Original article

Development of functional yogurt fortified with wheat germ and strawberry as functional ingredients

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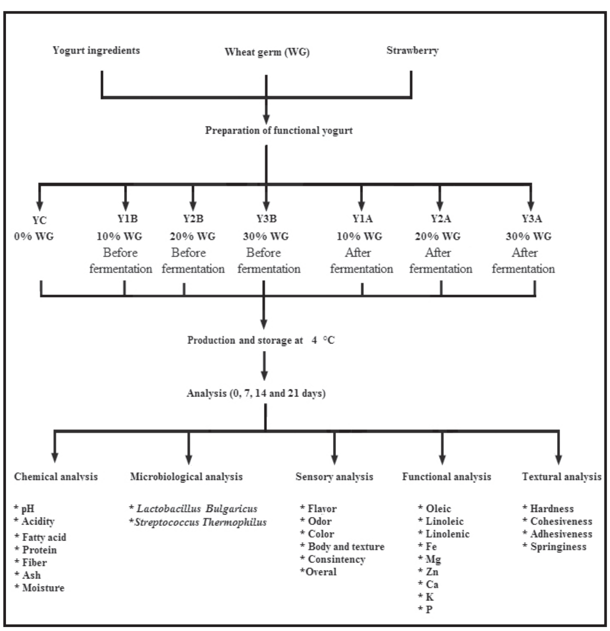
Summary. The aim of present study was to evaluate the effect of adding wheat germ to functional yogurt, which is well-known as a miraculous food around the world. For this purpose, seven functional formulations containing wheat germ (0, 10, 20 and 30% based on dry matter) were produced by adding wheat germ to yogurt samples after and before fermentation. The results showed that adding wheat germ to functional yogurt led to an increase in the amount of such fortified compounds as ferric, potassium, zinc, phosphorous, magnesium, unsaturated fatty acids and fiber. Moreover, the addition of wheat germ did not have a significant effect on the acidity profile and growth of starter culture. The results of sensory evaluation indicated that addition of wheat germ after fermentation significantly increased the acceptability of yogurt samples. It is thus concluded that the prepared functional yogurts can be highly effective in supplying the nutritional needs of the age groups under nine years-old.

Key words: Functional yogurt, wheat germ, mineral, strawberry, fatty acids profile



Graphical abstract

Introduction

Functional food refers to food with positive effects on human health which reduces the risk of diseases (1). Due to the importance of nutritional quality of foods and shortage of micronutrients, production and consumption of fortified foods have increasingly received increased attention. (2). Micronutrients deficiency could lead to learning disabilities, mental retardation, low work efficiency, blindness and early death. To struggle with the shortage of nutrients and elevate the society's healthcare, interfering strategies including diet improvement, supplementation and enrichment of nutrients are being employed (3).

Due to the high consumption of dairy products, fortified yogurt containing functional components with low level of lactose and high calcium concentration as well as active biological effects have received increasing attention (4). Moreover, many studies suggest that the consumption of yogurt increases the absorption of phosphorous, magnesium, and zinc more easily and reduces cholesterol level (5). Wheat germ is an important by-product of the flour production industry. Wheat germ is rich in unsaturated fatty acids, alpha-tocopherol, calcium, potassium, zinc, arsenic, magnesium, selenium, silica, thiamine, riboflavin, lysine and niacin (6), which can be employed for increasing the nutritional value of many food products after being processed (7). Regarding the advantages of wheat germ, it has been used in various cereal-based products including, cake (8), cookie (9) and toast bread (10). The published research works dealing with the fortification of dairy products with wheat germ are very limited. In this regards, Majzoobi et al. (11) studied the physicochemical properties of fresh chilled dairy dessert supplemented with wheat germ at different levels (0, 2.5, 5, 7.5 and 10%) and particle sizes (120, 210 and 354 μm). This study reported that the higher level of wheat germ and particle size increase hardness, cohesiveness and gumminess of the desserts while decrease springiness and water release of the samples. Determination of the sensory characteristics of samples showed that that addition of germ with no more than 5 % wheat germ with either of the particle sizes had positive general acceptability.

Strawberry is also a good source of fiber, antioxidants, vitamin A and C, folate, potassium, calcium, iron, phosphorus, tannin, mucilage, various sugars and salicylates (12). Thus, the addition of strawberry can improve the sensory properties and increase products acceptability.

The present study is aimed to investigate and evaluate the nutritional, physicochemical, microbial and sensory attributes of functional strawberry yogurt fortified with wheat germ.

Materials and Methods

Materials

Fortified strawberry yogurts with wheat germ were produced using pre-processed milk containing 12.5% none-solid fat and 2.5% fat (Pegah Fars Dairy, Shiraz, Iran). Moreover, wheat germ powder and strawberry puree were respectively provided from Sepidan Flour Company (Shiraz, Iran) and Holland Ingredients (Ketelskamp 8, 7942 KG Meppel, Netherlands).

Methods

Stabilization and preparation of wheat germ

Raw wheat germ contains several enzymes and anti-nutritional factors (9). Wheat germ stabilization was carried out by roasting them at 160 °C for 20 min. At this condition, heat treatment is high enough to deactivate the enzyme activity (13). Roasted wheat germ was milled in an electrical mill (Gosonic, Model GCG705, Shanghai, China) and then sieved manually using a 35 mesh-size (0.841 μm) stainless steel sieve (ASTME:11, Iran). Wheat germ was samples were stored at −18°C in a polyethylene package further use.

Physicochemical determination of wheat germ

Chemical composition of wheat germ

Moisture (method 19-44), fat (method 10-30), protein (method 10-46), ash (method 01-08) and fiber (method 05-32) were measured using AACC (14) standard method.

