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Biological Studies on Bio-Yoghurt Fortified with Prebiotic Obtained from *Jerusalem artichoke*

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Abstract

Inulin, an oligosaccharide produced by several plants, has been shown to enhance the viability of probiotic cultures in milk through storage. *Jerusalem artichoke* (*Helianthus tuberosus* L.) is an interested prebiotic because its tuber has risen content of inulin and fructo-oligosaccharides. This study was aimed to: 1) set the effect of *Jerusalem artichoke* in deferent concentrations (2.5% & 5%) on the growth of probiotic *Lb. acidophilus* P106 in the bio-yoghurt during cold storage at 5˚C and sensory evaluation of probiotic yoghurts; 2) study the effect of feeding with this synbiotic fermented milk on diabetic mice. It could be concluded that the *Jerusalem artichoke* influenced the growth of *Lb. acidophilus* P106 and 5% (w/v) *Jerusalem artichoke* was given the highest growth and sensory evaluation. On the other hand, no serious adverse effects were observed; the reduction of blood glucose was observed at the termination of empirical phase, also, high level (5%) of *Jerusalem artichoke* led to more reduction of blood glucose, cholesterol levels and total lipids compared with control.

Keywords

Functional Food, Probiotic, *Jerusalem artichoke*

1. Introduction

Yoghurt is a popular dairy product consumed in world. The addition of probiotic bacteria to yoghurt progresses

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its functionality and health effects. Probiotics are bacterial members of the normal human intestinal microbiota that promote several beneficial effects on human health. They produce short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer risk, improving the immune system and lowering serum cholesterol levels [1]-[3]. The efficiency of added probiotic bacteria depends on dose level, their viability must be maintained throughout storage, and they must survive in the gut environment [4] [5]. In order to improve these features of probiotic bacteria, fermented food should be supplemented with prebiotics. There are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity bacteria in the colon (probiotics). Fructo-oligosaccharides (FOS) and inulin are among the most famous prebiotic compounds [6] [7].

Here only a few species impotent in the food industry will be mentioned. *S. thermophilus* is used in the manufacture of yoghurt. Lactococci, primarily *L. lactis*, are associated with the dairy industry and the latter is actually used in dairy technology. Species of *Lactobacillus*, such as *L. acidophilus*, *L. delbruckii*. *L. plantarum*, and *L. bulgaricus*, etc. are known in food technology. Homofermentative lactic acid bacteria use the glycolysis, also known as the Embden-Meyerhof-Parnas pathway, for hexose fermentation [8]. The group consists of the *Lactobacillus* groups I and II, enterococci, lactococci, pediococci, streptococci, tetragenococci and vagococci. The pathway is characterized by the formation of fructose 1, 6-diphosphate (FDP) that is split by a FDP aldolase into dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate. Fermentation of 1 mol of glucose results in the formation of 2 mol of lactic acid and 2 mol of ATP.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a native plant of the North American plains cultivated for different purposes in many countries. *Jerusalem artichoke* is a natural raw material for the derivation of a number of functional food ingredients such as inulin, oligofructose and fructose [9] having both nutritional and functional attributes, particularly beneficial to individuals with Type 2 diabetes and obesity [10]. Its tuber contains high amount of dietary fiber namely inulin and fructo-oligosaccharides. Inulin is a polysaccharide. Chemically, it is a linear biopolymer o D-fructose units connected by β (2,1) glycosidic linkages, and terminated with one D-glucose molecule linked to the fructose chain by an α (2,1) bond. The degree of polymerization of inulin generally ranges from 2 to 60. To date, inulin has been increasingly used as functional ingredients in processed foods due to its unique characteristics [11]. Inulin also has other applications for functional food ingredients that are eligible for enhanced function claims and reduced risk of a colorectal cancer [12]-[14]. Furthermore, dietary fructans cause an increase of the amine production in the intestine of animals preventing pasture-associated laminitis disease [15] [16]. In addition, inulin and (FOS) improve bioavailability of minerals such as calcium, magnesium and iron, increase activity of beneficial live active cultures and inhibition of harmful bacteria in the digestive tract. Inulin facilitates the digestion of high protein diets, retards fat absorption, and provides roughage preventing constipation, remains in digestive tract providing satiety without carrying of extra calories, lowers blood cholesterol and triglycerides [17], helps with blood glucose control for diabetics [18] and decreases incidence of colon cancer [5]. Inulin is such a carbohydrate which has a high potential nutritional advantage as low energy dietary supplements. It can be used as a source of carbohydrates for diabetic patients and more generally as dietary fiber. Moreover, an improvement of glucose/insulin ratio has also been observed in rats receiving Oligofructose added in a high fructose diet (inulin). These substances are added to milk products in order to support the viability of probiotic strains to make these products, synbiotics, beneficial for consumer's health [19].

