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EFFECT OF SKIM MILK POWDER SUBSTITUTION WITH BARLEY FLOUR ON THE PROPERTIES OF PROBIOTIC FRUIT FLAVORED STIRRED YOGHURT

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ABSTRACT

This study aimed to investigate the effect of skim milk powder substitution with barley flour on chemical, microbiological and sensory properties of probiotic fruit flavored stirred yoghurt.

Skimmed milk powder was replaced by 25%, 50%, 75% or100% with barley flour, fatted with 3% coconut oil, homogenized, cultured with 3% mixed culture (1:1) *Lactobacillus acidophilus* ATCC4495 and *Bifidobacterium lactis* Bb-12 and stirred. Strawberry or mango juice containing 50% sugar was added to all treatments at level of 15% and cold storage at 4-5°C for 15days.

The obtained results indicated that, The fruit flavored stirred yoghurt contained 17.24-20.75% total solids (TS), 2.28-3.75% protein, 2.85-3.20% fat, 0.76-1.62% ash and 9.78-13.64% carbohydrate. Substitution of skim milk powder by barley flour decreased the total protein and increased the ash, carbohydrate and total solids contents, but didn't have any effect on the 3% added fat. Increasing the replacement percentage or storage period gradually increased the titratible acidity (TA)% and decreased the pH values. The highest TA% and the lowest pH values were recorded at the end of storage period (ranged from 1.12 to 1.67% and 3.71 to 4.12, respectively). Microbiologically, the log_{10} cfu/ml of both *L. acidophilus* and *Bif. lactis* initially(after one day) ranged from 7.82 to 8.45, 7.93 to 8.45, respectively, and gradually increased to reach the maximum then decreased thereafter in all treatments. Barley – containing treatments showed higher counts of lactic acid bacteria than control. Total microbial count, yeasts and moulds increased gradually by increasing both of substitution percentage or storage period, so maximum counts were observed in those of 75% and 100% barley treatments. Coliform bacteria were not found in any sample along the storage period. Organoleptically, All barley treatments recorded higher organoleptic scores than control, but that of 50% barley flour gained the highest one. Gradually decrement in the panel score was noticed by increasing the storage period of all treatments till the end of cold storage period.

In conclusion, 50% barley treatment of fruit flavored (whether with strawberry or mango) stirred yoghurt have more acceptable organoleptic properties and higher numbers of probiotic bacteria with moderate amounts of nutrients and total acidity.

Key words: stirred yoghurt, probiotic bacteria, Skim milk, barely flour.

INTRODUCTION

Yoghurt is a coagulated milk product obtained by lactic acid fermentation, through the action of microorganisms. There are two types of plain yoghurt, one which is stirred after fermentation (stirred style) to produce a semi-liquid consistency, while the other is not stirred and the end product has a firm gel structure (set style) (Riener *et al.,* 2009). In the manufacture of stirred yoghurts, a set-style yoghurt gel is mixed at the end of fermentation, then cooled and packaged. At all stages, shearing during mixing and pumping disrupts the yoghurt protein network, leading to stirred yoghurt (Sodini *et al.,* 2004; Tamime and Robinson,1999).