Fatty acids profile of wheat germ oil

In order to evaluate the fatty acids profile, a gas chromatograph (BPX 70, SGE Inc., China(equipped with a column of 30 m long, 0.25 μm diagonal and 0.25 μm thickness and FDT 1075, was used. Initially, 200 mg of pure oil sample was mixed with 10 ml of 5% solution of acetyl chloride/methanol and heated in oven at 85°C for 1 h. Then, 5 ml distilled water was added and mixed for 5 min, followed by centrifugation at 25°C at 4000g for 5 min. The initial temperature of the column was 80 °C and reached to 200 °C at a rate of 15°C/min, held at this temperature for 10 min. Then temperature increased to 220°C at a rate of 30°C/min and held at this temperature for 5 min. The temperatures of injection valve and detector were both 210°C and the carrier gas (helium) flow rate was 1 ml/min. Injection to GC was conducted by Split method (16).

Preparation of Functional Yogurts

Formulation optimization

In order to determine the best formulation for production of functional yogurt fortified with wheat germ, following parameters were considered: wheat germ particles size (125, 250 and 500 µm), final pH of yogurt samples (4.2, 4.5 and 4.8) at the end of fermentation, the percentage of strawberry's puree (5%, 10% and 15%), thermal processing conditions (roasting) of wheat germ (180 ˚C for 15 min, 160 ˚C for 20 min and 140 ˚C for 30 min), and the puree types (peach, apple and strawberry). According to the panelists, yogurts containing wheat germ with the size of 500 µm, final pH 4.2 at the end of fermentation, 7.5 % of strawberry's puree, and roasted at 160 ˚C for 20 min received the highest acceptance. Therefore, this optimum formulation used for further investigation of wheat germ percentages.

Yogurt Fermentation

The formulation of different stirred yogurt samples have shown in Table 1. The stirred yogurt samples were prepared according to the method of Hosseini *et al.* (17) with some modifications. First, the milk was pasteurized at 85˚C for 30 min and then cooled down to 42˚C. The starter (YoFlexÒ express 1.0, Chr. Hansen Inc., Milwaukee, WI) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus* was inoculated into the milk. The resultant mixture was poured into the cups and then incubated at 42±1˚C until the pH reached to 4.2 ± 0.1. The yogurt samples were immediately cooled and stored at 4 ±1˚C for 21 days. Due to adverse effects of wheat germ on the textural and sensory properties of yogurt, the impact of different concentrations of wheat germ (10, 20 and 30 % based on dry matter of yogurt) and the addition time (before and after fermentation) on the nutritional, physicochemical, textural, microbial and sensory properties of yogurt were investigated during a period of 21 days at 4 ±1˚C. Percent of dry matter and fat remained relatively constant in all samples at the level of 12.5% and 2.5%, respectively.

Physicochemical determination of functional yogurts
Titratable acidity and pH

Total titratable acidity and pH of yogurt samples were measured by AOAC (18) standard method. Titratable acidity was reported as percentage of lactic acid every 7 day over 21 days of refrigerated storage.

Minerals measurement of functional yogurt

Iron, calcium, magnesium and zinc were measured using atomic absorption spectrophotometer method (AA-670, Shimadzu) and phosphorus and potassium were measured using flame photometer method (Corning, 405) according to the AACC (15) standard method.

Viable counts of Lactic acid bacteria

For a viable number of starter cultures in the functional yogurt, *S. thermophilus* was enumerated using M17 agar acidified to pH 6.8 by 1 M HCl and subsequently incubated at 37 ± 1˚C for 48 h under an aerobic condition. The number of *L. bulgaricus* was determined using MRS agar acidified to pH 5.4 with 100% glacial acetic acid and incubated at 37 ± 1˚C for 72 h under an anaerobic condition (19). The number of lactic acid bacteria was reported during 21 days of refrigerated storage.

Texture profile analysis

Textural features such as hardness, cohesiveness, adhesiveness and springiness of the functional yogurts were investigated using texture analyzer (Brookfield, USA) by a double compression test. 8 g of each sam-

Table. 1. Formulation of control and functional yogurt supplemented with wheat germ

Yogurt	supplements			Treatments
	Skimmed milk powder (g*)	Cream powder (g)	Wheat germ (g)	Addition time of wheat germ
YC	10.02	4.98	-	-
Y1B	8.82	4.68	1.5	Before fermentation
Y2B	7.62	4.38	3	Before fermentation
Y3B	6.41	4.09	4.5	Before fermentation
Y1A	8.82	4.68	1.5	After fermentation
Y2A	7.62	4.38	3	After fermentation
Y3A	6.41	4.09	4.5	After fermentation

ple was compressed to 15 mm penetration depth with 2 mm/s pretest speed and 2 mm/s test speed using a cylinder probe of 25 mm diameter.

Sensory evaluation

Sensory evaluation was conducted by 10 well-trained panelists from Pegah Fars Company (Shiraz, Iran). This test was performed according to 9-points standard hedonic method (1 = dislike extremely, 3 = dislike moderately, 5 = neither like nor dislike, 7 = like moderately, and 9 = like extremely). For this purpose, the samples kept at 4 °C for 1 week prior sensory tests. All samples were coded with random numbers and given in a white plastic cup to each panelist in order to evaluate the characteristics including aroma, taste, color, texture, strength and general acceptance. (21). Sensory evaluation was conducted in testing booths with different light sources; day light illumination for color assessment and red light for other organoleptic properties. Panelists rinsed their mouth with tap water before begin tasting and also between each sample.

