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ORIGINAL ARTICLE

Immature wheat grain as a potential prebiotic ingredient in set-type yoghurts: impact on antioxidative, textural properties and survival of different probiotics

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Abstract The aim of this study was to investigate the effect of immature wheat grain (IWG) on the survival of *Lactobacillus acidophilus* NCFM (LNCFM), *Lactobacillus casei* 431 (L431) and *Lactobacillus acidophilus* 20079 (L20079) in yoghurts under cold storage. Furthermore, the impact of IWG on physicochemical, textural and antioxidative properties of yoghurts was evaluated. Fortification of yoghurt with IWG positively affected LNCFM and L20079 counts during cold storage whereas no statistical improvement was observed in the viability of L431. The addition of IWG clearly supported the antioxidative activity and total phenolic content in yoghurt. No statistical differences were discovered regarding syneresis and water holding capacity in all probiotic applications. Although, enrichment with IWG enhanced the firmness of probiotic yoghurts, it simultaneously reduced the cohesiveness and viscosity index. This study demonstrated that IWG may be used as a food additive for enhancing probiotic LNCFM and L20079 survival and providing functional aspects in yoghurt.

Keywords Immature wheat grain · Probiotic · Prebiotic · Yoghurt

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Introduction

Nowadays, consumers are demanding food products that are both nutritious and beneficial for health. This trend encourages the development of functional foods which provide potential health benefits to meet the demands. Functional foods can be defined as whole, fortified or enriched foods that provide therapeutic profit beside that the supply of essential nutrients if they are consumed at proper amounts as part of a diet (Aghajanpour et al. 2017). Probiotic bacteria defined as 'living microorganisms when administered in sufficient amounts provide health benefits on the host' has frequently been used in foods (Hill et al. 2014). It would not be wrong to say that yoghurt is the one of the most well-known probiotic carrier food products. Although wide range of lactic acid bacteria have been identified as probiotic, a clear majority of probiotic products available in the market comprise of *Lactobacillus* and *Bifidobacterium* species. There is no doubt that some specific strains of probiotic microorganisms have been employed more than the others in the food industry such as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium animalis* (Granato et al. 2010).

Prebiotics are food ingredient that offered several beneficial effects for health, namely enhanced bioavailability of mineral, stimulated activity and growth of profitable live

microorganisms in the colon and interfering with pathogen microorganisms in the gastrointestinal tract of host (Aryana and McGrew 2007). Numerous researches have been published on the enrichment of probiotic yoghurt with various stimulating supplements identified as 'prebiotic' which enhance the probiotic growth and survival (do Espírito Santo et al. 2010, 2012). The desired growth and activity of lactic acid bacteria with cereals bring to mind that incorporation of probiotics with cereal substrates under controlled conditions in various foods.

In recent years, cereals have also been investigated with regard to their potential use in advancing new functional foods especially as fermentable substrate for stimulating of probiotic microorganisms due to their content of non-digestible carbohydrates. In this context, many researchers used variety of cereals such as maize-based whole grain (Carvalho-Wells et al. 2010), wheat flakes (Connolly et al. 2012b), oat-based cereals (Connolly et al. 2012a) and wheat bran (Terpou et al. 2017) in yoghurt formulations for prebiotic potential.

Immature wheat grain (IWG) is obtained in the physiological stage named 'milky phase' which occurs 2–3 weeks after flowering while wheat culms are still green (D'Egidio et al. 2008). IWG contains more fiber, soluble sugar than mature wheat grain (D'Egidio et al. 2006). This grain markedly has fructo-oligosaccharides (FOS) and fructose-rich materials that provide many functional supports such as prebiotic, anticarcinogenic and immune stimulating effect (Maskan 2001). The positive influence of FOS-rich ingredients on the growth of bifidobacteria and lactobacilli and their potential prebiotic effect in vivo conditions was previously described by Campos et al. (2012). Consequently, the objective of the present study was to evaluate the prebiotic effect of IWG on the probiotic strains of *L. acidophilus* NCFM (LNCFM), *L. casei* 431 (L431) and *L. acidophilus* 20079 (L20079) survival throughout the shelf-life period at 4 °C in yoghurt samples. Moreover, the other aim of this study was to determine the impact of IWG addition on antioxidative properties, textural parameters, acidifying activities of bacteria, and sensorial evaluation.

