EVALUATION OF COMMERCIAL PREBIOTIC PRODUCTS ON THE GROWTH OF SELECTED PROBIOTIC BACTERIA STRAINS

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ABSTRACT

The commercial prebiotic in cereal-based products with bovine colostrum, namely Germa-Fit® and Extra-Fit® as culture media for the growth of selected probiotic lactic acid strains (grown separately): L. gasseri and L. rhamnosus were cultured in MRS (control) and commercial prebiotic products. The media were fermented for 48h at 37°C and analysed for viable cell count and changes in pH values. In Germa-Fit® medium, both probiotic lactic acid strains were attained the highest population (9.6 cfu/ml) after 16h of fermentation time (P≤0.05). With the addition of lactose at 0.5, 1.0 and 1.5% the viability of L. gasseri was improved by approximately 2.1, 2.3 and 1.7 log10 cycles respectively, compared to 3.0, 2.3 and 2.5 for L. rhamnosus after 12h of fermentation time. The effect of Extra-Fit® medium was more positive for growth L. gasseri than that of L. rhamnosus after 8-24h of inoculation time. Generally, Germa-Fit® medium in the presence lactose exhibited the highest growth for both prebiotic strains compared to Extra-Fit® and MRS media. The changes in pH of commercial media were in the optimal pH range (4.5-4.4) for growth of lactic acid bacteria. Therefore, this study suggested that the feasibility use of commercial prebiotic products based cereal with bovine colostrum in the place the widely used MRS for culture media of probiotic lactic acid bacteria to developing new synbiotic functional fermented dairy food products.

Keywords: prebiotic, media, growth, probiotic bacteria.

INTRODUCTION

There is an obvious potential synergy between probiotic and prebiotic ingredients and foods that contains both are termed "synbiotics functional foods" came into the market, which have focused on intense research activity in recent years due to numerous health benefits (Ziemera and Gibson, 1998; Jones and Jew, 2007). Lactic acid bacteria play an important role in this trend. Hence, probiotic (life microorganisms) such as Lactobacilli and Bifidobacteria are now popular choices for application in probiotic preparation and in fermented dairy products. (Sabiki and Mathur, 2001; Crittenden et al., 2005; Trachoo and Boudreux, 2006; Shah, 2007 and Wang et al., 2007).

Growth of lactic acid bacteria (LAB) remain as a difficult task which require complex culture media, from both a qualitative and quantitative point of view, owing to their fastidious nutritional requirements and the variability from a strain to another. Therefore, only one medium (MRS or M17) may not be convienment for a high number of LAB strains.

The prebiotic ingredients are known to stimulate substrates for cultivation of lactic acid probiotic strains; which provide for high growth potential metabolic products and cell viability during storage and stimulate the
growth in the colon, that can improve host health (Shin et al., 2000, Bruno et al., 2002, Bamba et al., 2002 and Tuohy et al., 2003, Lior et al., 2005).

Many workers focused on the oligosaccharides such as galactooligosaccharide, fructo-oligosaccharide, inulin and lactulose as potential prebiotic (Cummings et al., 2001 Sharaf et al., 2003, and Akalin et al., 2007, and Wehling and Hutkins 2007). But in recent years, cereals have been investigated regarding their potential use in novel functional foods. Their use is considered as a powerful tool to increase the number or the activity of the two main health-promoting bacteria groups, bifidobacteria and lactobacilli (Charalampopoulos et al., 2002a,b, 2003, Sadek et al., 2003 Patel et al., 2004, Michida et al., 2006;Huebener et al.2008, and Trachoo et al., 2008).

The wheat germ with bovine colostrum, product Germa-Fit® is a dietary supplement that provides concentrated nutrients of high biological value; such as octacosanol (fatty alcohol) that reduced blood lipid profile and control liver hyperlipidemia. Calmodulin, (calcium-binding protein) improves calcium intake and protects against oskopenia and oskoprosis. Agglutinin, is potent anti-cancer against many forms of human cancers. Omega3-fatty acid, protects against heart attack as well as antiathersclerotic and antithrombotic conditions. Soluble and insoluble fibers, control overweight and obesity, stimulates colonic prbiotic bacteria. In addition, its rich in vitamin E and folic acid, (Amado and Arrigoni, 1992, Matteuzzi et al., 2004 and Khaleel et al., 2008).