More recently, a renewed and rapidly growing interest is for the use of *Jerusalem artichoke* tubers which are rich in inulin as raw materials for bioethanol production. Multiple applications of *Jerusalem artichoke* are illustrated in **Figure 1**. These diverse applications along with low-cost of plantation render *Jerusalem artichoke* a promising biomass for the development of a bioeconomy [20].

Therefore, in this study, *Jerusalem artichoke* is selected to develop as a healthy food choice for people who are at risk for less dietary fiber consumption and chronic diseases, such as diabetes and production of new yoghurt with high biological value, and studying the health benefits of *Jerusalem artichoke* as prebiotic on diabetic and hyperlipidemic mice.

2. Materials and Methods

2.1. Starter Culture

A probiotic isolate *Lactobacillus acidophilus* P106 was identify by Mahrous *et al.* [21], was used in the pro-

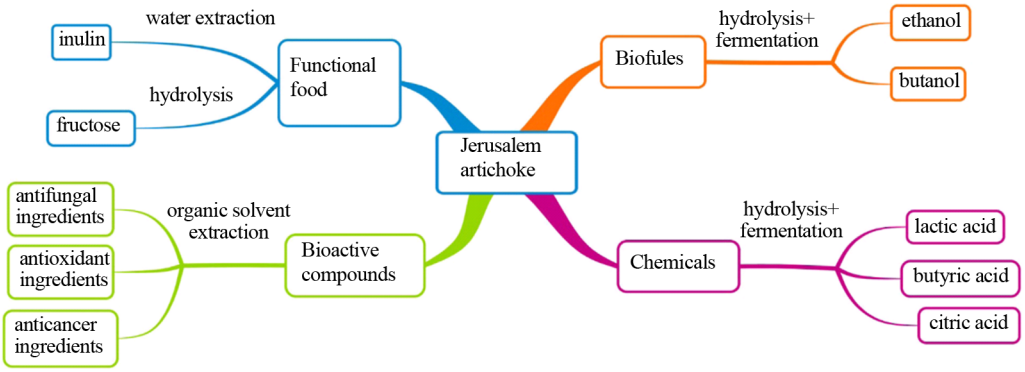

Figure 1. These diverse applications along with low-cost of plantation render *Jerusalem artichoke* a promising biomass for the development of a bioeconomy [20].

duction of bio-yoghurt. The strain was isolated from, breast-feeding infant (15 days old) and selected as probiotic in previous studies [22]. The strain was maintained on MRS-agar (E. Merck, Darmstadt, Germany) at 4˚C - 6˚C. The commercial lyophilized culture containing a mix of *Streptococcus salivarius* ssp. *thermophilus* (ST) and *Lactobacillus bulgaricus* (LB) strains. It prepared for direct inoculation of milk for yoghurt production.

2.2. *Jerusalem artichoke* Tubers

Jerusalem artichoke tubers were obtained from Sabahia Horticultural Research Station, Agric. Res. Center, Alexandria, Egypt. *Jerusalem artichoke* tubers were washed with tap water and any deteriorated parts were removed, than the tubers were sliced in dividedly to the reasonable thickness by conventional food slicing machine. The sliced tubers were immersed immediately in boiling water for 5 min. following by immediate dipping in cold citric acid solution (1%) to inhibit polyphenoloxidas activity. After that slices of tuber were dried in electronic air oven at 55˚C - 65˚C until samples reached constant weight. The recovered powder was preserved in tight polyethylene bags and stored under freezing until use.

