxidant activity in vitro by the DPPH method. Radical scavenging activity was calculated and expressed as percentages.

Texture profile analysis

Textural characteristics of yoghurt samples were detected using the texture analyzer (Multi test 1d Memes in, Food Technology Corporation, Slinfold, W. Sussex, UK), following the method described by Bhat et al. (2018). Briefly, texture profile analysis was carried out by means of a compression test that generated plot of force (N) versus time (s). A 25-mm-diameter perplex cylindrical probe was used to measure textural profile of the yoghurt samples at 10 ±0.5°C. In the first stage, the samples were compressed to 10 mm depth and the speed of the probe was fixed at 30 mm/min. during the pre-test, compression, and relaxation of the samples. The typical textural profile (force–time) curve was obtained with one complete run. Hardness, gumminess, adhesiveness, cohesiveness, chewiness and springiness of yogurt samples were calculated using the software programme (Texture Pro software, Brookfield Instruments). The data presented are the average of 5 replications

Viscosity and water-holding capacity

A Brookfield digital rotating viscometer (model DV-II +, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) with spindle No. 4 at 10 rpm for one minute was used to measure the apparent viscosity of yoghurt samples. Before being measured, the samples were allowed to temper at 25°C for ten minutes. The viscosity value was then expressed in centipoises (cP). The yoghurt's capacity for holding water was assessed using the methodology outlined by Hassan et al. (2022). The WHC was calculated using the following equation:

$$\text{WHC (\%)} = [(\text{yoghurt weight} - \text{supernatant weight}) / \text{yoghurt weight}] \times 100$$

Syneresis Index

The syneresis of yoghurt samples was performed according to the method described by Damin and Olteanu (2014). A portion of 100 ml of yoghurt samples was placed on a funnel lined with a Whatman filter paper No.1 at 4ºC. After 2 h of drainage, the separated whey was measured and used as an index of syneresis, according to the following equation.

$$\text{Syneresis (\%)} = \text{weight of the whey expelled} / \text{weight of initial yoghurt sample} \times 100$$

Determination of acetaldehyde and diacetyl

Acetaldehyde and diacetyl were determined as presented by Hassan et al. (2015). The absorption for acetaldehyde and diacetyl was measured at 224 nm and at 270 nm, respectively.

Microstructure visualization

The microstructure of the yoghurt samples was observed by SEM following the method described by Pang et al. (2016). The sample preparation for SEM followed this method. After the fixation of fat, samples were rewashed several times in 0.1 M PBS (pH 7.2) for 15-min. intervals. Samples were dehydrated and dried to the critical point using CO2 in a Critical Point Dryer (Polaron, Waterford, UK) and mounted on aluminum SEM stubs, sputter-coated with gold (Spi module sputter coater, spi supplies division of structure probe). The final voltage was 25 kV, with magnifications of 1,000 times observed by SEM.

Sensory assessment

Yoghurt samples with and without PHM were first assessed for their acceptability by general untrained staff and the question to be answered was whether this product is acceptable or not. The second stage was carried out by six well trained panelists from the staff members of the Dairy Science Department, Faculty of Agriculture, Cairo University to identify some characteristics of the resultant yoghurt, like appearance, flavour, texture, mouth feel, and overall acceptability. All the yogurt samples were evaluated within a random order in its high-density polyethylene plastic cups. The score card was designed as described by Hussein et al. (2011).

Statistical analysis

The results were shown as the mean ±standard deviation (SD) and three replicates of each parameter were carried out. A randomized complete block design and the analysis of variance of factorial methods were carried out using Mstat-C software. The significance level was established at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of psyllium husk mucilage (PHM)

Table 1 shows the chemical composition of psyllium husk mucilage. It contained 2.72% moisture with total solids reaching 97.28 ±0.92%, whereas the carbohydrate, fat, and protein contents were recorded as 87.08 ±0.52%, 1.19 ±0.01 %, and 3.89 ±0.01 %, respectively. The results were in agreement with Deepika et al. (2017). The contents of crude fibre and ash were 2.96± 0.02% and 2.16 ±0.01%, respectively. PHM contained a total phenolic content of 40.37± 0.62 µg/mg (Gallic acid equivalent) per gram of PHM and its Radical Scavenging Activity (RSA %) was 47.81 ±0.46%. PHM is clearly a nutrient-dense food that is a rich

Table 1. Chemical composition of psyllium husk mucilage PHM

Parameters	Psyllium husk mucilage
Total Solids	97.28 ±0.92%
Fat	1.19 ±0.01%
Protein	3.89 ±0.01%
Carbohydrate	87.08 ±0.52%
Crude fibre	2.96 ±0.02%
Ash	2.16 ±0.01%
Moisture	2.72 ±0.01%

