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Effect of Chicory Water Extract and Lactulose Syrup on Growth and Viability of *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss* in Skim Milk

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Abstract: The present research aimed to study the effect of chicory extract and lactulose syrup on the growth and viability of three probiotic organisms (*Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss*) and regular yoghurt cultures (*Streptococcus* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus) in skim milk. Three concentrations of probiotic inoculums and prebiotic ingredients (0.5, 1 and 1.5% for probiotic and 1, 3 and 5% for prebiotic) were used to select the suitable ratio in the application experiments. The tested cultures with or without prebiotic were incubated on 42 and 37°C. The obtained results showed that the growth of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*) were affected by the incubation temperature in all of the tested bacteria and inoculation percentage in all of probiotic strains individually or mixed with traditional yoghurt cultures. The percentage of viability in traditional yoghurt cultures were increased when cultures were incubated at 42°C compared with the cultures incubated at 37°C and different from the viability of *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss* which, were increased with incubation at 37°C compared with incubation at 42°C. Also, data found that the generation time of all probiotic cultures was decreased in the presence of chicory extract and lactulose syrup due to the enhancement of growth in probiotic cultures. The obtained results can help our further studies for selection the optimum inoculation percentage and incubation temperatures of tested strains individually and/or mixed with traditional yoghurt cultures in the application experiments.

Key words: Lactulose syrup, skim milk, yoghurt culture

INTRODUCTION

Probiotic bacteria have been defined as live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance (Fuller, 1989). Probiotic bacteria are increasingly utilised in human food as well as in animal feed products (Fuller, 1999; Sanders and Huis in't Veld, 1999). However, composition of the intestinal microbiota is poorly known, which hinders understanding of the probiotic functions (Tannock, 1999). A probiotic strain should be of host origin, acid- and bile-fast, adhere to the gut epithelial tissue, persist in the gastrointestinal tract for short periods, produce antimicrobial substances, modulate immune responses and influence the metabolic activities of the gut (Dunne *et al*., 1999).

Chicory known botanically as *Cichorium intybus* L., is a perennial member of the daisy family (Asteraceae). The major component of chicory root is inulin, which is a polymer of fructose with

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-(2-1) glycosidic linkages. Inulin belongs to the fructan family; naturally occurring fructans are important storage of carbohydrates, widely found in various flowering plants. Fructans are present in noticeable amounts in chicory, *Jerusalem artichokes* (up to 20%), salsify, asparagus and onions (Nilsson *et al.*, 1988; Rumessen *et al.*, 1990). Because inulin is soluble in water and not hydrolyzed by human digestive enzymes, it is expected to behave like a soluble fiber and to have a hypolipidemic effect. Both the fermentability and the bifidogenic effect of chicory fructooligosaccharides have been confirmed *in vivo* human studies that were performed by feeding human volunteers a standard diet containing chicory fructooligosaccharides (15 g day^{-1} for 15 day). A significant increase in the bifidobacteria population and a profound modification of the composition of the fecal flora were observed by Gibson *et al.* (1995). In human nutrition, inulin could constitute a promising source of soluble fiber either when present naturally in the food or when added to the diet (Roberfroid, 1996). In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose and reduce the levels of cholesterol and serum lipids (Coudray *et al.*, 1997).

Lactulose is a synthetic sugar, which does not occur naturally. The disaccharide lactulose (galacto-fructose) is synthesized from lactose (galacto-glucose) by isomerisation of glucose to fructose. Each molecule of galactose is linked to a molecule of fructose. These linked monosaccharides are the reason why lactulose cannot be degraded by animal or human enzymes and reaches the colon unchanged with only few available calories. Lactulose reaches the colon unchanged, where the bacteria of the intestines metabolise it, producing a number of short chain fatty acids. This process initiates a large number of effects in the gastrointestinal tract. Lactulose has been successfully applied in the treatment of constipation since 1959. Lactulose has proven its efficiency as a gentle as well as highly effective medication in the regulation of intestinal activity and regular bowels. It can also play an important role to support intestinal activities in the pre- and post-operative phases. Any foodstuff that reaches the colon, e.g., non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a candidate prebiotic. Certain non-digestible carbohydrates seem authentic prebiotics. Fructooligosaccharides (FOS) are β-d-fructans with various degrees of polymerisation. A number of other non-digestible oligosaccharides have now been developed, for which there is some evidence of their prebiotic effect. These include gluco-oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides,isomalto-ligosaccharides,xylo-oligosaccharidesandsoybean-oligosaccharides(Gibson, 2004; Ito *et al.*, 1993a, b; Imaizumi *et al.*, 1991).