Materials and methods

Materials

Raw cow milk was obtained from Selcuk University dairy farms located in Konya, Turkey. Medium-heat skim milk powder (34.5% protein, 3.5% moisture, 7.2% ash, 55% lactose, pH:6.55, 0.112% titratable acidity) was obtained from ENKA Dairy Co. Ltd. (Konya, Turkey). Commercial freeze-dried yoghurt starter cultures YF-L901 consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* supplied by Chr. Hansen-Peyma (Istanbul). Probiotic strain of L431 (Chr. Hansen) was obtained from Selcuk University, Food Microbiology Laboratory Collection, and LNCFM and L20079 were provided from Akdeniz University Dairy Products Laboratory Culture Collection as broth cultures. IWG was obtained from a Selcuk University farms in Konya (Turkey) and harvested at 36 days after anthesis. Following the harvest, it was dried at 35 °C for 24–30 h to reduce moisture from about 65 to 13% and thereafter, it was ground in a hammer mill (Falling Number-3100 Laboratory Mill, Perten Instruments AB, Huddinge, Sweden) equipped with a 0.5 mm opening screen.

Media, growth conditions and bacterial enumerations

MRS (deMan Rogosa and Sharpe) agar (Sigma-Aldrich, SL., USA) supplemented with bile (10 mg/L) (HiMedia Laboratories Pvt. Ltd, India) was used for the selective enumeration of L431, LNCFM and L20079 under anaerobic conditions at 37 °C for 48 h (Phillips et al. 2006). *L. delbrueckii* subsp. *bulgaricus* was enumerated in MRS agar (pH 5.4, Merck KGaA, Darmstadt, Germany) and plates were incubated anaerobic conditions at 45 °C for 72 h, whereas M-17 agar (7.2 ± 0.2, Merck KGaA, Darmstadt, Germany) was used for selective enumeration of *S. thermophilus* by incubating aerobically at 37 °C for 24 according to Tharmaraj and Shah (2003). Bacterial enumerations were carried out at 1, 7 and 14 days in duplicate of each experimental lots.

The probiotic cells were prepared by cultivating in 100 mL MRS broth at 37 °C for 24 h (Sousa et al. 2008). The culture was then centrifuged (7200 rpm) at 4 °C for 10 min. The cell pellet was washed twice with sterile ringer solution. After discarding of ringer solution, the pellet was re-suspended in ringer solution and it was used as a probiotic cell culture for production of probiotic yoghurts (Srisuvor et al. 2013).

Preparation of set-type yoghurts

Raw milk samples were adjusted to 12% of solid non-fat content with skim milk powder and then divided into 7 experimental lots containing as control, LNCFM, LNCFM + IWG, L20079, L20079 + IWG, L431, L431 + IWG. IWG were added at 1% concentration for all enriched treatments. Each mixture was subjected to heat treatment at 90 °C for 10 min and homogenized using a hand homogenizer at 14,000 rpm for 3 min. Next, mixtures were rapidly cooled in chilled water to 44 °C. The mixtures were inoculated with the freeze dried and thawed (30 °C)

starter culture and prepared fresh probiotic cultures (~ 8 log CFU/mL) at a concentration of 2% (1:1, v/v) and agitated uniformly. The mixtures were transferred into 200 mL sterile plastic cups and incubated at 42 °C until a pH was reached to 4.4–4.5. After fermentation, all yoghurt samples were sealed and stored at 4 °C. Storage periods were selected as 1, 7, and 14 days (Bonczar et al. 2002).

Physicochemical analysis

Moisture content, ash content, titratable acidity and pH of yoghurts were determined by AOAC methods (Horwitz and Latimer 2000). Furthermore, syneresis and water holding capacity of yoghurt samples were assessed according to the method of Isanga and Zhang (2009). Each treatment was replicated 3 times. Titratable acidity and pH were monitored during cold storage, while other physicochemical characteristics were observed on the 7th day of cold storage.

Determination of antioxidant activity and total phenolic content

The antioxidant activity of extracted samples was determined by the DPPH scavenging method described by Shetty et al. (1995) and the ABTS scavenging method conducted by Re et al. (1999). Total phenolic contents of experimental samples were analyzed according to the method of Tseng and Zhao (2013). All trials were carried out in duplicate.