source of antioxidants, phenolics, and flavonoids that have been linked to disease prevention including cancer and other non-communicable diseases (Shah et al., 2020). Table 2 summarizes the chemical composition of the heat-treated milk with PHM before fermentation. The addition of PHM showed no significant effect ($p > 0.05$) on the fat content. In contrast, the protein, and total solid contents of the yoghurt samples increased significantly ($p < 0.05$) upon the addition of PHM. These findings could be attributable to the high contents of total solids and protein in PHM. These results are in accordance with those of the previous reports (Mehanna, 2013; Saker, 2019). Moreover, fibre content was not detected in the control samples, while it increased gradually as the rate of PHM increased, reaching 2.14 ±0.23 with the addition of 100 mg PHM in T_3. Figure 2 shows the changes in acidity (a) and pH (b) of yoghurt during 15 days at 5 ±1°C. Interestingly, the yoghurt incubation time was found to increase as a result of the incorporation of PHM into the yoghurt. The control (C_1 and C_2) took 3 h to reach pH 4.6, whereas yoghurt with 100 mg PHM reached pH 4.6 in 3 h and 25 min, followed by the sample containing 66.6 mg (3 h 15 min) and the sample contain 33.3 mg PHM reached pH 4.6 in 3h and 10 min. However, Alwazeer et al. (2020) stated that the addition of plant extracts slightly stimulated the acidification activity of the LAB strain, and reduced the fermentation time when the milk sample was enriched with any type of plant extracts. An overall look at the data presented clearly shows that the titratable acidity recorded values

Table 2. Chemical composition of the standardized buffalo milk used in the production of low-fat yoghurt

PHM concentrations	Control (C_1)	Control (C_2)	T_1	T_2	T_3
Total solid, %	14.7 ±0.01[b]	14.20 ±0.01[e]	14.31 ±0.03[d]	14.51 ±0.03[c]	14.80 ±0.01[a]
Fat, %	3.00 ±0.01[a]	1.50 ±0.01[b]	1.50 ±0.01[b]	1.50 ±0.01[b]	1.50 ±0.01[b]
Protein, %	4.66 ±0.01[e]	4.94 ±0.03[d]	5.25 ±0.08[c]	5.48 ±0.22[b]	5.87 ±0.01[a]
Fibre, %	ND*	ND*	1.06 ±0.15[c]	1.34 ±0.12[b]	2.14 ±0.23[a]

*ND: not detected. Data means ±SD (n = 3). Different superscript letters in the same raw indicate significant difference ($p < 0.05$)
All values are means ± standard deviation for three replicates.

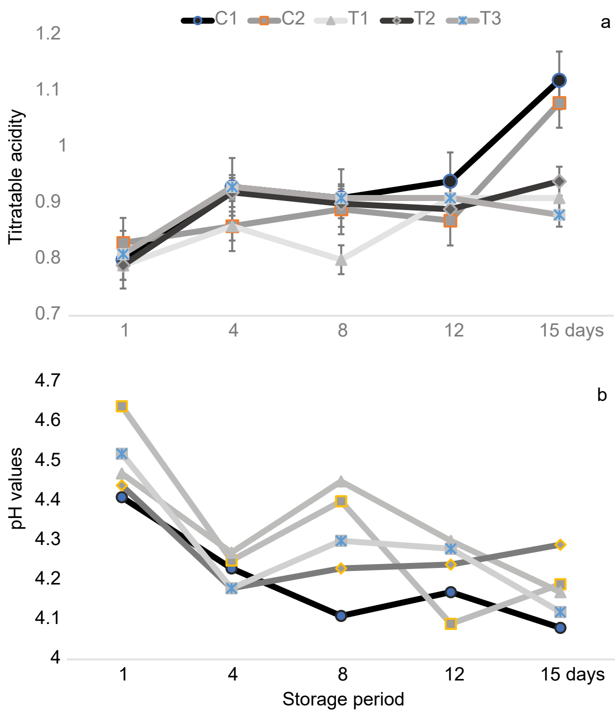

Fig. 2. Average titratable acidity (a) and pH values (b) of yoghurt treatments during cold storage, C_1 3.0% fat without PHM; C_2 1.5% fat without PHM; T_1, T_2, T_3 are low-fat yoghurt with 33.3 mg, 66.6 mg and 100 mg PHM, respectively

ranged from 0.79 to 0.83% after one day of cold storage, and it increased during cold storage in all treatments, to finish with final values ranging from 0.87 to 1.12%. The increase from the first day to the end of the storage time is compatible with the results reported by Srisuvor et al. (2013) and do Espírito Santo et al. (2012), who fortified yoghurt with banana purée and passion fruit peel powder, respectively. The highest mean values of acidity after 15 days of cold storage were 0.94 for the full-fat control yoghurt C_1 followed by low-fat control yoghurt C_2 then yoghurt with a high and medium concentration of PHM T_3 and T_2, which recorded 0.90, 0.89 and 0.88%, respectively. Statically no significant difference ($p > 0.05$) was detected between C_2, T_2 and T_3 but they differed significantly ($p < 0.05$) with C_1. On the other hand, T_1 recorded the lowest mean titratable acidity value at 0.85% with significant difference ($p < 0.05$) than all other treatments). The opposite trend to titratable acidity was observed in pH values. These values decreased after one day of cold storage and ranged from 4.41 for C_1 to 4.44 for T_2, with a slight fluctuation in T_1, T_2, and T_3 on the 8th day, and all values decreased, ending with 4.17 with C_1 and 4.29 for T_2, while C_2, T_1 and T_3 ended with 4.19, 4.17 and 4.12, respectively, after 15 days of cold storage. This finding is in agreement with those of Amiri et al. (2014), who reported that the pH of the low-fat formulations would be higher than that of the full-fat sample due to the dilution of acetic acid in the aqueous phase of the low-fat samples. Furthermore, Amini et al. (2018) stated that high viscosity induced by a high amount of PHM may slow down the mass transfer of energy-providing substrate and, consequently, alleviate the efficiency rate of fermentation or acidification process. The pH values of all treatments decreased slightly during storage. This effect may be attributed to the fibre-buffering capacity of psyllium husk resulting in no influential effect on lactic acid production by starter cultures, in addition to the metabolic activity of the starter culture, which produced lactic acids and other organic acids (Shori et al., 2018).

