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Original article

Improving the Probiotics Viability and Quality Characteristics of Yoghurt Enriched with Barley Bran

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Abstract

Results of current study reported that increase in addition of barley bran in yogurt containing bifidobacterium BB-12, L. bulgaricus and S. thermophillus significantly influence the probiotics numbers. Samples fortified with barley bran has more numbers of probiotics as compare to control samples during storage. Correlation between concentration of barley bran and bifidobacterium was optimistic. Maximum numbers of L. bulgaricus and bifidobacterium were found in yogurt containing 1.5% barley bran. Addition of barley bran increased the acidity percentage of yogurt and decreased viscosity value during 3 weeks of storage. Sensory scores of functional yogurt decreased with increased in barley bran. However sensory score of sample with (0.5% barley bran) did not significantly changed from control sample and number of bifidobacterium bacteria in this sample was higher than minimum standard level (log106 CFU/g) of world health organization (WHO). Syneresis value of control sample was significantly reduced with storage period but yogurt sample enriched with different concentration of barley bran were showed minimum decreased in syneresis percentage of yogurt. Barley bran level 1% can be added to yogurt successfully. Barley bran enrichment increased the color parameters of yogurt during storage this may be due to the results of blueness color of barley bran.

Keywords: Probiotics, Bran, Yoghurt.

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INTRODUCTION

Probiotics may be define as a live microorganisms which are introduce into the human body through food that provide some beneficial functions in human body through healthy way (Fuller, 1989). Throughout 21st century probiotics gain too much popularity from food makers, academic studies and functional food consumer. Probiotics history can be determined by the first use of cheese and fermented dairy products well known by the Romans and Greeks who suggested their use for beneficial purpose (Gismondo et al., 1999).Growing demand for probiotics has led to the need for more rigorous criteria for scientific substantiation of the putative benefits that microorganisms claim to be probiotics (Rijkers et al., 2011). Most of the dairy fermented products are produced from lactic acid bacteria (LAB) in dairy industries. Most LAB's are very important for human nutrition because of their probiotics properties (Tannock, 2005).

In last few years studies have shown that the probiotic bacteria e.g. Lactobacillus casei which is most common type of lactic acid bacteria (LAB) has contain many beneficial effects in biological processes such as intestinal pathogens inhibition, cardiovascular disease protection and beneficial properties to immune system of the human body (Awaisheh et al., 2013). Prebiotics are considered to be food of the probiotics which are define as non-digestible components of food and contain health promoting effects on the host because of their beneficial characteristics, they selectively stimulate the reproduction and development of bacterial colonies in the human colon (Oliveira et al., 2009). The word 'synbiotic food' refer to the food which contain both probiotics and prebiotics effects and the synbiotic properties is synergy between them (Pandey and Mishra, 2015).

There is only small indication of low quality research to show that probiotics are effective against the people which are the victim of milk allergy. In 2015 study found that probiotics administered directly to infants which are involves in eczema lower the risk of this disease in infants (Qamer et al., 2019). Approximately 11 to 40 per cent diarrhea occurring in infants are treated with antibiotics which result in the formation of Antibiotic Association Diarrhea (AAD) due to imbalance in the microbiota of the infants induced by antibiotic therapy. These modification in the microbial population contributes to change in the metabolism of carbohydrates which result in reduced absorption of short chain fatty acids and osmotic diarrhea. In adults there is scientific proof that some probiotics play an important role in reducing the Antibiotic Association Diarrhea (AAD) and treating some other diseases (Goldenberg et al., 2015).

Probiotics also play an important role in the immune function and protect human body from different infections most of the lactic acid bacteria (LAB) strains can kill pathogens through reasonable inhibition and most of the times evidence indicates that they can boost immunity of the body by raising the amount of plasma cells generating IgA and raising phagocytosis. The strains of lactic acid bacteria (LAB) increasing the number of natural killer cells in human body and raising the proportion of T

lymphocytes. The products which contain suitable amount of Lactic Acid Bacteria (LAB) help to reduce the disease of traveler diarrhea in adults and manage to reduce the risk of rotavirus infection in children. These products provide insufficient evidence that they reduce the occurrence of respiratory infections in adults and not reduce the risk of different kind of infections in older people (Wachholz et al., 2018).

Pakistan ranked 4th in worldwide production of milk with approximately 58 billion of milk produced in 2015. Due to developing and lack of modern technologies e.g. transportation, collection and processing in Pakistan thousands of liters of milk spoiled and discarded every year (GOP, 2015-2016). Milk is a white lacteal secretion which is fully nutrient rich, pure, liquid food provided by mammalian animals by their mammary glands. This is the main nutrient source which give strength to the immune system and provide growth to the baby mammals includes breast feed humans until they are able to absorb or digest other forms of food (Winckel et al., 2011).

There are different treatments provide to the raw milk in dairy industries for the manufacturing of different products. As raw milk is received and it stands for a longer time the taste of the milk will converted into sour taste. The lactose of the milk will breakdown by the action microorganisms and resulted in the formation of lactic acid by the biochemical changes of lactic acid bacteria (LAB). After sometime these unfavorable conditions will produce curdling in milk which changes the pH of the milk. Fermentation is the basic phenomenon for the production of fermented products. This process may be started with the inoculation of different bacterial culture such as (Leuconostoc sp., lactobacilli sp., Streptococcus sp., etc.) to manufacture thousands of different kinds of fermented milk products in dairy industries.