Statistical analysis

All experiments were performed in the form of completely random blocks and repeated three times. Comparison of means with Duncan's multiple range test was done at a significant level (p<0.05 (. The data were reported as mean ± standard deviation (22). Additionally, the quantitative data were statistically analyzed using SAS Statistical Software, Version 9.1 (SAS Institute Inc., 2000; Cary, NC, USA).

Results and discussion

Physicochemical determination of wheat germ

Chemical composition of wheat germ

The wheat germ used in the present study contained about 9.03% of fatty acid, 29.5% of protein, 7.36% of fiber, 4.31% of ash and 5.15% of moisture. These results in a good agreement with those reported by other researchers (10). The wheat germ protein is mainly composed of albumin and globulin (about 50%). Its fiber contains cellulose and hemicellulose and its ash is mainly composed of phosphorus, potassium and magnesium. The high amount of fiber in wheat germ is also essential for blood sugar and cholesterol control, intestinal health and detoxification of body (23). Iron, potassium, calcium, phosphorus, magnesium, and zinc content of wheat germ was 164.08, 10750, 2441.67, 8333.33, 2011 and 204.5, respectively.

Fatty acids profile of wheat germ oil (WGO)

Fatty acids profile of WGO was measured using gas chromatography. The amounts of unsaturated fatty acids containing oleic, linoleic and linolenic acids were 15.83%, 56.21% and 8.08%, respectively. It has been proven that unsaturated fatty acids, especially polyunsaturated fatty acids, resulted in decreasing cholesterol level and heart diseases (24). Partial replacement of milk fat with WGO will be greatly effective in reducing cholesterol and increasing the yogurt nutritional value. These fatty acids play an influential role in the organs' metabolism of which the human body is incapable of synthesis. WGO contains 81 % of unsaturated fatty acids and 19 % of saturated acids (25). Among fatty acids, alpha-linolenic acid decreases O_2 production and oxidase activities of NADPH and thus, has an anti-oxidative property (26). These compounds are the precursor of prostaglandins production that participate in the muscles contraction and fast healing of inflammations. Moreover, linolenic acids reduce cholesterol and act as the precursor of phospholipid cell wall (23). WGO is also one of the most fortified natural sources of alpha-tocopherol with the highest vitamin activity (7).

Physiochemical determination of functional yogurts

Major minerals content of functional yogurt

Major minerals (iron, potassium, calcium, phosphorus, magnesium, and zinc) content of control and functional yogurt supplemented with wheat germ are shown in Table 2. As can be seen, the control sample exhibited the lowest minerals content and increasing the wheat germ level significantly (P < 0.05) promoted the mineral contents of yogurt. The addition time of wheat germ had no significant effect on the minerals content. Addition of grape and blackberry molasses to yogurt also resulted in higher minerals (Fe, Cu, Mn, K, and Zn) content (27). Calcium and phosphorus work together to maintenance of bones health. Interestingly enough, due to the release of free water and partial disjoint of the structure as well as surface increase and porosity during the

Table. 2. Minerals (mg/kg) of control and functional yogurt supplemented with wheat germ

Yogurt	Fe	Mg	Zn	Ca	K	P
YC	17.825±0.04D*	169±1.41F	26.55±0.78E	1050.5±23.33G	1950±70.71E	1336±11.31D
Y1B	20.15±0.14C	212.25±1.77E	35.35±0.42D	1590±28.28F	6000±141.42D	1525±9.90C
Y2B	22.725±0.04B	376±1.41C	45.4±1.06C	1863.25±4.60D	6950±70.71B	1765.5±21.92B
Y3B	25.65±0.07A	476.25±1.77A	55.75±1.06A	2127.5±24.75C	7850±70.71A	2073.5±7.78A
Y1A	20.125±0.11C	235±1.41D	36±0.14D	1647.5±27.58E	6450±70.71C	1527±12.73C
Y2A	22.725±0.04B	383.75±2.47B	48.525±0.74B	1948.5±23.33B	7050±70.71B	1791.5±14.85B
Y3A	25.6±0.02A	478.5±2.12A	56.6±0.85A	2151.5±34.65A	7950±70.71A	2046.5±21.92A

* The results are shown as mean ± standard deviation. Values with capital letter superscripts within a column and different small letters within a row are significantly different (p <0.05)
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

heating procedure, minerals' structure changed and their tendency to exit the material's texture increased (29).

Acidic media of yogurt ionizes calcium and magnesium, hence facilitating their intestinal absorption (31). In addition, low pH of fermented dairy products such as yogurt can reduce the inhibitory effect of phytate on mineral absorption particularly calcium.

The pH value

Yogurt production has been relied on the activity of two homo-fermentative bacteria as well as the production of lactic acid and other aromatic compounds. The produced acid is the main reason of coagulation during the fermentation. One of the main reasons of unilateral selection of starters for yogurt preparation is the acid generation during 4 to 5 h of incubation (33).

Due to the lack of wheat germ in YC, Y1A, Y2A and Y3A (Figure 1) during fermentation, no significant change (p > 0.05) was observed in the pH value during the incubation time. While, Y2B and Y3B samples containing respectively 20 and 30% wheat germ showed the reduction of pH during incubation and the rate of pH reduction decreased with an increase in the amount of wheat germ. Results were not compatible with those attained by Espírito Santo *et al.* (34) and McCann *et al.* (35). They showed that the addition of carrot powder and passion fruit peel's powder lead to the acceleration of incubation time. However, Zibadi and Watson (36) reported that the presence of buffered substrates was probably efficient in slowing the acidification process and hence the increasing of the incubation time.

YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation).