Total phenolic content

The total phenolic content of the control and PHM-added samples is presented in Figure 3. The control yoghurts C_1 and C_2 had the lowest value of TPC, standing at 105.53 and 120.28 mg GAE/g, respectively. Yoghurt with PHM had a considerably greater TPC concentration ($p < 0.05$), which gradually increased as increasing amounts of PHM were added, which might be due to the release of more phenolic compound. At the end of cold storage, TPC concentration significantly ($p < 0.05$) decreased in all treatments to reach 95.83, 105.41, 112.70, 121.51, and 134.42 mg GAE/g for C_1, C_2, T_1, T_2 and T_3, respectively. Similar findings were obtained by Jambi (2018) and Muniandy et al. (2016), who reported that the TPC of yoghurt fortified with date seed powder (a rich source of polyphenols) decreased during storage. This may be due to the decomposition of polymeric phenolics in the presence of lactic acid bacteria during refrigerated storage.

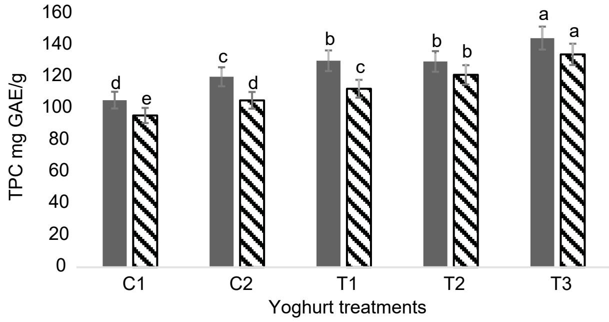

Fig. 3. Total phenolic content of different yoghurt treatments on day 1 ■ and day 15 ▧. The data are means ±SD; values of each parameter assigned identical letters do not vary significant

Radical scavenging activity (RSA %) assay

The antioxidant capacity of yoghurt was determined using the DPPH radical scavenging method and the results are illustrated in Figure 4. The highest significant ($p < 0.05$) RSA% was noted in yoghurt samples fortified with a high concentration (100 mg) of PHM either on day 1 or at the end of cold storage and the lowest ($p < 0.05$) value was observed in yoghurt without PHM (C_1) on day 1. These findings are attributed to the fortification of yoghurt with PHM, which contains considerable phytochemical contents as reported by Thompson et al. (2007). While low-fat yoghurt without PHM(C_2) recorded the lowest DPPH scavenging activity after two weeks of cold storage. In general, yogurt products exhibit antioxidant activities mainly due to their bioactive peptide contents produced by the action of lactic acid bacterial culture, as reported

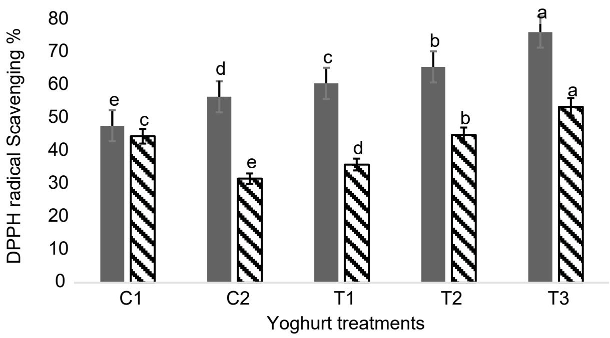

Fig. 4. DPPH radical scavenging activity of different yoghurt treatments on days 1 ■ and 15 ▧. C_1 3.0% fat without PHM; C_2 1.5% fat without PHM; T_1, T_2, $T_3$are low-fat yoghurt with 33.3 mg, 66.6 mg and 100 mg PHM, respectively. Data are means ±SD; values of each parameter assigned identical letters do not vary significant

by Demirci et al. (2017). This may explain the antioxidant activity of the control yogurt without PHM supplementation. As expected, the DPPH was significantly increased and in a direct and proportional relationship with the increase in the concentration of PHM as reported by Sah et al. (2015). In parallel with these findings, Xu et al. (2016) and Demirci et al. (2017) reported an increase in the antioxidant activity of probiotic yogurt due to the high polyphenol content in rice bran, pineapple waste powder. The reduction in antioxidant activity during storage may be due to the degradation of the phenolic compound as a result of the changes in microbial activity during storage and/or increasing milk protein-polyphenol interaction (Yildiz et al., 2009).

