

EFFECT OF LACTIC ACID STARTERS AND PROBIOTIC BACTERIA ON SOME ANTINUTRITIONAL COMPOUNDS ASSOCIATED WITH FOODS

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ABSTRACT

The effect of six strains of lactic acid starter bacteria (*Lactobacillus* (*Lb.*) *delbrueckii* ssp. *bulgaricus*, *Lb. fermentum*, *Lb. helveticus*, *Lb. plantarum*, *Lactococcus* (*Lc.*) *lactis* ssp. *cremoris* and *Streptococcus* (*S.*) *thermophilus*) and four strains of probiotic bacteria (*Lb. casei* ssp. *imunitass*, *Bifidobacterium* (*B.*) *adolescentis*, *B. infantis* and *B. longum*) was studied in liquid media in relation to some known antinutritional compounds. These compounds included phytic acid and catechin (naturally occurring in plant products) and furfural (mostly formed during food processing). Phytic acid and catechin did not interfere with the growth and acid development through the growth of the strains at concentrations commonly found in foods, while the effect of furfural was concentration and strain dependent. The examined bacteria eliminated phytic acid by 72.22% (*S. thermophilus*), while completely eliminated by *Lb. delbrueckii* ssp. *bulgaricus* and *Lb. plantarum*. Catechin was reduced to 62.40% by *Lb. helveticus*, while was completely eliminated by *Lactobacillus casei* ssp. *imunitass*. Furfural was reduced by 87.91% at 50 mg 100 ml⁻¹ (*Lb. plantarum*) and by 65.39% at 100 mg 100 ml⁻¹ (*Lb. casei* ssp. *imunitass*).

Therefore, the use of lactic acid starter and probiotic bacteria especially in foods containing these compounds is beneficial due to their capability of reducing or eliminating the harmful effects of antinutritional compounds.

Keywords: Fermented food, Starter, Probiotic, Lactic acid bacteria, Phytic acid, Catechin and Furfural, Elimination.

INTRODUCTION

Probiotic bacteria (bifidobacteria and lactic acid bacteria) are widely used as adjunct cultures for fermented dairy and many other food products (Vinderola *et al.*, 2002). The benefits gained from these bacteria are: absorption of calcium (Deguchi *et al.*, 1985), production of antimicrobial substances (Kang *et al.*, 1989), reduction of lactose intolerance (Hughes and Hoover, 1995), inhibition of ulcers and tumor formation (Midolo *et al.*, 1995), raising blood pressure (Hata *et al.*, 1996), enhancement of immune response (Hamburger *et al.*, 1997), lowering of total cholesterol and low density lipoprotein cholesterol (Taylor and Williams, 1998), maintenance of health intestinal flora (Ouweland *et al.*, 1999), decrement of diarrhea incidence (De Ross and Katan, 2000), counteraction of mutagenic and genotoxic effects in human organs (kailasapathy and Chin, 2000). Other benefits include inhibition of pathogenic bacteria and synthesis of β -vitamins (Bruno and Shah, 2002). These benefits are realized when the gastrointestinal tract is provided with elevated viable population (Bruno *et al.*, 2002).

These potential capabilities of probiotic bacteria give impetus to proceed forward to uncover other promising aspects of them. One of these aspects in their probable desirable action on some antinutritional compounds either naturally occurring (phytic acid and catechin) or formed upon processing (furfural). These compounds are known to have adverse effects on human health. Thus, phytic acid and phytates are antinutritional components of many cereal, nuts, oilseeds, legumes and soyabean milk (being present at 1 – 2%); they reduce the bioavailability of some essential elements particularly iron and inhibit the action of some important enzymes (Reddy et al., 1989; Liener, 1994; Feil, 2001 and Hurrell et al., 2003). Deshpande and Cheryan (1984) reported that the interaction of phytate with protein, vitamins and minerals is considered one of the factors that limit the nutritive value of plant foods. Moreover, Hiassa et al. (1992) studied the carcinogenicity of phytic acid in rats and found that phytic acid developed many tumors in rats.

Marklinder et al. (1995) and Ibrahim et al. (2002) reported that *Lb. plantarum* and *Rhizopus oligosporus* were able to decrease phytate in oat and cowpea. In general, fermentation caused a reduction of phytate due to the activity of the microbial phytase (Mahgoub and Elhag, 1998). Also, Greiner and Jany (1996) mentioned that phytate undergoes hydrolysis in the small intestine. Moreover, Marklinder et al. (1996) found that phytate levels were reduced in barley sour dough bread by a starter culture of lactic acid bacteria.

Giovanelli and Polo (1994) reported that the fermentation of dough with *Saccharomyces cerevisiae*, *Lb. brevis* and *Lb. plantarum* caused phytic acid to be completely or almost completely hydrolysed in the straight dough or sour dough process, respectively, when using wheat flour. For rye doughs, phytic acid levels were reduced more when the sourdough process was used.

Svanberg et al. (1993) mentioned that fermentation of cereal foods with a starter culture (containing *Streptococcus lactis*, a *Lactobacillus* strain and *Candida krusei*) reduced phytate content and may have potential in developing countries to improve iron nutrition.

Catechin is a bioflavonoid that is found in black tea, apples, grapes; cacao, chocolate, herbs and spices (originating from wood plants) and especially green tea which contains as high as 30% on dry basis (Schulz & Herrmann, 1980; Herrmann, 1998; Arts et al., 2001 and Rios et al., 2003). Some hazards are associated with catechin, as its combination with iron may cause red blood cell damage and other relevant problems (Salama and Mudler, 1987). Also, Guyot et al. (1995) mentioned that catechin is a major cause of quality degradation during handling, storage and processing of fruits. Moreover, catechin inhibits the enzymes concerned with starch hydrolysis in the digestive channel: α -amylase, amyloglucosidase and maltase (Bjoerck and Nyman, 1987).