Preparation of *Jerusalem artichoke* Tuber Extracts Solution

Jerusalem artichoke tuber was used in the preparing of bio-skimmed yoghurt as a prebiotic for the tested strains. Five gram of *Jerusalem artichoke* powdered were suspended with distilled water to give a final volume 100 ml, then agitated at 76˚C ± 1˚C for 20 min using shaker. The residue was separated by centrifugation at 6000 r.p.m/15 min, and then the supernatant was sterilized using sterile membrane filter.

2.3. Bio-Yoghurt Production

The bio-yoghurt was prepared as: Cow milk (with 3.2% fat), divided into four portions. The first portion was inoculated by yoghurt starter at 2% (V:V) and was denoted as a control yoghurt; the second portion was inoculated with 1% (V:V) yoghurt starter plus 1% (V:V) *Lb. acidophilus* P106 and was denoted as bio-yoghurt, while the third portions was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 2.5% (v/v) of the sterilized *Jerusalem artichoke* tuber extract solution was added before the inoculation step to be as a source inulin (prebiotic) beside adding the equal amount of skimmed powdered milk to replace the dilution resulted from the addition of the prebiotic. And the four potions was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 5% (v/v) of the sterilized *Jerusalem artichoke* tuber extract solution. The mix were placed in a glass jars and heated at 85˚C for 30 min [23]. After that cold to incubation temperature (40˚C - 42˚C), after incubation yoghurts were stored in 4˚C ± 1˚C for 21 days. Every 7 days each group of yoghurts was examined in order to determine the chemical and microbiological analysis.

2.3.1. Chemical Analysis

Determination of chemical composition

Analysis contents were carried out according to AOAC [24]. Moisture content was determined by air-oven drying at 105˚C overnight. The, total protein content was determined by Kjeldahl method (% protein = N ×

6.25). Fat content was determined by Soxhlet apparatus; using hexane as an organic solvent at 80˚C for 6 h. Crud fiber was determined by dilute acid and alkaline hydrolysis. Carbohydrate content was determined by differences of total contents (moisture, protein, fat and ash) from 100.

Determination of inulin

Preparation of extracts

Jerusalem artichoke tubers and samples of yoghurt at different storage period were homogenized with water (1:2 w/v) and heated at 120˚C for 20 min (1 atm.) in a vertical retort Luferco; the treated sample material were then filtered and subjected to chromatography.

Extract analysis

Sample analysis was performed using a Waters high performance liquid chromatograph (HPLC) under the following conditions: column (Aminex HPX-87C); detector-refractive index detector, Waters model 2414; eluent: water; flow rate: 0.3 ml/min; injected volume: 20 µl: column temperature: 80˚C; detector temperature: 40˚C [25]. For the inulin quantitation, a commercial standard (Fluka-BioChemika 57,614) was used.

Fractionation of sugars by HPLC

Preparation of extracts

Sugars and organic acids compositions of *Jerusalem artichoke* tubers and samples of yoghurt at different storage period were determined as described by [26]. The sample was diluted 1:10 (v/v) with Milli-Q water (type 1) and then filtered through a 0.22 µm filter membrane (Waters, Milford, MA, USA). An aliquot of 1.5 mL of these solutions was placed in vials for the analysis.

Extract analysis

Jerusalem artichoke tubers and sample analysis was performed using a Waters high performance liquid chromatography HPLC Hewllet Packared (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and quarter HP pump (Waters 2695 Alliance, Milford MA, USA), The column (Aminex HPX-87C) temperature was maintained at 80˚C and the detector at 50˚C. Sample detection was performed by comparing retention time's standards. Gradient separation was carried out with methanol and acetonitrile as mobile phase. The injection volume was 10 µL and the flow rate was 0.5 $mL \cdot min^{-1}$. The temperature of column was hold at 80˚C and the detector at 50˚C. Sample detection was performed by comparing retention time's standards.