In recent times, there has been an increased interest to adapt healthy diets, which help in preventing diseases, and as a consequence, the study and development of new functional foods has gained much importance. Food additives as probiotics and prebiotics may exert positive effects on the composition of gut microbiota and are subject of intensive research. The allergy to dairy products affect negatively some persons. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Traditions and economic reasons that limit the use of dairy fermented products in some developing countries promote the idea of reduction of milk components as vehicles for the probiotic agents. At present, some non-dairy probiotic beverages are being comercialized (Prado *et al.,* 2008). During the last two decades people began to be more aware of the close relationship between diet and health. This awareness dictated great interest in seeking elegant methods for improving food industry. Thus different types of nutraceuticals were introduced in this field such as soluble dietary fibers (SDF) which have been thought to have a great role in health promotion and disease prevention (Fagan *et al.,* 2006). Soluble dietary fibers were shown to have remarkable effects in relation to human health situation; such diets were shown to reduced the risk of the developing cardiovascular disease because they lead to a decrease in blood cholesterol levels, decreased carbohydrate digestion, and hence regulation of postprandial blood glucose and insulin level. Also, dietary fibers could be reduced and promote a healthy balance of gut micro flora. One SDF, which has attracted considerable interest, is barley β-glucan. The incorporation of β-glucan into low fat cheese products has been investigated. Moreover, inclusion of β-glucan in low fat yoghurt resulted in a product with similar firmness to that of a full fat control (Jenkins *et al.,* 2002; Tudorica *et al.,* 2002; Brennan and Tudorica., 2003). Anti-nutritive activity associated with dietary fiber components, mainly mixed-linked $(1\rightarrow3)/(1\rightarrow4)$-β-glucans were degraded during controlled short-time fermentation of wheat and barley whole meal flours (WMF), using a *Lactobacillus* strain (Skrede *et al.,* 2001, 2002).

Probiotic foods have steadily gained popularity over the past decades and a wide variety of foods are nowadays used as carriers for probiotic cultures, including fermented milk products (Coeuret *et al.,* 2005) and cereal bars (Ouwehand *et al.,* 2002). Although tremendous reports and researches were published in the field of the utilization of many legumes such as Soya in the manufacture of yoghurt and yoghurt like products, yet the work on the interaction of barley β-glucan and milk compounds is very scantly indeed (Shirai *et al.,* 2006; Salem and Abd El-Gawad 2007). However very few published reports on the preparation and quality characteristics of yoghurt and stirred yoghurt made along with use of barley β-glucan containing scattered information are available (Volikakis *et al.,* 2004).

Thus, the objective of this work was to study the effect of skim milk powder substitution with barley flour on the chemical, microbiological and sensory properties of probiotic stirred yoghurt flavored with strawberry or mango fruits along cold storage period for 15 days.

MATERIALS AND METHODS

1. Materials:

Holland spray dried skim milk (Type low heat, grade A) was obtained from Egyptian local market. It contained 36% protein, 51% lactose, 5% fat, 7.3% ash and 4% moisture. Hull-less barley was obtained from Institute of Field Crop Research, Agricultural Research Centre, Giza, Egypt. Sugar (sucrose), coconut oil, stabilizer (Lacta 501), strawberry and mango fruits used in this study were obtained from Egyptian local market.

Probiotic strains of *Lactobacillus acidophilus ATCC4495* and *Bifidabacterum lactis* Bb12 were obtained in order from American Type Culture Collection (ATCC; Rockport, MD, USA) and from Chr. Hansen laboratories, Copenhagen, Denmark, respectively. Lactic acid strains were grown in MRS medium at 40±2°C for 24h.

2. Experimental procedures:

2.1. Preparation of barley flour

Barley dehisced using a stake rice machine (Stacke Engineering Co. Ltd, Tokyo, Japan) following by grinding into a flour and sieving through a 25 um screen using a hammer mill, the flour was packed in plastic container and kept at refrigeration temperature until used.

2.2. Preparation of LAB starter:

Actively growing cultures were inoculated into 100ml of previously reconstituted and sterilized (121°C/ 15min) skimmed milk with 10% total solids (TS). This mixture was then incubated at 42°C until the onset of gelation. Two milliliters of culture from this passage were transferred into 100 ml of sterile skim-milk at 42°C and, once again, the culture was incubated until a gel had just formed. This second culture was used for the propagation of a bulk culture (1L) for inoculation of the different treatments. Bulk cultures were prepared 1 day before the production of yoghurt. The starter used was a blend of *L. acidophilus* and *Bif. lactis* at a rate 1:1.

