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MANUFACTURE OF A FUNCTIONAL FERMENTED BEVERAGE CONTAINING CEREAL EXTRACTS

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ABSTRACT

Growth and viability of five probiotic strains (*Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus acidophilus* and *Bifidobacterium bifidum*) was studied as into barley, wheat and maize extracts. In the barley extract, all strains attained the highest population (8.30-10.55 log_{10} cfu ml^{-1}, depending on the strain), but slightly lower (7.75-9.90 log_{10} cfu ml^{-1}) in the wheat and maize extracts, especially with *L. reuteri, L. acidophilus* and *B. bifidum*. Three cereal-based probiotic beverages were manufactured. Growth and viability of probiotic bacteria were determined in the products during refrigeration. Organoleptic properties were also tested immediately after processing. The pH values slightly decreased during refrigeration. The numbers of probiotic bacteria showed no marked change in the resulting beverages during refrigeration for 15 days. Although the reduction noticed in the probiotics population, its level after 15 days of storage was greater than 10^7 cfu ml^{-1}. The functional fermented beverage made with *L. gasseri* showed the highest score in barley extract followed by that made with *L. rhamnosus* with wheat extract. Finally, this approach would be the starting point for developing novel functional fermented beverages as economical and nutritious staples for children and adults.

Keywords: Functional beverages, Cereals, Probiotic bacteria

INTRODUCTION

The functional food research has moved progressively towards the development of dietary supplementation, introducing the concept of probiotics, which may affect gut microbial composition and activities (**Ziemer and Gibson, 1998**). New applications of probiotic microorganisms in foods have been introduced into the market or are still in the development phase, such as frozen yoghurt, soy yoghurt, dairy desserts, cheese, ice-cream, bread and chocolate (**DeVuyst, 2000**). In the dairy industry, a large variety of milk formulations have been used as delivery vehicles of probiotic lactic acid bacteria (**Scheinbach, 1998**). Also, probiotic-containing baby foods or confectionery formulations have been developed by adding the strains as additives (**Saarela *et al* 2000**).

In recent years, cereals have been investigated regarding their potential use in

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developing functional foods. Cereals are grown over 73% of the total world harvested area and contribute over 60% of the world food production providing dietary fibre, proteins, energy, minerals, and vitamins required for human health (**Charalampopoulos *et al* 2002a**). Cereals are suitable substrates for lactic acid bacteria growth, which has led to the commercialization of cereal-based probiotic products (**Charalampopoulos *et al* 2003**). Several technological aspects have to be considered in the design of such a novel food fermentation process, such as the composition and processing of the raw material, the growth capacity and productivity of the starter culture and the stability of the final product during storage (**Charalampopoulos *et al* 2002**). Additionally, cereals can be used as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and acts as prebiotics (**Charalampopoulos *et al* 2002a**). Cereals contain water-soluble fibre, such as β-glucan and arabinoxylan, oligosaccharides, such as galacto-and fructo-oligosaccharides and resistant starch, which have been suggested to fulfill the prebiotic concept (**Severson, 1998**). However, information concerning the effects of cereal composition on the growth of probiotic microorganisms is limited. **Charalampopoulos *et al* (2002)** reported that malt medium supported the growth of *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus reuteri* and *Lactobacillus acidophilus* more than barley and wheat media.

In the present study, the effect of each of barely, wheat and maize extracts on the viability of potentially probiotic *L. gasseri, L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum* strains was investigated. The main objective was aimed to achieve a novel functional fermented beverage based on cereals extract.

MATERIAL AND METHODS

A. Materials

I. Bacterial strains

The microorganisms used in this study were as follows: *Lactobacillus gasseri* B-14168, *Lactobacillus rhamnosus* B-445 and *Lactobacillus reuteri* B-14171. These strains were provided by Northern Regional Research Laboratory, Illinois, USA (NRRL). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Lab., Denmark. All strains had previously been shown to possess properties required of a probiotic microorganisms including bile salt tolerance, tolerance to low-pH values and antagonistic activity (**Amin *et al* 2002**). In addition, *Streptococcus thermophilus* was obtained from Chr. Hanson's Lab and used as starter in beverages.

