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SUSCEPTIBILITY OF LACTIC ACID STARTERS AND PROBIOTIC BACTERIA TOWARD SOME FOOD PRESERVATIVES AND ACIDULANTS

BY

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ABSTRACT

Food preservatives and acidulants used in the food industry can significantly influence the growth and cell viability of spoilage bacteria in the fermented products. The current study assess the growth and cell viability of lactic acid starter and probiotic cultures in the presence of food preservatives and acidulants. The behavior of six strains of lactic acid starter bacteria (*Lactobacillus* (*Lb.*) *fermentum, Lb. helveticus, Lb. plantarum, Lactococcus* (*Lc.*) *lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis* and *Streptococcus* (*S.*) *thermophilus*) and ten strains of probiotic bacteria (*Bifidobacterium* (*B.*) *aldolescentis, B. bifidum* Bb-11, *B. breve, B. infantis, B. lactis* Bb-12, *B. longum, Lb. acidophilus* La-5, *Lb. casei, Lb. paracasei*, and *Lb. casei* strain Immunitas) was studied in liquid media in the presence of some food preservatives and acidulants commonly used in food industry namely: sodium nitrite, sodium sulphite, sodium hypochlorite, capsicum, sodium benzoate, potassium sorbate, calcium propionate, formic, propionic and acetic acids.

The study revealed that some of these preservatives were not inhibitory to the valuable bacterial strains at the concentrations used for industrial manufacturing, while others, were of strain-dependent effects. Probiotic bacteria (*B. aldoscentis, B. infantis, B. lactis Lb. casei* and *Lb. paracasei*) were more resistant to food additives than lactic acid bacteria except *Lb. plantarum* and *Lb. fermentum*. Acetic, formic and propionic acids were ihhibitory at the concentrations used in food industry.

The tolerance of starters and probiotic bacteria to food preservatives or acidulants should be a selection criterion in order to achieve the best benefits.

Keywords: Fermented food, Probiotic, Lactic acid starters bacteria, Preservatives, Acidulants, bacterial growth, acid production.

INTRODUCTION

Probiotics are commonly defined as viable microorganisms that exhibit a beneficial effect on the health of the host when they are ingested. They are used in foods, especially in fermented dairy and food products, as well as in pharmaceutical preparations (Salminen *et al.*, 1998).

Some species and strains of the genus *Bifidobacterium* are considered probiotic and are used as active ingredients in functional food products. The health-promoting attributes of these microorganisms are numerous (Salminen *et al.*, 1999). To exert beneficial effects, these bacteria must overcome biological barriers, including acid in the stomach and bile in the intestine (Gilliland, 1978 and Lankaputhra & Shah, 1995), in order to at least temporally colonize specific parts of the intestinal tract. *Bifidobacterium*, a probiotic organism, and its β-galactosidase preparations are generally recognized as safe (GRAS) for use in foods and food systems

Probiotics are live microbial supplements, which beneficially affect the host by improving its intestinal microbial balance (Fuller, 1992). The health and nutritional benefits ascribed to bifidobacteria are many, including maintenance a healthy intestinal flora (Okamura *et al.*, 1986), synthesis of vitamin β-complex and absorption of calcium (Deguchi *et al.*, 1985), amelioration of diarrhea or constipation (Shornikova *et al.*, 1997 and Hilton *et al.*, 1997), antimicrobial production (Kang *et al.*, 1989) and immunity activation (Madara, 1997). Bifidobacteria also lower the levels of fecal bacterial enzymes, responsible for catalyzing the conversion of carcinogenic amines (Spanhaak *et al.*, 1998), reduction of the intestinal pH to reduce microbial activity (Gilliland, 1990), improve lactose utilization by lactose malabsorbers (Hughes and Hoover, 1995), reduce serum cholesterol levels (Tahri *et al.*, 1995) and reduce the antinutrients (phytate, catechins, and furfural) in foods (Shatta *et al.*, 2004).

The probiotic effects are not only influenced very strongly by the ability of the organism to survive in the host, but also to survive in the product. To achieve a beneficial effect, the amount of probiotic bacteria in the product has been prescribed as 10^6 cfu ml^{-1} (Dave & Shah, 1997, Pagano, 1998, Kurman & Rasic, 1991 and Kailasapathy & Chin, 2000) or the daily intake should be about 10^8 cfu ml^{-1} (Anonymous, 1992).