Furfural occurs naturally in many fruits and in tea, coffee and cocoa and mostly is formed during the processing and domestic preparation of a broad range of foods (Monti et al., 2000 and Murata et al., 2002). It is also carried over into food from its use as an extraction solvent or as a component of flavor mixtures. Furfural and its derivatives from consumption of foods in

which its occur naturally is approximately 0.3 (Stofberg and Grundschober, 1987) to 0-0.5 mg /kg /day (IARC, 1995). The highest concentrations of furfural in food have been reported in cocoa (55 - 255 ppm), wholegrain-bread (26 ppm), heated skim milk (230 ppm) and coffee (90 - 881 ppm) (Adams *et al.*, 1997).

Furfural is genotoxic to mouse liver (NTP, 1990), mutagenic (Kato *et al.*, 1989), beside being irritant (Cohen and Ellwein., 1988) and carcinogenic in male mice (Shane *et al.*, 1988). Therefore, carry-over into food should be reduced to the lowest extent technologically feasible. (NAS, 1989).

As seen from the about review almost all trials of reduction or elimination of the hazardous effects of antinutritional compounds were mainly confined to phytic acid.

The present work aims at investigating the effect of lactic acid starter and probiotic bacteria on some of the above-reviewed, antinutritional compounds namely, phytic acid, catechin and furfural.

MATERIALS AND METHODS

Bacterial strains

Lactobacillus (Lb.) delbrueckii ssp. bulgaricus, *Lb. helveticus*, *Lactococcus (Lc.) lactis ssp. cremoris* and *Streptococcus (S.) thermophilus*, were obtained from Chr. Hansen's Lab., Denmark. Other bacteria used in this study, namely *Lb. plantarum* (DSM 20205, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany), *Lb. fermentum* (3025162 1M, FDRCK, Germany), *Lb. casei ssp. imunitass* (isolated from biofermented milk), *B. adolescentis* (ATCC 15704), *B. infantis* (ATCC 15637) and *B. longum* (ATCC 15707) were obtained from American Type Culture Collection, Rockville, Maryland, USA.

Antinutritional compounds

The antinutritional compounds examined were phytic acid (dodecasodium salt) at 150 mg 100 ml⁻¹, catechin at 20 mg 100 ml⁻¹ (Sigma Chemical Co., P.O. Box 14508 St. Louis No. 63178, USA) and furfural at 50, 100 and 150 mg 100 ml⁻¹ (Merck, Darmstadt, Germany).

Determination of Antinutritional compounds

Phytic acid was estimated by the procedure of Wheeler and Ferrel (1971), catechin by vanillin-HCl assay according to Sun *et al.* (1998) and furfural by the procedure of Ranganna, (1977).

Culture media

MRS broth medium (Biolife) was used as a growth medium for lactobacilli and bifidobacteria, while M17 broth medium (Biolife) for lactococci and streptococci.

Methodology and incubation conditions

Solutions of antinutritional compounds were sterilized by filtration through a sterile 0.45 µm cellulose nitrate filter (Sartorius, AG. 37070 Goettingen, Germany) and then added at different concentrations (mentioned above) to the bacterial growth media. The media were inoculated with 1%, v/v of bacterial culture (initial count, $10^6 - 10^7$ cfu mL⁻¹). The inoculated media were incubated at 37°C, aerobiosis for lactococci, streptococci, lactobacilli and anaerobiosis for bifidobacteria (GasPak System-Oxoid, Basingstoke, Hampshire, England). The growth was measured by absorbance at 600 nm on a Spectronic 20D spectrophotometer (Milton Roy Company, USA) at intervals (0, 6, 9, 12, 24, 48 and 72 hrs). The growth was expressed as normal ($A_{600nm} > 70\%$ of the control culture), weak ($30\% < A_{600nm} < 70\%$ of the control culture) and none ($A_{600nm} < 30\%$ of the control culture).

The initial bacterial count was determined using M17 agar medium (Biolife) for lactococci and streptococci after incubation at 37°C for 48 hrs (aerobiosis), while MRS agar medium (Biolife) was used for lactobacilli (aerobiosis) and bifidobacteria (anaerobiosis) after incubation at 37°C for 48 hrs.

RESULTS AND DISCUSSION

Culture blends containing probiotic bacteria (bifidobacteria as well as lactic acid starters) are used in a wide variety of food products as sour cream, buttermilk, yogurt, powdered milk, low fat and diet yogurt, hard cheese, ice cream, frozen desserts, fruit juices, mayonnaise, dry fermented sausages, fermented meats or fish, fermented mackerel minces, lactic acid-fermented beverages (Khalil and Mansour, 1998; Pszczola, 2002 and Yin *et al.*, 2002). However, there are some problems associated with incorporating probiotic bacteria into the starter used to make cultured products. One of them is the slow growth of these bacteria, probably, due to their low proteolytic activity. Another problem is the suppressive action of the traditional starter culture on probiotic bacteria (McComas and Gilliland, 2003). Also, the viability of these bacteria decreases by time under the influence of progressive acidity of the product, storage period and temperature. Therefore, their viability in fermented product is crucial (Dave and Shah, 1997). Moreover, health benefits from these bacteria are largely dependent on the presence of sufficient viable numbers at the time of consumption (Dave and Shah, 1997; Shin *et al.*, 2000 and McComas and Gilliland, 2003). How far the presence of some antinutritional factors would affect the viability of these bacteria or be affected by them was the target of the present investigation. Herein it should be mentioned that the levels of each of phytic acid, catechin and furfural used in our experiments was selected on the basis of probable occurrence in food products. One level was tried for each of phytic acid (150 mg 100 ml⁻¹) and catechin (20 mg 100 ml⁻¹), while three levels (50, 100 and 150 mg 100 ml⁻¹) for furfural as its formation in processed food varies according to processing conditions.

Results of the research are given in Tables (1-4) and Figures (1- 10).