The word “yoghurt” is basically derived from Turkish word which means “to knead or coagulate” sometime also called yoghurt or yogourt. Yoghurt is mainly a fermented dairy product which is produce as a result of fermentation of milk in specific condition under the presence of suitable bacterial culture. The mechanism behind the production of yoghurt is to convert the lactose which is milk carbohydrate change into lactic acid under the presence of bacterial culture (FDA, 2018).

Bran also play an important role in metabolic activity they show great resistance against type 2 diabetes, cardiovascular problems and obesity. Apart from this dietary fiber and cereal bran play an important role to balance the satiety and slow the absorption time of nutrients (Slavin, 2013). In most of the cases the antioxidants, resistance starch and dietary fiber are key factors for controlling the following factors such as release of insulin, glucose absorption, lower the risk of cardiovascular diseases (CVD) and control body weight. Adequate intake of bran and fiber report great result to control diabetes (Cho et al., 2013). Ghrelin hormone excrete from empty stomach which produce the sensation of food desire and hunger in human body (Engel and Jerlhag, 2014).

Research indicates that barley bran has a greater efficiency to cure the induced hypercholesterolemia and hyperlipidemia in rates. Rats were fed with approximately 1 % cholesterol and its significantly lower the lipid level and promote the high density lipoprotein (HDL) cholesterol, enhance the histopathology of kidney, maintain antioxidant enzymes and liver problems (Rabey et al., 2013). Scientifically β-glucan which is dominantly present in barley bran maximized liver actives and increased high density lipoprotein (HDL) cholesterol in diabetic mice (Zhao et al., 2014). In this context the current study was designed to improve and enhance the quality characteristics and probiotic viability of yoghurt by adding barley bran in various proportions.

MATERIALS and METHODS

Procurement of Raw Material

For the preparation of yoghurt standardized cow milk (Carbohydrate, Protein, fat, pH and acidity) purchased from milk collection center near Shujabad Road Multan, Pakistan. Barley bran was purchased from local flour mill located near Khanewal Road Multan, Pakistan. Fresh freeze dried commercial starter culture (YC-X11) of Lactobacillus delbrueckii spp bulgaricus and Streptococcus thermophiles was purchased from (Christian Hansen, Dubai, United Arab Emirates). The probiotic culture of Bifidiobacterium BB-12 also purchased from (Christian Hansen, Dubai, United Arab Emirates).

Physicochemical analysis of milk

Analysis of cow milk (Carbohydrates, Protein, fat, Ash, pH and acidity) was performed according to the method of (AOAC, 2016).

Physicochemical analysis of barley bran

Chemical analysis (moisture, ash, fat, acidity and pH) of barley bran was determined by the method of (AOAC, 2016).

Determination of total plate count

For measurement of microbial count in barley bran 10 fold serial dilution method was used with little modification. 10 fold dilutions of bran was prepared and 0.1 ml of each dilution was plated on petri plates containing nutrient agar by the method of surface plating. Plates were placed in incubator (Isotemp Incubator 637D, Fisher Scientific Pittsburgh, USA) for approximately 24 ± 1 hours at 37 ˚C. After incubation plates heaving range from 30-300 colonies were selected for microbial count. Conducted results were reported as total plate count (CFU/g) (AOAC, 2016).

Determination of mold and yeast

For the determination of yeast and mold in barley bran 10 fold serial dilution method was used. First of all 10 fold dilutions of bran was be prepared and 0.1 ml of each dilution were plated on potato

dextrose agar by the method of surface plating. The plates was placed in the incubator (Isotemp Incubator 637D, Fisher Scientific Pittsburgh, USA) approximately for 3-5 days at 25 ˚C. After incubation plates heaving range from 15-150 colonies were selected for microbial count. The conducted results were reported as mold and yeast count (CFU/g) (AOAC, 2016).

Titratable acidity of yoghurt

For the determination of titratable acidity of yoghurt 10 g of sample was mixed with approximately 20 ml of distilled water. Mixture was titrate with 0.1 N NaOH. Phenolphthalein indictor was used as an indicator and titration of sample was continued until pink colored appeared (Hasani *et al*., 2017).

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$$\text{Acidity (\%)} = \frac{0.009 \times \text{volume of NaOH used}}{\text{weight of sample}} \times 100$$

pH of yoghurt

The pH of the yoghurt was determined by pH meter (Mettler, Tolegado AG, Model E120, Switzerland) by the method of (Zanhi and Jideani, 2012).

Color measurement

The color of the yoghurt was determined by chroma meter (Konica Minolta, CR-400 Head Series, Japan). The b, a and L value of the yoghurt was measured. L value measured the lightness, a value measured the redness and the b value measured the blueness of the sample. The instrument was calibrated by using white standards plates before the measurement (Barkallah et al., 2017).

Determination of syneresis

Syneresis value was determined with little modification by the method of (Gouraji et al., 2019). 25 g of sample was filter through Whatman paper for 2 hours at 4˚C. The result was calculated using the following equation.