2.3.2. Microbiological Analysis

Serial dilutions in sterile peptone water (0.1%) were prepared from every groups of yoghurt (1 g sample). Then 1 ml of dilution was plated over selected culture media (BA-sorbitol agar) for *Lactobacillus acidophilus* P106 in two repetitions. Plates were incubated anaerobically (GasPak System—Oxoid) in 37˚C for 48 h.

2.3.3. Consumer Panel

Ten volunteer participated on the panel evaluated appearance, mouthfeel, flavor, and overall quality of yoghurt gropes on a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely). Panelists were served five samples at a time and asked to rinse their mouths between samples.

2.4. Biological Experiments

2.4.1. Animals and Conditions

Fifty male mice, approximately 4 week-old with the average body weight of (25.9 ± 1.50) g were obtained from Faculty of Science, Department of Zoology, Alexandria University, Alexandria, Egypt. All mice were examined for health status and acclimated to laboratory conditions for 2 weeks prior to use. The temperature was hold at 23˚C ± 2˚C, and relative humidity at approximately 50%, with a 12 h: 12 h light: dark photoperiod. Animals were housed in stainless-steel cages and given standard diet and water throughout the study period. Preparation of diabetic mice by intraperitoneal injection of alloxan (150 mg/kg body weight) according to the method is described by [27].

2.4.2. Probiotic Feeding

Mice were randomly assigned to treatment groups according to an approximately equal mean body weight to 5 treatment groups of 10 each. The treatments were: 1) group A, were fed by the normal yoghurt (as control negative group); 2) group B diabetic mice (were fed by normal yoghurt plus 1.0% (w/w) cholesterol; 0.2% (w/w)

oxgal) (as control positive group); 3) group C diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); 4) group D diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 2.5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal; and 5) group E diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal). The experiment was carried out for four weeks (5 days week-1, 20 days) by oral gavages; dose level 10^7 - 10^8 CFU mL^{-1}. The administered volume of each dose was 1.0 $mL \cdot kg^1 \cdot day^{-1}$, adjusted daily for recorded body weight changes during the treatment period. At the end of experiment, the mice were fasted for 12 hours before blood collection.

2.4.3. Animal Observations

Health status of treated mice was monitored daily throughout the experimental period. The Mice body and organ body weight gain were recorded daily.

2.4.4. Mice Blood Collection

After dosing (20 days), mice were anesthetized by using diethyl ether. Mice blood was obtained by cardiac puncture via aspiration through polyethylene tubing attached to a heparinized microhematocrit capillary tube which had been flamed and pulled to a fine point.

2.4.5. Blood Sugar; Serum Cholesterol and Total Lipids

Triglycerides; cholesterol and sugar were determined in blood serum of each group. Biochemical determinations were made as: Glucose was determined by enzymatic methods using kits according to Trinder [28]. Determination of total lipids in serum was determined by colorimetric method according to Schimit [29]. Total cholesterol was determined by colorimetric method according to Allain [30].

2.5. Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of replicates from the different groups and the control.

3. Results and Discussion

3.1. Chemical Composition of *Jerusalem artichoke*

Chemical composition of *Jerusalem artichoke* tubers percentages were calculated as dry weight (**Table 1**). Data obtained from this table showed that, *Jerusalem artichoke* had a low level of moisture content, there was 6.8 ± 0.11 g/100 g also, from the same table that *Jerusalem artichoke* tubers seems to have total carbohydrate content, Curd protein, Curd fat, Curd fiber and Ash were 84.6 ± 0.11, 2.6 ± 0.02, 0.8 ± 0.11, 4.4 ± 0.03 and 5.2 ± 0.01 g/100 g, respectively. Our results are in line with those of Sahar [31], who reported that chemical composition of *Jerusalem artichoke*, Moisture, total carbohydrate, crude protein; crude fiber and ash were 6.50, 86.21, 7.40, 7.52 and 5.30 g/100 g, respectively. Also, these results are slightly with those of Fleming and Groot-Wassink [32]; Guiraud *et al.* [33] and Rashwan [34], who reported that, *Jerusalem artichoke* tubers contained 85.95% carbohydrates that were recovered mainly in the form of inulin. From the previous results, it could be concluded that, *Jerusalem artichoke* tubers have level of inulin high enough to be utilized commercially.