The present research aimed to study the effect of chicory extracts and lactulose syrup on the growth and viability of three probiotic strains (*Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss*) and traditional yoghurt cultures (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*) in skim milk for selection the optimum percentages from probiotic and prebiotic with economic advantages and beneficial effect with application experiments in yoghurt.

MATERIALS AND METHODS

Media and Chemicals

Spray dried skim milk (low heat) was obtained from Valio, Finland. This study was conducted during the period extending between 2004 and 2006 through M.Sc. project under supervision of Dairy Science Departments in Faculty of Agriculture, Cairo University and National Research Centre, Egypt.

Preparation of Chicory Water-Soluble Extract

Chicory water-soluble extract was prepared according the methods described by Kim and Shin (1998). After extraction chicory extract was added to the experimental media.

Lactolose syrup (52.40% lactulose, 4.3% lactose and 2.5 galactose) was obtained was manufactured by Egyptian International Pharmaceutical Industries Company (EIPICO), Cairo, Egypt.

Table 1: Addition percentage for inoculation and incubation temperature design of different starter cultures

Incubation temperature	Cultures addition (%)		Culture	
42 and 37 °C	3	YC	This is the recommended percentage which used in most of dairy factories	
	0.5	Lp	Lc	Lr
		Lp+YC	Lc +YC	Lr+YC
	1	Lp	Lc	Lr
		Lp+YC	Lc +YC	Lr+YC
	1.5	Lp	Lc	Lr
		Lp+YC	Lc +YC	Lr+YC

YC: - Traditional yoghurt cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Lp:- *Lactobacillus plantarum* Lc:- *Lactobacillus casei* Lr:- *Lactobacillus rhamnosuss*

Table 2: Experimental design using prebiotic ingredients

Prebiotic	Concentration (%)	Cultures 3% YC + (0.5, 1 and 1.5%) Lp, Lc and Lr
Lactulose syrup	1	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
	3	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
	5	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
Chicory extract	1	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
	3	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
	5	YC-Lp-Lc-Lr individual
		YC+Lp-YC+Lc-YC+Lr
None		Control 1- Control 2 - Control 3

YC: Traditional yoghurt cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Lp:- *Lactobacillus plantarum* Lc:- *Lactobacillus casei* Lr::- *Lactobacillus rhamnosuss*, Control:- cultures without any prebiotic

Traditional and Probiotic Cultures

Starter cultures of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from Chr. Hansen`s Lab., Copenhagen Denemark. Strains of *Lactobacillus plantarum* B-531, *Lactobacillus casei* B-444 and *Lactobacillus casie* subsp. *rhamnosuss* B-445 were provided by Northern Regional Research Laboratory, Illinois, USA (NRRL).

Experimental Design for Selection of the Optimum Addition Percentage of Cultures and Incubation Temperature

Table 1 show the addition percentage for inoculation and incubation temperature design of different starter cultures. Three inoculation percentage of three probiotic cultures (0.5, 1 and 1.5%, respectively) were used in the experiments. One of inoculation percentage of yoghurt cultures was used (3%). This is the recommended percentage which used in most of dairy factories.

Microbiological Analysis

The counts of *Streptococcus thermophilus* were enumerated on M17 agar according to Dave and Shah (1996). MRS agar (Oxoid, UK adjusted to pH 5.2 and aeaerobic incubation at 42°C for 72 h were used for the differential enumeration of *Lb. delbrueckii* subsp. *bulgaricus*. Total probiotic organisms were enumerated on medium according methods described by Spencer and de-Spencer, 2004.

Determination of % Viability and Mean Doubling Time of Probiotic Organisms in the Presences of Lactulose Syrup and Chicory Extract

The enumeration of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss* (Donkor *et al*., 2005; Ravula and Shah, 1998; Young and Seung, 2006).

RESULTS AND DISCUSSION

The composition of the chicory roots glucidic fraction by the analysis using HPLC with the column Sugar Pak were reported by Beirão-da-Costa *et al.* (2004) in Table 3.

The results revealed that the growth of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures (*Streptococcus thermophilus, Lactobacillus bulgaricus*) were affected with different incubation temperatures in all of the tested bacteria and inoculation percentage in all of probiotic strains individually or mixed with traditional yoghurt cultures. The initial viability corresponding to 100% for all strains (Table 4).