Phytic acid did not interfere with the growth of lactic acid starter and probiotic bacteria strains as evident from Table 1 and 2 and Fig 1 and 2. As seen from Table (3 and 4), the elimination of phytic acid by lactic acid starter ranged from 72.22% (*S. thermophilus*) to 100% (*Lb. delbrueckii* ssp. *bulgaricus* and *Lb. plantarum*). For probiotic bacteria the elimination of phytic acid varied from 81.03% (*B. longum*) to 98.56 (*Lb. casei* ssp. *imunitass*). These data affirm that fermentation by starters may completely remove or remarkably lessen phytic acid and its hazardous effect on human body.

Table (1): Growth and pH value of lactic acid starter in liquid media (37°C for 3 days) in the presence of phytic acid, catechin and furfural.

Strain		Control	Phytic acid 150 mg 100 ml ⁻¹	Catechin 20 mg 100 ml ⁻¹	Furfural		
					50 mg 100 ml ⁻¹	100 mg 100 ml ⁻¹	150 mg 100 ml ⁻¹
<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i>	Growth	+	+	+	+	+	None
	pH	4.10	4.16	3.99	4.02	4.05	6.01
<i>Lb. fermentun</i>	Growth	+	+	+	+	Weak	None
	pH	4.24	4.45	4.25	4.48	4.73	5.99
<i>Lb. helveticus</i>	Growth	+	+	+	Weak	None	None
	pH	4.04	4.80	4.77	4.72	4.87	6.12
<i>Lb. plantraum</i>	Growth	+	+	+	+	+	None
	pH	4.13	4.43	5.10	4.34	4.35	5.95
<i>Lc. lactis</i> ssp. <i>cremoris</i>	Growth	+	+	+	+	+	None
	pH	3.71	3.77	3.70	3.84	3.87	5.84
<i>S. thermophilus</i>	Growth	+	+	+	+	Weak	None
	pH	4.23	4.40	4.17	4.43	4.71	6.05

+: Normal ($A_{600nm} > 70\%$ of the control culture), Weak ($30\% < A_{600nm} < 70\%$ of the control culture), None ($A_{600nm} < 30\%$ of the control culture). All the results are means of triplicates.

In this connection, Marklinder *et al.* (1995 and 1996) and Ibrahim *et al.* (2002) reported that *Lb. plantarum* and *Rhizopus oligosporus* were found to be able to decrease phytate in cereals and pulses. We are inclined to the opinion that fermentation caused a reduction of phytate due to the activity of the microbial phytase (Mahgoub and Elhag, 1998). Also, Greiner and Jany (1996) mentioned that phytat is further hydrolysed in the small intestine. Shirai *et al.* (1994) found that phytic acid did not impede the growth twelve strains of lactic acid bacteria on the contrary it was degraded under the influence of the bacteria. The degradation of phytate from the medium was not solely due to phytases as a fall in pH (due to lactic acid production by the bacteria) also resulted in co-precipitation of phytic acid with low molecular weight proteins in the medium.

Table (2): Growth and pH value of probiotic bacteria in liquid media (37°C for 3 days) in the presence of phytic acid, catechin and furfural.

Strain	Control	Phytic acid 150 mg 100 ml ⁻¹	Catechin 20 mg 100 ml ⁻¹	Furfural			
				50 mg 100 ml ⁻¹	100 mg 100 ml ⁻¹	150 mg 100 ml ⁻¹	
<i>B. adolescentis</i>	Growth pH	+ 3.58	+ 3.85	+ 3.52	+ 3.91	+ 4.05	- 5.96
<i>B. infantis</i>	Growth pH	+ 3.66	+ 3.90	+ 3.82	+ 4.60	+ 4.63	- 6.00
<i>B. longum</i>	Growth pH	+ 3.61	+ 3.80	+ 3.58	+ 4.08	+ 4.21	- 5.60
<i>Lb. casei</i> ssp. imunitass	Growth pH	+ 3.52	+ 3.63	+ 3.47	+ 3.54	+ 3.90	- 5.95

+: Normal ($A_{600nm} > 70\%$ of the control culture), Weak ($30\% < A_{600nm} < 70\%$ of the control culture), None ($A_{600nm} < 30\%$ of the control culture).

All the results are means of triplicates.

Catechin also exhibited no inhibitory effect on the growth of bacteria and acid production (Table 1, 2 and Fig 4, 5). Elimination of this compound by lactic acid starter varied from 62.40% (*Lb. helveticus*) to 98% (*Lc. lactis* ssp. *cremoris*). As for probiotic bacteria the elimination percentage ranged from 72.20% (*B. adolescentis*) to complete removal (*Lb. casei* ssp. *imunitass*).

Furfural at the low concentration (50 mg 100 ml⁻¹) was devoid of any apparent adverse effect on the growth and acid production of the tested cultures (Table 1, 2 and Fig 5, 6). However, at 100 mg 100 ml⁻¹ remarkable was the effect on the growth and acid production of all bacteria (Table 1, 2 and Fig. 7, 8). At 150 mg 100 ml⁻¹, the growth was suppressed (Table 1, 2 and Fig. 9, 10) and the pH in all cases was around 6.0 indicating low acid production.

Elimination of furfural ranged from as low as 3.48 (*Lb. fermentum*) to as high as 87.91% (*Lb. plantarum*) at 50 mg 100 ml⁻¹, while from zero (*Lb. delbrueckii* ssp. *bulgaricus*, *Lb. helveticus*, *S. thermophilus*) to 61.16% (*Lb. plantarum*) at 100 mg 100 ml⁻¹.

On the basis of our results it could be stated that probiotics were not affected by the antinutritional compounds, on the contrary they exerted a desirable reducing or eliminating effects on these compounds. Particular attention should be paid to *Lb. plantarum*. This bacterium could be recommended for fermented food products containing furfural.

In general, these results add a new benefit to lactic acid and probiotic bacteria as represented as by their ability to eliminate a great part of the tested antinutritional compounds and thereby, their harmful effect on human body.