Texture profile analysis

Textural characteristics of yoghurt samples were determined by texture profile analysis (TPA) according to the method of Sandoval-Castilla et al. (2004) with some modifications. For TPA, the yoghurt samples were kept into plastic cylindrical containers (80 mm diameter, 50 mm height) of 200 mL to a depth of 45 mm. Firmness, consistency, cohesiveness and viscosity index were measured with using TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, England) equipped with a 500 N compression load cell and operating at 1 mm/s head speed. The probe was a 25 mm acrylic cylinder moved speed of 5 mm/s and test speed of 1 mm/s through 30 mm within the sample. The results were expressed as the average of three measurements. Firmness, consistency, cohesiveness and viscosity index were stated as g, g s, g and g s, respectively. Textural characteristics were examined on the 7th day of cold storage.

Sensory evaluation

Sensory acceptability test of yoghurt samples was appraised by a trained panel of seven members using nine-point hedonic scale (Aryana and McGrew 2007). Panelists evaluated color and appearance, consistency, odor, taste and overall acceptance of all yoghurt samples on the 7th day of cold storage.

Statistical analysis

The data were analyzed by using Minitab software version 17 (State College, USA) according to the appropriate experimental designs. The results were provided as mean ± standard deviation, which were compared by the Tukey test with a confidence interval set at 95%.

Results and discussion

pH and titratable acidity

The results of pH and titratable acidity throughout the shelf-life of the yoghurts are presented in Table 1. After the first day of cold storage, pH values varied from 4.30 to 4.41 amongst the treatments. The IWG yoghurts with LNCFM strain had lower pH ($p < 0.05$) than corresponding control without IWG. It was observed slight but not significant difference between the IWG yoghurts with and without L431. Higher level ($p < 0.05$) of pH values were detected at day 1 in LNCFM without IWG when compared with IWG yoghurt. Titratable acidity data ranged from 0.51 to 0.66% lactic acid. The increase in titratable acidity induced by the addition of IWG was statistically significant in all the yoghurt samples at day 1 ($p < 0.05$).

After 7 days of shelf-life, the pH of yoghurt samples ranged from 4.23 to 4.41. It was observed that pH values of all IWG enriched probiotic yoghurts, excepting sample with LNCFM, remained low compared to corresponding probiotic yoghurt samples without IWG. After one week of shelf-life, all probiotic yoghurt samples with or without IWG showed an increase in titratable acidity apart from yoghurt co-fermented by L431 without IWG, but this increment was only statistically significant in yoghurts containing L431 with IWG, L20079 without IWG and LNCFM without IWG ($p < 0.05$). The higher values of average titratable acidity were detected in probiotic yoghurts with IWG compared with their respective controls without IWG (Table 1).

After 2 weeks of cold storage, pH values of yoghurt samples varied from 4.19 to 4.38 amongst the all treatments (Table 1). Surprisingly, IWG yoghurts containing L431 and L20079 seemed a significantly higher pH

Table 1 pH and titratable acidity changes during storage of yoghurt samples

	pH			Titratable acidity (lactic acid %)		
	D1	D7	D14	D1	D7	D14
Control	4.32 ± 0.00^{cA}	4.23 ± 0.01^{eB}	4.32 ± 0.00^{cA}	0.54 ± 0.00^{eB}	0.64 ± 0.02^{bA}	0.62 ± 0.00^{bA}
L431	4.36 ± 0.00^{bB}	4.39 ± 0.01^{abA}	4.27 ± 0.01^{dC}	0.61 ± 0.00^{cB}	0.58 ± 0.00^{cC}	0.63 ± 0.00^{bA}
L431 + IWG	4.35 ± 0.01^{bA}	4.35 ± 0.01^{cA}	4.34 ± 0.00^{bA}	0.62 ± 0.00^{bC}	0.64 ± 0.00^{bA}	0.63 ± 0.00^{bB}
L20079	4.35 ± 0.01^{bB}	4.41 ± 0.01^{aA}	4.34 ± 0.01^{bB}	0.51 ± 0.00^{fB}	0.53 ± 0.00^{dA}	0.50 ± 0.00^{cB}
L20079 + IWG	4.41 ± 0.01^{aA}	4.37 ± 0.01^{bcB}	4.38 ± 0.01^{aB}	0.61 ± 0.00^{cA}	0.63 ± 0.00^{bA}	0.63 ± 0.00^{bA}
LNCFM	4.35 ± 0.00^{bA}	4.31 ± 0.02^{dA}	4.19 ± 0.00^{eB}	0.57 ± 0.00^{dB}	0.65 ± 0.00^{abA}	0.66 ± 0.02^{bA}
LNCFM + IWG	4.30 ± 0.00^{dA}	4.28 ± 0.02^{dA}	4.19 ± 0.00^{eB}	0.66 ± 0.00^{aB}	0.67 ± 0.00^{aB}	0.71 ± 0.00^{aA}