Potential applications of culture blends containing probiotic bacteria are numerous, e.g. sour cream, buttermilk, yogurt, powdered milk, spreads, frozen desserts, fruit juices, mayonnaise, dry fermented sausages, fermented meats or fish, deep fried LAB-fermented carrot chips, Japanese miso-fermented rice or soya bean, fermented mackerel minces, probiotic sunflower spread and peanut butter (Aukrust *et al.*, 1994, Gab-Alla & Gad, 2001, Khalil and Mansour, 1998, Lukow *et al.*, 2005, Pszczola, 2002, Wyers, 2004, and Yin *et al.*, 2002).

After several hundred years of safe use in fermented foods (Ballongue, 1998; Naidu *et al.*, 1999), lactic acid bacteria (LAB) are drawing increasing attention of both medical (Fuller, 1991 and Saavedra, 1995) and nutritional scientists (Lee & Salminen, 1995 and O'Sullivan *et al.*, 1992). Nowadays, lactic acid starter bacteria are widely used in combination with probiotic (*Bifidobacterium, Lactobacillus*) bacteria to manufacture fermented products.

Food additives are indispensable for (and sometimes characteristic of) milk drinks and other food products (Speer, 1998). These additives become a part of food in order to provide some very specific and precisely defined sensory characteristics such as taste, appearance, consistency, or shelf life (Nakazawa & Hosono, 1992, Gilliland, 1998 and Speer, 1998). Combinations of different bacterial strains belonging to the genera *Lactobacillus, Streptococcus* and *Bifidobacterium*, have been used traditionally in fermented products to promote human health (Prasad *et al.*, 1998 and Dunne *et al.*, 1999). These microorganisms are selected on the basis of medical, scientific and technological criteria (Collins *et al.*, 1998). The cultures used must tolerate the manufacturing process which they are to undergo so as to prepare a bioproduct (Charteris *et al.*, 1998) and maintain cell viability during storage. Strain survival in the product will depend on many factors such as pH, presence of preservatives (Charteris *et al.*, 1998) and even the occurrence of potential microbial growth inhibitors (Collins *et al.*, 1998).

Food processors use routinely various preservative like sulfur dioxide, sodium benzoate, potassium sorbate, formic acid and propionic acids. The effects of preservatives or acidulants on the growth of lactic acid starter and probiotic bacteria have not been extensively studied.

Therefore, the aim of the present study is to determine the influence of preservatives and acidulants commonly used in food industry on the growth of lactic acid starter bacteria (*Lactobacillus* (*Lb.*) *fermentum, Lb. helveticus, Lb. plantarum, Lactococcus* (*Lc.*) *lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis* and *Streptococcus* (*S.*) *thermophilus*) and probiotic bacteria (*Bifidobacterium* (*B.*) *aldolescentis, B. bifidum* Bb-11, *B. infantis, B. lactis* Bb-12, *B. longum. B. breve* and *Lb. acidophilus* La-5, *Lb. casei, Lb. paracasei*, and *Lb. casei* strain Immunitas)

MATERIALS AND METHODS

Bacterial strains

Bifidobacterium (*B.*) *bifidum* Bb-11, *B. lactis* Bb-12, *Lactobacillus* (*Lb.*) *acidophilus* La-5, *Lb. helveticus, Lactococcus* (*Lc.*) *lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris* and *Streptococcus* (S.) *thermophilus* were obtained from Chr. Hansen's Lab., Denmark. Other bacteria, *B. aldolescentis* (ATCC 15704), *B. infantis* (ATCC 15637), *B. longum* (ATCC 15707) were obtained from American Type Culture Collection, Rockville, Mich., USA. *Lb. casei* strain Immunitas (isolated from biofermented milk), *Lb. casei* (LS/B 32, Zahn Kl. Univ., Würzburg, Germany). *B. breve* and *Lb. fermentum* (3025162 1M) were obtained from Milchforschung Institute, Kiel, Germany. *Lb. paracasei* (DSM 5622) and. *Lb. plantarum* (DSM 20205) were provided by The German Collection of Microorganisms, Braunschweig, Germany. It should be emphasized that all these strains were checked up by the authors.