Texture profile analysis

Texture profile analysis (TPA) is a technique commonly used in the food industry to assess the texture attitude of food, as it might reveal sensory qualities. Furthermore, texture is an important factor in determining the quality of food products, and it is fully influenced by food composition (Fundo et al., 2018). The effect that adding PHM has on the texture attributes is listed in Table 3. Full-fat yoghurt C_1 was characterized by higher significant values of hardness, adhesiveness, gumminess, and chewiness when compared with the low-fat one (C_2). On the other hand, springiness and

Table 3. Texture profile analysis of different yoghurt treatments* fortified with PHM

	Storage days	C_1	C_2	T_1	T_2	T_3
Hardness, N	1	11.14 ±0.11^b	4.20 ±0.02^e	8.13 ±0.01^d	9.30 ±0.02^c	7.47 ±0.01^e
	15	12.00 ±0.01^a	11.00 ±0.09^b	5.93 ±0.05^f	8.37 ±0.01^d	5.80 ±0.00^g
Adhesiveness, N s^{-1}	1	0.98 ±0.01^e	0.87 ±0.01^f	1.03 ±0.01^d	1.20 ±0.01^b	1.05 ±0.00^c
	15	1.07 ±0.02^d	0.88 ±0.0^f	1.11 ±0.00^c	1.47 ±0.01^a	1.25 ±0.00^b
Cohesiveness	1	0.51 ±0.01^e	0.82 ±0.01^b	0.87 ±0.00^a	0.55 ±0.00^c	0.52 ±0.01^d
	15	0.43 ±0.01^h	0.53 ±0.00^d	0.49 ±0.01^f	0.51 ±0.00^e	0.47 ±0.01^g
Gumminess, N	1	5.77 ±0.03^a	3.47 ±0.02^h	5.20 ±0.01^b	4.20 ±0.00^e	3.80 ±0.02^g
	15	5.20 ±0.02^b	4.77 ±0.05^c	4.67 ±0.01^d	4.02 ±0.01^f	2.80 ±0.01^i
Springiness	1	7.93 ±0.01^c	8.16 ±0.01^b	8.19 ±0.02^a	6.93 ±0.01^d	5.58 ±0.03^g
	15	5.45 ±0.010^i	5.18 ±0.00^j	6.66 ±0.01^e	6.04 ±0.01^f	5.30 ±0.01^h
Chewiness, N	1	45.51 ±2.15^a	28.02 ±1.35^d	31.58 ±1.35^b	29.13 ±1.15^c	21.31 ±1.21^g
	15	28.23 ±1. 4^d	29.31 ±1.41^c	27.08 ±0.95^e	24.89 ±1.25^f	17.32 ±1.30^h

*Abbreviations are:C_1 Control, 3.0% fat without PHM; C_2 Control, 1.5% fat without PHM, T_1,T_2, T_3 are low-fat yoghurt with 33.3 mg, 66.6 mg and 100 mg PHM, respectively. Data means ±SD; values of each parameter with the same superscript letters do not vary significantly.

cohesiveness recorded lower values when compared with C_2. Upon fortification with PHM, hardness values increased in all treatments when fresh, with no direct relationship with the addition ratio. By the end of storage, the hardness values had decreased significantly in all treatments. This decrease in hardness could be due to the disrupting effect of fibre on the gel and may be justified with the viscosity effect of PHM. In higher concentrations, PHM might show higher viscosity and thus may have a film forming action, which restricts the interaction of culture medium and milk components and desired texture of yogurt could not be attained at higher levels of PHM supplementation. Hence, at higher levels, PHM decreased the hardness of yoghurt. While with adhesiveness, the values increased in direct relationship with the PHM concentration added. This increase in adhesiveness values might be due to the effect of the branches of hydrocolloids that exist in the PHM, which leads to adhesion strength. On the other hand, cohesiveness values decreased as PHM concentration increased. This decrease might be due to the negative correlation between adhesiveness and cohesiveness parameters. As for gumminess, springiness and chewiness, the recorded values in T_1 increased when compared with C_2 and then decreased as the addition ratio increased when fresh. By the end of the storage period, all parameters measured and, in all concentrations, added had decreased significantly. These results are compatible with the results of viscosity and WHC and with what was previously reported by Hussein et al. (2011) and Bhat et al. (2018).

Acetaldehyde and diacetyl content

The changes in the flavour compounds of yoghurt samples, as measured by the concentration of acetaldehyde and diacetyl through the storage period as affected by the ratio of PHM added, are given in Table 4. There was a fluctuation in the results due to the volatile nature of both compounds. Where the higher addition ratio of PHM in T_3 led to a significant increase in acetaldehyde content as compared with the control yoghurt samples C_1, C_2, and T_1&T_2. This increment was concentration-dependent. During cold storage, its values decreased significantly with a clear fluctuation in all treatments. These results may be due to the conversion of acetaldehyde to ethanol as a result of the presence of enzyme alcohol dehydrogenase produced by yoghurt cultures (Güler et al., 2009) or its oxidation to acetic acid (Guzel-Seydim et al., 2005). Diacetyl clearly showed an opposite trend to that of

Table 4. Acetaldehyde and diacetyl content of yoghurt treatments fortified with PHM