II. Cereals

Barley, wheat and maize grains were purchased from the local market in Giza governorate.

III. Milk

Raw buffaloes milk was obtained from the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Cairo.

B. Experimental

I. Cereal-based fermentation media

The following method (as described by **Charalampopoulos *et al* 2002**) was used to prepare the fermentation media. The grains were ground in a Laboratory Falling Number hammer mill with a sieve of size 0.5 mm. A sample (50g) of the flour obtained was mixed with 450 ml tap water. The resulting extract was centrifuged (6000g) for 30 min at room temperature. The starch-free supernatant fluid was collected and immediately sterilized at 121°C for 45 min. Sedimentation of solids was observed after sterilization. The extraction and sterilization procedures were repeated four times. Probiotic strains (*L. gasseri, L. rhamnosus, L. reuteri, L. acidophilus* and *B. bifidum*) were inoculated into the resulting cereal extracts at 2% (v/v) in 500 ml screw-capped bottles. In all cases, the initial bacterial concentration was approximately 10^7 cfu ml^{-1}. Fermentation processes were performed at 37°C with no pH control and no agitation. MRS broth (Oxoid) was used as a control medium for the same probiotic strains. Samples were collected at intervals of 3h during the first 12h of the fermentation process, then at intervals of 6-12h during the next 36h. All fermentations were performed in duplicate.

II. Fermented milk-cereal extract manufacture

Fresh buffalo's whole milk was subjected to thermal treatment at 90°C during 10 min, and cooled to 45°C. Milk was divided into three equal portions. Each portion was mixed with sterilized extract of barely, wheat or maize prepared as described previously at level 45% milk and 30% cereal extract. Then, each mixture was divided into four equal parts and poured into 500-ml Erlenmeyer flasks. Starters were added as follows:

1. *S. thermophilus* + *L. gasseri*
2. *S. thermophilus* + *L. reuteri*
3. *S. thermophilus* + *L. rhamnosus*
4. *S. thermophilus* + *L. acidophilus* + *B. bifidum*

Streptococcus thermophilus was added at the level of 0.5%, while all probiotic strains were added at an inoculum level of 1% (v/v). Each inoculated mixture was incubated at 42°C until pH 4.3 was reached. Then each fermented mixture of milk and cereal extract was divided into three portions.

III. Functional cereal beverages manufacture

The functional cereal beverages, each of three replicates, were manufactured on a laboratory scale by mixing 75% fermented mixture of milk and cereal extract and 5% sucrose with:

a) 20% strawberry juice, flavoring agents, ascorbic acid and Arabic gum.
b) 20% apple juice, flavoring agents, ascorbic acid and Arabic gum
c) 0.02% vanilla flavor, flavoring agents, ascorbic acid and Arabic gum

A magnetic stirrer did the mixing at 10°C and at 300 rpm for 15 min. The prepared beverages were packed into sterilized bottles and stored at refrigerator for 15 days. Beverages samples were taken at 0, 7 and 15 days of storage and analyzed for probiotic bacteria viability and pH values. Organoleptic properties were done immediately after processing.

C. Methods

Bacterial enumeration

Fermentation samples were decimally diluted in sterile quarter-strength Ringer's solution, and appropriate dilutions were pour-plated. Plate counts on MRS agar (Oxoid) were performed to determine the growth of *L. gasseri, L. rhamnosus* and *L. reuteri*. *Lactobacillus acidophilus* was determined on lactobacillus selective agar plus 0.2% oxgall (LBSO) (**Gilliland and Walker, 1990**). Enumeration of *B. bifidum* was done according to **Blanchette *et al*** (1996) using modified MRS agar (Oxoid) supplemented with 0.05% L. cysteine-HCl (Merck, Germany). All plates were incubated at 37°C for 48 h under anaerobic conditions. Colony-forming units were counted (cfu ml^{-1}) and the results expressed as their log_{10} values.

pH measurement

pH values were measured using a digital pH meter model Hanna HT4817 at room temperature.