[a–e]Different superscript lowercase letters denote significant differences between formulations for the same sampling period of the in vitro assay ($p < 0.05$); [A–C]different superscript capital letters denote significant differences between formulations for the different sampling periods of the in vitro assay for same formulation ($p < 0.05$)

($p < 0.05$) compared with their respective controls without IWG (Table 1). However, such a scenario was reported by do Espírito Santo et al. (2010) about probiotic yoghurts fermented with açai pulp for detecting their prebiotic effects. Likewise, according to a study of do Espírito Santo et al. (2012) who assessed effect of the passion fruit peel powder on probiotic bacteria, this behavior collaborated with their findings. These authors explained this circumstance by simultaneous occurrence of fatty acid consumption as carbon source. On day 14, higher levels ($p < 0.05$) of average titratable acidity were detected in IWG yoghurt co-fermented LNCFM and L20079 compared with their respective controls without IWG. However, titratable acidity levels showed no significant difference ($p < 0.05$) due to IWG presence in yoghurt samples co-fermented L431 at day 14. Considering the whole cold storage period, it was determined that average titratable acidity in IWG yoghurts co-fermented LNCFM and L20079 was higher than in their controls without IWG unlike in the yoghurts containing IWG and L431.

Viability of microorganisms

Streptococcus thermophilus counts varied from 7.21 and 7.93 log CFU/g after first day of cold storage and the highest counts ($p < 0.05$) exhibited in the IWG yoghurts co-fermented L20079 strain. On day 1, IWG yoghurts containing both L20079 and L431 showed higher ($p < 0.05$) *S. thermophilus* counts compared with their respective controls without IWG, but lower counts were observed in IWG yoghurts co-fermented LNCFM. The counts of *S. thermophilus* on day 14 ranged from 8.29 to 8.60 log CFU/g. In the period between 1 and 14 days, an apparent increase occurred in all the yoghurt samples. These results are in accordance with the observations of do Espírito Santo et al. (2010) who pointed to an increase between first day and 14 days of cold storage. After 14 days of shelf-life, *S. thermophilus* population showed no significant difference ($p > 0.05$) among all treatments.

Lactobacillus bulgaricus counts ranged from 7.35 to 8.21 log CFU/g at the first day of cold storage and the lowest counts were obtained in the IWG yoghurts with L20079 which had the highest *S. thermophilus* counts after the first day of shelf-life. As opposite to increase in *S. thermophilus* counts ($p < 0.05$) during shelf-life, a reduction ($p < 0.05$) in the viability of the *L. bulgaricus* was obvious in all the yoghurt types excluding IWG yoghurt with co-fermented L20079. Similar results were presented by do Espírito Santo et al. (2012) who further observed a decrease in yoghurts containing passion fruit peel powder co-fermented *L. acidophilus* L10 and NCFM after first day to end of the cold storage. On day 14, *L. bulgaricus* counts varied from 6.00 to 6.47 log CFU/g and significantly higher viability of *L. bulgaricus* was observed in IWG enriched yoghurts co-fermented LNCFM and L20079 compared to control without IWG. Thus, it appeared that the presence of IWG had a positive influence upon the counts of starter *L. bulgaricus* in yoghurts at day 14 agree with the findings of do Espírito Santo et al. (2010) who reported a high viability in *L. bulgaricus* counts when produced yoghurts with açai pulp co-fermented *B. longum* B105 compared to plain yoghurt.

Probiotic counts in yoghurts, with or without IWG, after 14 days of cold storage are presented in Fig. 1. On day 1, the addition of IWG had positive significant effect ($p < 0.05$) on the growth of L431 and L20079 strains, however, the presence of IWG had a negative impact upon viability of LNCFM compared with their respective controls without IWG. It was showed that the higher probiotic counts were in IWG yoghurts with L431 and L20079 compared with the other yoghurt types on day 1.