Culture media and incubation conditions

Lactobacilli (aerobiosis) and bifidobacteria (anaerobiosis), GasPak System-Oxide. Basingstoke, Hampshire, England) were cultured in MRS broth (Biolife) at 37°C. Lactococci and streptococci were grown in M17 (Biolife) at 37°C, aerobic incubation. For lactobacilli and bifidobacteria, cell enumerations were carried out on MRS agar (Biolife, 48 h at 37°C, aerobiosis and anaerobiosis, respectively). For lactococci and streptococci, viable counts (48 h at 37°C, aerobiosis) were performed on M17 agar.

Test materials and methodology

Preservatives:

Food preservatives, acidulants and their concentrations used in this study are shown in Table 1. Solutions of food preservatives and acidulants were sterilized by filteration through a sterile 0.45 μm cellulose nitrate filter (Sartorius, AG. 37070 Goettingen, Germany) and then added at the concentrations mentioned above to the growth media. The concentrations of these compounds are permissible according to GRAS (Lück, 1980 and Rahman, 1999). The effect of them on strain growth was assessed by the growth –in - liquid-medium assay (GLM assay) as follows: overnight broth cultures were inoculated (1%) (initial count, 10^6 to 10^7 cfu ml^{-1}) in test tubes containing MRS or M17 plus the preservatives or acidulants. The inoculated media were incubated at 37°C, aerobiosis for lactococci, streptococci, lactobacilli and anaerobiosis for bifidobacteria (GasPak System-Oxoid, Basingstoke, Hampshire, England). The relative growth (after 24 h) of the strains in the presence of each chemical was assessed and expressed as a percentage relatively to the control culture of optical density at 600 nm (O.D. $_{600nm}$) on a Spectronic 20D spectrophotometer (Milton Roy Company, USA) at intervals of 0, 3, 6, 12, 24, 48 and 72 hrs. Values of a relative growth were expressed as normal (A_{600nm} > 70%), weak (30% < A_{600nm} < 70) and none (A_{600nm} <30) (Vinderola *et al.*, 2002).

Table (1): The concentration (%) of some food preservatives and acidulants used in the present study

Food additives		Concentration (%)
Preservatives	Sodium nitrite (Merck)	0.006 and 0.2
	Sodium sulfite (Merck)	0.01 and 0.025 (0.0050 and 0.0127 % available SO_2 respectively)
	Sodium hypochlorite (Merck)	0.1 - 0.3% (available chlorine)
	Capsicum (Ransom)	0.01 and 0.03
	Potassium sorbate (Merck)	0.05 and 0.2
	Sodium benzoate (Merck)	0.05 and 0.2
	Calicum propionate (Merck)	0.1 and 0.3
Acidulants	Formic acid (Merck)	0.1 - 0.4
	Propionic acid (Merck)	0.1-0.3
	Acetic acid (Merck)	0.05 and 3

All food preservatives and acidulants were obtained from Merck (Food grade, Merck, Darmstadt, Germany), except concentrated capsicum, from Ransom (William Ransom & Sonple Hilchin, Herts, SG5 ILY, England)

The pH value was also measured as a criterion of bacterial growth using a pH meter (Jenway 3305, England).

Statistical analysis

The analysis of variance (ANOVA) and LSD were performed as described by Ott (1984).

RESULTS AND DISCUSSION

From tables (2- 7) it is seen that:

Capsicum at 0.01-0.03% did not interfere with the growth and acid production of lactic acid starter and probiotic bacteria expect *Lb. casei* strain Immunitas (at 0.03%). The same results, came with calcium propionate at 0.1-0.3% except *B. breve* at 0.3%. *B. longum* and *Lb. acidophilus* gave a weak growth at 0.3%. These differences between the results are significant ($p>0.01$).

Potassium sorbate at 0.05 did not affect the growth of all strains, while at 0.2% it inhibited the growth of *B. bifidum, B. lactis,* and *B. longum*. These differences between the results are significant ($p>0.01$).

Sodium benzoate at 0.05% did not exhibit any effect on the growth of all strains. At 0.2%, the growth of *Lb. helveticus, Lc. lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis, Str. themrophilus, B. berve, B. longum* and *Lb. casei* strain Immunitas was weak. These differences between the results are significant ($p>0.01$).

Sodium nitrite at 0.006 and 0.02% did not exhibit any affect on the growth and acid production of all stains except *B. breve*. Another exception was noted for *Lc. lactis* ssp. *lactis* and *B. bifidum* which grew weakly at 0.02%. These differences between the results are significant ($p>0.01$).