2.3. Strawberry and mango fruits preparation:

Mango and strawberry fruits were washed well using tab water, cleaned and cut into small pieces about 10mm cubes. Fruit cubes were weighted to calculate the added sugar. Sugar was added by ratio 50% from fruit weight (w/w), mixed well by high speed blender, then heat treated at 85°C for 15min . This fruit syrup was added to yoghurt at the level of 15% to give 10% fruit and 5% sugar in the final product.

2.4. Manufacture of stirred yoghurt.

Reconstituted skim milk (10% TS), fatted with 3% coconut oil and stabilized with 0.5%Lacta 501 was used for yoghurt production (control treatment). Skim milk powder was substituted with 25% of barley flour (25% barley treatment), 50% of barley flour (50% barley treatment), 75% of barley flour (75% barley treatment) and 100% of barley (0% skim milk) flour (100% barley treatment). All mixtures were heated to 60°C and homogenized using APV GAULIN Inc., model 53 M3-5TBS, USA homogenizer, then heat treated at 85°C for 30min and rapidly cooled to 45°C. Active culture of both *L. acidophilus* and *Bif. lactis* were added at the rate of 3% (w/v) as starter culture. The inoculated mix was filled into 1.7 kg plastic containers and incubated at 43°C. Incubation was terminated when gel formed. At this point, the yoghurt was transferred to store in a refrigerator (4±1°C) overnight.

Strawberry or mango fruit syrup was added at the level of 15% (w/w). The yoghurt treatments are stirred, then filled into 250g plastic cups and stored in the refrigerator for 15 days as described by Küçüköner and Tarakçi, (2003). Three replicates of yoghurt samples were carried out.

3. Analytical methods:

Total solids (TS), total protein = (total nitrogen of skim milk portion×6.38) + (total nitrogen of barley flour portion×5.70), fat, ash as well as titratable acidity (TA,as lactic acid) contents were determined according to AOAC, (2000). Total carbohydrates were calculated by differences. The pH value was measured using digital pH meter (JENWAY 3510)

L.acidophilus was enumerated using *lactobacillus* selective agar plus 0.2 Oxgell (LBSO) according to Gilliland and Walker, (1990). Plates were incubated at 37°C for 48h. *Bif. lactis* was determined in MRS-OG mixture solution of (0.02% Oxgall and 0.03% Gentamince), incubated anaerobically at 37°C for 48h. as the method described by Lim *et al.* (1995). Total bacterial counts of all samples were determined using plate count agar method according to Lonner *et al.* (1986). Plates were incubated at 30°C for 72h. Yeasts and moulds were enumerated using potatoes dextrose agar. Plates were incubated at 25°C for 3-5 days. Coliforms were determined using violet Red Bile agar (VRBA). The plates were incubated at 37°C for 48hr according to Marshall, (1992).

Stirred-yoghurt samples were organoleptically evaluated for its flavor (60 points), body and texture (10 points), appearance (30 points) and overall acceptability according to the methods of Nelson and Trout (1981). Sensory properties were tested by ten trained panelists from staff of Food Technology Research Institute (FTRI), Agriculture Research Center (ARC), Giza, Egypt. Organoleptic data were statistically analyzed according to Snedecor and Cochran (1989). Least significant difference (LSD) test at 0.05% level of significance was used to compare between the means.

RESULTS AND DISCUSSION

1. Chemical properties:

The results in Table (1) revealed that total protein of final stirred yoghurt ranged from 2.28 to 3.75%. Protein content decreased gradually by increasing the substitution percent, this may be due to the lower protein content of barley flour (about 12%) *versus* skim milk powder (about 36%). Whereas, higher protein content was in control treatments either flavored with mango (3.70%) or strawberry (3.75%), while the lowest protein content recorded in 100% barley treatments, it was 2.54% in mango yoghurt or 2.48% in strawberry yoghurt. Similar trends were observed by Basyony *et al.* (2002). On the other hand fruits and sugar addition led to reduce the protein content as reported by Cinbas and Yazici (2008) who mentioned that the mass fraction of protein in yoghurts with added fruit was significantly lower than of plain yoghurts. Due to the previous adjustment of the level of the coconut oil added (3.0%) to all treatments, there were no negative effect of skim milk substitution with barley flour on the product fat content.