Sensory evaluation

All samples were evaluated according to **Ahmed *et al* (1992)** for flavor (40 points), appearance (40 points) and colour (20 points) by ten panelists of the experienced staff members of Dairy Science Department, National Research Centre.

RESULTS AND DISCUSSION

Viability of probiotic bacteria in barley, wheat and maize media

Figure (1-a,b,c,d) demonstrates the evaluation of *L. gasseri, L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum* during 48 h in barley, wheat, maize and MRS (control) media. In the control experiments, MRS media without cereal extracts, *L. gasseri* cell population decreased 0.39 log_{10} cycle, while *L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum* showed a 0.23, 0.11, 1.25 and 0.34 log_{10} cycles reduction in their cell population, respectively (Fig. 1-a).

An exponential growth phase of 6-9 h was observed for *L. gasseri, L. rhamnosus, L. acidophilus* and *B. bifidum*, while *L. reuteri* grew exponentially until 12 h of fermentation in control and all cereal extract media. The viable cell densities of *L. gasseri, L. reuteri* and *L. rhamnosus* declined slightly during 12-48 h of fermentation, however, the cell population of *L. acidophilus* and *B. bifidum* declined quite rapidly. Since all fermentations were performed under no pH control, the organic acids formed via the metabolic pathways decreased the pH of media (**Charalampopoulos *et al* 2002**).

In barely medium, *L. gasseri* exhibited a higher maximum growth rate and population density than *L. reuteri* (Fig. 1-b). *L. rhamnosus, L. acidophilus* and *B. bifidum* reached the highest population densities (9.90, 8.30 and 10.55 log_{10} cfu ml^{-1}, respectively) after 6-9 h of fermentation. At the end of the exponential phase (9 and 12 h), pH values dropped from 5.45 to 4.03, 3.90, 3.85, 3.93 and 4.1 for *L. gasseri, L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum*, respectively. In general, the barely medium supported well the growth of *B. bifidum, L. rhamnosus, L. gasseri* and *L. reuteri*, which showed increases in their cell populations of 3.38, 2.39, 1.91 and 1.57 log_{10} cfu ml^{-1}, respectively, at the end of the exponential phase (9 and 12 h). This