Syneresis Percentage (%)=(Whey weight)/(Sample weight)×100

Water holding capacity

The water holding capacity was determined according to the method of (Demerci et al., 2017) with little modification. 10 gram of yoghurt (X) sample was placed in centrifugal machine (Eppendorf

Centrifuge Mini Plus, Hamburg, Germany) and run at 5000 rpm for 20 minutes at 4˚C. After centrifugation the whey (Y) expelled was drained and weigh. The result was calculated according to the following equation.

$$\text{Water Holding Capacity (\%)} = (\text{Clear supernatant (Y)})/(\text{Initial weight (X)}) \times 100$$

Viscosity determination

Viscosity of functional yoghurt was evaluated using Brookfield Viscometer (RVDV2, Brookfield, MA, USA) by using R4 spindle. The reading was carried out at 80 rpm for 60 seconds. Reading was calculated in (cP) centipoise (Hasani et al., 2017).

Growth response of bifidiobacterium BB-12

The response of the bifidobacterium BB-12 was evaluated at the time of interval week 1, 2 and 3. For the enumeration of probiotic bacteria MRS (deMan Rogosa and Sharpe) agar (Sigma Aldrich, USA) was used by the method of surface plating. The plates was placed in incubator (Isotemp Incubator 637D, Fisher Scientific Pittsburgh, USA) approximately for 72 hours at 37 ˚C under strict anaerobic condition. Enumerated colonies were observed for plate counting. Conducted result was reported as (CFU/g) (Allgeyer et al., 2010).

Growth response of the L. delbrueckii ssp. bulgaricus

The colony counting of the probiotic bacteria was determined by the method of (Demirci et al., 2017). MRS (deMan Rogosa and Sharpe) agar (Sigma Aldrich, USA) was used by the method of surface plating. The petri plates was incubated (Isotemp Incubator 637D, Fisher Scientific Pittsburgh, USA) nearly at 37 ˚C for 24 ± 1 hours in anaerobic condition. Enumerated colonies was observed for plate counting. Conducted result was reported as (CFU/g). The response was evaluated at the interval of week 1, 2, and 3.

Sensory evaluation of yoghurt

Sensory evaluation of yoghurt enriched with barley bran was assessed by the students of Institute of Food Science & Nutrition, Bahauddin Zakariya University Multan. All yoghurt samples were assessed by using 9 points of hedonic scale (Ozcan, 2013). In hedonic scale one was the lowest score and nine was the highest score. Yoghurt was evaluated for its color, taste, flavor, texture, appearance and overall acceptability.

Statistical analysis

All the characteristics of functional yoghurt were calculated by (General Linear Model ANOVA) using Statistix 8.1® software (Statsoft, Tulsa, OK, USA) (Santo et al., 2012)

RESULTS and DISCUSSION

Four treatments were made for the production of functional yoghurt and each was incorporated with different concentration of barley bran (0%, 0.5%, 1% and 1.5%). The quality characteristics of the functional yoghurt was assessed by performing sensory, microbial and physicochemical analysis. All samples of the yoghurt were evaluated for their textural, microbial and physicochemical characteristics at the interval of 1, 7, 14 and 21 days.

Physicochemical analysis of milk

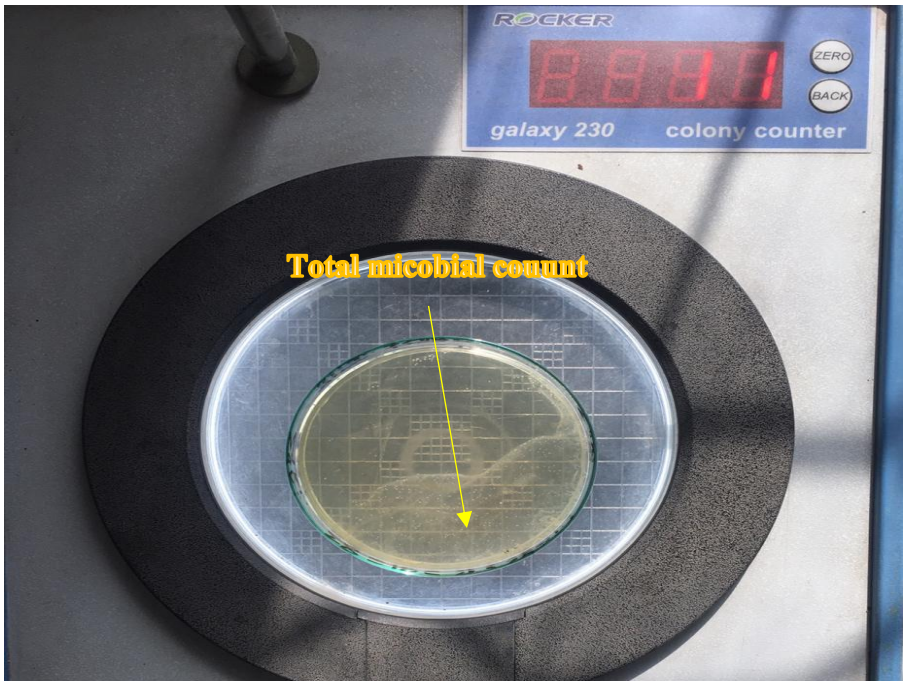
Milk was evaluated for its physicochemical factors. Fat, protein, solid not fat, acidity and pH values of milk were 3.27%, 3.13%, 7.17%, 0.29% and 6.68 respectively.