Table (1): Chemical composition of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Chemical composition (%)				
	Protein	Fat	Ash	Carbo. *	TS**
Mango flavored:					
Control	3.70	2.90	0.76	9.88	17.24
25% Barley	3.33	3.00	1.08	10.42	17.83
50% Barley	3.01	2.89	1.15	11.04	18.09
75% Barley	2.85	3.00	1.33	12.22	19.40
100% Barley	2.54	2.85	1.47	13.64	20.50
Strawberry flavored:					
Control	3.75	3.00	0.83	9.78	17.36
25% Barley	3.50	2.88	1.10	10.39	17.87
50% Barley	3.08	3.00	1.28	11.96	19.32
75% Barley	2.75	3.15	1.43	12.07	19.40
100% Barley	2.48	3.20	1.62	13.45	20.75

*: Carbohydrates **: Total solids

Concerning the ash content of stirred yoghurt the results showed that ash content gradually increased by increasing the substitution ratio, where the highest ash content was observed in 100% barley flour treatment flavored either with mango (1.47%) or strawberry (1.62%), while, control of mango or strawberry treatments recorded the lowest ash content (0.76% and 0.83% respectively). Similar findings were reported by Ghadge *et al.* (2008).

Carbohydrate content of fruit flavored stirred yoghurt ranged from 9.78 to 13.64%.

Increasing the substitution of skim milk powder with barley flour led to increase the total carbohydrate. The highest carbohydrate content recorded by 100% barley treatment either flavored with mango (13.64%) or strawberry (13.45%). In addition, TS% of stirred yoghurt increased gradually by increasing the substitution percentage as shown in Table (1). These results are in coincidence with those of Küçüköner and Tarakçi (2003) and Cinbas and Yazici (2008).

The results given in Table (2) showed that, increasing the replacement percentage of skim milk powder with barley flour increased gradually the TA %, since 100% barley treatment (completely replacement) contained the highest TA% than all other treatments, while control treatment contained the lowest TA%. These results may be explained with regard to the growth rate of *L. acidophilus* and *Bif. lactis* (Table 4 and 5), where the increase in the lactic acid bacteria counts associated with increasing barley flour portion let consequently to increase the acid production. Moreover, TA% gradually increased by increasing the storage period and recorded the maximum at the end of storage. These results are in agreement with those of Küçüköner and Tarakçi (2003) who found that TA % of the fruits yoghurt samples increased during storage. Also, Vahedi *et al.* (2008) found that acidity increased until 14th day of storage in fruit yoghurt.

Table (2): Titratable acidity (TA%) of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	0.75	0.80	0.91	1.12
25% Barley	0.66	0.88	0.96	1.20
50% Barley	0.85	0.90	1.11	1.31
75% Barley	0.89	1.05	1.20	1.46
100% Barley	0.97	1.14	1.31	1.64
Strawberry flavored:				
Control	0.78	0.82	0.92	1.14
25% Barley	0.70	0.84	0.97	1.24
50% Barley	0.88	0.96	1.19	1.37
75% Barley	0.90	1.06	1.20	1.46
100% Barley	0.95	1.17	1.38	1.67

The results in Table (3) indicated that, the increasing of the replacement percentage of skim milk powder with barley flour gradually decreased the pH values, where completely replacement (100% barley) recorded the lowest pH value than all other treatments, while control treatment recorded the highest pH value, whether when frish or during 15 days of cold storage period. This may be due to the increase in the *L. acidophilus* and *Bif. lactis* counts those associated with increase of barley flour portion as shown in Tables (4 and 5). The pH values were in the same range obtained by Ghadge *et al.* (2008) who reported that pH values of fruits fortified yoghurt varied from 4.10 to 4.32pH values gradually decreased by increasing the storage period till the end.