On day 7, L431 counts in yoghurts with or without IWG showed dramatic decrease ($p < 0.05$) about 1.5–2 log CFU/g. Similar remarkable reduction ($p < 0.05$) of *L.*

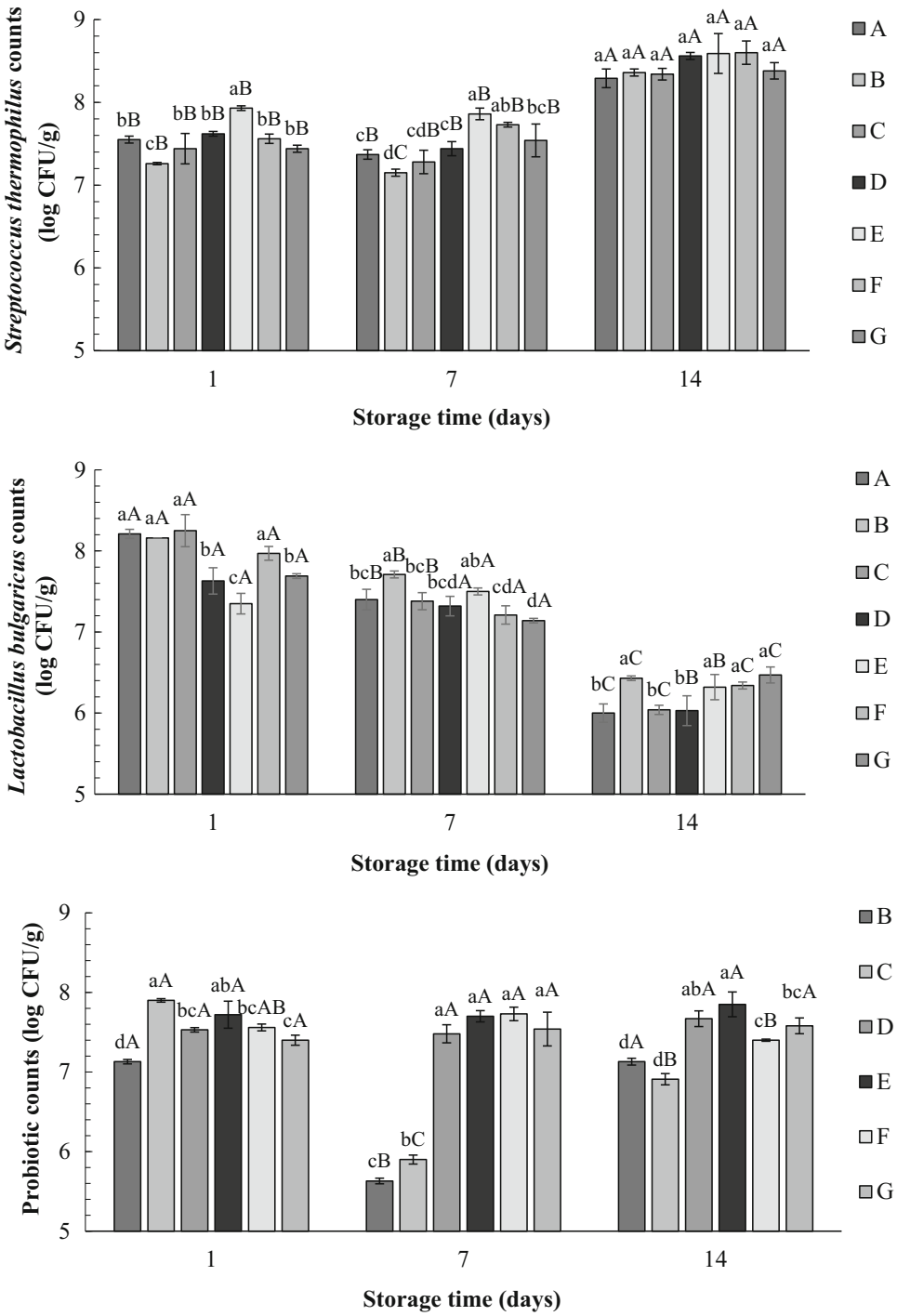

Fig. 1 Survival of probiotic and starter bacteria in yoghurt samples during cold storage for 1, 7, and 14 days. For the same cold storage period, a–d different superscript lowercase letters denote significant differences between formulations for the same sampling period of the in vitro assay ($p < 0.05$); A–C different superscript capital letters denote significant differences between formulations for the different sampling periods of the in vitro assay for same formulation ($p < 0.05$) (A: Plain, B: L431, C: L431 + IWG, D: L20079, E: L20079 + IWG, F: LNCFM, G: LNCFM + IWG)

acidophilus L10 and *B. longum* B105 viability in yoghurt towards the middle of the cold storage period were reported by do Espírito Santo et al. (2010). However, no significant difference was observed between LNCFM and L20079 counts from the first day to seventh day of cold storage ($p > 0.05$). Significantly higher ($p < 0.05$) viability of L431 was observed in IWG yoghurt compared to yoghurt that without IWG on day 7, but same situation was not appeared for LNCFM and L20079 counts. The differences of the counts of LNCFM and L20079 between with and without IWG yoghurt were not statistically significant ($p > 0.05$) on day 7.