Bovine colostrum is the most effective food currently recognized and used as food supplement that meets most criteria allowing classification as prebiotics. It is the most ideal integrated nourishment which contains immunoglobulins growth factor, antioxidant anticancer, minerals and vitamins (Horreini et al., 2001 and Abd El-Messih, 2007).

Extra-Fit® is a cereal mix (baby food) contains rice with mixed fruits and bovine colostrum, it ensures optimum growth protection against anemia and systemic infections supports bone, regulates nervous system responses (www.nutra-fit.int.com).

Furthermore, fruits contain compounds are called antioxidants (flavounoids, vitamins). Potential effect of these compounds include anticancer effect, lowering cholesterol and prevention of cardiovascular diseases (Arvantoyannis and Houwelingen-Koukaliaroglou, 2005).

Earlier, few paper are available which recommended the use of some cereal products as the main compounds of the media for growth lactic acid bacteria (Arrigoni et al., 2002 Helland et al., 2004, Patel et al., 2004, Djeghri-Hoine et al., 2006, Trachoo et al., 2006b, Novik et al., 2007, Kedia et al., 2007 and Wang et al., 2007).

Therefore, the aim of this study was to evaluate the potential culture media e.g. commercial available prebiotics based on cereal with bovine colostrum (Germa-Fit® or Extra-Fit® ) on the viability of selected probiotic bacteria, Lactobacillus gasseri and Lactobacillus rhamnosus for production novel fermented dairy products

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MATERIAL AND METHODS

The microorganisms used in this study were as follows: *Lactobacillus gasseri* B-14168 and *Lactobacillus rhamnosus* B-445. These strains were provided by Northern Regional Research Laboratory, Illinois, U.S.A (NRRL). Both strains had previously been shown to possess properties of a probiotic microorganisms including bile salt tolerance and tolerance to low pH values (Amin et al., 2002).

The commercial prebiotics in cereal- based fermentation media, Germa-Fit® and extra-Fit® were purchased from Arab Company for medical food (Medi Food) under license of BONA VITAL for health and immunity food (Munich-Germany for Nutra Fit International (Egypt). Germa-Fit® is wheat germ with bovine colostrum. Average analysis (Carbohydrate: 50-55%, Protein: 25-30%, fat: 1-3%, minerals: 5-7% and moisture: 3-5%) Extra-Fit® is rice flour mixed with fruits and bovine colostrums. Average analysis (carbohydrate: 74-75%, protein: 15%, fat: 3.5%, minerals 12.5% and moisture: 4-5%). Both commercial probiotics fortified with vitamins (A,K,E,D) and minerals (Ca, Fe, P).

MRS agar (Oxoid) was used as a control medium for the cultivation of probiotic strains.

The following method as described by Charalampoulos et al., (2002a) was used to prepare the fermentation media. A sample (50g) of Germa-Fit® was mixed with 1000 ml of tap water. The resulting mixture was divided into two equal portions. The first portions was divided to three equal portions, and supplemented with 0.5, 1.0 and 1.5% lactose respectively. The second portion was not supplemented with lactose. The Extra-Fit® medium was not supplemented with lactose because lactic acid strains (*Lactobacillus* and *bifidobacterium*) had the amylase activity that do grow well on the nutrient rich starch medium such as rice flour (Crihttenden et al., 2001 and; Lee et al., 2001;Sanni et al., 2002). A sample (50g) of Extra-Fit® was also mixed with 1000 ml of tap water. Each portion of commercial prebiotics were sterilized at 121°C/15min. probiotic strain, *L. gasseri* and *L. rhamnosus* were inoculated into each media at 2% (v/v). In all cases, their initial bacterial concentration was approximately 10^7 cfu/ml. Fermentation process were performed at 37°C/48h with no pH control and no agitation. MRS broth (Oxoid) was used as control medium for the same probiotic strains.