Charalampopoulos *et al.* (2002) reported that, decreasing the pH values of fruit flavored stirred yoghurt may be due to bacterial fermentation of sucrose during the exponential phase resulting in the accumulation of lactic acid (1.06-1.99 g/liter), which progressively decreased the pH of the medium as. This decrement was also reported by many investigators, Cinbas and Yazici (2008) found that pH values of the yoghurts with fruit samples significantly declined throughout storage and addition of a sugar source may help lactic acid bacteria to produce more acid. Likewise, Renan *et al.* (2009) found that pH of fruit stirred yoghurt decreased during storage.

Table (3): pH value of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	4.88	4.61	4.43	4.12
25% Barley	4.62	4.30	4.16	3.92
50% Barley	4.30	4.20	4.00	3.99
75% Barley	4.23	3.98	3.90	3.88
100% Barley	4.16	3.99	3.87	3.81
Strawberry flavored:				
Control	4.63	4.41	4.28	4.03
25% Barley	4.45	4.18	4.07	3.89
50% Barley	4.17	4.11	3.88	3.84
75% Barley	4.13	3.87	3.79	3.71
100% Barley	4.10	3.84	3.79	3.72

2. Microbiological situation:

2.1. *Lactobacillus acidophilus* counts:

Data given in Table (4) reveal that as skim milk powder was substituted with barley flour the count of *L. acidophilus* increased in the resultant stirred yoghurt. Strain count initially ranged from 7.93 to 8.45 $\log_{10}$ cfu/ml (as shown in Table 4) and gradually increased by increasing the stirred-yoghurt storage period at 4-5°C reaching the maximum after 3 days for 25% and 50% barely flour flavored with either mango or strawberry fruits (8.69 and 8.49; and 8.47 and 8.50 $\log_{10}$ cfu/ml, respectively), and after 7dayes for control (8.54 and 8.47), 75% barely (8.73 and 8.58 $\log_{10}$ cfu/ml) and 100% barely (8.58 and 8.71 $\log_{10}$ cfu/ml) for mango and strawberry flavored yoghurt, respectively. The numbers then decreased gradually till the end of storage. These results are in agreement with those obtained by Canganella *et al.* (1998) and Vahedi *et al.* (2008) who mentioned that *Lactobacillus* spp. decreased vigorously during storage, until the end. Moreover, Bekers *et al.* (2001) reported that cereals stimulate the growth of lactobacilli and bifidobacteria. In addition, Bacha *et al.* (1999) reported that heterofermentative and homofermentative lactobacillus spp. were the major groups of LAB with final counts of 10^9 cfu/ml in barley malt (a traditional ethiopian fermented beverage). On the other hand, Charalampopoulos *et al.* (2002) found that all lactic acid strains included *L. Acidophilus* attained high maximum cell populations (8.10-10.11 $\log_{10}$ cfu/ml) in the barley and wheat media. They added that cereals are suitable substrates for the growth of potentially probiotic lactic acid bacteria.

2.2. *Bifidobacterium lactis* counts:

Data presented in Table (5) indicated that, substitution of skim milk powder with barley flour led to relatively slight increase in the count of *Bif. lactis*, since all barley added treatments reached maximum higher counts of lactic acid bacteria than control except for 25% barley treatment. These results are confirmed with those obtained by Bacha *et al.* (1999) who mentioned that barley malt in a traditional ethiopian fermented beverage contributed most of the lactic acid bacteria (LAB) which were most important to the fermentation and dominated the fermentation flora reaching final counts of 10^9 cfu/ml. On the other hand, Charalampopoulos *et al.* (2002) found that all strains of lactic acid attained high maximum cell populations (8.10-10.11 $\log_{10}$ cfu/ml) in the barley media. In addition, cereals are suitable substrates for the growth of potentially probiotic lactic acid bacteria. Although the reduction occurred in the probiotic population, because of storage prolonging, the counts stilled higher than 10^7 cfu/ml even at the end of cold storage period (15 days). Furthermore, *Bif. lactis* counts were gradually increased by increasing the storage period till reached the maximum then decreased in all treatments. *Bif. lactis* was decreased at early stage (7 days) of storage for both 25%