3.2. Inulin, Sugars and Organic Acids of *Jerusalem artichoke*

Table 2 includes the content of Inulin, sugars and organic acids of *Jerusalem artichoke* tubers. The data from the HPLC method was used for determination of inulin content in *Jerusalem artichoke* tubers (21.46 g/100 g dry weight). The water extracts of *Jerusalem artichoke* tubers, contained three major sugars: sucrose (4.33 g/100 g dry weight), fructose (3.25 g/100 g dry weight) and glucose (2.77 g/100 g dry weight), but lactose was not present in detectable amounts (**Table 2**). Compared to our observations on *Jerusalem artichoke tubers*, high levels of fructose were found in *Jerusalem artichoke* tubers [35]. Elsewhere, the presence of sucrose, glucose, fructose and maltose were also reported in *Jerusalem artichoke* tubers [36]. Sorbitol (1.55 g/100 g dry weight)

Table 1. Chemical composition of *Jerusalem artichoke* tubers (as dry weight).

Components (g/100 g)	*Jerusalem artichoke* tuber
Moisture	6.8 ± 0.11
Total solids	93.20 ± 0.01
Curd protein	2.6 ± 0.02
Curd fat	0.8 ± 0.11
Ash	5.2 ± 0.01
Curd fiber	4.4 ± 0.03
Total carbohydrate	84.6 ± 0.11

Data are presented as mean ± SD.

Table 2. Inulin, sugars and organic acids content in water extracts of *Jerusalem artichoke* tubers, g/100 g dry weight.

Inulin and sugar content	(g/100 g)
Inulin	**21.46**
Sucrose	4.33
Fructose	3.25
Glucose	2.77
Galactose	1.63
Ribose	2.74
Mannose	0.68
Sorbitol	1.55
Mannitol	1.18
Glucuronic acid	4.31
Galacturonic acid	0.60
Lactose	ND

and Mannitol (1.18 g/100 g dry weight) were detected in *Jerusalem artichoke* tubers. Mannitol is formed from inulin via hydrolysis followed by catalytic hydrogenation [37].

3.3. Inulin, Sugars and Organic Acids in Yoghurt

Some lactic acid bacteria such as *Streptococcus thermophilus* transport lactose by a lac permease transport system, followed by an intracellular hydrolysis and phosphorylation [38]. The growth of *Lactobacillus* and *Bifidobacterium* were observed in media with the addition of prebiotics. Other authors reported that galacto-oligosaccharides and fructo-oligosaccharides with lower DP are best in supporting the growth of bifidobacteria and carbohydrates with high DP are poor substrates for bifidobacteria [39]. **Table 3** & **Figure 2** showed the inulin, sugars and organic acids content in water extracts in yoghurt, g/100 g dry weight after 0, 7, 14 and 21 days from production. The *obtained Jerusalem artichoke* could be used as an additive in different concentrations (2.5% & 5%) in kinds of functional foods like yoghurt that the biochemical composition showed there were the development in the concentrations of all sugars which were determined. The obtained results showed there were increase in a lot of determined sugars especially in group C & D like inulin; Fructose; glucose; galactose; ribose and mannose & organic acids compared with control and group B during the storage time.

Glucose was converted into sorbitol and fructose into mannitol as well as its isomer sorbitol. Mannitol production by fermentation with microorganisms, and food-grade microorganisms in particular, may therefore be an interesting alternative. A fermentation process could have several advantages compared to the chemical synthesis,

Table 3. Inulin, sugars and organic acids content in water extracts in yoghurt, g/100 g dry weight.