Total number of *L. gasseri* and *L. rhamnosus* in the fermentation samples were enumeration by technique has been described by Ravula and Shah, (1998). Briefly, fermentation samples decimally diluted in sterial quarter-strength Ringer’s solution, and appropriate dilutions were pour-plated. Plate counts on MRS agar (Oxoid) of *L. gasseri* and *L. rhamnosus*. All plates were incubated at 37°C for 0, 4, 8, 12, 16, 24 and 48h. under anaerobic conditions. Colony-forming units were counted (CFU/ml) and the results expressed as their log10 values.
pH values were measured using a digital pH-meter Model 5.1, Portugal (UK) at room temperature. Experiment was carried out in triplicate and each analysis in duplicate. The results were analysed statistically using Analysis System Version 8.0 (SAS, 2000) software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means from triplicate analysis at (P≤0.05) were determined by Duncan’s Multiple range test.

RESULTS AND DISCUSSION

Fig. (1) shows the evaluation viability of probiotic bacteria, L. gasseri and L. rhamnosus during 48h fermentation in MRS (control medium) and commercial prebiotic media e.g. Germa-Fit® and Extra-Fit®. In the control medium (MRS), L. rhamnosus cell population was significantly higher (P≤0.05) than that of L. gasseri during fermentation period (8-48h). However, the highest viable count of L. gasseri and L. rhamnosus were observed in the 16th of fermentation time (P≤0.05). L. gasseri and L. rhamnosus showed a 1.6 and 2.3 log10 cycles increase in their cell population respectively. These results are contrary to those of Sadek et al., (2003) who reported that, in the control experiments MRS media without cereal extracts, L. gasseri and L. rhamnosus showed a 0.39 and 0.11 log10 cycles reduction in their cell population respectively.

Fig (1) also shows the viable count of L. gasseri and L. rhamnosus in Germa-Fit® medium with or without lactose supplement (0.5, 1.0 and 1.5%). The viable population of both probiotic bacteria was improved in Germa-Fit® medium as compared to MRS medium. Improved viability could be to higher carbohydrates content about 50-55% present in Germa-Fit® medium and both probiotic bacteria metabolized all mostcarbohydrates present. Thus promoting their growth. These observations are in good agreement with Helland et al., (2004). They appeared that L. rhamnosus GG preferentially utilized glucose as an easily metabolized carbohydrate source. Arrigoni et al., (2002), and Matteuzzi et al., (2004) showed that the consumption of viogerm® PBI or Biogerm®, a highly nutritious wheat germ preparation, has prebiotic effect. It is rich in raffinose and other undigestible polysaccharide, which are available for microbial fermentation and modify the colonic microflora by lowering some Germ-negative bacteria (coliforms), and increasing potentially health-promoting bacteria (bifidobacteria and lactobacilli). Hartemink et al., (1996) used raffinose-bifidobacterium agar, a new selective medium for bifidobacteria. Also, germination causes many changes in nutritional composition of plant seed sugar, protein and free amino acids which available for microbial fermentation (Kanauchi et al., (2003), Trachoo et al., (2006). However, in Germa-Fit® medium without lactose, L. gasseri and L. rhamnosus reached the highest population density 9.6 log10 cfu/ml after 16h of fermentation. This viable cell count was well above the suggested minimum limit of 6 log10 cfu/g for efficacy of a probiotic product (Gopal et al., 2005). Sadek et al., (2003) indicated that the barley medium supported well
the growth of *L. gasseri*, *L. rhamnosus* and *L. reuteri* which showed increases in their cell population of 1.91, 2.39 and 1.57 log$_{10}$ cfu/ml respectively at the end of experimental phase (9 and 12h). This could be attributed to the simultaneous presence of considerable amount of monosaccharide (glucose and fructose) and disaccharides (maltose and sucrose) in the barley medium and these strains did not grow well in wheat and maize media ascribed to the low total fermentable sugar and free amino nitrogen concentrations (Charalampopoulos, 2002 a,b). Furthermore, Helland et al., (2004) observed that probiotic bacteria such as *L. reuteri*, *L. rhamnosus* and *L. acidophilus* (LAS and 1748) reached maximum cell count after 12-h fermentation (7.2-8.2 log$_{10}$ cfu/g with a pH blew 4.0) in maize porridge with added malted barley and the protective effect on bacteria viability was mainly attributed to carbohydrates in the cereals. While, Kedia et al., (2008) indicated that the highest cell concentration of *L. plantarum* was observed in white oat flour (9.16 log$_{10}$ cfu/ml) and the lowest in the bran (8.17 log$_{10}$ cfu/ml).