	Storage days	C_1	C_2	T_1	T_2	T_3
Acetaldehyde, ppm	1	99.57 ±0.31^{e}	121.17 ±0.60^{c}	111.45 ±0.59^{d}	125.79 ±0.61^{b}	136.10 ±0.37^{a}
	4	88.20 ±0.26^{c}	82.17 ±0.38^{d}	81.10 ±0.29^{e}	113.5 ±0. 52^{a}	98.87 ±0.43^{b}
	8	108.13 ±0.50^{b}	106.42 ±0.48^{c}	96.24 ±0.34^{d}	91.35 ±0.4^{e}	117.41 ±0.61^{a}
	12	52.01 ±0.20^{e}	67.92 ±0.13^{b}	62.96 ±0.23^{c}	61.45 ±0.32^{d}	104.81 ±0.60^{a}
	15	58.22 ±0.16^{e}	101.14 ±0.41^{b}	103.06 ±0.58^{a}	98.55 ±0.41^{c}	90.30 ±0.31^{d}
Diacetyl, ppm	1	20.0 ±0.04^{a}	18.0 ±0.03^{b}	14.0 ±0.01^{c}	10.0 ±0.01^{d}	7.0 ±0.01^{e}
	4	18.0 ±0.01^{a}	11.0 ±0.02^{e}	14.0 ±0.00^{c}	12.0 ±0.02^{d}	15.0 ±0.01^{b}
	8	15.0 ±0.04^{c}	13.0 ±0.02^{d}	15.5 ±0.02^{b}	12.0 ±0.00^{e}	18.5 ±0.01^{a}
	12	6.0 ±0.01^{e}	15.5 ±0.01^{b}	14.5 ±0.01^{c}	12.0 ±0.00^{d}	18.0 ±0.02^{a}
	15	17.0 ±0.05^{c}	17.0 ±0.01^{c}	17.0 ±0.01^{c}	21.5 ±0.03^{a}	19.0 ±0.03^{b}

*Abbreviations are: C_1 Control, 3.0% fat without PHM; C_2 Control, 1.5% fat without PHM; T_1, T_2, T_3 are low-fat yoghurt with 33.3 mg, 66.6 mg and 100 mg PHM, respectively. Data are means ±SD, values with different superscript lowercase letters in the same row are significantly different.

acetaldehyde, as the diacetyl values decreased in all treatments when compared to the control sample (C_1) and this decrease was found in direct relationship to the concentration added of PHM on day 1 of cold storage. During cold storage, diacetyl content decreased gradually in control samples C_1, C_2 and increased by the end of storage period to reach 17 ppm. On the other hand, diacetyl content gradually increased in PHM treatments (T_1, T_2, T_3) to reach 17, 21.5 and 19 ppm, respectively. The decline in the content of diacetyl may be due to the slow conversion of diacetyl to acetoin, as reported by Driessen and Puhan (1988), while Sahan et al. (2008) and Hassan et al. (2015) demonstrated that the addition of guar gum to yoghurt led to a slight increase in the diacetyl concentration of yoghurt samples during the storage time.

Syneresis Index and WHC

The changes that occurred in the syneresis value (%) and water-holding capacity (WHC) of low-fat yoghurts fortified with PHM as compared with traditional ones were noted on days 1, 4, 8 and 12, and after 15 days of cold storage (Fig. 5). It is obvious from the data presented here that the syneresis values were higher in all yoghurt samples when fresh (after 24 h of cold storage) and it began to decline significantly ($p < 0.05$) but gradually during cold storage until it ended in low values, especially in T_2 and T_3. It should be noted that at a lower temperature, stronger bonds can be formed between the particles of the gel, and it is possible that their number increases. This can be assumed when swollen particles become linked to each other in a broader space. On days 8, 12 and 15, there were significant ($p < 0.05$) differences between the

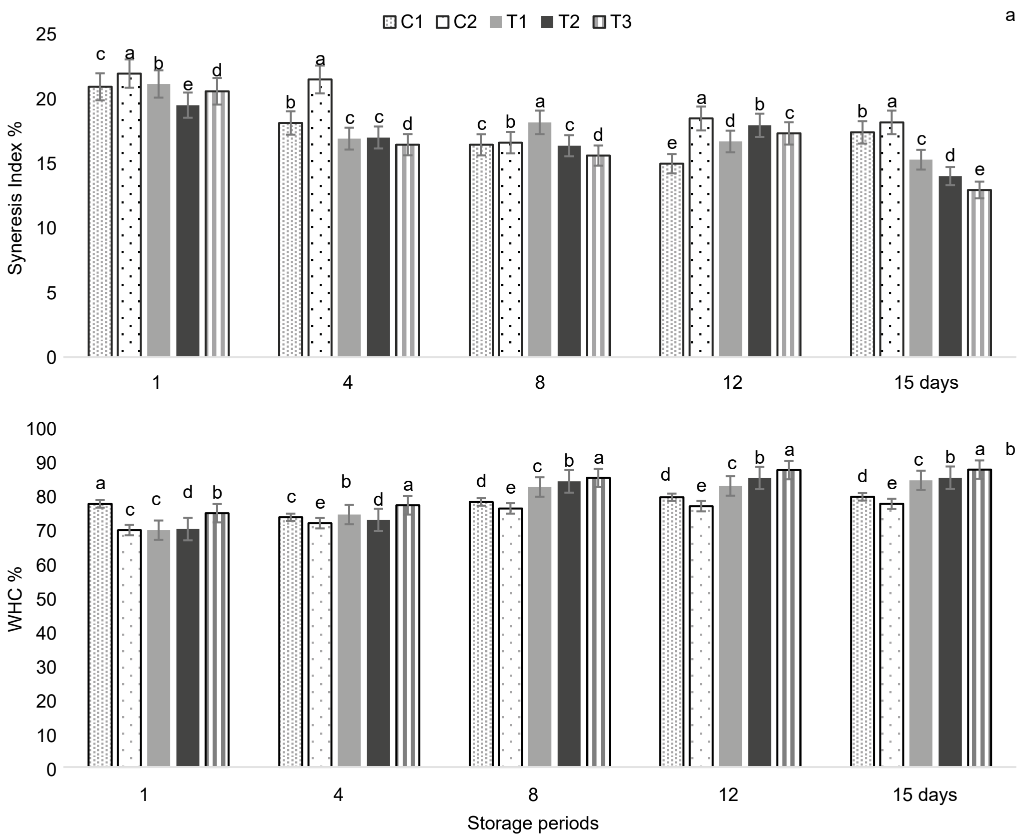

Fig. 5. Syneresis Index (a) and water-holding capacity (WHC; b) of different yoghurt treatments, data means ±SD; values of each parameter assigned identical letters do not vary significantly