The percentage of viability in yoghurt cultures (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*) were increased when cultures were incubated at 42°C and the viability value was 45.84±12.72 compared with the cultures incubated at 37°C and the viability value was 37.11± 8.56. In the contrary, the percentage of viability in probiotic cultures (*Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss*) were increased when cultures were incubated at 37°C compared with the cultures incubated at 42°C.

The viability values of *Lactobacillus plantarum* individually were 104.29±84.85, 79.67±62.70 and 80.62±56.57 at 37°C and 96.49±81.91, 57.46±41.67 and 67.47±55.65 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively, while the viability values of *Lactobacillus plantarum* mixed with traditional yoghurt cultures were 39.64±22.22, 11.19±6.66 and 9.05±5.57 at 37°C and 35.17±21.46, 9.972±6.29 and 8.86±5.72 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively.

Table 3: Composition of the chicory roots glucidic fraction, analyzed by HPLC with the column Sugar Pak I

	Gf	Ff	S	Gt	Ft	Gi	Fi	Gi+Fi	Inulin
	g/100 g dry matter								
Mean	0.10	0.80	2.10	5.60	70.40	4.40	68.50	72.90	65.60
SD±	0.08	0.14	0.05	0.19	2.79	0.12	2.84	2.95	2.66

SD = Standard deviation (n = 3) Gf-free glucose, Ff-free fructose, S-sucrose, Gt-total glucose, Ft-total fructose, Gi- glucose from inulin hydrolysis, Fi-fructose from inulin hydrolysis

Table 4: Percentage viability of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures grown in skim milk during incubation with different inoculation percentage and temperature

		Viability (%)					
Cultures	Inoculation (%)	37°C	SD(±)	CV	42°C	SD (±)	CV
YC	3	37.105	8.56	23.06	45.84	12.72	27.74
LP	0.5	104.29	84.85	81.35	96.49	81.91	84.88
	1	79.67	62.70	78.69	57.46	41.67	72.52
	1.5	80.62	56.57	70.16	67.47	55.65	82.48
LC	0.5	72.33	48.55	67.12	70.08	49.14	70.11
	1	73.52	52.41	71.28	65.58	47.01	71.60
	1.5	75.28	47.53	63.13	64.92	36.02	55.48
Lr	0.5	102.91	58.34	56.69	93.76	66.61	71.04
	1	111.67	68.35	61.20	99.63	66.66	66.90
	1.5	82.35	45.75	55.55	73.47	45.68	62.17
YC +LP	0.5	39.64	22.22	56.05	35.17	21.46	61.017
	1	11.19	6.66	59.51	9.972	6.291	63.08
	1.5	9.05	5.57	61.54	8.86	5.72	64.55
YC+LC	0.5	70.00	26.40	37.71	56.74	27.45	48.37
	1	65.83	20.82	31.62	53.75	30.44	56.63
	1.5	36.76	12.89	35.06	33.84	12.92	38.17
YC+Lr	0.5	44.09	21.856	49.57	41.39	20.60	49.77
	1	47.72	23.78	49.83	44.27	22.11	49.94
	1.5	43.26	23.66	54.69	40.38	21.75	53.86

YC = Traditional yoghurt cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Lp: *Lactobacillus plantarum* Lc: *Lactobacillus casei* Lr: *Lactobacillus rhamnosuss*, SD: Standard Deviation, CV: Coefficient Variation

The viability values of *Lactobacillus casei* individually were 72.33±48.55, 73.52±52.41 and 75.28±47.53 at 37°C and 70.08±49.14, 65.58±47.01 and 64.92±36.02 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively, while the viability values of *Lactobacillus casei* mixed with traditional yoghurt cultures were 70.0±26.40, 65.83±20.82 and 36.76±12.89 at 37°C and 56.74±27.45, 53.75±30.44 and 33.84±12.92 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively. Also, The viability values of *Lactobacillus rhamnosuss* individually were 102.91±58.34, 111.67±68.35 and 82.35±45.75 at 37°C and 93.76±66.61, 99.63±66.66 and 73.47±45.68 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively, while the viability values of *Lactobacillus rhamnosuss* mixed with traditional yoghurt cultures were 44.09±21.86, 47.72±23.78 and 43.26±23.66 at 37°C and 41.39±20.60, 44.27±22.11 and 40.38±21.75 at 42°C with inoculation percentage of 0.5, 1 and 1.5%, respectively.