In this current study, LNCFM and L20079 populations remained above 7 log CFU/g between first and the 14th day of shelf-life in yoghurt samples with or without IWG. After 14 days, IWG addition showed statistically significant

increase on the counts of LNCFM and L20079 compared with its control without IWG. This improved viability in IWG yoghurts may have been due to the availability of its fructo-oligosaccharide (FOS) content and the other fiber components, although same trends in relation to the yoghurts samples with IWG were not statistically observed in the case of L431 over cold storage. Paradiso et al. (2006) stated that maximum collecting of FOS in wheat kernel determined between second and third weeks of flowering at the physiological stage, thereafter it quickly decreases. They noted that increased level of fructans in IWG seems available for the usage of kernels harvested at the mentioned stage to functional properties such as prebiotic effect. Hence, improving effect for the viability of LNCFM and L20079 counts in yoghurt samples containing IWG over cold storage was not surprising, however, supplementation with IWG showed no satisfactory effect more than 1 log after 14 days of cold storage. Any positive survival influence of IWG did not observed for L431 in yoghurt matrix at the end of the 14 days of cold storage. Overall, these results may demonstrate that improving effects of IWG are selective for probiotic strains. Similarly, in a previous study has shown that FOS may increase the growth of specific bacteria such as some *L. acidophilus* and *Bifidobacterium* strains but does not support the growth or survival of the other bacteria (Kaplan and Hutkins 2000).

Physicochemical, antioxidative and textural properties

The physicochemical properties of yoghurt samples are shown in Table 2. The syneresis and water holding capacity are the most important factors which can influence final product quality for the yoghurt samples. There were no differences ($p > 0.05$) between yoghurt formulations with different co-fermented probiotic strain on syneresis and water holding capacity. This fact may be associated with the small amount of exopolysaccharide (EPS) production, which is normally affect retaining water in yoghurt gel structure, of all probiotic bacteria used. Also, adding the IWG led to no distinction in syneresis and water holding of yoghurt samples among all samples no matter of the probiotic strain, which may also be contributed to not being effective amount of IWG added (1%) for changing these parameters. Similarly, our data are in agreement with Aryana and McGrew (2007) who reported no any statistically variation in syneresis values when used short chain prebiotic in yoghurt with co-fermented *L. casei* in comparison to control, whereas our results disagree with the observation of Pimentel et al. (2012) who reported that spontaneous whey separation values statistically differed among probiotic (only with *L. paracasei*) synbiotic (with inulin and *L. paracasei)* and control yoghurts.

Regarding total phenolic contents (TPC) and DPPH radical scavenging activity (RSA), not surprisingly an enhancement was observed in these parameters when IWG added to yoghurt. Supplementation of IWG was helpful in improving the RSA and TPC in all IWG enriched yoghurt samples. These results are compatible to the findings of Jenkins et al. (2011) who reported higher numbers in immature wheat kernels than in mature one regarding vitamin C and hydrophilic antioxidant activity. Corroborating this affirmation Paradiso et al. (2006) reported that immature wheat kernels contain considerable amount of vitamin C and antioxidant molecules. Similar result has been reported earlier by Aktaş et al. (2015) who found a significant improvement in the RSA and TPC when used wheat grain at pre-ripening stages for making bread and tarhana, respectively.

As expected, total solids (TS), total soluble solids (TSS) and ash contents of the yoghurt samples increased with adding IWG regardless the strain type of probiotic bacteria. Similar trend was found by Srisuvor et al. (2013) who pointed out an increase in TS and TSS parameters of yoghurt samples when added inulin and polydextrose as a prebiotic. The increase in TSS was found to be statistically significant whereas this in TS and ash content was not. Any statistical variation between different type probiotic yoghurts without IWG were not determined for these parameters depending upon probiotic strain.

The texture profiles of the yoghurt formulation are shown in Table 2. The firmness values of the yoghurts with only probiotic bacteria no matter of the bacteria strain was significantly lower than plain sample in contrast to findings of Pimentel et al. (2012) who reported that the addition of probiotic bacteria resulted in firmer products when used *L. paracasei* subsp. *paracasei* as a probiotic strain unlike our strains. However, our results are in accordance with the observations of Bonczar et al. (2002) who pointed to a lower firmness values in probiotic yoghurt containing *L. acidophilus* and *Bifidobacterium* ssp. comparing to control. At the same time, in the present study the firmness values of the probiotic yoghurt samples containing IWG were significantly higher than their probiotic respective controls without IWG. Different firmness results of IWG yoghurts and respective probiotic controls may have been result of positive interaction FOS and fructose-rich materials present in IWG with proteins. This is in an agreement with the statement of De Souza Oliveira et al. (2011) who reported that FOS could have produced extra energy to potentiate EPS biosynthesis, hence improving firmness of yoghurt.