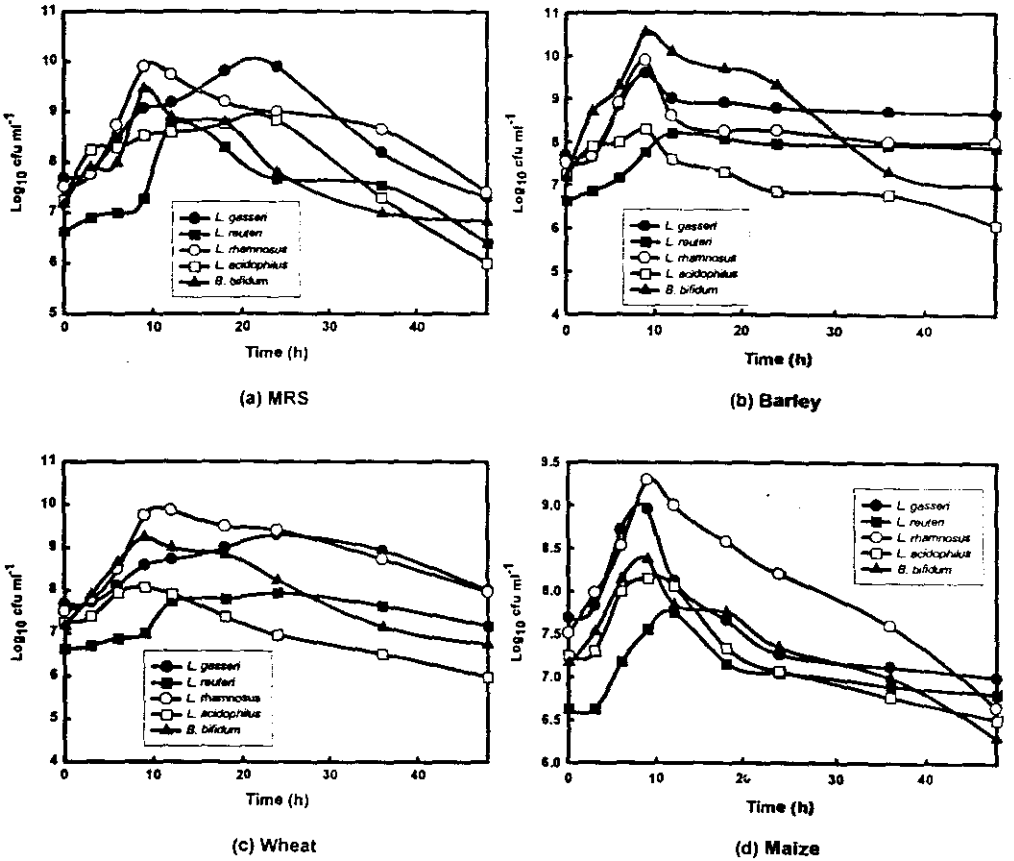


Fig. 1. Viability of probiotic bacteria in (a) MRS, (b) Barley, (c) Wheat and (d) Maize media

could be attributed to the simultaneous presence of considerable amounts of monosaccharides (glucose and fructose) and disaccharides (maltose and sucrose) in the barely medium (**Charalampopoulos *et al* 2002a**). Regarding *L. acidophilus*, the small increase in cell populations (1.05 $\log_{10}$ cfu ml^{-1}) could be explained by the possible absence of specific nutrients in the malt medium, such as free amino acids, B-vitamins or minerals. This species has complex growth requirements (**Gomes and Malcata, 1999**), usually exhibiting poor growth in synthetic media without the addition of large amounts of supplements, such as yeast extract and peptone (**Taillandier *et al* 1996** and **Elli *et al* 1999**). Since all experiments were performed under uncontrolled conditions, the accumulation of lactic and acetic acids produced via the metabolic pathways progressively decreases the pH of the medium. These organic acids can inhibit microbial growth in their undissociated form, dissociated form or indirectly by the protons (H^+) that

are released in the medium (**Passos *et al* 1993**). *L. acidophilus* entered directly into the decline phase, presumably due to its inability to withstand the low pH of the medium (3.93) at the end of the exponential phase. Similar results were reported by **Charalampopoulos *et al* (2002)**.

Lactobacillus gasseri, L. rhamnosus and *B. bifidum* attained high cell populations growing in wheat medium (Fig. 1-c). However, *L. reuteri* and *L. acidophilus* did not grow well. The increase in their cell population was 1.31 and 0.82 $\log_{10}$ cfu ml^{-1}, respectively. At the end of the exponential phase, pH values decreased from 5.65 to 4.60, 4.46, 4.52, 3.84 and 4.14 for *L. gasseri, L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum*, respectively.

In the maize medium, the five lactic acid bacteria strains displayed similar growth patterns to those observed in wheat medium except *B. bifidum* (Fig. 1-d). The growth of *L. reuteri, L. acidophilus* and *B. bifidum* was inhibited, leading to an increase in cell population of only 1.12, 0.90 and 1.21 $\log_{10}$ cfu ml^{-1}. At the end of the exponential phase, pH values dropped from 5.46 to 4.25, 4.14, 4.10, 3.78 and 4.16 for *L. gasseri, L. reuteri, L. rhamnosus, L. acidophilus* and *B. bifidum*, respectively.