When lactose was present in the Germa-Fit®, the cell count of *L. gasseri* increased by 2.1, 2.3 and 1.7 log$_{10}$ cycles at 0.5, 1.0 and 1.5% respectively after 12-h fermentation as compared to 3.0, 2.3 and 2.5 log$_{10}$ cycles with strain *L. rhamnosus*. Novik et al., (2007) demonstrated that supplementation of the media containing protein or polysaccharide fractions of the barley spent grain with lactose and mineral salts was favorable for the growth lactic acid bacteria and bifidobacteria. In Extra-Fit® medium as baby food (rice flour with bovine colostrum and fruits) *L. gasseri* showed significantly higher viability (P≤0.05) as compared to control medium (MRS) at 8-24h of incubation time, while *L. rhamnosus* exhibited opposite trend (Fig.1). However, *L. gasseri* and *L. rhamnosus* reached the highest population 9.6 and 9.2 log$_{10}$ cfu/ml respectively during 16h of fermentation period. The promotion of Extra-Fit® for growth lactic acid bacteria could be due to increase level of nutrients and bioactive compounds of nutrient requirements for growth lactic acid bacteria. These compounds include protein, amino acids, sugar, vitamins, gamma oryzanol, gamma amino butyric acid, tocopherols and other phytochemical substances (Foster, 2004 and Tain et al., 2004). Trachoo et al., (2006) indicated that the growth of probiotic bacteria *L.acidophilus* and *L. plantarum* in media containing germinated rough rice powder was greater (P≤0.05) than that in media containing rice powder. On other hand, colostrum apparently provides a more readily available source of peptides and amino acids needed for growth of probiotic bacteria (Coppa et al., 2006 and Akalin et al., 2007). Roberts et al., (1992) observed that bifidobacteria grow well in human milk than bovine milk because of Lactoferrin and transferin. Also, Dubey and Mistry (1996) indicated that *B. bifidum* and *B. longum* had greater variability in growth characteristics in infant formulas than in non-fat milk.

An increase of metabolic activity of probiotic bacteria would have contributed to decrease the pH of media. On other words, the final activity in the fermented medium has a major impact on the microbial viability during the shelf-life of the product (Martesson et al., 2002).
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Cereal extract from malt, wheat and barley were reported to protect *L. acidophilus*, *L. plantarum* and *L. reuteri* in acidic conditions (Charalampopoulou *et al.*, 2003 and Michida *et al.*, 2006).

In control medium (MRS), pH values always over 4.5 for both probiotic bacteria at the end of fermentation time, but below 4.5 in the prebiotic media (Fig.2) the pH values are in the optimal pH range (4.5-4.4) for growth lactic acid bacteria (Salminen and Wright (1993) and the buffering capacity of media was the major affecting the variation in the pH of fermented product (Salamin et al., 2005). They also suggested that addition WPC to yoghurt increased the buffering capacity around pH 4. Helland *et al.*, (2004) demonstrated that pH of maize porridge with added malted barley for *L. rhamnosus* and *L. reuteri* reaching a pH as low as 3.1 after 20-h fermentation. While Trachoo et al., (2008) illustrated that the inoculum system with lactic acid bacteria containing soy bean powder had the highest buffering capacity (free amino acid, soluble non-reducing sugars), therefore it potentially protects the bacteria from undesirable acidic conditions and improve their survival during cryopreservation and storage.