Physicochemical analysis of barley bran

Physicochemical analysis of barley bran was performed because barley bran was incorporated in milk to manufactured functional yoghurt. pH, moisture, fat, ash and acidity of barley bran was determined and their mean values were 6.32, 9.58%, 8.13%, 6.07% and 0.53% respectively.

Microbial analysis of barley bran

Barley bran was tested for the total microbial count and mold count and their mean values were 4.4×10^2 CFU/g, and 5.7×10^2 CFU/g, respectively. The amount of total viable microorganisms are important during storage of yoghurt because their numbers are significantly influence the quality and sensorial characteristics of yoghurt. Greater the amount of microbial organisms greater will be the chances of spoilage of yoghurt.



Colonies of total plate count

Colonies of mold and yeast count

Water Holding Capacity (WHC) of functional yoghurt

Water holding capacity of functional yoghurt has been exhibited in table 1 output of water holding capacity of functional yoghurt was in agreement to the results of (Demirci *et al.*, 2017) who studied antioxidative and microbial properties of yoghurt with different concentration of rice bran. According

to (Randheera *et al.,* 2012) water holding capacity of yoghurt greatly affected by the fat globule of milk during different days of storage. Water holding capacity percentage of treatment T_0 (control sample with 0% barley bran) were 41.50, 37.83, 34.50 and 30.83 for the storage period of day 0 to 21 with the interval period of 7 days. Highest value of water holding capacity for treatment T_0 was noted as 41.57 at day 0 and lowest value was observed 30.91 at day 21. Overall mean value of this treatment was 36.17. Our results was in agreement to the findings of (Yadav *et al.,* 2017) who observed declined in water holding capacity of yoghurt after 9 and 18 days during refrigerated storage.

Treatment T_1 (yoghurt sample with 0.5% barley bran) mean values for the water holding capacity percentage was 43.17, 41.33, 36.83 and 32.83 during storage period of 21 days with 7 days of time interval. During analysis of T_1 maximum value 43.21 showed at day 0 and lowest value 32.87 was observed at day 21 during 3 weeks storage of functional yoghurt. Same declined in water holding capacity percentage of chickpea fortified yoghurt was observed by (Sidhu *et al.,* 2020) during first week of storage. Treatment T_2 (yoghurt sample) which was manufactured with addition of 1 % barley bran presented the water holding capacity percentage values 44.77, 42.17, 39.83 and 37.33 during 3 weeks of refrigerated storage. Water holding capacity was declined as the day passes during storage of functional yoghurt. Highest value of functional yoghurt was observed 44.77 at day 0 and lowest value was noticed 37.33 at day 21. For this treatment 40.96 was the overall mean value of functional yoghurt. Study showed that water holding capacity of yoghurt related to the capability of fat globule and protein to retain water (Hongyu *et al.,* 2000). Water holding capacity percentage values for treatment T_4 (yoghurt sample with 1.5% barley bran) was 45.67, 43.23, 41.51 and 40.17 during 3 weeks of refrigerated storage. Cumulative mean of this treatment was 42.63. Highest value of yoghurt enriched with 1.5% barley bran was noticed 45.67 at day 0 and lowest value was showed at day 21. Reduction in Water holding capacity percentage with the passage of time is totally independent on fortified substances because all treatments showed decreased in water holding capacity over storage period (Riakos *et al.,* 2020).

Table 1. Water Holding Capacity (WHC) of yoghurt during storage

Treatment	Storage Days			
	0	7	14	21
$T_\circ$	41.57 ± 1.5^{cd}	37.91 ± 1.7^{ef}	34.53 ± 1.5^{gh}	30.91 ± 1.9^{i}
T_1	$43.21\pm1.2^{a-c}$	41.42 ± 2.4^{cd}	36.81 ± 2.6^{fg}	32.87 ± 1.2^{hi}
T_2	44.77 ± 1.8^{ab}	$42.17\pm1.3^{b-d}$	39.83 ± 3.3^{de}	$37.33\pm2.5^{e-g}$
T_3	45.67 ± 1.4^{a}	$43.23\pm0.7^{a-c}$	41.51 ± 1.5^{cd}	40.17 ± 1.5^{de}

means with standard deviation (n=3); T0= Control sample; T1= 0.5% barley bran; T2= 1% barley bran: T3= 1.5% barley bran

Syneresis of functional yoghurt

Syneresis is the amount of whey which is spontaneously separated from yoghurt due to gel shrinkage. During this process the whey portion comes on the surface of the yoghurt and described as syneresis percentage (Garcia et al., 2005). In all type of yoghurt syneresis is a complex problem which influence the different characteristics of yoghurt during storage (Randheera et al., 2012). During 21 days of storage the syneresis results of functional yoghurt was recorded in agreement with the results obtained by (Zare et al., 2011) who studied different characteristics of plain yoghurt with addition of different concentration of lentil flour during 3 weeks of storage. Table 2 indicated overall values of yoghurt syneresis. Treatment T0 (control sample) has showed lowest value 12.27 of syneresis percentage at day 0 and highest value of syneresis value was examined at day 21 during 3 weeks of refrigerated storage. According to (Santiago et al., 2010) who studied stirred yoghurt enriched with soluble dietary fiber, increased in syneresis value of functional yoghurt is associated with the arrangements of conjugated network of casein protein. Overall values of treatment T0 were 12.27, 14.29, 16.5 and 19.21 at day 0, 7, 14 and 21 respectively.