and 50% barley treatments, while control treatment (100% skim milk powder) and both of 75% and 100% barley showed the counts reduction at the end of storage period (15 days). This decrement of *Bif. lactis* during later periods of storage may be due to nutrient consumption and/or accumulation of the microbial metabolism products. The results are in full concord with the results obtained by Gomes and Malcata, (1999) who has attributed the decrease in the viability of *Bif. lactis* Bb-12 to the sensitivity of bifidobacteria to low pH value.

Table (4): Counts of *Lactobacillus acidophilus* ($\log_{10}$ cfu/g) of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	7.93	8.39	8.54	8.20
25% Barley	8.42	8.69	8.30	8.10
50% Barley	8.28	8.49	7.96	7.89
75% Barley	8.42	8.55	8.73	8.53
100% Barley	8.45	8.51	8.58	8.18
Strawberry flavored:				
Control	8.13	8.20	8.47	8.12
25% Barley	8.14	8.47	8.22	8.15
50% Barley	8.41	8.50	8.31	8.02
75% Barley	8.40	8.41	8.58	8.16
100% Barley	8.19	8.50	8.71	8.25

Table (5): Counts of *Bifidobacterium lactis* ($\log_{10}$ cfu/ml) of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	7.93	8.38	8.26	8.09
25% Barley	8.45	8.67	7.98	7.90
50% Barley	8.29	8.48	8.51	8.25
75% Barley	8.44	8.57	8.54	8.27
100% Barley	8.41	8.49	8.76	8.55
Strawberry flavored:				
Control	8.11	8.22	8.51	8.16
25% Barley	8.17	8.46	8.24	8.17
50% Barley	8.45	8.51	8.39	7.93
75% Barley	8.42	8.48	8.67	8.19
100% Barley	8.21	8.51	8.73	8.44

2.3. Total microbial counts

Table (6) showed that replacing the skim milk powder with barley flour have no negative effect on the total microbial counts, since initially all barley added treatments recorded total microbial counts similar to other treatments and to control treatment. Total microbial counts increased gradually during cold storage period by increasing the substitution percent, so maximum log counts were observed in 100% barley at the end of storage and recorded 8.54 and 8.35 for mango and strawberry flavored stirred yoghurt, respectively. These results are confirmed with those obtained by Basyony *et al.* (2002). $\log_{10}$ of total microbial counts (cfu/ml) of samples ranged initially from 7.38 to 7.71 and from 7.72 to 8.12 for mango and strawberry flavorred stirred yoghurt. Total microbial count increased gradually by increasing the cold storage period and reached the maximum at 7 days of storage, then decreased thereafter at 15 days of storage. The total microbial count changes of a stirred yoghurt took the same trend of lactic acid bacteria (*L. acidophilus* and *Bif. lactis*) as shown in Tables (4 and 5), which represented most of micro flora occurred in a stirred yoghurt. These results are in agreement with those obtained by Canganella *et al.* (1998) and Küçüköner and Tarakçi (2003) who reported that total mesophilic bacteria count were increased during storage period in fruit yoghurts during storage. Also, Vahedi *et al.* (2008) found that total count of apple yoghurt increased until 21st day. But in the case of strawberry yoghurt the total count peaked at 7th day of storage.