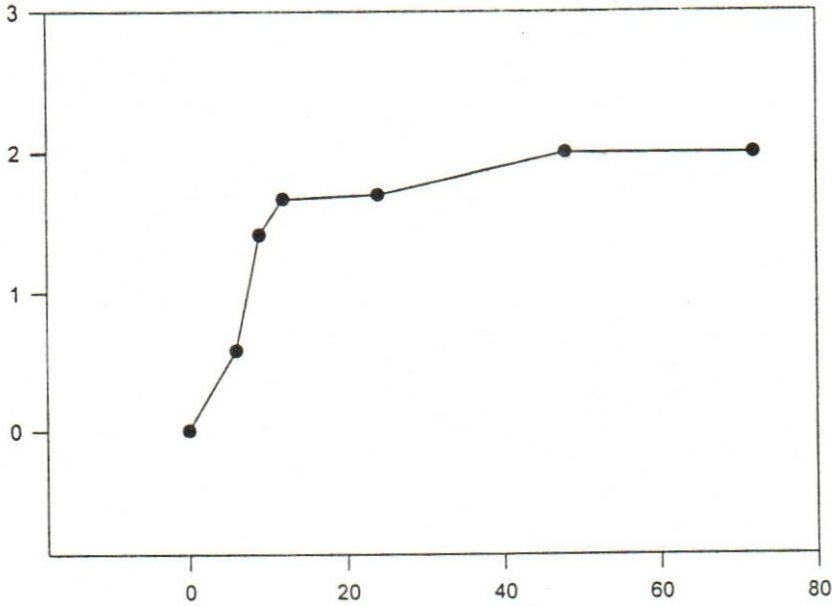


Fig. (1): Effect of phytic acid ($150 \text{ mg } 100 \text{ ml}^{-1}$) in MRS or M17 broth medium on the growth of lactic acid starter at 37°C .

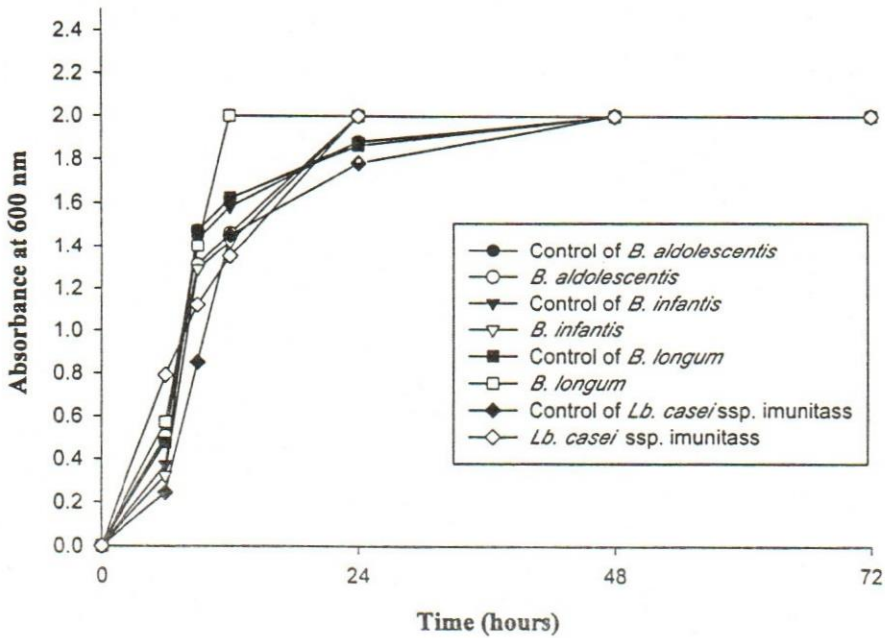


Fig. (2): Effect of phytic acid ($150 \text{ mg } 100 \text{ ml}^{-1}$) in MRS broth medium on the growth of probiotic bacteria at 37°C .

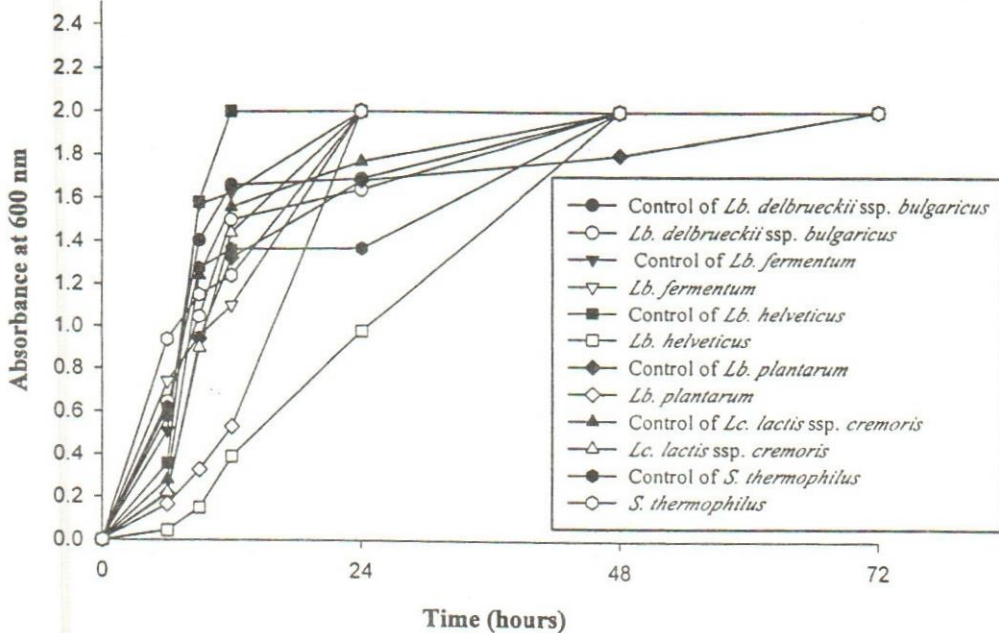


Fig. (3): Effect of catechin (20 mg 100 ml⁻¹) in MRS or M17 broth medium on the growth of lactic acid starter at 37°C.