Sodium hypochlorite at 0.1 and 0.3 % was devoid of any effect on the growth of all strains. Similar results were recorded for Sodium sulfite at 0.01-0.025% (Free SO_2 is 0.005 and 0.0127%, respectively) i.e. it did not interfere with the growth of lactic acid starter or probiotic bacteria except *B. breve* at 0.0250 % (0.0127% free SO_2).

For acidulants, acetic acid at 0.5 and 3% inhibited the growth of all stains expect *B. infantis* which was not affected at 0.5%. These differences between the results are significant ($p>0.01$) (Tables 5 and 6). This results are in agreement with the conclusion given by Lock and Board (1994) who reported that the numbers of *B. bifidum* and *B. infantis* decreased markedly in mayonnaise; This decrease might be attributed to the bactericidal activity of acetic acid (vinegar) in mayonnaise.

Formic acid at 0.1 and 0.4% exhibited an inhibitory effect on almost all strains under our investigation; however *Lb. plantarum, B. aldoscentis* and *B. infantis* were not affected by formic acid at 0.1%, but at 0.4%, they were inhibited.

Propioninc acid at 0.1% did not interfere with the growth of lactic acid starter and probiotic bacteria with the exception of *Lb. helveticus, B. bifidum, B. breve, B. longum* and *Lb. acidophillus* La-5 which grew weakly. At 0.3% propioninc acid showed an inhibitory effect on all strains expect *Lb. plantarum, B. aldoscentis, B. infantis* and *Lb. casei* strain Immunitas These differences between the results are significant ($p>0.01$) (Tables 5 and 6).

The inhibition action of organic acids is related to the undissociated acid molecule. because the anions of the acids had no effect on survival of bacterial population (Corlett and Brown, 1980)

Table (2): Growth and pH value of lactic acid starter in liquid media in the presence of some food preservatives.

Food additives	Concentration %	*Lb. fermentum* Growth	*Lb. fermentum* pH	*Lb. helveticus* Growth	*Lb. helveticus* pH	*Lb. plantarum* Growth	*Lb. plantarum* pH	*Lc. lactis* ssp. *cremoris* Growth	*Lc. lactis* ssp. *cremoris* pH	*Lc. lactis* ssp. *lactis* Growth	*Lc. lactis* ssp. *lactis* pH	*S. thermophilus* Growth	*S. thermophilus* pH	LSD 0.01
Control[a]		100	4.12	100	4.27	100	3.56	100	3.79	100	3.65	100	4.22	
Capsicum	0.01	100^{a}	4.31	97^{a}	4.27	97^{a}	3.52	88^{b}	3.83	96^{ab}	3.65	98^{a}	4.20	8
	0.03	100^{a}	4.26	96^{ab}	4.34	88^{bc}	3.66	83^{c}	3.70	89^{bc}	3.76	97^{ab}	4.32	9
Calcium propionate	0.1	95^{a}	4.29	85^{ab}	4.31	87^{ab}	3.65	77^{b}	4.04	84^{ab}	3.88	90^{a}	4.29	11
	0.3	90^{a}	4.39	72^{c}	4.47	83^{ab}	3.78	73^{bc}	4.14	82^{abc}	4.10	76^{bc}	4.42	10
Potassium sorbate	0.05	97^{a}	4.16	92^{ab}	4.31	83^{bc}	3.57	81^{c}	3.82	83^{bc}	3.81	94^{a}	4.31	10
	0.2	84^{a}	4.50	73^{ab}	4.56	77^{ab}	3.57	70^{b}	4.01	73^{ab}	4.12	72^{b}	4.53	11
Sodium benzoate	0.05	100^{a}	4.19	99^{a}	4.19	83^{bc}	3.62	76^{c}	3.65	83^{bc}	3.80	97^{ab}	4.20	14
	0.2	77^{a}	4.53	65^{bc}	4.53	71^{ab}	3.98	66^{bc}	4.07	62^{c}	4.20	67^{bc}	4.62	6
Sodium hypochlorite	0.1	100^{a}	4.19	97^{a}	4.18	92^{ab}	3.57	83^{b}	3.77	96^{a}	3.52	100^{a}	4.15	10
	0.3	100^{a}	4.15	96^{a}	4.29	88^{ab}	3.59	79^{b}	3.57	89^{ab}	3.53	97^{a}	4.18	13
Sodium nitrite	0.006	95^{ab}	4.30	96^{ab}	4.34	91^{b}	3.56	71^{c}	3.93	78^{c}	3.90	100^{a}	4.38	8
	0.02	94^{a}	4.57	84^{b}	4.61	84^{b}	3.75	70^{c}	4.14	69^{c}	4.27	89^{ab}	4.63	5
sodium sulfite	0.01	90^{ab}	4.48	91^{ab}	4.21	93^{a}	3.58	87^{b}	3.56	91^{ab}	3.52	92^{ab}	4.19	5
	0.025	90^{a}	4.20	87^{ab}	4.41	83^{ab}	3.60	79^{b}	3.76	85^{ab}	3.79	89^{ab}	4.43	10