control samples C_1 and C_2 (which showed the highest rate of syneresis) and the samples with added psyllium husk mucilage. A higher level of syneresis was found in yoghurts with lower fat content, this result may be due to the effect of the fat ratio on the syneresis value, so the low-fat products due to body and appearance defects are usually produced with the addition of some ingredients as fibre in order to improve the textural properties. The following happened: as the addition of psyllium husk mucilage led to a decrease in the syneresis values with very clear significant differences from the two control samples. This is due to the role of fibre in trapping the water inside the yoghurt curd or inside the protein matrix. These results are in agreement with what was reported by Damin and Olteanu (2014), who used pea fibre, and with Ünal et al. (2003), who used locust bean gum. Similar reductions in whey syneresis among yoghurt samples have been reported by enriching yoghurts with quince seed mucilage, cress seed mucilage, and flaxseed mucilage (Razmkhah et al., 2010). An increasing trend in WHC was observed in low-fat yoghurts fortified with PHM compared with control samples. Moreover, yoghurts fortified with PHM had significant ($p < 0.05$), higher WHC during storage. This increase in the water-holding capacity of fortified yoghurt is due to the higher water-holding capacity of soluble fibre in the mucilage, which also provides strength to the yoghurt coagulum network and aids water retention. In this respect, Yekta and Ansar (2019) found that the addition of Jujube mucilage as a potential stabilizer in stirred yoghurt reinforced the WHC of low-fat yoghurt.

Microstructure visualization

The microstructures of yoghurt treatments on day 1 and after 15 days of cold storage are shown in Figure 6. Different yoghurt treatments' scanning electron micrographs revealed variations in casein micelles,

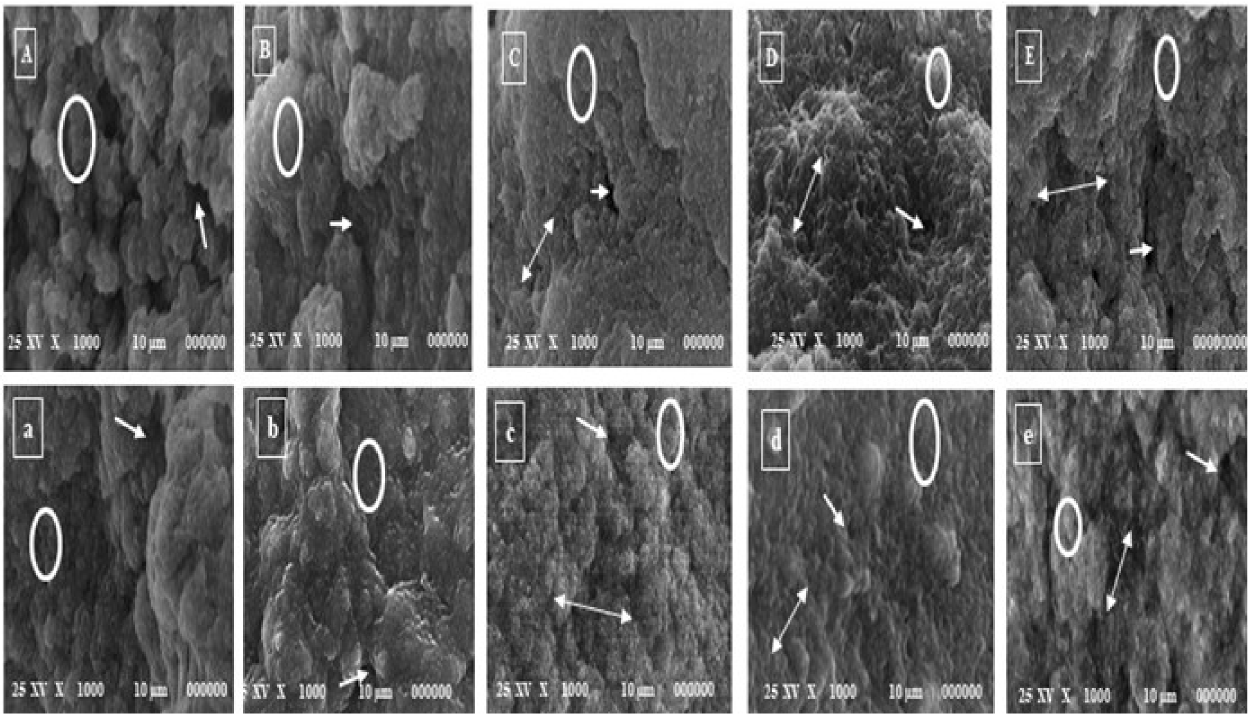

Fig. 6. Scanning electron micrographs (1000×) of yoghurt treatments; capital letters for fresh yoghurt, while small letters for stored yoghurt treatments at 4°C for 15 days (A, a) full-fat control yoghurt, (B, b) low-fat control yoghurt, (C, c; D, d and E, e) low-fat yoghurt fortified with 33.3, 66.6, 100 mg PHM /100 gm, respectively. Void space contains whey (marked with white arrows), PHM effect on protein aggregates and structure marked with double arrows and casein network marked with white ovals. Scale bar is 10 µm