The pH of control and functional yogurt during storage are shown in table 3. The results showed that the pH

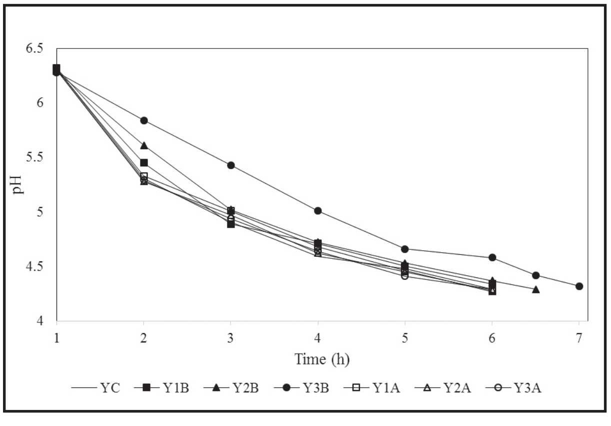


Figure 1. Evolution of pH of control and functional yogurt supplemented with wheat germ during incubation period
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

of all yogurt samples is significantly ($p<0.05$) decreased with a stable pace (0.1). Similarly, Tseng and Zhao (37) reported pH decreased during the storage time for in yogurts fortified with black grape pomace (37). The results attained by Temiz *et al* (38) also showed the effect of storage time on the reduction of pH for fruit yogurt (38). Moreover, the yogurt samples in which the wheat germ had been added to the milk before fermentation showed higher pH values compared to those fortified with gem after fermentation. It can be attributed to the presence of protein's buffer and wheat germ's fiber.

The amount of added wheat germ had no significant ($p<0.05$) effect on the pH value which was similar to the results obtained by Zomorodi (39). Zomorodi (39) stated that the addition of 5 levels of wheat fiber had no effect on the pH of fruit yogurt which could probably be attributed to the presence of buffered compounds that inhibit considerable changes of pH.

The pH reduction has also attributed to the production of ß-galactosidase enzyme by starters. Moreover, the application of remained carbohydrates by survived microorganisms over the storage time causes the production of lactic acid, formic acid and CO_2 (40).

Titratable acidity

The titratable acidity of control and functional yogurt are shown in table 4. The acidity of all functional yogurts has been significantly increased with a stable

Table. 3. Evolution of pH of control and functional yogurt supplemented with wheat germ during storage

Yogurt	Storage time (days)			
	0	7	14	21
YC	4.22±0.03 Ca*	4.18±0.02 Cab	4.15±0.03 Abc	4.13±0.02 Bc
Y1B	4.3±0.04 Aa	4.28±0.03 Aa	4.26±0.03 Bab	4.21±0.03 Ab
Y2B	4.22±0.04 Ca	4.17±0.05 Ca	4.16±0.04 Ba	4.14±0.02 Ba
Y3B	4.34±0.03 Aa	4.28±0.04 ABb	4.25±0.02 Abc	4.21±0.04 Ac
Y1A	4.24±0.04 Ca	4.20±0.05 Cab	4.18±0.02Bab	4.15±0.03 Bb
Y2A	4.25±0.03 BCa	4.20±0.04 Cab	4.19±0.04 Bab	4.16±0.03 ABb
Y3A	4.26±0.03 BCa	4.21±0.02 BCb	4.19±0.03 Bb	4.18±0.02 ABb

* The results are shown as mean ± standard deviation. Values with capital letter superscripts within a column and different small letters within a row are significantly different ($p<0.05$)
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation).

Table. 4. Evolution of acidity of control and functional yogurt supplemented with wheat germ during storage

Yogurt	Storage time (days)			
	0	7	14	21
YC	1.068±0.014 Ad*	1.095±0.005 Ac	1.128±0.005 Ab	1.143±0.000 Aa
Y1B	1.050±0.010 Ac	1.080±0.009 Bb	1.110±0.005 BCa	1.122±0.005 Ca
Y2B	1.059±0.010 Ab	1.062±0.009 Cb	1.125±0.009 Aba	1.137±0.005 Aba
Y3B	1.050±0.010 Ad	1.083±0.005 Ac	1.104±0.005 Cb	1.125±0.009 CBa
Y1A	1.056±0.005 Ad	1.077±0.005 Bc	1.101±0.005Cb	1.131±0.010 ACBa
Y2A	1.056±0.014 Ad	1.080±0.009 Bc	1.116±0.009 ABCb	1.137±0.005 Aba
Y3A	1.062±0.009 Ac	1.080±0.009 Bc	1.113±0.014 ABCb	1.140±0.005 Aa

* The results are shown as mean ± standard deviation. Values with capital letter superscripts within a column and different small letters within a row are significantly different ($p<0.05$)
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

pace over the storage period. Results were consistent with those reported by Bakirci & Kavaz (41), Singh & Muthukumarappan (42), Temiz *et al.* (38), and Tseng & Zhao (37). Singh & Muthukumarappan (42) explained that the enrichment of yogurt with calcium resulted in the more yogurt acidity during storage time. The results reported by Temiz *et al.* (38) also showed the increase of acidity in fruit yogurt upon storage time. The addition time and amount of wheat germ had no effect on the yogurt's acidity. The pH reduction and increase of titratable acidity during the storage time can be attributed to the activity of starter microorganisms. In fact, the consumption of sugar and producing organic acids by microorganisms might cause a decrease in pH and an increase in acidity level (43).

Viability of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus

Although there are many standards for the number of starters in yogurt, the acceptable number is usually reported as 10^7 cfu/g for both starters (44). Table 5 shows the number of *Lactobacillus delbrueckii* subsp. *bulgaricus* during the storage. As it can be seen, the survival of this bacterium has been significantly decreased during the storage time. Eskandari *et al*. (45) reported that the count of starter bacteria decreased during the storage period. The obtained results suggested that the amount of wheat germ content and applied treatment (time of adding wheat germ) had no effect on the survival of bacteria.