a: In MRS broth medium for lactobacilli or M17 broth medium for lactococci and streptococci without additives.

Normal (A_{600nm} > 70%), Weak (30% < A_{600nm} < 70%), None (A_{600nm} <30%) of the control culture 10^{8}- 10^{9} cfu ml^{-1}

All the results are means of triplicates.

Means having the same letter within each row are not significantly different ($p > 0.01$).

Table (3): Growth and pH value of probiotic bacteria (*Bifidobacterium* ssp.) in liquid media in the presence of some food preservatives.

Food additives		Probiotic bacteria												LSD 0.01
		B. aldoscentis		*B. bifidum*		*B. breve*		*B. infantis*		*B. lactis*		*B. longum*		
	Concentration %	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	
Control[a]		100	3.46	100	4.12	100	4.75	100	3.49	100	4.13	100	4.19	
Capsicum	0.01	97^{a}	3.56	85^{c}	4.18	100^{a}	4.38	100^{a}	3.53	94^{a}	4.16	85^{b}	4.16	8
	0.03	87^{bc}	3.68	83^{b}	4.31	91^{ab}	4.73	98^{a}	3.68	90^{abc}	4.29	81^{c}	4.18	9
Calcium propionate	0.1	91^{ab}	3.49	81^{bc}	4.29	70^{c}	4.48	100^{a}	3.50	87^{b}	4.28	81^{bc}	4.28	11
	0.3	88^{b}	3.63	73^{c}	4.41	4^{e}	5.60	100^{a}	3.62	74^{c}	4.35	59^{d}	4.41	10
Potassium sorbate	0.05	84^{cd}	3.60	100^{a}	4.25	92^{abc}	4.53	98^{ab}	3.51	89^{bc}	4.27	77^{d}	4.25	10
	0.2	77^{ab}	3.84	61^{c}	4.53	70^{bc}	4.70	88^{a}	3.81	69^{bc}	4.60	60^{c}	4.62	11
Sodium benzoate	0.05	80^{b}	3.59	92^{ab}	4.17	92^{ab}	4.35	91^{ab}	3.52	100^{a}	4.18	88^{ab}	4.17	14
	0.2	70^{c}	3.99	91^{a}	4.50	54^{e}	5.01	73^{bc}	4.00	78^{b}	4.18	63^{d}	4.55	6
Sodium hypochlorite	0.1	92^{ab}	3.56	89^{b}	4.18	100^{a}	4.66	100^{a}	3.63	96^{ab}	4..18	92^{ab}	4.14	10
	0.3	91^{a}	3.57	84^{ab}	4.19	77^{b}	4.87	95^{a}	3.66	92^{a}	4.20	87^{ab}	4.19	13
Sodium nitrite	0.006	88^{b}	3.60	82^{b}	4.46	0.5^{c}	5.67	100^{a}	3.61	89^{b}	4.36	82^{b}	4.45	8
	0.02	87^{b}	3.69	69^{d}	4.80	0^{e}	5.83	96^{a}	3.81	81^{c}	4.63	77^{c}	4.72	5
sodium sulfite	0.01	89^{b}	3.57	89^{b}	4.19	97^{a}	4.59	100^{a}	3.57	89^{b}	4.21	87^{b}	4.16	5
	0.025	86^{b}	3.61	74^{c}	4.57	52^{d}	4.64	98^{a}	3.57	87^{b}	4.44	75^{c}	4.47	10

a: In MRS broth medium for lactobacilli or M17 broth medium for lactococci and streptococci without additives.