Data present in Fig.(2) show the pH of Germa-Fit® and Extra-Fit® media was significantly different (P≤0.05) compared to MRS medium (control). At the end of fermentation time, the changes pH values of Germa-Fit® medium with or without lactose supplement when inoculated with *L. gasseri* were significantly higher (P≤0.05) than those media cultivated with *L. rhamnosus* at 16-48h. However, supplement lactose had no significant effect (P≥0.05) on the changes pH of medium for both probiotic bacteria. No significantly differences (P≥0.05) were found between changes pH values of Extra-Fit® medium inoculated with *L. gasseri* and *L. rhamnosus* at end of incubation time. Generally, the changes of pH values of Extra-Fit® medium cultivated with both probiotic strains at end fermentation time were higher than those of MRS and Germa-Fit® media.

Because of shortage, expensive increasing costs of lactic acid bacteria culture media and available commercial prebiotic in cereal-based products contain potentially functional compounds in market, therefore, this study demonstrates the feasibility use of commercial prebiotic products namely Germa-Fit® and Extra-Fit® in place of nutriental requirements (meat, yeast extracts, peptones and sugar) of culture media for specific prebiotic lactic acid bacteria (*L. gasseri* or *L. rhamnosus*) as a functional fermented foods. Also, the results of this study can be use for further studies in vivo experiments (Bielecka *et al.*, 2002) for the mechanisms of prebiotic &probiotic activity of cereal and lactic acid bacteria for developing novel technologies of cereal processing that enhance their health potential and innovative products in market to meet and exceed the expectation of today's health-conscious consumer, infants or adults. (Manzi *et al.*, 2007 and Reid 2008, Hekmat *et al.*, 2009).
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تقييم المحفزات التجارية لنمو بعض السلالات الميكروبية الداعمة للحيوية

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نظراً للأهمية الصحية والتغذوية للكثير من الدوامات الدائمة للحيوية فقد استهدف هذا البحث تقييم استخدام الحبوب المحيطة مع مرصوب اللين البقرى (جنيت النحل) و L. gasseri ( فوق الأرز) كمحفزات لنمو نوع من البادات الدائمة للحيوية وهماشبعتينة الاستخدام ونمو بكتيريا حامض اللاكتيك وقد أظهرت النتائج إلى MRS كمدل لبيئة rhhamnos ما يلي:

1. عند استخدام Germa-Fit® تم الحصول على أقصى عدد للخلايا L. gasseri بتركيزات متكررة 10^5 و10^6 لغرام حويضة/مل عند 12 ساعة من التحضين.
2. عند إضافة اللاكتوز إلى البينة بتركيزات مرتبتين في البينة بتركيزات 21.7 و21.8 لغرام حويضة/مل، وبدوره بتركيزات 2.6، 2.7، و1.3. و1.4 لجرام حويضة/مل، وذلك بعد 12 ساعة من وقت التحضين، L. rhamnosus عن سلالات L. gasseri لذا تكون أكثر إيجابية في معدل نمو سلالات.
3. كما كانت النتائج على أن: بيئة Extra-Fit® لم تتأثر أكثر إيجابية في معدل نمو سلالات L. rhamnosus عن سلالات L. gasseri
4. كانت بيئة MRS وExtra-Fit® الدائمة للحيوية بالمقارنة يخلص من بيئة Germa-Fit® المضافة إلى اللاكتوز لها النتائج في ارتفاع معدل نمو السلالتين لامتصاص الببتيدات، و вещات التغيرات في pH للكثير من البادات في حدوت المدى المطلوب لنمو بكتيريا حامض اللاكتيك.
5. ونظراً لارتفاع أسعار بعض مكونات بيئة MRS يعد استخدام هذه المحفزات التجارية لاحتياج اللب إلى كميات كبيرة من العناصر الغذائية (المواد الكربوهيدراتية – البروتينية والببتيدات) للحصول على X.1 لجرام حويضة/مل للتحقيق من الخصائص التغذوية، ونسبة ماء، ونسبة الدهون والتمايل بينها ذات الخصائص التغذوية والصحية العالية التي تتناسب مع رغبات جميع أعمار المستهلكين.