The similar fermentation patterns observed in wheat and maize media for all the strains tested could be attributed to the low total fermentable sugar and the low free amino nitrogen concentration (**Charalampopoulos *et al* 2002**). In accordance with existing reports (**Salovaara and Valjkka, 1987** and **Bvochora *et al* 1999**), the concentrations of these constituents were significantly lower than those in malt medium. Interestingly, the microbial growth ceased at higher pH values than those observed for malt fermentations, which suggests that the growth-limiting factor was not only pH but that deficiency in nutrients also contributed to growth limitation. A deficiency in specific vitamins or minerals could contribute to growth limitation but wheat and maize contain significant amounts of these nutrients (**Palmer, 1989**). Therefore, the poor growth of *L. reuteri* (1.31 and 1.12 $\log_{10}$ cfu ml^{-1} increase in wheat and maize, respectively), *L. acidophilus* (0.82 and 0.90 log cfu ml^{-1} increase in wheat and maize, respectively) and *B. bifidum* (1.21 $\log_{10}$ cfu ml^{-1} increase in maize) could be attributed to the low concentrations of sugar (mainly glucose and fructose) and free amino nitrogen (**Charalampopoulos *et al* 2002a**). These observations are in good agreement with the literature (**Marklinder and Lönner, 1992**). In the case of *L. gasseri* and *L. rhamnosus*, the final cell counts were lower when compared with barley fermentation. However, these cell populations, ranging between 7 and 8 log cfu ml^{-1}, still enhance the potentially probiotic activities of these strains. The increase in the cell population of *L. gasseri* and *L. rhamnosus* suggests that these strains are less fastidious than *L. reuteri, L. acidophilus* and *B. bifidum* strains, being able to grow under nutrient-limiting conditions.

Functional fermented beverages containing cereals extracts

1. Changes in pH values

Functional fermented beverages at zero time had pH values ranged from

4.48 to 5.29 (Fig. 2-a, 3-a and 4-a). The differences between initial pH values of the products could be related to the conditions of manipulation during probiotic beverages manufacture, which allowed a little post-acidification (**Oliveira *et al* 2002**).

pH decreased noticeably (0.12 to 0.38 pH units) during the first week of storage (Fig. 2-a, 3-a and 4-a). **Oliveira *et al* (2002)** found similar results for lactic beverages containing probiotic cultures, and **Ibrahim *et al* (2002)** for flavoured whey beverages. Therefore, pH slightly decreased (less than 0.7 pH units) and could be considered stable. Similar results were reported by **Oliveira *et al* (2002)** and **Wang *et al* (2002)**.