Normal (A_{600nm} > 70%), Weak (30% < A_{600nm} < 70%), None (A_{600nm} <30%) of the control culture 10^{8}- 10^{9} cfu ml^{-1}.

All the results are means of triplicates.

Means having the same letter within each row are not significantly different ($p > 0.01$).

Table (4): Growth and pH value of probiotic bacteria (*Lactobacillus* ssp.) in liquid media in the presence of some food preservatives.

Food additives	Concentration %	Probiotic bacteria (*Lactobacillus* ssp.)								LSD 0.01
		Lb. casei		***Lb. paracaei***		***Lb. casei* strain. Immunitas**		***Lb. acidophilus***		
		Growth	pH	Growth	pH	Growth	pH	Growth	pH	
Control[a]		100	4.19	100	4.23	100	3.66	100	3.94	
Capsicum	0.01	100[a]	4.25	97[a]	4.25	100[a]	3.64	100[a]	3.95	NS
	0.03	99[a]	4.31	94[a]	4.31	68[b]	3.74	93[a]	3.98	9
Calcium propionate	0.1	89[a]	4.30	88[a]	4.30	95[a]	3.79	76[b]	4.32	11
	0.3	82[a]	4.44	80[a]	4.45	90[a]	3.79	58[b]	4.37	10
Potassium sorbate	0.05	91[ab]	4.35	89[b]	4.32	88[b]	3.71	100[a]	3.92	10
	0.2	73[bc]	4.50	70[c]	4.46	82[ab]	3.85	86[a]	4.11	11
Sodium benzoate	0.05	100[a]	4.18	98[a]	4.20	83[b]	3.74	98[a]	3.96	14
	0.2	71[a]	4.51	70[a]	4.62	59[b]	3.95	75[a]	4.32	6
Sodium hypochlorite	0.1	100[a]	4.12	100[a]	4.14	100[a]	3.57	89[b]	4.10	10
	0.3	100[a]	4.13	95[a]	4.19	93[ab]	3.58	81[b]	4.18	13
Sodium nitrite	0.006	91[a]	4.32	91[a]	4.34	92[a]	3.70	88[a]	4.05	NS
	0.02	86[a]	4.57	82[a]	4.64	82[a]	4.01	87[a]	4.19	NS
sodium sulfite	0.01	93[ab]	4.17	88[bc]	4.18	98[a]	3.61	83[c]	4.11	5
	0.025	81[b]	4.44	83[b]	4.42	96[a]	3.66	80[b]	4.39	10

Table (5): Growth and pH value of lactic acid starter bacteria in liquid media in the presence of acetic, formic and propionic acids

Bacteria	Control[a]	Acetic acid		Formic acid		Propionic acid	
		0.5%	3%	0.1%	0.4%	0.1%	0.3%
Lb. fermentum							
Growth	100	3.0[d]	3.0[a]	15[d]	3.0[a]	79[ab]	10[c]
H	4.12	4.40	3.64	4.62	3.79	4.12	4.59
Lb. helveticus							
Growth	100	5[d]	1[ab]	11[d]	0.1[b]	68[c]	8[c]
pH	4.27	4.46	3.66	4.66	3.79	4.11	4.58
Lb. plantarum							
Growth	100	40[a]	2[ab]	78[a]	2[ab]	83[a]	79[a]
pH	3.56	4.05	3.62	3.65	3.77	3.50	3.52
Lc. lactis* ssp. *cremoris							
Growth	100	10[c]	2[ab]	40[c]	0.3[b]	70[bc]	57[b]
pH	3.79	4.34	3.64	4.24	3.79	3.76	3.96
Lc. lactis* ssp. *lactis							
Growth	100	22[b]	0.5[b]	60[b]	1.0[ab]	79[ab]	64[b]
pH	3.65	4.29	3.62	3.96	3.84	3.70	3.84
S. thermophilus							
Growth	100	5[d]	1.0[ab]	13[d]	0.0[b]	75[abc]	11[c]
pH	4.22	4.45	3.62	4.67	3.82	4.15	4.59
LSD at 0.01		4	2	8	2	9	7

a: In MRS broth medium for lactobacilli or M17 broth medium for lactococci and streptococci without additives.

Normal (A_{600nm} > 70%), Weak (30% < A_{600nm} < 70%), None (A_{600nm} <30%) of the control culture 10^8- 10^9 cfu ml^{-1}.

