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# 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.gasseria* and *L.rhamnosus* were cultured in MRS (control) and commercial prebiotic products. The media were fermented for 48h at 37ºC 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 $16^{h}$ of fermentation time (P≤0.05). With the addition of lactose at 0.5, 1.0 and 1.5% the viability of *L.gasseria* was improved by approximately 2.1, 2.3 and 1.7 $\log_{10}$ 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.gasseria* 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, Crihttenden *et al*., 2005 ; Trachoo and Boudreuy, 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 quantitive 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 converniment 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, Liong *et al*., 2005).

Many workers focused on the oligosaccharides such as galactooligossacharide fracto-oligassacharride, 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 hyperlipdemia. 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 antiathersclerrotic and antithromobotic conditions. Soluble and insoluble fibers, control overweigh and obesity, stimulates colonic pribiotic 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 *Lacctobacillus rhamnosus* for production novel fermented dairy products

## 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 Charalampopoulos *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 121ºC/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 37ºC/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 37ºC 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 $\log_{10}$ values.

pH values were measured using a digital pH- meter Model 5.1, Porlugal (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 ($P \leq 0.05$) were determined by Duncan's Multiple range test.

## RESULTS AND DISCUSSTION

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 ($P \leq 0.05$) 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 $16^{th}$ of fermentation time ($P \leq 0.05$). *L. gasseri* and *L. rhamnosus* showed a 1.6 and 2.3 $\log_{10}$ 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 $\log_{10}$ 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 nutrious wheat germ preparation, has prebiotic effect. It is rich in raffainose and other undigestible polysaccharide, which are available for microbial fermentation and modify the colonic microflora by lowing 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 $\log_{10}$ cfu/ml after 16h of fermentation. This viable cell count was well above the suggested minimum limit of 6 $\log_{10}$ 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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).

Cereal extract from malt, wheat and barley were reported to protect *L. acidophilus, L. plantarum* and *L. reutri* in acidic conditions (Charalampopouls *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 (Salatin *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. rahmanosus* 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 cryopresevation and storage.

Data present in Fig.(2) show the pH of Germa-Fit® and Extra-Fit® media was significantly different ($P \leq 0.05$) 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 ($P \leq 0.05$) than those media cultivated with *L. rhamnosus* at 16-48h. However, supplement lactose had no significant effect ($P \geq 0.05$) on the changes pH of medium for both probiotic bacteria.No significantly differences ($P \geq 0.05$) 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 the use of commercial prebiotic products namely Germa-Fit® and Extra-Fit® in place of nutrienal 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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# تقيم المحفزات التجارية لنمو بعض السلالات الميكروبية الداعمة للحيوية

**إســـماعيل حســـين إســـماعيل عبـــد الغنـــى*، ســـحر حســـين صـــلاح محمـــد** و فاطمة على متولي رمضان***

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نظرا للأهمية الصحية والتغذوية لكثير من المواد كمحفزات للبادئات الداعمة للحيوية فقد استهدف هذا البحث تقيم استخدام الحبوب المحضرة مع سرسوب اللبن البقرى ®Germa-Fit (جنين القمح) و ®Extra-Fit (دقيق الارز) كمحفزات لنمو نوعين من البادئات الداعمة للحيوية وهما *L. gasseri* ، *L. rhamnosus* كبديل لبيئة MRS الشائعة الاستخدام في نمو بكتريا حامض اللاكتيك وقد أظهرت النتائج إلى ما يلي:

١- باستخدام ®Germa-Fit تم الحصول على أقصى عدد لخلايا *L. gasseri* ، *L. rhamnosus* ٩,٦ لوغاريتم خلية/مل عند ١٦ ساعة من التحضين

٢- عند إضافة اللاكتوز الى البيئة Germa-Fit بتركيزات ٠,٥ ، ١ ، ١,٥% زاد معدل النمو لسلالة *L. gasseri* بمقدار ٢,١ ، ٢,٣ ، ١,٧ (دورة لوغاريتمية بينما زاد ٣ ، ٢,٣ ، ٢,٥ دورة لوغاريتمية لسلالة *L. rhamnosus* وذلك عند ١٢ ساعة من وقت التحضين

٣- كما دلت النتائج على أن : بيئة ® Extra-Fit لها تأثير أكثر ايجابية في معدل نمو سلالة *L. gaseri* عن سلالة *L. rhamnosus*

٤- كانت بيئة ®Germa-Fit المضاف إليها اللاكتوز لها اثر فعال في ارتفاع معدل نمو السلالتين الداعمة للحيوية بالمقارنة بكل من بيئة ®Extra-Fit و MRS

٥- وكانت التغيرات في pH تلك البيئات في حدود المدى الملائم لنمو بكتريا حامض اللاكتيك

ونظرا لارتفاع أسعار خامات بيئة MRS يوصى هذا البحث باستخدام هذة المحفزات التجارية لاحتوائها على كثير من العناصر الغذائية (المواد الكربوهيدراتية – البروتينية والأملاح والفيتامينات) للحصول على ١٠^٧ خلية/جم للمنتج ذو الخواص الوظيفية مما يساهم في تطوير كثير من المنتجات البنية ذات الخواص التغذوية والصحية العالية التي تتلائم مع رغبات جميع أعمار المستهلكين.