2. Viability of probiotic bacteria

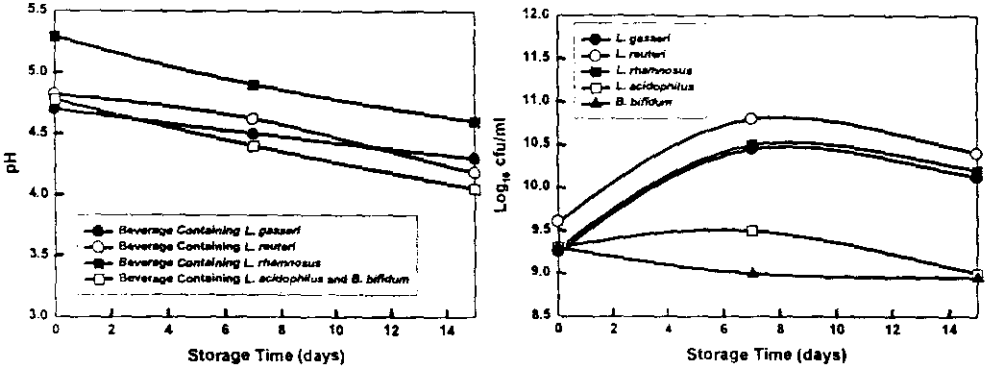
Figures (2-b, 3-b and 4-b) show the viability of probiotic bacteria in functional fermented barley, wheat and maize beverages, respectively. Data in Fig. (2-b) indicate that the counts of *L. gasseri, L. reuteri* and *L. rhamnosus* in functional fermented barley beverage exhibited excellent increase during the first 7 days and then slightly decrease till the end of storage period as compared with *L. acidophilus* and *B. bifidum*. Our results are in line with **Oliveira *et al* (2002)** who reported that until 14 days of storage of fermented lactic beverage, counts of *L. rhamnosus* and *L. acidophilus* remained stable, also, storage time affected the counts of lactobacilli only after 21 days at 4°C. This decrease was more pronounced in the case of *L. acidophilus* than in the case of *L. rhamnosus*. Results in Fig. (3-b) demonstrate that viability of all probiotic bacteria in fermented beverage containing wheat were similar. The highest increase in viable count was observed in fermented beverage containing *L. gasseri* and *L. rhamnosus*. On the other hand, from Fig. (4-b), it is clear that fermented beverage prepared with maize using *L. gasseri* had gained the highest counts for probiotic bacteria.

Our study shows that barley, wheat and maize beverages can be fermented with *L. gasseri, L. rhamnosus, L. reuteri, L. acidophilus* and *B. bifidum* and still have higher numbers of viable organisms after 15 days of storage. **Wang *et al* (2002)** studied viability of *Bifidobacteria* and *L. acidophilus* in cultured soy milk drinks during storage at 5°C. Their study indicated that no marked changes in numbers of both bacteria were observed during 10 days of storage at 5°C. Also, **Usman and Hosono (1999)** studied survival of *L. gasseri* in unfermented milks. They found that *L. gasseri* decreased after 14 days of storage at 4°C, however, viable cells were still at 10^8 cfu/ml after 28 days of storage.

Although viability of probiotic bacteria decreased during storage, it was observed that the products contained 8.7 $\log_{10}$ cfu ml^{-1} of probiotic, in average, after 15 days of storage. These counts are higher than 10^7 cfu/ml, which is the level suggested by some authors to have health promoting effect (**Vinderola & Reinheimer, 1999** and **Wang *et al* 2002**).

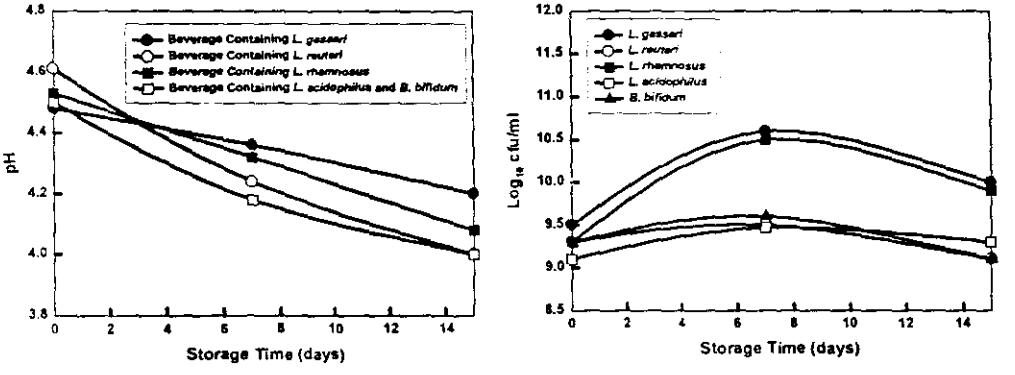
3. Sensory evaluation

Table (1) shows the averages of sensory attributes colour, appearance and flavours of functional fermented beverages after processing (fresh). Functional fermented beverages present a homogeneous appearance after mixing. According to the panelists, beverage prepared