As reported in Table 5, the survival of *Streptococcus thermophilus* is significantly (around 1 log) decreased during the storage time. However, it was noted that the amount of wheat germ had no significant effect on the number of *Streptococcus thermophilus*.

Salwa *et al.* (46) proposed that the addition of 5 to 20 % carrot juice did not affect the growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in yogurt. With respect to the number of *Lactobacillus delbrueckii* subsp. *bulgaricus* in stirred yogurt during the storage time, Senaka Ranadheera *et al.* (47) found that the storage time led to a significant decrease in the survived population of this bacterium. Trigueros *et al.* (48) also reported the reduction of *Lactobacillus delbrueckii* subsp. *bulgaricus* during the storage time.

According to Table 5, it is observed that the amount of survived population at the end of storage period was more than $10^6 – 10^7$ cfu/g. The results were consistent with those reported by Salwa *et al.* (46). The reduction of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* during the storage time was attributed to their activity and as a result, consumption of the existing carbohydrate and lactic acid production. It causes a decrease in the pH of produced samples and a simultaneous increase in titratable acidity related to post-acidification (49). Decrease in the number of bacteria can be attributed to their antagonistic effect which produces such antimicrobial compounds as bacteriocins during the storage time (50).

Textural properties

Rheological evaluation can be divided into two sensory and instrumental methods. The present study used TPA in order to evaluate the textural properties of samples. Table 6 demonstrates different textural properties of samples including hardness, cohesiveness, adhesiveness and springiness.

Hardness represents the maximum force of the first compression cycle (20). According to Table 6, the addition of wheat germ in yogurt formulations significantly decreased the hardness as compared to the control sample and this reduction was more pronounced for adding wheat germ into yogurt after fermentation. Moreover, increasing wheat germ content before fermentation caused the reduction of hardness which can be attributed to the denaturation of wheat germ protein during pasteurization. While, by increasing germ level after fermentation, the hardness increased which can be due to the germ ability for water absorption and the presence of undenatured proteins. The highest level of hardness in fortified samples was related to Y3A (21.00 g) and the lowest hardness level was attributed to Y3B (14.50). Similar results were reported by Garcia-Perez *et al.* (51) with the addition of orange fiber into yogurts mix less than 1%.

Adhesiveness is the required force to separate the probe from the sample by the first push (52). This characteristic is influential in making a favorable sensory feeling and improving the histological features of the product (53). According to Table 6, the addition of

Table. 5. Evolution of survival *Lactobacillus Delbrueckii subsp. Bulgaricus* and *Streptococcus Thermophilus* of control and functional yogurt supplemented with wheat germ during storage

	Yogurt	Storage time (days)			
		1	7	14	21
Lactobacillus delbrueckii subsp. bulgaricus	YC	8.90±0.074Aa*	8.66±0.147ABb	7.85±0.122Ac	7.88±0.127Ac
	Y1B	8.68±0.1046BCa	8.81±0.159Aa	7.87±0.063Ab	7.82±0.102Ab
	Y2B	8.85±0.058Aba	8.51±0.080Bb	7.78±0.097Ac	7.77±0.111Ac
	Y3B	8.81±0.055Aba	8.44±0.056Bb	7.77±0.067Ac	7.73±0.070ABc
	Y1A	8.88±0.108Aba	8.59±0.092Bb	7.77±0.124Ac	7.75±0.129Ac
	Y2A	8.62±0.227Ca	8.82±0.066Aa	7.77±0.085Ab	7.73±0.117ABb
	Y3A	8.82±0.067Aba	8.51±0.121Bb	7.71±0.146Ac	7.72±0.124Bd
Streptococcus thermophilus	YC	8.96±0.106Aa*	8.85±0.113Ab	8.05±0.062Ac	7.90±0.126Ac
	Y1B	8.91±0.088Aa	8.79±0.092Aa	8.02±0.059Ab	7.90±0.088Ab
	Y2B	8.76±0.124Aba	8.78±0.076Aa	7.81±0.080BCb	7.91±0.106Ab
	Y3B	8.82±0.085Aba	8.65±0.214Aa	7.84±0.148BCb	7.80±0.151Ab
	Y1A	8.94±0.052Aa	8.74±0.153Ab	7.99±0.054ABc	7.90±0.065Ac
	Y2A	8.89±0.120Aba	8.68±0.099Aa	7.76±0.116Cb	7.84±0.152Ab
	Y3A	8.69±0.160Ba	8.75±0.096Aa	7.83±0.123BCb	7.80±0.062Ab

* The results are shown as mean ± standard deviation. Values with capital letter superscripts within a column and different small letters within a row are significantly different (p <0.05).
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

Table. 6. Texture profile analysis of control and functional yogurt supplemented with wheat germ

Yogurt	Springiness (mm)	Cohesiveness	Adhesiveness(g)	Hardness (g)
YC	67.08±2.52 A*	0.46±0.16 B	10.25±1.06 A	23 ±0.71 A
Y1B	68.57±1.72 A	0.67±0.04 AB	7.5±0.70 B	20.25±1.06 B
Y2B	71.19±0.74 A	0.68±0.04 AB	4.75±0.35 C	14.75±0.35 D
Y3B	69.34±0.88 A	0.99±0.09 A	4.5±0.70 C	14.5±0.71 D
Y1A	56.02±10.35 B	0.47±0.29 B	6.75±1.06 B	17.75±0.35 C
Y2A	68.67±3.87 A	0.8±0.04 AB	6.25±1.06 BC	16.75±0.35 CD
Y3A	61.04±0.54 AB	0.69±0.07 AB	7.75±0.35 B	21 ±2.12 AB