Treatment T1 (yoghurt sample with 0.5% barley bran) of functional yoghurt has shown not significantly difference as compare to treatment T0. Lowest value 10.90 of syneresis percentage was recorded at day 0 and highest value 18.17 was examined at day 21 with the interval of 7 days. Collective values of this treatment were 10.91, 12.07, 14.17 and 18.17 at day 0, 7, 14 and 21 correspondingly. Findings of (Amal et al., 2016) was in accordance with our results who examined plain yoghurt with different concentration of dried fruits has decreased the syneresis value of yoghurt during 15 days of storage. Syneresis percentage of treatment T2 (yoghurt sample with 1% barley bran) of functional yoghurt was noticed completely different as compare to T1 because this sample has more concentration of barley. Lowest value of this treatment was observed at 10.07 and highest value 13.57 was noticed at day 21 during 3 weeks of storage. Overall values of T2 were 10.07, 11.17, 12.27 and 13.57 during 3 weeks of storage with 7 days of interval. Syneresis behavior of yoghurt also related to the quantity of solid in fruit that absorbed most of yoghurt water which decreased syneresis value of yoghurt (Mahmood et al., 2008) which was observed in our study. Treatment T3 (yoghurt sample with 1.5% barley bran) of yoghurt sample has shown minimum changed in syneresis percentage during overall storage period. Lowest value 8.17 has noticed at day 0 and maximum value of syneresis for this treatment was 12.03 at day 21 during 3 weeks. Overall values of syneresis for this treatment were 8.17, 9.53, 10.77 and 12.03 at day 0, 7, 14 and 21 respectively.

Table 2. Synersis of yoghurt during storage

Treatment	Storage Days			
	0	**7**	**14**	**21**
T_0	12.27 ± 0.91^{de}	14.29 ± 0.61^{c}	16.5 ± 0.66^{b}	19.21 ± 0.83^{a}
T_1	10.91 ± 0.95^{fg}	12.07 ± 0.73^{ef}	14.17 ± 0.76^{c}	18.17 ± 0.85^{a}
T_2	10.07 ± 0.81^{gh}	$11.17\pm0.71^{e-g}$	12.27 ± 0.81^{de}	13.57 ± 0.75^{cd}
T_3	8.17 ± 0.86^{i}	9.53 ± 0.53^{h}	$10.77\pm0.85^{f-g}$	12.03 ± 0.91^{ef}

means with standard deviation (n=3); T0= Control sample; T1= 0.5% barley bran; T2= 1% barley bran: T_3= 1.5% barley bran

Viscosity (cP) of functional yoghurt

Viscosity values of functional yoghurt as reported in table 3 indicated that during 3 weeks of storage treatment control sample has examined maximum value of viscosity and yoghurt which was manufactured with highest concentration of barley bran has showed the lowest value of viscosity at the end of research. The results obtained in our study was completely in compliance with the results of (Tseng and Zaho, 2013) who suggested that increase in amount of grape pomace in yoghurt as a source of prebiotics decreased the viscosity value of yoghurt during refrigerated storage. According to (El-Said et al., 2014) who studied physical properties of stirred yoghurt stated that increasing amount of pomegranate peel extracts with different concentration in yoghurt lower the apparent viscosity value during storage and he associated these output with influence of extract on the aggregation network of yoghurt by electro-statistic interface. On the other hand results recorded by the (Donkor et al., 2007) showed that yoghurt fortified with different levels of peach dietary fiber and inulin exhibited significantly higher value of viscosity as compared to plain yoghurt.

Table 3. Viscosity of yoghurt during storage

Treatment	Storage Days			
	0	**7**	**14**	**21**
T_0	2364 ± 22.1^{a}	1814 ± 19.1^{b}	1564 ± 13.1^{c}	1247 ± 27.2^{e}
T_1	2143 ± 16.2^{d}	1666 ± 20.3^{f}	1314 ± 21.2^{h}	1011 ± 16.5^{k}
T_2	1916 ± 23.5^{g}	1353 ± 18.3^{i}	1057 ± 14.5^{l}	889 ± 17.1^{o}
T_3	1710 ± 26.8^{j}	1146 ± 19.6^{m}	834 ± 12.7^{n}	639 ± 10.6^{p}

means with standard deviation (n=3); T0= Control sample; T1= 0.5% barley bran; T2= 1% barley bran: T_3= 1.5% barley bran

L* value of functional yoghurt

Color of dairy products has great impact on consumer acceptability and show different behavior of pigment concentration in yoghurt during storage (Donmez *et al.,* 2017). Table no 4 has indicated significant change in L* value of yoghurt during 21 days of refrigerated storage. Huge difference in L* value has examined in control sample and samples which are fortified with different concentration of barley bran. Output for L value of color in this study was in obedient to findings of (Scibisz *et al.*, 2019) who studied color properties of yoghurt during storage. L attributes of yoghurt for T_0 was lower at day

0 and it was recorded the lowest value during whole study. Overall mean values for T_0 were 78.25, 80.34, 81.44 and 83.39 at day 0, 7, 14, and 21 respectively. Highest value of L was noticed in T_3 (yoghurt sample with 1.5 % barley bran) increase in L value of this treatment due to higher concentration of bran and its color greatly influence the color of yoghurt during storage this results was in accordance to the results of (Hasani and Sharifi, 2012) who reported that lightness of yoghurt fortified with barberry increased during storage. Mean square value has examined for T_3 were 89.34, 91.42, 92.55 and 94.03 during 21 days of storage. On the results of (Costa *et al.,* 2015) described that L value of color is decreased when yoghurt fortified cupuassu pulp which are opposite to the results of our study.