All the results are means of triplicates.

Means having the same letter within each row are not significantly different (p > 0.01).

pH:

The pH dropped during the fermentation periods in all cases. After 24 h, the pH it was below 4 for *Lb. plantarum. Lc. lactis ssp. cremoris, Lc. lactis ssp. lactis, B. aldoscetis, B. infantis* and *Lb. casei* strain Immunitas, while for the rest of stains it was above 4.0. The fastest and greatest reduction was seen for *Lb. plantarum.*

Table (6): Growth and pH value of probiotic bacteria in liquid media in the presence of acetic, formic and propionic acids

Bacteria	Control[a]	Acetic acid		Formic acid		Propionic acid	
		0.5%	3%	0.1%	0.4%	0.1%	0.3%
B. aldolescentis							
Growth	100	68 [b]	4.0 [a]	80 [b]	1.0 [a]	86 [b]	84 [b]
pH	3.46	**3.81**	**3.63**	**3.58**	**3.72**	**3.40**	**3.42**
***B. bifidum* Bb-11**							
Growth	100	4.0 [c]	1.0 [a]	2.0 [d]	1.0 [a]	65 [cd]	5 [c]
pH	4.12	**4.45**	**3.73**	**4.76**	**3.80**	**4.12**	**4.63**
B. breve							
Growth	100	2.0 [c]	1.0 [b]	2.0 [d]	0.2 [a]	5.0 [e]	0.6 [c]
pH	4.75	**4.50**	**3.65**	**4.88**	**3.78**	**5.17**	**4.69**
B. infantis							
Growth	100	83 [a]	1.0 [b]	98 [a]	2.0 [a]	100 [a]	100 [a]
pH	3.49	**3.62**	**3.66**	**3.57**	**3.77**	**3.35**	**3.45**
***B. lactis* Bb-12**							
Growth	100	2.0 [b]	1.0 [c]	12 [c]	1.0 [a]	71 [c]	4.0 [c]
pH	4.13	**4.45**	**3.63**	**4.68**	**3.80**	**4.07**	**4.61**
B. longum							
Growth	100	**3.0 [c]**	**1.0 [b]**	**4.0 [cd]**	**1.0 [a]**	**59 [d]**	**6.0 [c]**
pH	4.19	4.45	3.62	3.76	3.76	4.07	4.59
***Lb. acidophilus* La-5 Growth**	100	1.0 [b]	1.0 [b]	7.0 [c]	0.60 [a]	67 [c]	3.0 [c]
pH	3.94	**4.49**	**3.57**	**4.74**	**3.76**	**4.09**	**4.53**
***Lb. casei* Growth**	100	3.0 [b]	2.0 [b]	11 [bc]	0.7 [a]	77 [b]	11 [b]
pH	4.19	**4.44**	**3.63**	**4.63**	**3.77**	**4.08**	**4.58**
***Lb. casei* strain Immunitas**							
Growth	100	44 [a]	4 [a]	71 [a]	2.0 [a]	87 [a]	70 [a]
pH	3.66	**4.12**	**3.62**	**3.98**	**3.77**	**3.64**	**3.77**
Lb. paracasei							
Growth	100	2.0 [b]	2.0 [b]	16 [b]	0.2 [a]	75 [bc]	13 [b]
pH	4.23	**4.51**	**3.66**	**4.67**	**3.81**	**4.09**	**4.61**
LSD 0.01		**4**	**2**	**8**	**NS**	**9**	**7**

a: In MRS broth medium for lactobacilli or M17 broth medium for lactococci and streptococci without additives.

Normal (A_{600nm} > 70%), Weak (30% < A_{600nm} < 70%), None (A_{600nm} <30%) of the control culture 10^8- 10^9 cfu ml^{-1}

All the results are means of triplicates

Means having the same letter within each row are not significantly different ($p > 0.01$).

The main inhibitory action of acetic (pk_a 4.75) and propionic acids (pk_a 4.87) is due to neutralization of the electrochemical potential of cell membranes and lowering the intracellular pH. The acetic acid has been found more effective in synergistic combinations and under anaerobic conditions (ICMSF, 1980). Since the pk_a of acetic acid is < 5.0, it exists mostly in the dissociated form in broth media.