The obtained results showed that the pH values were decreased with increasing of the incubation time in all the tested microorganisms (Table 5).

Table 6 and 7 show the generation time of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and traditional yoghurt cultures in the presence of chicory extract and lactulose syrup. The obtained results found that the generation time of all tested cultures were decreased either in the presence of chicory extract or lactulose syrup.

The mean values of generation time (GT) of *Lactobacillus plantarum* individually were 111.52±0.671 and 225.42±0.11 at 37 and 42°C, without the addition of chicory extract or lactulose syrup, but the mean values of GT in the presence of chicory extract and lactulose syrup (1, 3 and 5%) were 66.10±38.70, 50.36±32.40 and 50.11±27.80 at 37°C and 119.44±26.4, 86.58±3.28 and 84.44±4.44 at 42°C in presence of chicory extract and were 92.76±8.591, 69.90±7.921 and 60.100±9.64 at 37°C and 103.87±1.705, 80.14±10.38 and 74.61±14.74 at 42°C in presence of lactulose syrup, respectively.

The mean values of generation time of *Lactobacillus casei* individually were 136.20±28.01 and 150.35±0.57 at 37 and 42°C, for respectively, without the addition of chicory extract or lactulose syrup, but the mean values of GT in the presence of chicory extract and

Table 5: Changes of pH values during incubation of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* with different inoculation percentage and temperature

		pH					
		37°C			42°C		
Cultures	Inoculation (%)	Initial	4 h	24 h	Initial	4 h	24 h
YC	3	6.50	4.60	4.56	6.50	4.50	4.45
Lp	0.5	6.50	5.20	5.05	6.50	5.30	5.10
	1	6.50	5.10	5.00	6.50	6.20	5.00
	1.5	6.49	5.10	5.00	6.50	5.10	5.00
Lc	0.5	6.50	5.39	5.10	6.63	6.43	5.30
	1	6.50	5.30	5.10	6.60	6.30	5.20
	1.5	6.50	5.22	5.10	6.50	6.20	5.10
Lr	0.5	6.50	6.30	5.10	6.52	6.40	5.17
	1	6.45	6.20	5.00	6.50	6.30	5.10
	1.5	6.30	6.00	5.00	6.50	5.10	5.00
YC + Lp	0.5	6.20	5.10	4.60	6.20	4.50	4.30
	1	6.00	5.00	4.56	6.00	4.51	4.32
	1.5	6.00	5.00	4.46	6.00	4.48	4.25
YC + Lc	0.5	6.20	5.00	4.50	6.20	4.70	4.50
	1	6.01	5.00	4.50	6.10	4.50	4.40
	1.5	6.00	4.90	4.40	6.00	4.46	4.36
YC + Lr	0.5	6.20	5.00	4.60	6.10	4.61	4.52
	1	6.01	5.00	4.56	6.00	4.56	4.43
	1.5	6.00	4.80	4.50	6.00	4.50	4.30

YC: Yoghurt Cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, Lp: *Lactobacillus plantarum* Lc: *Lactobacillus casei*, Lr: *Lactobacillus rhamnosuss*

Table 6: Generation time of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures during incubation time in the presence of chicory extract

		Generation time					
		Chicory extract					
		37°C			42°C		
Cultures	Prebiotic (%)	Mean	SD	CV	Mean	SD	CV
YC	-	139.31	63.20	45.36	140.40	0.56	0.390
	1	99.77	44.27	44.37	129.18	23.78	18.400
	3	79.51	40.10	50.43	111.20	0.28	0.250
	5	58.74	28.50	48.51	90.55	0.636	0.700
Lp	-	111.52	0.671	0.60	225.42	0.11	0.040
	1	66.10	38.70	58.54	119.44	26.40	22.100
	3	50.36	32.40	64.33	86.58	3.28	3.788
	5	50.11	27.79	55.45	84.44	4.43	5.240
Lc	-	136.20	28.01	20.56	150.35	0.57	0.370
	1	82.83	31.01	37.43	92.25	12.05	13.060
	3	49.26	26.40	53.59	95.80	14.79	15.430
	5	54.85	19.16	34.93	94.19	1.32	1.400
Lr	-	139.48	0.68	0.48	186.43	139.48	74.810
	1	86.68	32.18	37.12	165.27	34.65	20.960
	3	74.72	29.25	39.14	104.32	16.63	15.940
	5	51.44	22.26	43.27	90.02	7.23	8.030
YC + Lp	-	124.15	24.02	19.34	114.28	0.30	0.260
	1	64.44	35.527	55.13	105.58	1.20	1.130
	3	62.43	36.54	58.52	78.91	9.24	11.700
	5	51.93	21.81	41.99	75.55	9.07	12.000
YC + Lc	-	95.20	45.80	48.10	126.43	0.31	0.240
	1	70.96	56.31	79.35	84.06	0.23	0.270
	3	59.10	33.36	56.44	86.62	0.87	1.010
	5	60.59	32.40	53.47	76.119	0.80	1.0510
YC + Lr	-	135.11	56.11	41.52	183.01	94.02	51.370
	1	90.77	39.74	43.78	108.36	0.10	0.100
	3	75.50	32.17	42.60	80.73	8.42	10.430
	5	74.63	5.61	7.51	71.30	7.82	0.400