* The results are shown as mean ± standard deviation. Values with capital letter superscripts within a column and different small letters within a row are significantly different (p <0.05)
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

wheat germ reduced the adhesiveness of treated samples compared to the control. The highest adhesiveness level was related to the control sample (10.25 g) and the lowest level was related to Y2B (4.74 g). There was no significant difference between YC, Y1A, Y2A and Y3A (p<0.05). Fadela *et al.* (44) was stated that adhesiveness and cohesiveness are related to the produced exopolysaccharide by starters. There is a direct relation between hardness and adhesiveness (20). In a study by Hashim *et al.* (54), it was shown that the addition of a minimum of 1.5% palm fiber had no impact on textural parameters of the yogurt. However, they reported a decrease in the adhesiveness values by adding 3% palm fiber and 1.5% wheat fiber.

Springiness is representative of the returning speed of a sample to its initial or unchanged condition after disposal of deformative forces. As can be seen in Table 6, there was no significant difference between YC and Y1B, Y2B, Y3B, Y2A and Y3A regarding elasticity. However, the lowest springiness level was related to Y1A (56.02 mm). Similarly, Hashim *et al.* (54), showed that the addition of 1.5% of date fiber had no effect on the springiness.

According to Table 6, a significant increase was observed in the cohesiveness value for fortified sample compared to the control. However, this increase was significant only for Y3B. Similarly, it was reported that the addition of at least 1.5% palm fiber had no effect on cohesiveness of yogurt (54). In another study conducted by Kumar-Mishra (55), it was specified that the addition of soy milk or mango's pulp increases the cohesiveness of yogurt.

Stability is one of effective factors on the product physical characteristics which is influenced by different factors like the amount of solid matter, protein content, thermal process, homogenization, temperature and pH of stirring (56), starter bacteria, the amount of acidity, type and amount of exopolysaccharide, protolithic activity of bacteria (57) and storage conditions (58). In case of all above conditions, a desired product with a desired tissue will be achieved.

Sensory evaluation

Critical sensory attributes of the samples including color, odor, texture, flavor and consumer acceptance were determined by panelists. Figure 2 indicates the diagram of sensory properties of the prepared samples. The samples containing 20 % and 30% wheat germ added after fermentation received the highest texture and consistency scores compared to other samples. Flavor, color and odor texture of the samples were affected negatively when wheat germ was added particularly when 30% germ was used before fermentation. The results indicated that the general acceptability of control sample was higher than fortified samples. However, samples at all levels of wheat germ used after fermentation had higher acceptability then those fortified with germ before fermentation.

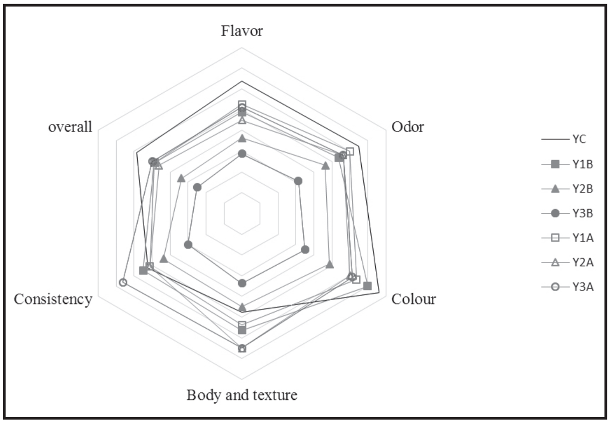


Figure 2. Sensory evaluation of control and functional yogurt supplemented with wheat germ
YC (Yogurt control), Y1B (Yogurt + 10 % wheat germ added before fermentation), Y2B (Yogurt + 20 % wheat germ added before fermentation), Y3B (Yogurt + 30 % wheat germ added before fermentation), Y1A (Yogurt + 10 % wheat germ added after fermentation), Y2A (Yogurt + 20 % wheat germ added after fermentation), Y3A (Yogurt + 30 % wheat germ added after fermentation)

Conclusions

In this work, our hypothesis was the fortification of yogurt via cereal-based by-product. Therefore, the possibility of producing functional yogurt using wheat germ due to its high nutritional value was investigated in the present study. The results suggested that wheat germ addition lead to a considerable increase in mineral content as well as unsaturated fatty acids and fiber in fortified samples. Thus, it is generally accepted that wheat germ can improve the nutritional quality of dairy products. The fortified samples became firmer as the level of wheat germ increased after fermentation. However, the increase of wheat germ level before fermentation showed lower hardness values. Moreover, changing the level of wheat germ had minor effects, while the storage time had a great impact on the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* of the yogurts. The sensory evaluation of the yogurts were affected by the presence of wheat germ and its addition time. The most acceptable fortified samples were produced with wheat germ added after fermentation. Therefore, fortification of yogurts with wheat germ needs future investigation for improving the sensory level of acceptability for samples containing higher levels of germ.