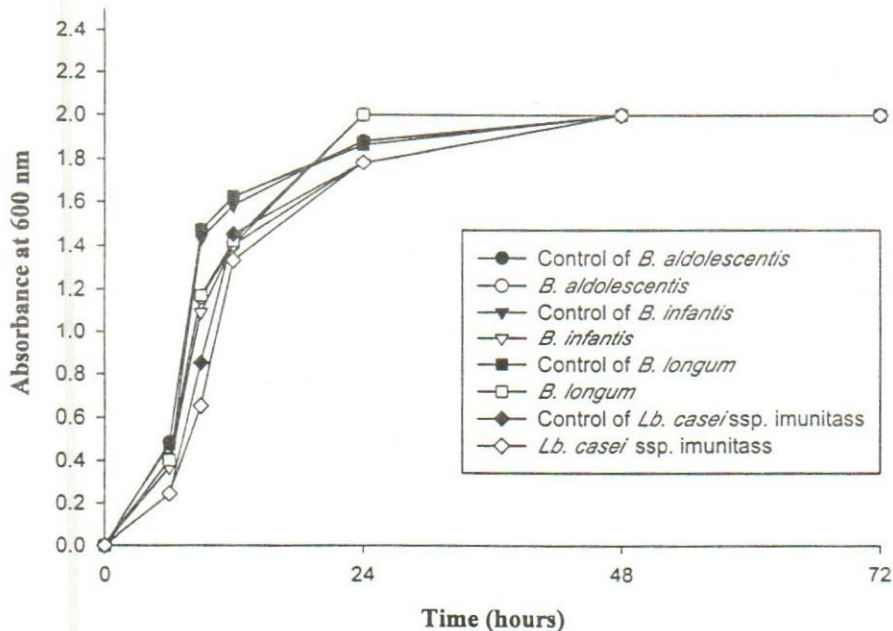


Fig. (4): Effect of catechin (20 mg 100 ml⁻¹) in MRS broth medium on the growth of probiotic bacteria at 37°C.

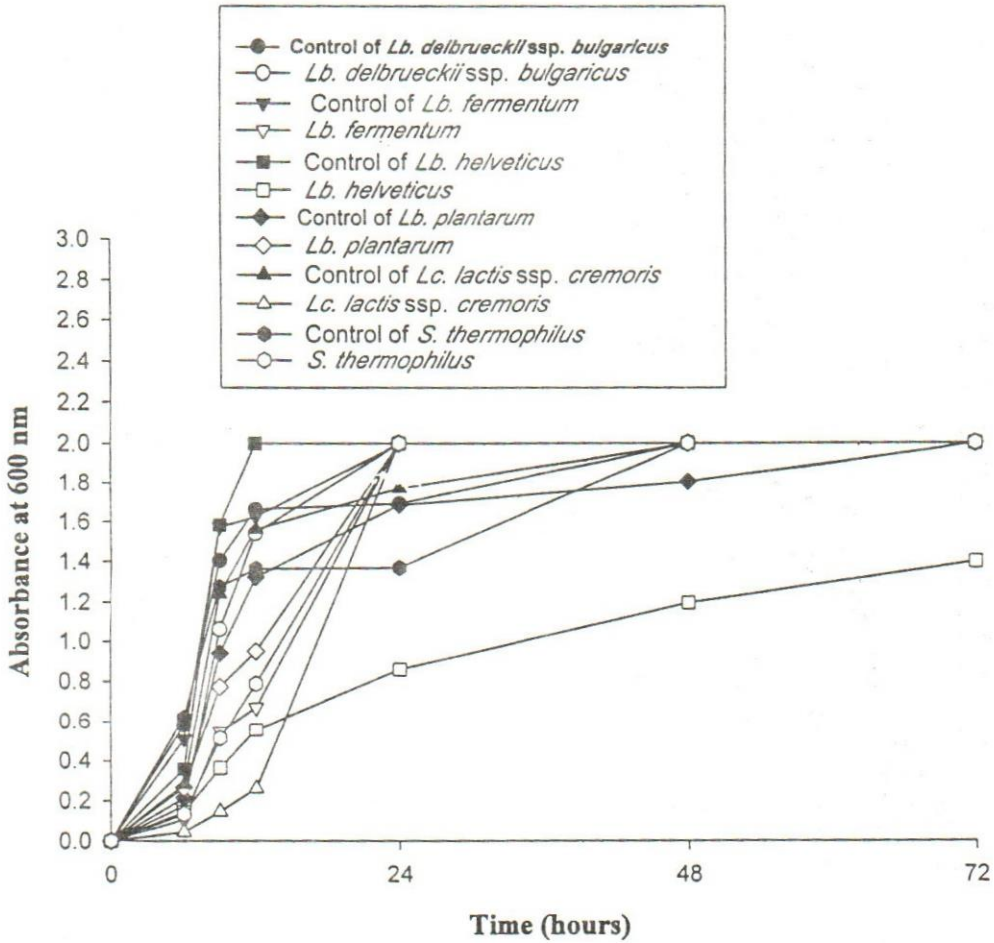


Fig. (5): Effect of furfural (50 mg 100 ml⁻¹) in MRS or M17 broth medium on the growth of lactic acid starter at 37°C.