Components (g/100 g)	Storage (days)	A	B	C	D
Inulin	0	5.44	8.03	11.69	14.32
	7	6.61	6.02	12.10	15.02
	14	5.02	6.47	16.31	14.95
	21	5.58	4.36	10.39	14.05
Lactose	0	32.97	28.31	24.19	29.71
	7	22.78	33.51	27.82	25.57
	14	24.14	29.77	26.16	35.65
	21	21.19	33.77	28.47	19.98
Fructose	0	1.84	0.37	1.53	4.19
	7	0.71	0.82	0.29	2.02
	14	0.58	2.19	0.84	0.50
	21	1.19	0.53	1.67	1.28
Glucose	0	2.42	1.84	1.96	4.44
	7	1.67	0.91	4.90	2.71
	14	1.76	5.74	0.79	1.79
	21	1.58	1.34	1.11	3.27
Galactose	0	2.91	2.93	4.84	4.18
	7	1.68	3.51	2.11	4.10
	14	4.47	1.15	3.04	2.21
	21	4.00	4.24	3.23	3.54
Glucuronic acid	0	1.32	0.82	2.33	2.70
	7	1.32	2.40	0.45	2.36
	14	1.77	2.77	3.51	3.42
	21	1.57	0.66	3.70	0.36
Galacturonic acid	0	0.57	1.76	1.42	2.27
	7	1.18	2.46	1.06	1.76
	14	0.62	1.90	1.11	2.69
	21	0.63	0.84	1.09	0.88
Mannitol	0	0.55	0.46	0.47	0.71
	7	0.37	0.42	0.33	1.00
	14	0.74	2.70	1.07	0.49
	21	0.82	0.73	0.95	1.56
Sorbitol	0	0.84	0.38	0.43	0.26
	7	0.35	0.60	0.19	0.60
	14	0.35	2.25	0.63	0.33
	21	0.44	0.37	0.88	0.64
Ribose	0	0.09	0.31	0.30	0.19
	7	0.04	0.31	0.65	0.17
	14	0.07	0.71	0.15	0.12
	21	0.39	0.28	0.21	0.42
Mannose	0	1.43	1.37	2.48	2.10
	7	2.37	1.63	1.10	2.71
	14	2.19	2.42	1.42	3.61
	21	1.87	1.00	0.70	1.91

A: was inoculated by yoghurt starter at 2% (V:V) (control skimmed yoghurt); B: was inoculated with 1% (V:V) yoghurt starter plus 1% (V:V) *Lb. acidophilus* P106 and was denoted as (bio-yoghurt); C: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106with 2.5% (v/v) of the sterilized *Jerusalem artichoke* and D: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 5% (v/v) of the sterilized *Jerusalem artichoke*.

such as a complete conversion of fructose to mannitol, absence of side products (like sorbitol) that are difficult to remove, moderate production conditions and no requirement of highly purified substrates [40]. Mannitol is a polyol or sugar alcohol that is produced by several organisms. Mannitol is assumed to have several beneficial effects, as an antioxidant (protection against oxidative damage by oxygen radicals) and as a non-metabolizable sweetener. Mannitol-producing lactic acid bacteria may directly be applied in the manufacture of foods and this may lead to fermented food products with an extra nutritional value. Mannitol is applied as a food additive (E421) as a sweet tasting bodying and texturing agent and it is used as a sweet builder in “sugar free” chewing