References

1. Saarela M. Functional foods Concept to product, Second edition, *Woodliead Publishing Limited*, Sawston, Cambridge. 2011.
2. Hahsemi Gahruie H, Eskandari MH, Mesbahi G, and Hanifpour MA. Scientific and technical aspects of yogurt fortification: A review. *Food Sci Human Well* 2015, **4**:1-8.
3. Lotfi M, Venkatesh Mannar M, Merx RJ, and Heuvel PN-vd. *Micronutrient fortification of foods: current practices, research, and opportunities*: The Micronutrient Initiative. 1996.
4. McKinley M. The nutrition and health benefits of yoghurt. *Int J Dairy Technol* 2005, **58**;1-12 .
5. Butriss J. Nutritional properties of fermented milk products. *Int J Dairy Technol* 1997, **50**:21-27.
6. Krings U, El-Saharty YS, El-Zeany BA, Pabel B, and Berger RG. Antioxidant activity of extracts from roasted wheat germ. *Food Chem* 2000, **71**:91-95.
7. Šramková Z, Gregová E, and Šturdík E. Chemical composition and nutritional quality of wheat grain. *Acta Chimica Slovaca* 2009, **2**:115-138.
8. Majzoobi M, Darabzadeh N, Farahnaky A. Effects of Percentage and Particle Size of Wheat Germ on Some Properties of Batter and Cake. *J Agricul Sci Technol* 2012. **14**:827-83.
9. Arshad MU, Anjum FM, and Zahoor T. Nutritional assessment of cookies supplemented with defatted wheat germ. *Food Chem* 2007, **102**:123-128.
10. Sidhu JS, Al-Hooti SN, and Al-Saqer JM. Effect of adding wheat bran and germ fractions on the chemical composition of high-fiber toast bread. *Food Chem* 1999, **67**:365-371.
11. Majzoobi M, Ghiasi F, Farahnaky A, Physicochemical assessment of fresh chilled dairy dessert supplemented with wheat germ. *Int J Food Sci Technol* 2016, **51**:78-86.
12. Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B, and Battino M. The strawberry: composition, nutritional quality, and impact on human health. *Nutrition* 2012, **28**:9-19.
13. Krings U, Johansson L, Zorn H, and Berger RG. In vitro DNA-protective activity of roasted wheat germ and fractions thereof. *Food Chem* 2006, **97**:712-718.
14. AACC. "Approved Methods of the AACC (10thed)". American Association of cereal Chemists, st Paul, (Methods 44-19, 30-10, 46-10, 32-05, 08-01) 2000.
15. Bubert H, and Hagenah WD. Detection and measurement. In P. W. J. M. Boumans (Ed.), *inductively coupled plasma emission spectroscopy*. New York: Wiley Interscience. 1987.
16. Gahruie HH, Ziaee E, Eskandari MH, Hosseini SMH. Characterization of basil seed gum-based edible films incorporated with Zataria multiflora essential oil nanoemulsion. *Carbohyd polym* 2017, **166**:93-103.
17. Hosseini SMH, Gahruie HH, Razmjooie M, Sepeidnameh M, Rastehmanfard M, Tatar M, Naghibalhossaini F, Van der Meeren P. Effects of novel and conventional thermal treatments on the physicochemical properties of iron-loaded double emulsions. *Food chem* 2019, 270:70-77.
18. Helrich K. (15th ed.). Official methods of analysis of the AOAC, agricultural chemicals, contaminants, drugs, vol. 2. Wilson Boulevard, Arlington: AOAC Inc. 1990.
19. Supavititpatana P, Wirjantoro TI, Apichartsrangkoon A, and Raviyan P. Addition of gelatin enhanced gelation of corn–milk yogurt. *Food chem* 2008, **106**:211-216.
20. Gauche C, Tomazi T, Barreto PLM, Ogliari PJ, Bordignon-Luiz MT. "Physical properties of yoghurt manufactured with milk whey and transglutaminase". *LWT - Food Sci Technol* 2009, **42**:239–243.
21. Shahbazi H, Hashemi Gahruie H, Golmakani MT, Eskandari MH, Movahedi M. Effect of medicinal plant type and concentration on physicochemical, antioxidant, antimicrobial, and sensorial properties of kombucha. *Food Sci Nutr* 2018,
22. Hashemi Gahruie H, Hosseini SMH, Taghavifard MH, Eskandari MH, Golmakani M-T, Shad E. Lipid oxidation, color changes, and microbiological quality of frozen beef burgers incorporated with shirazi thyme, cinnamon, and rosemary extracts. *J Food Quality* 2017.
23. Piras A, Rosa A, Falconieri D, Porcedda S, Dessi MA, and Marongiu B. Extraction of oil from wheat germ by supercritical CO2. *Molecules* 2009. **14**:2573–2581.
24. Mialon V, Clark M, Leppard P, and Cox DN. The effect of dietary fibre information on consumer responses to breads and "English" muffins: a cross-cultural study. *Food Qual Prefer* 2002, **13**:1-12.
25. Eisenmenger M, and Dunford NT. Bioactive components of commercial and supercritical carbon dioxide processed wheat germ oil. *J Am Oil Chem Soc* 2008, **85**:55–61.
26. Alessandri JM, Extier A, Al-Gubory KH, Harbeby E, Lallemand MS, Linard A, Lavialle M, and Guesnet P. Influence of gender on DHA synthesis: The response of rat liver to low dietary a-linolenic acid evidences higher ω3 ω4- desaturation index in females. *Euro J Nutr* 2011, **51**:199-209 .
27. Berkay Karaca O, Bas I, Saydam A, and Guven M. Physicochemical, mineral and sensory properties of set-type yoghurts produced by addition of grape, mulberry and carob molasses (*Pekmez*) at different ratios. *Int j dairy technol* 2012, **56**:111-117.
28. Bilgiçli N, Elgün A, Herken EN, ErtaωN, and ȸanogˇlu . Effect of wheat germ/bran addition on the chemical, nutritional and sensory quality of tarhana, a fermented wheat flour-yoghurt product. *J Food Eng* 2006, **77**:680-68.
29. Olanipekun EO. Kinetics of leaching laterite. *Int J Mineral Process* 2000, 60: 219–221.
30. Schaafsma G, Dekker P, and Waard HD. Nutritional aspects of yogurt, 2: bioavailability of essential minerals and trace elements. *Netherlands Milk Dairy J (Netherlands)*. 1988, **42**:135–46.
31. Bronner F, and Pansu D. Nutritional aspects of calcium absorption. *The J nutr* 1999, **129**:9-12.
32. Rosado JL, Díaz M, Gonzalez K, Griffin I, Abrams SA, and Preciado R. The addition of milk or yogurt to a plant-based diet increases zinc bioavailability but does not affect iron bioavailability in women. *The J nutr* 2005, **135**:465-468.