whey aggregation, and pore size. As shown in Figure 6, a compact and dense protein matrix (grey area) formed a continuous phase that was infiltrated by an amorphous system of voids, with spherical fat globules of various sizes dispersed in the continuous phase and distributed throughout the protein matrices. The gel network in the full-fat control sample C_1 was irregular, with large pores and short individualized casein filaments. The images showed heterogeneous in the clusters or in pore size. Additionally, there were some openings and fat globules; fewer pores were detected in C_2. The images C, D, and E showed that the PHM effect on protein aggregates leads to the formation of a fibrous structure and a decrease in the number and diameter of voids. A more filament structure and compact networks were obtained with increasing PHM proportions, resulting in a denser network and a lower tendency to syneresis. It is worth noting that the results of microstructure were in harmony with the syneresis index and TPC of the resultant yogurt, since increasing PHM addition led to a significant decrease in whey drainage. Further, the addition of PHM, which contains phenolic components, may lead to greater retention of whey in the yoghurt structure. In this respect, Oliveira et al. (2015) reported that dairy products incorporated with some polyphenol-rich compounds have improved physical properties resulting from the interaction between polyphenols and proteins. In addition, it might be attributed to the combined interaction of PHM and casein micelle via electrostatic and hydrophobic interaction in the pH range of 3.5–4.8, as reported by Tuinier et al. (2002). This microstructural arrangement results in less protein rearrangement and reduced syneresis susceptibility, making yoghurt fortified with PHM more stable during storage (Fig. 6 c, d, and e). In addition, the microstructure of yoghurt fortified with PHM during storage reveals a homogeneous systematic protein aggregation network and the protein matrix appeared as a smooth continuous phase of aggregated micelles with a compact fusion and a dense structure containing fewer voids. Moreover, the presence of PHM obscures the finer details of pores and strands. The diameter of these pores varies considerably with small pores in milk supplemented with PHM. The characteristics described in these SEM images are consistent with Dong et al. (2022), who stated that microstructural arrangement results in less protein rearrangement and reduced syneresis susceptibility, making yogurt supplied with soluble dietary fibre (SDF) more stable during storage. Also, the fact that complex micelle aggregations of adsorbed SDFs and casein form large networks might contribute to the stability of the yoghurt.

Apparent viscosity

Yoghurt's apparent viscosity was evaluated over 15 days of refrigerated storage and the data are shown in Figure 7. Apparent viscosity is affected by different

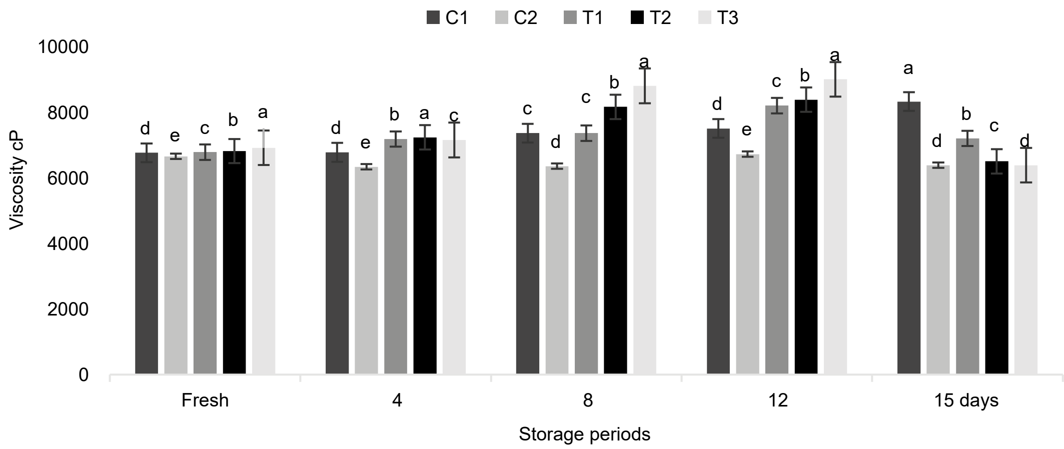

Fig. 7. Apparent viscosity of traditional and fortified low-fat yoghurt as affected by psyllium husk mucilage (PHM) percentages. Data means ±SD; values of each parameter assigned identical letters do not vary significantly