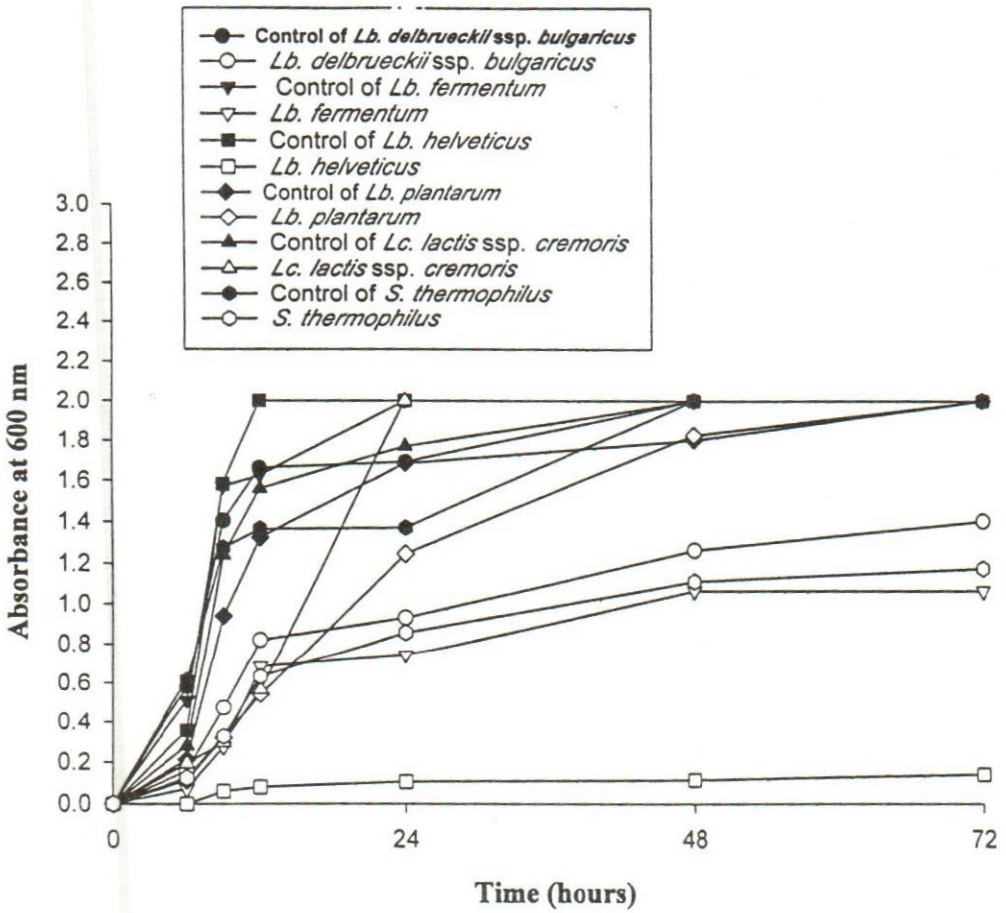


Fig. (6): Effect of furfural (100 mg 100 ml⁻¹) in MRS or M17 broth medium on the growth of lactic acid starter at 37°C.

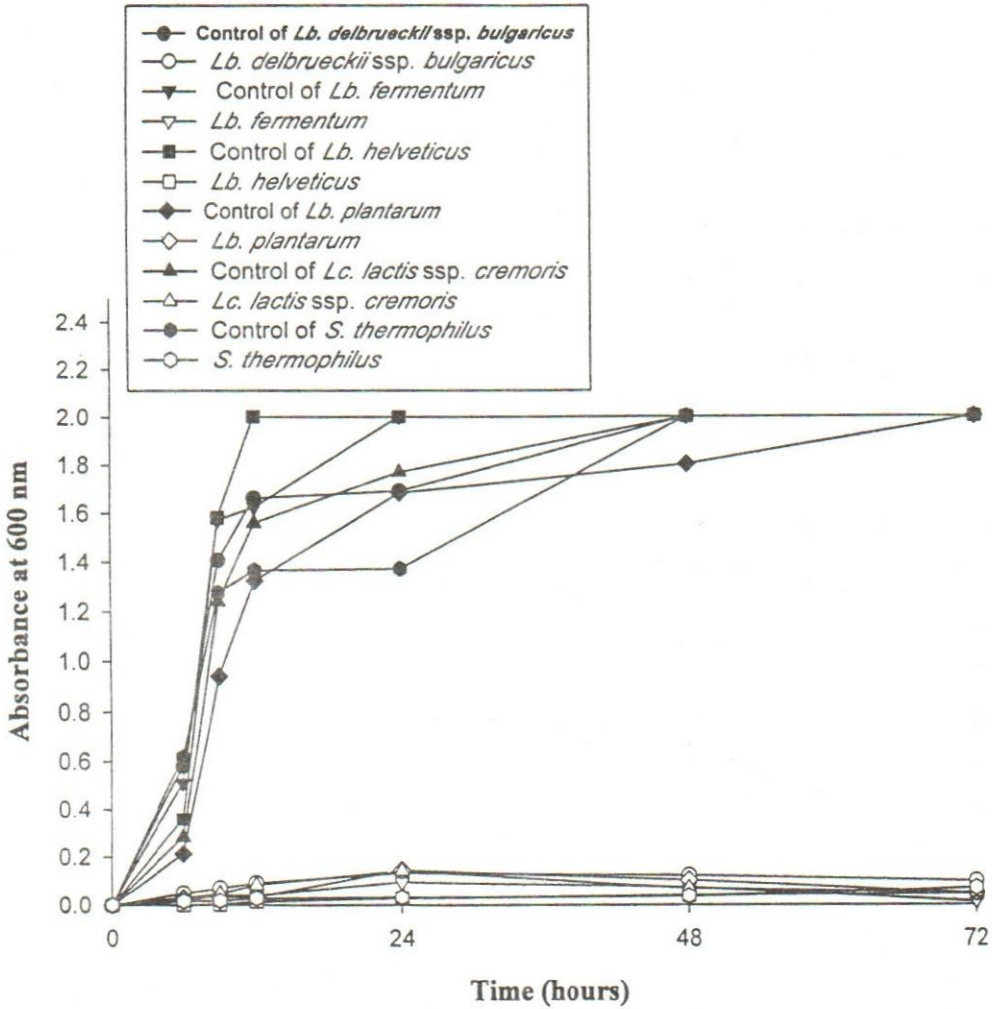


Fig. (7): Effect of furfural (150 mg 100 ml⁻¹) in MRS or M17 broth medium on the growth of lactic acid starter at 37°C.