33. Çelik ES. Determination of aroma compounds and exopolysaccharids formation by lactic acid bacteria isolated from traditional yoghurts. (Thesis submitted in fulfillment of the requirements for the degree of Master of Applied Sciennce in Biotechnology). Izmir Institute. Turkey. 2007.
34. Espírito Santo AP, Perego P, Converti A, and Oliveira MN. Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts. *LWT - Food Sci and Technol* 2012, **47**:393-399.
35. McCann TH, Fabre F, and Day L. Microstructure, rheology and storage stability of low-fat yoghurt structured by carrot cell wall particles. *Food Res Int* 2011, **44**:884-892.
36. Zibadi S, and Watson RR. Passion fruit (*Passiflora edulis*): composition, efficacy and safety. *Evidence Based Integrative Medicine* 2004, **1**:183-187.
37. Tseng A, and Zhao Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. *Food Chem* 2013, **138**:356-365.
38. Temiz H, Tarakci Z, Karadeniz T, and Bak T. The effect of loquat fruit (*Eriobotrya japonica*) marmalade addition and storage time on phsico-chemical and sensory properties of yogurt. *J Agricul Sci* 2012, **18**:329-338.
39. Zomorodi SH. Physicochemical, rheological and sensory properties of stirred fruit yoghurt fortified by wheat fiber. *J Food Res* 2013, **22**:443-454.
40. Panesar PS, and Shinde C. Effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, *and Bifidobacterium Bifidum* count of Aloe vera fortified probiotic yoghurt. *Curr Res Dairy Sci* 2012, **4**:17-23.
41. Bakirci I, and Kavaz A. An investigation of some properties of banana yogurts made with commercial ABT-2 starter culture during storage. *Int J Dairy Technol* 2008, **61**:270-276.
42. Singh G, Muthukumarappan K. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. *Food Sci Technol* 2008, **41**:1145-1152.
43. Lourens-Hattingh A, Viljoen BC. "Yogurt as probiotic carrier food". *Int J Dairy Technol* 2001, **11**:1-17.
44. Fadela C, Abderrahim C, and Ahmed B. Sensorial and physico-chemical characteristics of yoghurt manufactured with ewe's and skim milk. *World J Dairy Food Sci* 2009, **4**:136-140.
45. Eskandari MH, Baroutkoub A, Roushan Zamir M, Beglarian R, Ghasemkhani I, and Shekarforoush S. Effect of milk supplementation on growth and viability of starter and probiotic bacteria in yogurt during refrigerated storage. *Iran J Veterinary Res* 2012, **13**:195-202.
46. Salwa AA, Galal EA, and Elewa NA. Carrot yoghurt sensory, chemical, microbiological properties and consumer acceptance. *Pakistan J Nutr* 2004, **3**:322-330.
47. Senaka Ranadheera C, Evans CA, Adams MC, and Baines SK. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chem* 2012, **135**:1411-1418.
48. Trigueros L, Viuda-Martos M, Perez-Alvarez JA, and Sendra E. Low fat yoghurt rich in pomegranate juice. *Milchwissenschaft* 2012, **67**:177-180.
49. Daneshi M, Ehsani MR, Razavi SH, Labbafi M, and Sheykh Rezaee M. Effect of cold storage on viability of probiotic bacteria in carrot fortified milk. *J Nutr Food Sci* 2012, **2**:1-4.
50. Ganesh S. A novel yogurt product whit *Lactobacillus acidophilus*. (Thesis submitted in fulfillment of the requirements for the degree of Master of Science).Louisiana State University. United States. 2006.
51. Garcıa-Pérez FJ, Lario Y, Fernández-López J, Sayas E, Pé rez-Alvarez JA, and Sendra E. Effect of orange fiber addition on yogurt color during fermentation and cold storage. *Indusrial Application* 2005, **30**:457-463.
52. Breene WM. Application of texture profile analysis to instrumental food texture evaluation. Review. *J Texture Stud* 1975, **6**:53-82.
53. Zhao OZ, Wang JSH, Zhao MM, Jiang YM, and Chun C. Effect of casein hydrolysates on yogurt fermentation and texture properties during storage. *Food Technol Biotechnol* 2006, **44**:429-434.
54. Hashim IB, Khalil AH, and Afifi HS. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. *J Dairy Sci* 2009, **92**:5403-5407.
55. Kumar P, and Mishra HN. Effect of mango pulp and soymilk fortification on the texture profile of set yoghurt made from buffalo milk. *J Texture Stud* 2003, **34**:249-269.
56. Rasic JL, and Kurmann JA. Yogurt - scientific grounds, technology, manufacture, and preparations. *Technical Dairy Publishing House* 1978, 466.
57. Walstra P, Geurts TJ, Noomen A, Jelleman A, and Van Boekel MAJS. *Dairy Technology.* Marcel Dekker, Inc. 1999.
58. Tamime AY, and Robinson RK. *Yoghurt: science and technology*: Woodhead Publishing. 1999.

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