factors, such as milk composition, heat treatment of milk, and additives. An overall look at the presented data clearly indicates that the apparent viscosity of the full-fat control yoghurt C_1 recorded higher values than the low-fat control C_2 without psyllium husk when fresh (one day cold storage), while treatments T_1, T_2 and T_3 recorded higher values, mainly due to the effect of PHM. The higher viscosity value of C_1 is due to the effect of high fat content in this sample. During storage, the viscosity value increased significantly ($p < 0.05$) in all treatments till the end of storage except T_3, which recorded lower values starting from the twelfth day. This might be due to the higher concentration of psyllium husk (100 mg), which is likely to lead to an interaction between protein and fibre, and which leads to the formation of a weak gel. It is worth noting that the results of texture profile analysis (TPA) were in harmony with apparent viscosity. Since all parameters of T_3 had decreased, the hardness value, which was lower at the end of the storage period, led to a decrease in viscosity value. Preliminary experiments, on the other hand, have shown that increasing PHM beyond the reasonable limit leads to counterproductive results. From the data presented, one can observe that the addition of PHM led to a pronounced increment in yoghurt viscosity in all treatments and the increment was not proportional to the amount of PHM added, as the highest increment was recorded with T_1 followed by T_2 then T_3. These results are in conformity with those of Sahan et al. (2008), who reported that the addition of wheat bran led to a significant increase in the apparent viscosity of yoghurt. Furthermore, Aryana (2006) reported that the apparent viscosity of fibre-fortified yoghurts was comparable to the control when different fibres were added separately at the 0.02% level.

Sensory attributes

Sensory attributes were evaluated, namely appearance, flavour, texture, mouthfeel, and overall acceptability. Texture was evaluated by both visual controls with a spoon or mouthfeel. The results for the experimental yoghurt are presented in Figures 8a (d 1), and b (d 15). Control sample C_2 had the significantly lowest preference values in all sensory perceptions. Less significant differences were detected between C_1, T_1 and T_3 in both flavour and mouthfeel but all were better with a significant difference than C_2. In contrast, the addition of psyllium husk mucilage obviously exhibited a positive effect on all sensory attributes of their respective yoghurt treatments. Moreover, panelists described the yoghurt fortified with psyllium husk mucilage 66.6 mg (T_2) by its excellent smooth texture and

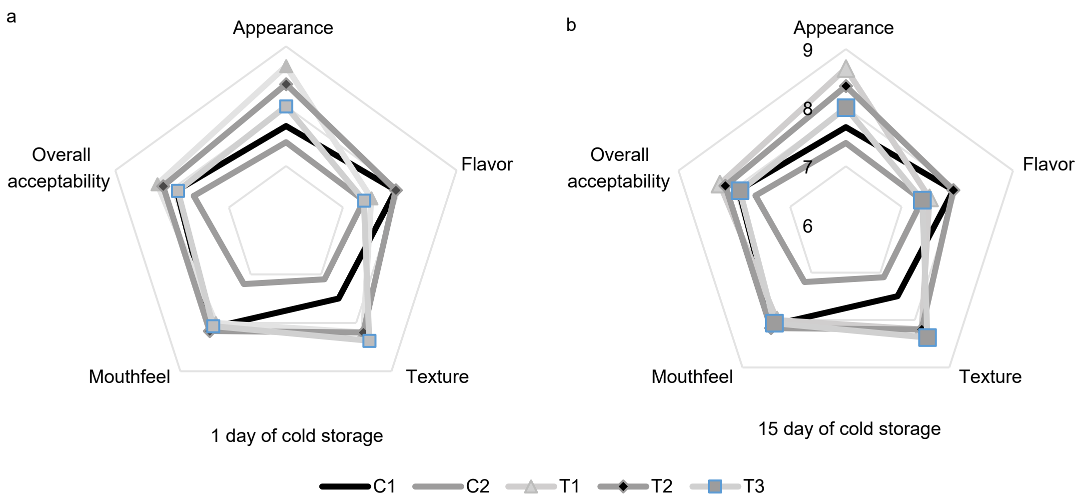

Fig. 8. A hedonistic scale of traditional and fortified low-fat yoghurt as affected by psyllium husk mucilage (PHM) percentages (a) fresh yoghurts, (B) 15-day-old yoghurts; (C_1) control full-fat yoghurt (C_2) control low-fat; T_1, T_2, T_3 are low-fat yoghurt with 33.3 mg, 66.6 mg and 100 mg PHM, respectively

mouthfeel. Our results agree with those of Hussein et al. (2011), who found that the addition of polysaccharides (fibre) improves body, texture, appearance, and mouthfeel and retards the syneresis of low-fat yoghurt. It is worth noting that in our study, no off-flavours were detected in any of the experimental yoghurt as indicated by the assessors.

CONCLUSION

The aim of this work was to fortify low-fat (1.5% fat) yoghurt with fibre in the form of psyllium husk and to study the effect of psyllium husk mucilage on some properties of the resultant yoghurt. The findings showed that fortifying yoghurt with either 66.6 mg/100 g (T_2) or 100 mg/100 g (T_3) psyllium husk mucilage had a positive effect on the texture properties, water-holding capacity and radical scavenging activity of resultant yogurt. Additionally, it enhanced the viscosity and showed high acceptability ratings overall with no significant differences between both treatments. Therefore, from the economic point of view, the addition of psyllium husk mucilage at 66.6 mg/100 g milk (T_2) is recommended for the development of fibre-fortified yoghurt as a novel ingredient for improving the properties of low-fat yoghurt. Also, psyllium husk mucilage to fortify yoghurt is a promising tool to improve the physiochemical, rheological and organoleptic characteristics of low-fat yoghurt by increasing the uptake of antioxidants by consumers. Further studies are needed that apply PHM in other fermented dairy products like low-fat cheese, allowing manifold applications with added market value.

DATA AVAILABILITY

Datasets from the current study are available from the corresponding author upon request.

DECLARATIONS

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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