Table (6): Total microbial counts ($\log_{10}$ cfu/ml) of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	7.63	8.18	8.47	7.55
25% Barley	7.63	8.40	8.56	7.75
50% Barley	7.71	8.46	8.63	7.77
75% Barley	7.38	8.46	8.63	7.80
100% barley	7.63	8.53	8.75	8.54
Strawberry flavored:				
Control	7.72	8.22	8.54	7.66
25% Barley	7.79	8.34	8.57	7.93
50% Barley	8.10	8.43	8.61	8.11
75% Barley	8.12	8.50	8.60	8.19
100% barley	7.98	8.48	8.69	8.35

2.4. Yeasts and moulds counts:

Concerning yeasts and moulds counts Table (7) revealed that increasing the substitution percentage led to increase the yeasts and moulds counts, so 75% and 100% barley treatments recorded the highest yeasts and moulds counts at the end of storage period while control treatment recorded the lowest one at the same period, this may be due to the breakdown of nutrients of the substrates, by the starter organisms, and to high acidity and low pH in barley treatments (Tables 2 and 3). These results are in agreement with those of Bacha *et al.* (1999) who mentioned that barley malt (in traditional ethiopian fermented beverage) contributed most of the yeasts and dominated the fermentation flora.

Increasing the storage period of stirred yoghurt products led to increase in the yeasts and moulds counts in all treatments. The log count remained low till the 3rd day of storage ranged from 2.03 to 3.43, but the numbers rapidly increase thereafter by increasing the storage period, this may be due to a synergistic effect to occur between yeasts and moulds and lactic acid bacteria since both population continued higher without inhibition by the other as reported by Simsek *et al.* (2006). These results are in similar to those obtained by Salji *et al.* (1987), Küçüköner and Tarakçi (2003) and Vahedi *et al.* (2008), who found that in strawberry yoghurt, yeast counts increased until 7th day of storage.

2.5. Coliform bacteria:

Coliform bacteria were not found in any sample during all storage periods up to 15 days of storage at 4-5°C (data not shown), this may be due to the long heat treatment (85°C/ 30min), to refrigeration conditions which was used for yoghurt storage and to pH reduction that can make undesirable condition for coliforms to growth. Jai, (1990) and Frazier and Westhhoff, (1995) revealed that competition with LAB cause difficult situation for coliforms activity so these microorganisms were absent. These results are in accordance with those obtained by Küçüköner and Tarakçi

(2003) who reported that yoghurt samples contained coliform group bacteria of <1.0 cfu/g during storage time, and in agreement with those obtained by Bacha *et al.* (1999) found that coliforms and other *enterobacteriaceae* were eliminated after 16h fermentation of barley ethiopian beverage.

3. Organoleptic profile:

Regarding the organoleptic scores illustrated in Table (8), there are significant increases were recorded as the potion of barley flour instead of skim milk powder raised up till 50%, then the panel score declined as the substitution level was exceeded 50% whether stirred yoghurt was flavored with mango or strawberry and whether when fresh or along cold storage period, which caused, also, significantly gradual reduction in the sensory scores. These results are in agreement with those obtained by Küçüköner and Tarakçi (2003) and Cinbas and Yazici (2008)

Table (7): Yeasts and molds count (log_{10} cfu/ml) of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	1.63	2.03	2.63	3.71
25% Barley	1.00	2.14	2.78	3.75
50% Barley	1.72	2.48	3.19	4.10
75% Barley	1.38	3.08	3.37	4.48
100% Barley	2.07	3.34	4.04	4.76
Strawberry flavored:				
Control	1.24	2.22	2.57	3.19
25% Barley	1.02	2.48	2.69	3.66
50% Barley	1.63	2.50	3.21	3.67
75% Barley	1.69	2.82	3.54	3.83
100% Barley	1.03	3.43	3.61	4.11

Table (8): Organoleptic score of fruit flavored stirred yoghurt as affected by the skimmed milk substitution with barley flour.