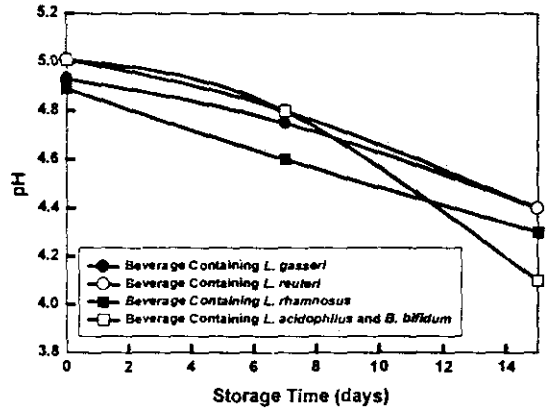
(a) Changes in pH Values (b) Viability of Probiotic Bacteria

Fig. 2. Viability of probiotic bacteria and changes in pH n functional barley beverage during refrigeration for 15 days

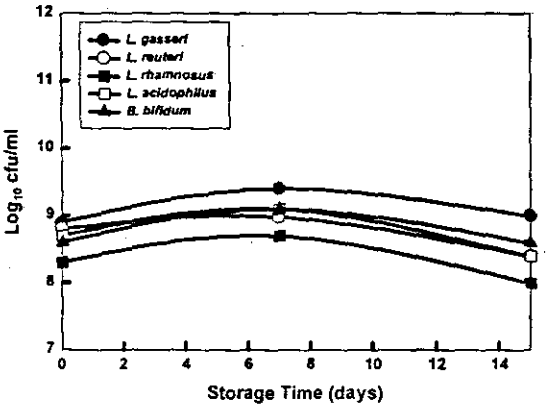


(a) Changes in pH Values (b) Viability of Probiotic Bacteria

Fig. 3. Viability of probiotic bacteria and changes in pH in functional wheat beverage during refrigeration for 15 days



(a) Changes in pH Values



(b) Viability of Probiotic Bacteria

Fig. 4. Viability of probiotic bacteria and changes in pH in functional maize beverage during refrigeration for 15 days

Table 1. Sensory evaluation of functional fermented milk beverages with different probiotic bacteria and cereal extracts

Probiotic Starter	Colour (20)	Appearance (40)	Flavour (40)	Total (100)
Functional Fermented Milk/Barley Beverage				
L. gasseri	20	37	39	96
L. reuteri	17	31	32	80
L. rhamnosus	17	25	24	86
L. acidophilus + *B. bifidum*	16	32	34	82
Functional Fermented Milk/Wheat Beverage				
L. gasseri	16	32	38	86
L. reuteri	17	32	32	81
L. rhamnosus	18	35	37	90
L. acidophilus + *B. bifidum*	17	30	35	82
Functional Fermented Milk/Maize Beverage				
L. gasseri	18	34	37	89
L. reuteri	17	31	32	80
L. rhamnosus	17	30	36	83
L. acidophilus + *B. bifidum*	18	31	35	84

with barley and apple juice fermented with *S. thermophilus* and *L. gasseri* received a higher average for appearance (37) followed by that manufactured with wheat and strawberry juice and fermented with *S. thermophilus* and *L. rhamnosus* and the beverage made with maize and vanilla flavor fermented with *S. thermophilus* and *L. gasseri*, although with no sharp differences between them. On the other hand, there were clear differences between other beverages (Table, 1). Analyzing colour, the panelists found great differences between apple barley beverage fermented with *S. thermophilus* and *L. gasseri* and that prepared with wheat and strawberry juice fermented with *S. thermophilus* and *L. rhamnosus*, being first beverage the most acceptable. In relation to taste, there were inferior differences between beverage contained maize and vanilla, which fermented with *S. thermophilus* and *L. gasseri* and the other one that contained wheat and strawberry juice and fermented with *S. thermophilus* and *L. rhamnosus*, being the former one, which received higher scores for this attribute (Table, 1). The traditional foods made from grains usually lack flavor and aroma (**Chavan and Kadam, 1989**). Lactic acid fermentation improves the sensorial value, which is

very much dependent on the amount of the end product. Consequently, the organoleptic properties of cereal-based and dairy fermented foods produced by lactic acid fermentation are very much dependent on the amounts of organic acids produced. In general, the main differences between the treatments were in their flavor scoring.