Reported results of T_1 (yoghurt sample with 0.5 % barley bran) for functional yoghurt has showed slightly higher as compared to T_0. L value of sample T_1 has ranged from 82.43 to 88.66 same results were obtained by (Sert *et al.,* 2017). Overall mean values for treatment T_1 were 82.34, 84.22, 86.32 and 88.66 at 21 days of storage with 7 days of interval indicated in table. Lowest value 82.34 of L parameter for this treatments was noticed at 0 and highest value was examined at day 21. Output of treatment T_2 was has showed increased in L value of yoghurt due to higher amount of barley bran as compared to T_1 and T_2. Overall mean values of this treatment were 85.20, 87.41, 89.30 and 91.48 at 3 weeks of storage condition. Outcome of this treatment was in accordance to the results of (Aghajani, *et al.,* 2019) whom studied low fat yoghurt fortified spirulina platensis during cold storage. They suggested that increased in L value of yoghurt due to the presence of large particles of casein micelles which increased light reflection. According to (Aryana *et al*., 2007) suggested that when casein micelles and retentate are accumulate they creat complex linkages and denser structure in milk which increased the intensity of L value of yoghurt.

b* value of functional yoghurt

All treatments of functional yoghurt including control sample has showed significant increase in b* value of yoghurt during storage. Fortified samples of yoghurt were exhibited more yellowish color as compared T_0 this behavior of fortified yoghurt is due to the yellowish color of barley bran this finding has resemblance with the results of (Hashim *et al.,* 2009) whom has same observation in b value when they fortified yoghurt with wheat bran. Concluded results of functional yoghurt for all treatments has indicated in table 5. Noticeable difference has examined between T_0 to T_3. Lowest value 6.19 was found at day 0 of treatment T_0 and highest value 14.28 of b parameter for yoghurt was examined at day 21. This results of b* parameters was in accordance with the results of (Demirci *et al*., 2017). b* value of color is increasing with the passage of time.

Treatment T_0 (yoghurt sample with 0% barley bran) showed lowest value of yellowness parameter as compared to all other fortified samples. Control sample value of b* for functional yoghurt were 6.19, 7.31, 7.84 and 8.23 during 3 weeks of refrigerated storage with 7 days of interval. Overall means values of control sample were 6.19, 7.31, 7.84 and 8.23 correspondingly. Sample T_1 showed higher change in

b value during whole study as compared to control sample. Range of b* value for this treatment was 7.65 to 9.39. Exhibited results of this treatment showed that addition of barley bran increased b* value of fortified sample this results was in agreement to the findings of (Hussein *et al.*, 2017) who studied characteristics of full fat rice bran and plain yoghurt fortified it. Overall mean values for treatment T_1 were 7.65, 8.14, 8.77 and 9.39 at 21 days of storage with interval of 7 days. Table 4.10. Indicated that there is significant increase in b* value of treatment T_2 (sample with 1% of barley bran) with the passage of time. Highest value for this treatment conducted at day 21 and lowest value was observed at day 0. Significant increase in b* value was also observed in this treatment. Collective mean value of this treatment were 10.18, 10.86, 11.64 and 12.29 at day 0, 7, 14 and 21 correspondingly. According to (Mousavi *et al.*, 2019) who studied quality characters of yoghurt enrich with flaxseed suggested that increase amount of flaxseed concentration showed higher results of b* value. Overall mean value for T_3 were 12.27, 12.66, 13.29 and 14.28 during storage.

Table 4. L value of yoghurt during storage

Treatment	**Storage Days**			
	0	**7**	**14**	**21**
T_0	78.25 ± 0.1^{n}	80.34 ± 0.5^{m}	81.44 ± 0.3^{l}	83.39 ± 0.5^{j}
T_1	82.43 ± 0.3^{k}	84.22 ± 0.2^{i}	86.32 ± 0.2^{g}	88.66 ± 0.7^{e}
T_2	85.2 ± 0.1^{h}	87.41 ± 0.3^{f}	89.3 ± 0.1^{d}	91.48 ± 0.3^{c}
T_3	89.34 ± 0.2^{d}	91.42 ± 0.2^{c}	92.55 ± 0.3^{b}	94.03 ± 0.6^{a}

Results of treatment T0 (control sample with 0 % barley bran) in compliance with the results of (Demirci et al., 2017) who studies different characters of yoghurt with the addition of rice bran. Findings of (Gouraji et al., 2019) who studied antibacterial properties of yoghurt enriched with phycocanin described that the highest value of plain yoghurt as compared to other treatments because of high concentration of solids form complex microstructure which breakdown the gel network of yoghurt results in lower viscosity due to reduce surface tension. Highest value 2364 of viscosity was observed at day 1 and lowest value 1247 was noticed at day 21 during 3 weeks of storage with interval of 7 days. Complete values of viscosity for T0 were 2364, 1814, 1564 and 1247 at day 0, 7, 14 and 21 respectively. Treatment T1 (yoghurt with 0.5% barley bran) of functional yoghurt has examined minimum change in viscosity as compared to treatment T0. Maximum value 2143 of was reported at day 0 and minimum value 1011 was observed at day 21 with the interval of 7 days. Overall mean values of treatment T1 was 2143, 1666, 1314 and 1011 at day 0, 7, 14 and 21 respectively.