The addition of probiotic bacteria resulted in a significant increase cohesiveness values of yoghurt comparing to plain yoghurt in contrast to the findings of Pimentel et al. (2012) who reported that probiotic addition to yoghurt had no effect on the cohesiveness. Bonczar et al. (2002) have

Table 2 Physicochemical properties of yoghurt samples

Physicochemical properties	Control	L431	L431 + IWG	L20079	L20079 + IWG	LNCFM	LNCFM + IWG
Water holding capacity (%)	37.60 ± 1.69^{a}	39.95 ± 0.91^{a}	38.75 ± 1.20^{a}	39.25 ± 1.06^{a}	39.40 ± 1.97^{a}	39.40 ± 0.84^{a}	39.50 ± 0.98^{a}
Syneresis (% v/w)	27.98 ± 4.15^{a}	27.88 ± 0.22^{a}	28.68 ± 1.92^{a}	30.90 ± 1.78^{a}	26.20 ± 2.26^{a}	28.58 ± 3.70^{a}	26.22 ± 6.30^{a}
Total phenolic content (mg GAE/g)	112.97 ± 0.02^{b}	112.64 ± 3.04^{b}	118.18 ± 8.58^{ab}	112.66 ± 1.44^{b}	117.79 ± 0.11^{ab}	120.55 ± 11.41^{ab}	128.80 ± 0.56^{a}
DPPH (% inhibition)	2.19 ± 0.07^{f}	2.33 ± 0.12^{f}	2.94 ± 0.08^{e}	3.88 ± 0.14^{c}	5.03 ± 0.05^{a}	3.23 ± 0.08^{d}	4.32 ± 0.02^{b}
Brix (%)	9.46 ± 0.04^{c}	9.19 ± 0.12^{d}	10.05 ± 0.02^{b}	9.32 ± 0.00 ^{cd}	10.20 ± 0.09^{ab}	9.31 ± 0.05 ^{cd}	10.22 ± 0.00^{a}
Total solids (%)	14.80 ± 0.04^{a}	14.30 ± 0.04^{a}	15.09 ± 0.63^{a}	14.19 ± 0.41^{a}	14.94 ± 0.09^{a}	13.28 ± 0.41^{b}	14.98 ± 0.41^{a}
Ash content (%)	0.94 ± 0.01^{a}	0.94 ± 0.00^{a}	0.96 ± 0.00^{a}	0.93 ± 0.00^{a}	0.95 ± 0.00^{a}	0.91 ± 0.07^{a}	0.94 ± 0.00^{a}
Firmness (g)	479.73 ± 2.83^{b}	310.20 ± 0.00^{c}	485.16 ± 0.00^{a}	256.90 ± 0.65^{f}	286.61 ± 2.83^{d}	276.35 ± 0.04^{e}	280.79 ± 2.83^{d}
Consistency (g s)	9297.13 ± 0.14^{a}	8053.01 ± 2.81^{c}	8417.84 ± 2.81^{b}	6434.44 ± 0.60 ^{g}	6823.24 ± 2.83^{e}	6845.96 ± 0.02^{d}	6490.34 ± 2.82^{f}
Cohesiveness (g)	107.28 ± 0.02^{e}	165.32 ± 0.02^{a}	99.54 ± 2.82^{f}	140.74 ± 0.02^{c}	131.78 ± 0.28^{d}	158.20 ± 0.00^{b}	142.43 ± 2.85^{c}
Index in viscosity (g s)	122.57 ± 0.02 ^{g}	487.31 ± 0.02^{a}	195.45 ± 0.00^{f}	289.42 ± 0.02^{c}	259.00 ± 0.00^{d}	300.31 ± 0.00^{b}	235.46 ± 2.83^{e}

^{a,b,c}Values in same row having different superscripts differ significantly ($p < 0.05$)