Treatment	Storage Period (Day)			
	1	3	7	15
Mango flavored:				
Control	88.66	87.16	87.00	82.00
25% Barley	91.00	91.66	89.00	85.66
50% Barley	92.66	92.50	90.66	88.00
75% Barley	90.00	90.00	89.33	87.00
100% Barley	91.66	91.33	86.66	85.00
Strawberry flavored:				
Control	89.33	88.50	87.50	81.66
25% Barley	91.66	90.33	90.00	86.50
50% Barley	93.66	93.00	91.66	89.33
75% Barley	92.00	90.33	89.66	86.00
100% Barley	90.50	90.00	88.50	84.66
L.S.D.	**2.5100**	**1.8166**	**1.1402**	**2.0083**

In conclusion, 50% barley treatment of fruit flavored stirred yoghurt have more acceptable properties concerning with organoleptic characteristics, have suitable population of probiotic bacteria included *L. acidophilus* and *Bif.lactis* with considerable health benefits.

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تأثير استبدال اللبن الفرز بدقيق الشعير على خواص الزبادي المقلب و المنكهة بالفواكه

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تهدف هذه الدراسة لبحث أثر استبدال مسحوق اللبن الفرز المجفف بدقيق الشعير على الخواص الكيميائية والميكروبيولوجية والحسية لمشروب الزبادي بالفواكه الملقح بسلالتي *Lactobacillus acidophilus* ATCC4495 و *Bifidobacterium lactis* Bb-12 خلال ١٥ يوم من التخزين على ٤-٥°م. وقد استبدل اللبن الفرز المجفف بنسب ٢٥% ، ٥٠% ، ٧٥% و ١٠٠% بدقيق الشعير. وقد أضيف عصير

الفراولة أو المانجو المحتوي على ٥٠% سكر لكل المعاملات بنسبة ١٥%. واحتوى مشروب زبادي الفواكه على ٢,٢٨-٣,٧٥% بروتين، ٢,٨٥-٣,٢٠% دهن، ٠,٧٦-١,٦٢% رماد، ٩,٧٨-١٣,٦٤% كربوهيدرات و١٧,٢٤-٢٠,٧٥% جوامد كلية. وأدى استبدال اللبن الفرز بدقيق الشعير على انخفاض البروتين الكلي وزيادة نسبة الرماد والكربوهيدرات والجوامد الكلية وكان عديم الأثر على الدهن المضاف (٣%). وقد ازدادت الحموضة الكلية وانخفض رقم الحموضة تدريجيا بزيادة نسبة الاستبدال وكذلك بزيادة فترة التخزين وسجلت أعلى حموضة كلية (١,١٢-١,٦٧%) وأقل رقم حموضة (٣,٧١-٤,١٢) في نهاية فترة التخزين. وأظهرت النتائج الميكروبيولوجية تراوح لوغاريتم أعداد بكتريا *L. acidophilus* و *Bif. lactis* في البداية بين ٧,٨٢-٨,٤٥ و٧,٩٣-٨,٤٥ على التوالي، وازدادت تدريجيا حتى وصلت الحد الأقصى ثم انخفضت في نهاية فترة التخزين في كل المعاملات. وأظهرت كل معاملات الشعير ارتفاع في أعداد بكتريا البروبيوتك وبقيت أعداد بكتريا *L. acidophilus* و *Bif. lactis* مرتفعة حتى نهاية فترة التخزين وكانت ٧,٨٩-٨,٥٣ و ٧,٩٠-٨,٥٥. كما أظهرت النتائج ازدياد العدد الكلي للميكروبات وأعداد الفطريات والخمائر تدريجيا بزيادة كل نسبة الاستبدال وفترة التخزين وكانت أعلى الأعداد لمعاملات ٧٥% و١٠٠% دقيق شعير. وخلت جميع العينات من بكتريا القولون خلال كل فترات التخزين. وانخفضت الخواص الحسية تدريجيا بزيادة فترة التخزين حتى نهايتها، وسجلت كل معاملات دقيق الشعير خواص حسية مرتفعة عن الكونترول واختلفت عنها معنوياً.

وقد حازت معاملة ٥٠% شعير من مشروب الزبادي على أعلى قبول حسي كما احتوت على أعداد مناسبة من بكتريا البروبيوتيك ذات الفوائد الصحية المتعددة.