Output of treatment T2 (yoghurt with 1% barley bran) has showed slightly changed in viscosity values as compared to treatment T1. Highest value 1916 for this treatment was examined at day 0 and

lowest value 889 was observed at day 21 during 3 weeks of storage with 7 days of interval. Overall mean values for this treatment were 1916, 1353, 1057 and 889 at day 0, 7, 14 and 21 correspondingly. Treatment T4 (yoghurt with 1.5 barley bran) of functional yoghurt has showed the lowest value 639 of viscosity as compared to other treatments during overall research. Highest value 1710 was noticed at day 0 and lowest value was examined at day 21.

b* value of functional yoghurt

All treatments of functional yoghurt including control sample has showed significant increase in b* value of yoghurt during storage. Fortified samples of yoghurt were exhibited more yellowish color as compared T_0 this behavior of fortified yoghurt is due to the yellowish color of barley bran this finding has resemblance with the results of (Hashim *et al.,* 2009) whom has same observation in b value when they fortified yoghurt with wheat bran. Concluded results of functional yoghurt for all treatments has indicated in table 5. Noticeable difference has examined between T_0 to T_3. Lowest value 6.19 was found at day 0 of treatment T_0 and highest value 14.28 of b parameter for yoghurt was examined at day 21. This results of b* parameters was in accordance with the results of (Demirci *et al*., 2017). b* value of color is increasing with the passage of time.

Treatment T_0 (yoghurt sample with 0% barley bran) showed lowest value of yellowness parameter as compared to all other fortified samples. Control sample value of b* for functional yoghurt were 6.19, 7.31, 7.84 and 8.23 during 3 weeks of refrigerated storage with 7 days of interval. Overall means values of control sample were 6.19, 7.31, 7.84 and 8.23 correspondingly. Sample T_1 showed higher change in b value during whole study as compared to control sample. Range of b* value for this treatment was 7.65 to 9.39. Exhibited results of this treatment showed that addition of barley bran increased b* value of fortified sample this results was in agreement to the findings of (Hussein *et al.,* 2017) who studied characteristics of full fat rice bran and plain yoghurt fortified it. Overall mean values for treatment T_1 were 7.65, 8.14, 8.77 and 9.39 at 21 days of storage with interval of 7 days. Table 5. Indicated that there is significant increase in b* value of treatment T_2 (sample with 1% of barley bran) with the passage of time. Highest value for this treatment conducted at day 21 and lowest value was observed at day 0. Significant increase in b* value was also observed in this treatment. Collective mean value of this treatment were 10.18, 10.86, 11.64 and 12.29 at day 0, 7, 14 and 21 correspondingly. According to (Mousavi *et al.,* 2019) who studied quality characters of yoghurt enrich with flaxseed suggested that increase amount of flaxseed concentration showed higher results of b* value. Overall mean value for T_3 were 12.27, 12.66, 13.29 and 14.28 during storage.

Table 5. b* value of yoghurt during storage

Treatment	Storage Days			
	0	**7**	**14**	**21**
T_0	6.19 ± 0.1^{m}	7.31 ± 0.1^{l}	7.84 ± 0.1^{k}	8.23 ± 0.4^{j}
T_1	7.65 ± 0.5^{k}	8.14 ± 0.2^{j}	8.77 ± 0.1^{i}	9.39 ± 0.1^{h}
T_2	10.18 ± 0.1^{g}	10.86 ± 0.1^{f}	11.64 ± 0.2^{e}	12.29 ± 0.1^{d}
T_3	12.27 ± 0.3^{d}	12.66 ± 0.3^{c}	13.29 ± 0.1^{b}	14.28 ± 0.2^{a}

L. delbrueckii ssp. bulgaricus of functional yoghurt

Results showed that different quantity of barley bran and storage time had noticeable effect on viability of probiotics. On 0 day of research and after incubation the amount of L. bulgaricus in fortified sample of yoghurt is higher than control sample. Fortified sample with 1.5% barley bran has showed highest number of L. bulgaricus on day 0 and lowest number of probiotics was observed in control sample. In general higher the concentration of barley bran increased the total number of probiotics in yoghurt. This results was in agreement to the results of (Hasani et al., 2017) who studied effect barley bran concentration on viability of L. acidiphillus during refrigerated storage. Results of control sample of yoghurt were log CFU/g 7.25, 7.17, 7.10 and 7.06 at day 0, 7, 14 and 21 respectively. Decrease in number of probiotics was observed during whole storage. This results was in favor to the results of (Hasani et al., 2016) who studied viability of probiotics in stirred yoghurt fortified with rice bran. Results of treatment T1 (yoghurt sample formulated with 0.5%) barley bran has showed higher amount of L. bulgaricus during storage as compared to T0. Highest value of L.bulgaricus was noticed at day 0 and lowest value was noticed at day 21. Overall mean values of this treatment was indicated in table 6. Reduction in probiotics during study due to increase in acidity value of yoghurt which called as over acidification and creation of hydrogen peroxide by thermophilic culture.