YC: Traditional yoghurt cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Lp: *Lactobacillus plantarum*, Lc: *Lactobacillus casei*, Lr:: *Lactobacillus rhamnosuss*, SD: Standard Deviation, CV: Coefficient variation

lactulose syrup (1, 3 and 5%) were 82.83±31.01, 49.26±26.40 and 54.85±19.16 at 37°C and 92.25±12.05, 95.80±14.79 and 94.19±1.32 at 42°C in presence of chicory extract, while the mean values were 109.49±32.25, 72.43±0.61 and 63.09±12.93 at 37°C and 105.18±1.97, 92.05±13.27 and 86.63±6.34 at 42°C in presence of lactulose syrup. Moreover, the mean values of generation time of *Lactobacillus rhamnosuss* individually were 139.48±0.68 and 186.43±0.52 at 37 and 42°C, respectively, without the addition of chicory extract or lactulose syrup, but the mean values of G.T. in the presence of chicory extract and lactulose syrup (1, 3 and 5%) were 86.68±32.18, 74.72±29.25 and 51.44±22.26 at 37°C and 165.27±34.65, 104.32±14.79 and 90.02±7.23 at 42°C in presence of chicory extract, while the mean values were 110.88±2.23, 93.13±0.18 and 89.25±23.40 at 37°C and 133.67±10.82, 90.64±0.49 and 90.48±18.23 at 42°C in presence of lactulose syrup, respectively.

Also, Data in Table 6 and 7 showed that the mean values of generation time of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* individually or mixed with traditional yoghurt cultures at 37°C were decreased rapidly compared with these values at 42°C in the in the presence of chicory extract and lactulose syrup.

The fermentability of various dietary components has been studied in vitro using mixed faecal culture, with the predominant culturable bacterial groups, including bacteroides, clostridia, lactobacilli and bifidobacteria being enumerated (Wang and Gibson, 1993). Bifidobacteria selectively fermented the fructans, in preference to other carbohydrate sources such as starch, fructose, pectin and

Table 7: Generation time of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures during incubation time in the presence of lactulose syrup

		Generation time					
		Lactulose syrup					
		37°C			42°C		
Cultures	Prebiotic (%)	Mean	SD	CV	Mean	SD	CV
YC	-	185.51	0.679	0.36	114.41	0.440	0.38
	1	85.905	0.1342	0.15	90.706	0.009	0.009
	3	81.44	0.64	0.78	89.10	0.148	0.16
	5	81.23	0.75	0.92	87.35	0.01	0.01
Lp	-	111.52	0.671	0.60	225.42	0.11	0.04
	1	92.763	8.591	9.26	103.87	1.705	1.64
	3	69.90	7.921	11.33	80.14	10.38	12.95
	5	60.100	9.64	16.03	74.61	14.74	19.75
Lc	-	136.20	28.01	20.56	150.3507	0.57	0.37
	1	109.49	32.25	29.45	105.18	1.97	1.87
	3	72.43	0.61	0.84	92.05	13.27	14.41
	5	63.09	12.93	20.49	86.63	6.34	7.31
Lr	-	139.46	0.678	0.48	186.43	0.52	0.27
	1	110.88	2.23	2.01	133.67	10.82	8.09
	3	93.13	0.18	0.19	90.64	0.49	0.54
	5	89.25	23.40	26.21	90.48	18.23	20.14
YC + Lp	-	124.15	24.02	19.34	114.28	0.30	0.26
	1	85.69	7.42	8.65	81.6	5.27	6.45
	3	80.18	6.61	8.24	72.08	1.00	1.38
	5	64.75	2.41	3.72	72.02	2.44	3.38
YC + Lc	-	95.20	45.80	48.109	126.43	0.31	0.24
	1	82.46	11.66	14.14	93.56	16.68	17.82
	3	71.26	2.26	3.17	75.68	6.52	8.61
	5	67.43	6.79	10.06	72.32	0.75	1.03
YC + Lr	-	135.11	56.11	41.52	183.08	94.02	51.35
	1	98.14	17.05	17.37	113.46	34.37	30.29
	3	81.96	7.38	9.00	78.79	7.49	9.50
	5	67.21	6.63	9.86	69.14	11.04	15.96