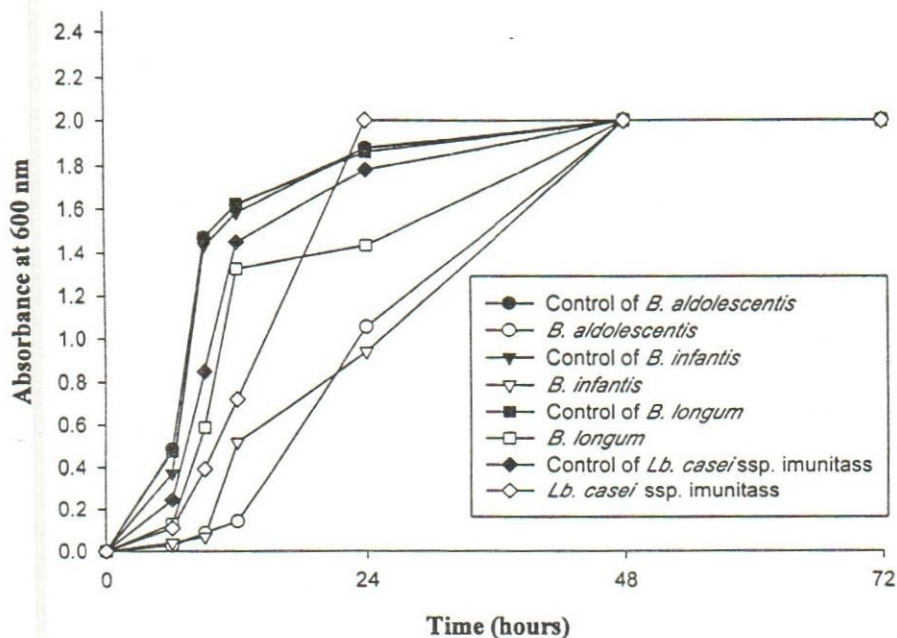


Fig. (8): Effect of furfural (50 mg 100 ml⁻¹) in MRS broth medium on the growth of probiotic bacteria at 37°C

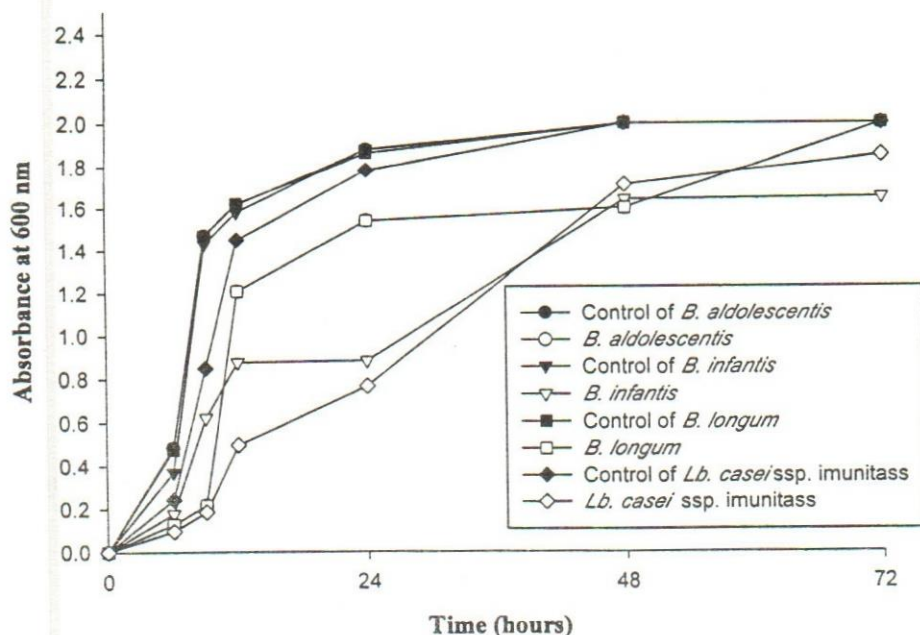


Fig. (9): Effect of furfural (100 mg 100 ml⁻¹) in MRS broth medium on the growth of probiotic bacteria at 37°C.