Treatment T2 (yoghurt with 1% barley bran) has examined higher value of L. bulgaricus as compared T0 and T1. This results recorded same as results of (Demirci et al., 2017). Highest value of L. bulgaricus was noticed log CFU/g 7.39 at day 0 and lowest value log CFU/ g 7.23 was conducted at day 21. Reduction in numbers of L. bulgaricus from day 0 to day 21 was log CFU/g 0.16 which was lower as compared T2. Treatment T3 (yoghurt sample with 1.5% barley bran) has showed highest numbers of L. bulgaricus in comparison to all treatments this may be due to the nitrogenous and starchy compounds present in barley bran and presence of structural polysaccharides e.g beta glucan (Desai et al., 2004; Makars et al., 2005). Mean values of this treatment were log CFU/g 8.18, 8.12, 7.35 and 7.29 during 3 weeks of storage with 7 days of interval.

Table 6. *L. delbrueckii ssp. bulgaricus* of yoghurt during storage

Treatment	Storage Days			
	0	**7**	**14**	**21**
T_0	$7.25\pm0.2^{c-f}$	$7.17\pm0.2^{f-h}$	7.10 ± 0.1^{gh}	7.06 ± 0.1^{h}
T_1	$7.32\pm0.1^{b-d}$	$7.23\pm0.1^{d-f}$	$7.19\pm0.4^{e-g}$	7.11 ± 0.3^{gh}
T_2	7.39 ± 0.3^{b}	$7.32\pm0^{b-d}$	$7.27\pm0.1^{c-f}$	$7.23\pm0.1^{d-f}$
T_3	8.18 ± 0.1^{a}	8.12 ± 0.1^{a}	7.35 ± 0.1^{bc}	$7.29\pm0.2^{b-e}$

Sensory evaluation of functional yoghurt

Sensorial evaluation of plain yoghurt was made by using 100 % cow milk as control sample and other samples formulated by the enrichment of 0.5%, 1% and 1.5% of the barley bran were resolute the best replacement level of barley bran for made best quality functional yoghurt. 10 panelists of Food Department were assessed the all samples of yoghurt and results are exhibited in table 7. All results of sensorial properties (Color, taste, texture, flavor, appearance and overall acceptability) showed significant difference from one and another.

Overall mean values of sensory assessment of has examined that color score of functional yoghurt was decreased with the addition of barley bran. Score of color parameter of functional yoghurt was observed highest in 0% control sample and lowest score was noticed in 1.5% barley bran, this observation was in favor to the results of (Demrici *et al.*, 2017) who formulated yoghurt with different concentration rice bran. Yoghurt with 0.5% and 1% barley bran showed good results of color. Taste value of functional yoghurt showed satisfactory score in control, 0.5% 1% sample but 1.5% of barley bran decreased the sensorial attributes of taste, lowest value 5.33 of taste was examined in 1.5% barley bran. This observation was in agreement to the results of (Hasani *et al.*, 2017) who observed decreased the quantity of barley bran increased taste score of low fat yoghurt. Flavor of yoghurt sample of showed acceptable score except treatment T_3. Flavor score of all samples were 7.67, 6.77, 6.27 and 5.73 of T_0, T_1, T_2 and T_3. Satisfactory scores of functional for texture were conducted in sample T_0, T_1 and T_2. Highest score 8.20 of satisfaction was noticed in control sample. Results of 0.5% and 1% bran also showed noticeable score. Our results indicated that increased in concentration of barley bran lower the texture properties of yoghurt which are opposite to the results of (Graccia and McGregor, 1997) suggested that increase quantity of fiber improved the texture and consistency of yoghurt.

Table 7. Effect of barley bran on sensory attributes of yoghurt

Treatments	Color	Taste	Flavor	Texture	Appearance	Overall acceptability
T_o	7.47±0.5^{a}	8.6±0.4^{a}	7.67±0.6^{a}	8.20±0.6^{a}	8.47±0.4^{a}	8.72±0.3^{a}
T_1	7.41±0.6^{a}	7.30±0.4^{b}	6.77±0.2^{ab}	7.27±0.2^{ab}	8.18±0.2^{a}	7.81±0.2^{a}
T_2	6.63±0.6^{ab}	6.43±0.2^{b}	6.27±0.2^{b}	6.07±0.1^{bc}	6.23±0.3^{b}	6.47±0.5^{b}
T_3	5.57±0.7^{a}	5.33±1^{c}	5.73±1.2^{b}	4.93±1.2^{c}	5.03±1.5^{b}	4.83±0.8^{c}

Conclusion

Results of appearance indicated that yoghurt made from highest concentration of bran showed unsatisfactory to the panelists. Overall score of appearance for yoghurt were decreased with increased of barley bran. Maximum score 8.47 was conducted in 0% barley bran. Overall acceptability of sample with 1.5% barley bran was poor as compared to all other samples. Sample with 0.5% and 1% bran showed good acceptance by the all panelists.

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