YC: Traditional yoghurt cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, Lp: *Lactobacillus plantarum*, Lc: *Lactobacillus casei*, Lr: *Lactobacillus rhamnosuss*, SD: Standard Deviation, CV: Coefficient variation

polydextrose. This was subsequently confirmed in a volunteer trial, which examined the bifidogenic effect of fructo-oligosaccharides (Gibson *et al*., 1995). Gluco-oligosaccharides (GOS) have as yet not been extensively investigated. Although they are thought to be bifidogenic, one study fed gnotobiotic rats a diet of 40 g day^{-1} of GOS and found little effect on the bacterial groups. However, they did modify certain glycolytic activities. Transgalacto-oligosaccharides (TOS) are manufactured from lactose by transglycosylation reactions and consist of galactosyl derivatives of lactose with b1-3 and b1-6 linkages. Bifidobacterial numbers were significantly increased in the faeces of rats fed TOS (Djouzi and Andrieux, 1997).

The present study showed that the percentage of viability in probiotic cultures (*Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosuss*) were increased and the generation time decreased in the in the presence of chicory extract or lactulose syrup. So, this study is in agreement with finding reported by Ito *et al*. (1993a, b), which used transgalactosylated disaccharides in a human volunteer trial to determine their effects on the faecal flora. They showed that the prebiotic increased bifidobacteria and Lactobacillus numbers. Also, the used chicory water extracts contains fructose and glucose, which can used by the lactobacilli. The fructose/Glucose ratio in chicory extracts which represents by the referred bibliography was 85-90% fructose and 10-15% glucose (Januário, 1999; Macrae *et al*., 1993; Biedrzycka and Bielecka, 2004). The present study are consistent with previous studies which showed a stimulatory effect of inulin on the growth of human fecal bacteria (Roberfroid *et al*., 1998), Bifidobacteria (Akalin *et al*., 2004; Brunto *et al*., 2002) and lactobacillus strains (Desai *et al*., 2004). As a prerequisite to their survivability, these bacteria require a carbohydrate source for fermentation that has not been metabolized by the human digestive system before reaching the colon. Selective non-digestible carbohydrate food sources that promote the proliferation of bifidobacteria and lactobacilli have been defined as prebiotics (Gibson and Roberfroid, 1995). Prebiotics that would have potential use, as ingredients in foods should be non-digestible, very shelf stable, require no refrigeration, be easily and effectively incorporated into processed foods and nourish all endogenous beneficial bacteria. The combined form of a probiotic and prebiotic, as described by Gibson and Roberfroid (1995) is termed a synbiotic.

The lactulose syrup which, used as pharmaceutical drug contain monosaccharide such as fructose and glucose, which can used by probiotic and traditional yoghurt cultures. Also, another study on growth of lactobacilli in the presence of prebiotics were carried out by Makras *et al*. (2005) using the agar plate assay, Lactobacillus strains that fermented the prebiotics tested as the sole energy source gave a yellow zone against a purple background, due to the production of significant amounts of organic acids, while the non-fermenting strains did not cause any color change of the agar medium. They found the tested strains such as *L. acidophilus*, *Lb. amylovorus*, *Lb. casei shirota*, *Lb. fermentum*, *Lb. johnsonii*, *Lb. gasseri*, *Lb. paracasei* subsp. *paracasei*, *L. plantarum* and *L. rhamnosus* GG fermented the monosaccharides glucose, fructose and galactose Moreover, all lactobacilli fermented lactulose and TOS, with the exception of *Lactobacillus rhamnosus GG*, which did not ferment lactulose, while TOS were slightly fermented.

The data from the percentage of viability of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosuss* and yoghurt cultures grown in skim milk during incubation with different inoculation percentage and temperature and generation time in the presence of chicory extracts and lactulose syrup can help us to select the optimum percentages from probiotic and prebiotic with economic advantages and beneficial effect with application experiments in yoghurt.

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