Table 3 Mean scores of sensory attributes of yoghurt samples

Sensory properties	Control	L431	L431 + IWG	L20079	L20079 + IWG	LNCFM	LNCFM + IWG
Color and appearance	8.46 ± 1.14^{a}	8.46 ± 1.07^{a}	7.02 ± 1.19^{b}	8.66 ± 1.07^{a}	6.48 ± 1.60^{c}	8.55 ± 1.81^{a}	7.00 ± 1.19^{b}
Consistency	7.92 ± 1.22^{ab}	8.37 ± 1.54^{ab}	7.17 ± 1.43^{c}	8.28 ± 1.83^{b}	7.41 ± 1.19^{c}	8.58 ± 1.11^{a}	7.20 ± 1.27^{c}
Odor	7.89 ± 1.86^{b}	$8.82 \pm .1.96^{a}$	7.56 ± 1.18^{b}	7.92 ± 2.04^{b}	6.53 ± 1.00^{c}	8.64 ± 1.17^{a}	7.53 ± 1.17^{b}
Taste	7.38 ± 1.19^{a}	7.83 ± 1.14^{a}	5.40 ± 1.16^{c}	7.83 ± 1.28^{a}	5.22 ± 1.42^{c}	7.56 ± 1.42^{a}	6.21 ± 1.59^{b}
Overall acceptability	8.46 ± 2.04^{a}	8.46 ± 1.51^{a}	7.06 ± 1.67^{c}	7.74 ± 1.24^{b}	6.50 ± 1.40^{d}	8.46 ± 1.14^{a}	6.48 ± 1.13^{d}

[a,b,c]Values in same row having different superscripts differ significantly ($p < 0.05$)

reported that a similar accelerating effect of probiotic cultures on cohesiveness values in yoghurt in parallel with our results. Significant decrease in cohesiveness values was detected in probiotic yoghurts fortified with IWG comparing to their respective controls without IWG. Sendra et al. (2010) observed that the consistency values of yoghurts enriched with orange fiber were significantly higher than control yoghurt, which is similar to our findings for yoghurts enriched with IWG except yoghurts containing LNCFM. On the other hand, in the current study probiotic yoghurts without IWG had the higher viscosity values than the other formulations regardless which probiotic strain was used. This may be a consequence of presence of EPS which probably produced by probiotic strains and could increase the viscosity resulting an improved rigidity of gel structure in yoghurt in accordance with the statements of Duboc and Mollet (2001).

Sensory quality

The sensory evaluations of yoghurt samples are shown in Table 3. The taste scores of all probiotic yoghurts without IWG were greater than plain yoghurt (but not statistically significant). These findings are consistent with the research reported by Uysal-Pala et al. (2006) who found that using probiotic cultures positively influenced intensities of desirable taste attributes comparing to plain yoghurt. The higher overall acceptability scores for the probiotic yoghurts without IWG were in accordance with some physical attributes and they diminished by IWG addition which led to decreases in L^{*} values and viscosity and increases in b^{*} values. Considering only the probiotic yoghurts, no significant differences were observed in overall preference scores between yoghurts with L431, LNCFM and plain yoghurt, but yoghurts containing only L20079 had lower scores in comparison to the other probiotic yoghurts.

The scores for color and appearance, consistency, odor, taste and overall acceptance exhibited that the addition of IWG negatively influenced the sensorial parameters, that is to say IWG seemed to yield the product with worse sensory properties. All parameters examined without any exception was greater in plain and probiotic yoghurts without IWG than IWG enriched probiotic yoghurts. Similarly, Fernández-García and McGregor (1997) reported lower overall flavor and texture scores comparing to conventional product when used to fortify yoghurt with seven types of insoluble dietary fiber from different sources (soy, rice, oat, corn and sugar beet). Unfortunately, consumers are not interested in functional foods if the added ingredients resulted in unsuitable flavors to the product quality even if consumers aware of the health benefits of these ingredients. Thus, further studies may be managed to reduce unpleasant characteristics of IWG for the usage of development new functional foods.

Conclusion

The results of this study demonstrate that IWG have a selectively prebiotic influence on the number of probiotic bacteria in yoghurt over a 14 days cold storage. The addition of IWG into yoghurt favored an increase in LNCFM and L20079 counts at the end of the shelf-life while there was no statistically improvement in number of L431 in the presence of IWG. The higher increase in mean titratable acidity in IWG containing probiotic yoghurts suggests that probiotic bacteria used IWG. Besides, DPPH radical scavenging activity and total phenolic contents were greatly influenced by IWG enrichment. This study indicates that enhancing effect of IWG is probiotic strain-dependent so that different probiotic cultures can be endeavored in the future studies.

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