CONCLUSION

Fermented milk beverages containing barley or wheat extracts as fermented with *S. thermophilus* and *L. gasseri* or *L. rhamnosus* can be considered as functional foods. In addition, presence of cereal extracts would obviously provide not only good environment for the probiotic lactic acid bacteria in the human gut, but also provide potentially prebiotic and highly nutrition compounds.

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إنتاج مشروب متخمر ذو وظيفة صحية محتوى على مستخلص الحبوب [٤٧]

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تم فى هذا البحث اختبار مدى حيوية خمسة سلالات من البادئات المدعمة للحيوية وهى *Lactobacillus gasseri* و *Lactobacillus reuteri* و *Lacobacillus rhamnosus* و *Lactobacillus acidophilus* و *Bifidobacterium bifidum* عند تنميتها فى مستخلصات الشعير ، القمح، الذرة . وقد دلت النتائج على أن مستخلص الشعير أدى إلى تشجيع نمو جميع السلالات المختبرة حيث تم الحصول على أقصى أعداد للخلايا (يتراوح بين ٨,٣٠ - ١٠,٥٥ لوغاريتم خلية/مل ويعتمد هذا على نوع السلالة) . أما فى حالة بيئات مستخلصات القمح والذرة فقد بلغت أقصى أعداد لهذه السلالات ما بين ٧,٧٥ - ٩,٩٠ لوغاريتم خلية/مل وكانت منخفضة عنها فى حالة بيئة مستخلص الشعير ، وخاصة سلالات *L. acidophilus* و *L. reuteri* و *B. bifidum*.

ولتقييم مدى إمكانية استخدام مستخلصات الحبوب فى إنتاج مشروب متخمر فقد تم تصنيع مشروبات ذات وظيفة صحية باستخدام مستخلصات الحبوب السابق اختبارها وبادئات المدعمات الحيوية وتم اختبار مدى حيوية هذه البادئات فى هذه المشروبات أثناء الحفظ فى الثلاجة بجانب قياس درجة الـ pH واختبار صفاتها الحسية.

وقد لوحظ أن قيم الـ pH تقل ببطء أثناء فترة الحفظ بالثلاجة . أما أعداد بكتريا البادئات المدعمة للحيوية فقد لوحظ حدوث انخفاض طفيف فى أعدادها طوال فترة حفظها فى الثلاجة ويرجع ذلك إلى طبيعة التركيب الكيماوى لمستخلصات هذه الحبوب والتى تمتاز بوجود السكريات الذائبة بجانب احتوائها على كميات قليلة من النيتروجين الأمينى الحر والتى تجعلها تقوم بحماية بادئات المدعمات الحيوية . وبالرغم من ذلك فقد كانت جميع المشروبات التى تم تصنيعها تحتوى على أعداد أكثر من 10^7 خلية/مل وهى الأعداد الموصى بها لكى يكون المنتج ذو وظيفة صحية . وقد أظهرت نتائج التحكيم للمشروبات أن المشروب المصنع بمستخلص الشعير والمتخمر ببادىء

S. thermophilus و *L. gasseri* كان أفضلها من حيث الطعم والقوام واللون والمظهر يليه المشروب المحتوى على مستخلص القمح والمتخمر ببادىء *L. rhamnosus* و *S. thermophilus* .

ويعتبر هذا البحث نقطة بداية لإنتاج مشروب صحى متخمر ذو تكلفة اقتصادية منخفضة وقيمة غذائية عالية للأطفال والبالغين .

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