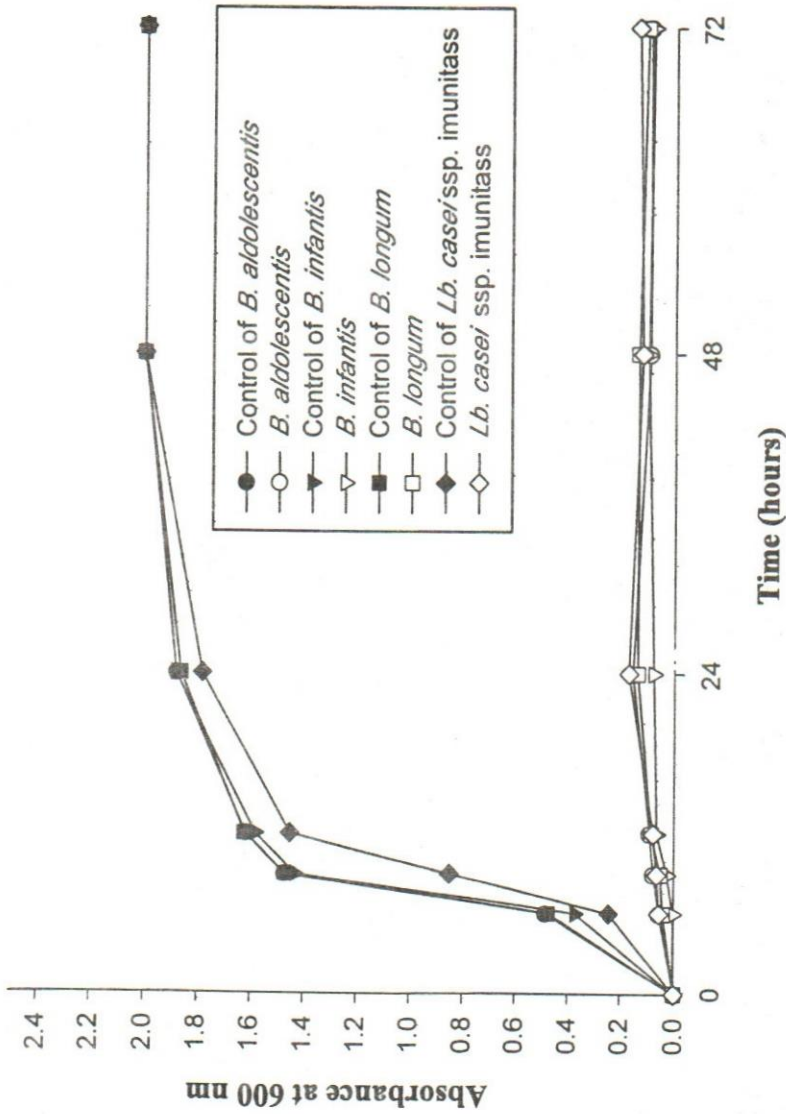


Fig. (10): Effect of furfural (150 mg 100 ml⁻¹) in MRS broth medium on the growth of probiotic bacteria at 37°C.

Table (3): Residue (mg 100 ml⁻¹) and % elimination of phytic acid, catechin and furfural as a result of growth of lactic acid starter in liquid media (37 ° C for 3 days)

Strain	Phytic acid			Catechin			Furfural *					
	150 mg 100 ml ⁻¹			20 mg 100 ml ⁻¹			50 mg 100 ml ⁻¹			100 mg 100 ml ⁻¹		
	Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)	
<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i>	0.00	100		0.35 ± 0.04	96.50		42.50 ± 0.24	15.00		100		0.00
<i>Lb. fermentum</i>	12.92 ± 0.02	87.08		0.34 ± 0.03	96.60		48.08 ± 0.07	3.84		97.59 ± 0.02		2.41
<i>Lb. helveticus</i>	19.39 ± 0.07	80.61		3.76 ± 0.02	62.40		41.92 ± 0.01	16.16		100		0.00
<i>Lb. plantarum</i>	0.00	100		2.28 ± 0.02	77.20		6.05 ± 0.08	87.91		38.84 ± 0.03		61.16
<i>Lc. Lactis</i> ssp. <i>cremoris</i>	7.72 ± 0.02	92.28		0.2 ± 0.02	98.00		40.96 ± 0.05	18.08		86.05 ± 0.04		13.95
<i>S. thermophilus</i>	27.78 ± 0.07	72.22		1.88 ± 0.02	81.20		46.71 ± 0.02	61.59		100		0.00

* At 150 mg 100 ml⁻¹: no growth

All results are means of three replicates

Table (4): Residue (mg 100 ml⁻¹) and % elimination of phytic acid, catechin and furfural as a result of growth of probiotic bacteria in liquid media (37 ° C for 3 days)

Strain	Phytic acid			Catechin			Furfural *					
	150 mg 100 ml ⁻¹			20 mg 100 ml ⁻¹			50 mg 100 ml ⁻¹			100 mg 100 ml ⁻¹		
	Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)		Residue (mg 100 ml ⁻¹)	Elimination (%)	
<i>B. acidolascensis</i>	12.19 ± 0.02	87.91		2.78 ± 0.06	72.20		15.19 ± 0.16	69.62		61.54 ± 0.08		38.46
<i>B. infantis</i>	17.15 ± 0.04	82.55		0.76 ± 0.04	92.40		36.34 ± 0.28	27.32		79.12 ± 0.10		20.88
<i>B. longum</i>	19.97 ± 0.04	81.03		0.88 ± 0.05	91.20		16.44 ± 0.16	67.12		64.84 ± 0.03		34.16
<i>Lb. casei</i> ssp. <i>imunitass</i>	1.44 ± 0.02	98.56		0.0	100		15.1 ± 0.08	69.80		34.61 ± 0.16		65.39

* At 150 mg 100 ml⁻¹: no growth.

All results are means of three replicates.

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منتجات الأغذية المتخمرة:

٢- تأثير بادئات حامض اللاكتيك والبكتريا الحيوية على بعض مضادات التغذية المصاحبة للأغذية

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درس تأثير ستة من بادئات حامض اللاكتيك (*Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactococcus lactis* ssp. *cremoris*, *Streptococcus thermophilus* و *Bifidobacterium adolescentis*, *Bifidobacterium infantis*) أربعة من البكتريا الحيوية (*Bifidobacterium longum*, *Lactobacillus casei* ssp. *imunitass*) على بعض المركبات الضارة الموجودة بالأغذية سواء بصورة طبيعية (حامض الفيتيك، الكاتشين) أو المتكونة نتيجة للمعاملات التصنيعية (الفرفيورال).

لم يؤثر أى من حامض الفيتيك والكاتشين بالتركيزات المختبرة على نمو البكتيريا وإنتاجها للحامض. أما الفرفيورال فقد أعتمد تأثيره على التركيز والسلالة المختبرة. فعند تركيز ٥٠ و ١٠٠ مجم ١٠٠ مل^{-١} لم يكن له تأثير على نشاط البكتيريا. كما تمكنت بكتيريا *Lactobacillus plantarum* من إزالة ٨٧,٩ و ٦١,١٦% من الفرفيورال على التوالي. تصيف هذه النتائج فائدة جديدة لبكتريا حامض اللاكتيك والبكتريا الحيوية نظرا لما تحدثه من تأثير خافض لبعض المواد الضارة المصاحبة للمواد الغذائية وبالتالي تقلل من تأثيرها على الجسم.