For formic acid, the percentage of undissociated form at pH 4.96 and 4.76 is 0.094 and 0.361, respectively. The undissociated acid is considered to have a greater effect on the microbial growth than the dissociated one. Generally, the undissociated acid at 0.1% is adequate to inhibit the microbial growth in lab media when conditions are near optimal (Booth and Kroll, 1989, Chichester and Tanner, 1972). The rest of preservatives or acidulants are shown in Table (7).

Conclusively, the tested substances namely sodium benzoate, sodium nitrite, sodium hypochlorite, capsicum, sodium sulphite, potassium sorbate, and propionic acid and calcium propionate did not interfere with the growth of the lactic acid starter and probiotic bacteria strains used in this study, at the concentrations permissible in food industry. Some other acidulants (formic acid and acetic acid) were inhibitory, but only at the highest concentrations tested.

Table (7): Calculated undissociated proportions of some preservatives and acidulants at the pH of MRS or M17 broth.

Preservatives or acidulants	pH medium	pk_a	Concentration (%)	Undissociated form
Calcium propionate	5.61 5.79	4.87	0.10 0.30	0.082 0.264
Potassium sorbate	5.69 5.79	4.80	0.05 0.20	0.044 0.180
Sodium benzoate	6.20 5.90	4.20	0.05 0.20	0.049 0.196
Sodium nitrite	5.88 6.02	3.29	0.006 0.02	9.99×10^{-3} 0.019
Sodium sulphite	6.02 6.09	1.8	0.01 0.025	9.99×10^{-3} 0.025
Acetic acid	4.44 3.61	4.76	0.5 3.0	0 0
Formic acid	4.96 4.76	3.75	0.10 0.40	0.094 0.361
Propionic acid	5.32 4.77	4.87	0.10 0.30	0.065 0

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حساسية بعض بادئات بكتيريا حمض اللاكتيك والبكتيريا الحيوية تجاه بعض المواد الحافظة والاحماض العضوية المضافة للاغذية

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تستخدم المواد الحافظة والاحماض العضوية كثيرا في صناعة الغذاء لما لها من تأثير على نمو وحيوية البكتيريا المسببة للتلف في المنتجات المتخمرة.لذلك كان الهدف من الدراسة هو معرفة مدى حساسية ستة من بادئات بكتيريا حمض اللاكتيك وهى:

Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus plantarum, Lactococcus lactis ssp. *lactis, Lactococcus lactis* ssp. *cremoris*, and *Streptococcus thermophilus*.

وعشر سلالات من البكتريا الحيوية:

Bifidobacterium aldolescentis, Bifidobacterium bifidum Bb-11, *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium lactis* Bb-12 *Bifidobacterium longum, Lactobacillus acidophillus* La-5, *Lactobacillus casei* strain Immunitas, *Lactobacillus casei* and *Lactobacillus paracasei*.

وذلك فى بيئة سائلة فى وجود بعض المواد الحافظة مثل بنزوات الصوديوم، كبريتيت الصوديوم، هيبوكلوريت الصوديوم، مستخلص الفلفل الحار (الكابسيكم)، سوربات البوتاسيوم، بروبيونات الكالسيوم، حمض الخليك، الفورميك و البروبيونيك بتركيزات تستخدم بالفعل فى الصناعة.

أوضحت النتائج إن إضافة مثل هذه المواد الحافظة السابق الإشارة إليها ليست مثبطة أو موقفة لنمو البكتيريا النافعة بالتركيزات المستخدمة فى الصناعة وان توقف تأثيرها على حسب السلالة المختبرة.

كانت البكتيريا الحيوية (*Bifidobacterium aldolescentis,* *Bifidobacterium infantis, Lactobacillus casei* and *Lactobacillus paracasei*) أكثر مقاومة لهذه الإضافات الغذائية من بادئات بكتيريا حامض اللاكتيك باستثناء (*Lactobacillus fermentum, Lactobacillus plantarum*). بينما كان للأحماض العضوية تأثير مثبط أو موقف للنمو و ذلك على حسب السلالة المختبرة.

مما سبق يمكن القول بأن مقاومة البكتريا الحيوية وبادئات حامض اللاكتيك للمواد الحافظة والاحماض العضوية المضافة للغذاء تعتبر مقياسا لابد أن يؤخذ فى الاعتبار عند استخدامها فى صناعة الغذاء أو عند التغذية لتحقيق التأثيرات النافعة لهذه البكتيريا.