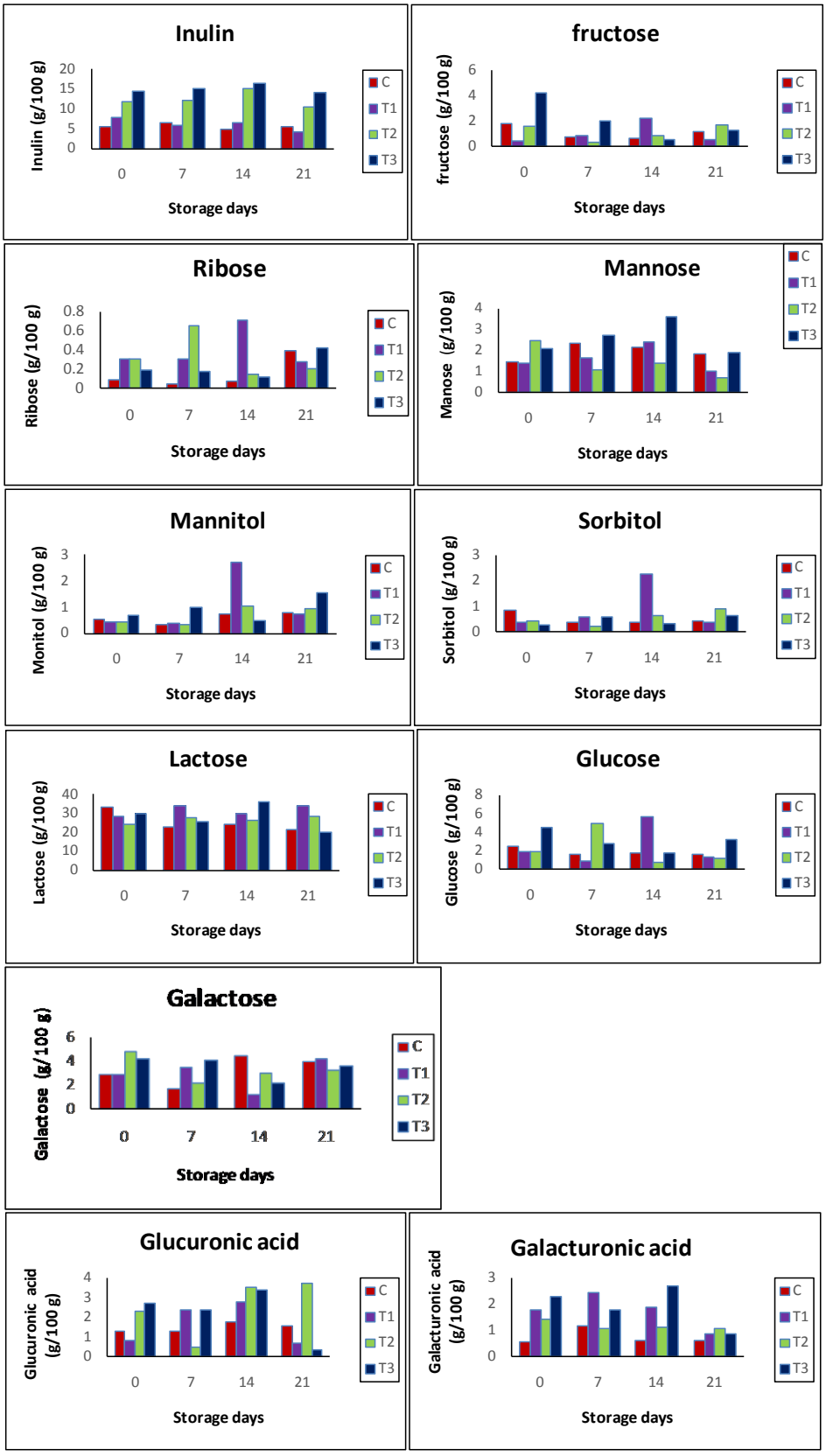

Figure 2. Inulin, sugars and organic acids content in water extracts in yoghurt, g/100 g dry weight.

gum and in pharmaceutical preparations. Mannitol has some laxative properties and the daily intake of mannitol should therefore not exceed 20 g [41]. This results need to advanced study.

3.4. Probiotic Content

It was found that *Jerusalem artichoke* influenced the growth of *Lb. acidophilus* P106 (**Table 4**), the fermentation of different concentration shows that 5% (w/v) *Jerusalem artichoke* give the highest growth of *Lb. acidophilus* P106, reaching population $8.4 \times 10^8 \pm 0.12$ cfu/ml after 14 days as compared to $8.5 \times 10^7 \pm 0.15$ cfu/ml for control. The lactic acid content of the yoghurt supplemented with 2.5% *Jerusalem artichoke* was also increased for $5.2 \times 10^8 \pm 0.16$ after 14 days. The same observation had also shown by Cardarelli *et al.* [42] on their petit-Suisse cheeses supplemented with oligofructose and inulin.

3.5. Organoleptic Evaluation

Organoleptic evaluation of the different manufactured yoghurt presented in **Table 5**. The data indicated that, bio-yoghurt prepared with *Jerusalem artichoke* (2.5% & 5%) had the highest values of aroma, color, texture, sourness and overall acceptability comparing to those prepared as control and bio-yoghurt as judged by a group of panelists. In addition, it should be noted from obtained data that, there were no differences between group C & group D (*Jerusalem artichoke* 2.5% & 5%) except in the dark color which was observed in group D.

3.6. Adverse Clinical Signs & Body and Body Weight Gain

No serious adverse effects were observed for control and other groups. These results are in agreement with those

Table 4. Probiotic content in the deferent groups of bio-yoghurts.

Days	Group B	Group C	Group D
Zero	$2.1 \times 10^7 \pm 0.11$	$3.2 \times 10^7 \pm 0.21$	$4.5 \times 10^7 \pm 0.11$
7	$3.3 \times 10^7 \pm 0.01$	$5.1 \times 10^7 \pm 0.20$	$6.4 \times 10^7 \pm 0.21$
14	$8.5 \times 10^7 \pm 0.15$	$5.2 \times 10^8 \pm 0.16$	$8.4 \times 10^8 \pm 0.12$
21	$5.5 \times 10^7 \pm 0.11$	$3.6 \times 10^7 \pm 0.12$	$4.9 \times 10^7 \pm 0.01$

Data are presented as mean ± SD. B: was inoculated with 1% (V:V) yoghurt starter plus 1% (V:V) *Lb. acidophilus* P106 and was denoted as (bio-yoghurt); C: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 2.5% (v/v) of the sterilized *Jerusalem artichoke* and D: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 5% (v/v) of the sterilized *Jerusalem artichoke*.

Table 5. Sensory evaluation of probiotic bio-yoghurts.

Properties	Mean scores			
	Group A	Group B	Group C	Group D
Aroma	7.90 ± 0.01	8.00 ± 1.2	8.31 ± 1.02	8.32 ± 0.12
Color	7.88 ± 0.11	7.93 ± 0.1	7.99 ± 0.11	7.85 ± 0.01
Texture	7.9 ± 1.02	8.01 ± 1.2	8.2 ± 0.12	8.1 ± 1.12
Sourness	7.53 ± 0.12	7.86 ± 0.01	8.12 ± 0.2	8.2 ± 0.03
Overall acceptability	7.9 ± 1.11	8.1 ± 0.14	8.3 ± 0.01	8.2 ± 0.11

Data are presented as mean ± SD. Group A: was inoculated by yoghurt starter at 2% (V:V) (control yoghurt); B: was inoculated with 1% (V:V) yoghurt starter plus 1% (V:V) *Lb. acidophilus* P106 and was denoted as (bio-yoghurt); C: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 2.5% (v/v) of the sterilized *Jerusalem artichoke* and D: was combination with 1% (V:V) yoghurt starter and 1% (V:V) *Lb. acidophilus* P106 with 5% (v/v) of the sterilized *Jerusalem artichoke*.

reported previously [43].

Mice body and organ body weight gain were presented in **Table 6**. Body weight gain was increased in the C; D&E groups at the end of the treatment compared to the control groups A & B. No deferent were observed in body weights in the groups D & E treated groups. This indicates no significant effect of the concentrations 2.5% & 5% of *Jerusalem artichoke* [44].

3.7. Hematological Analysis

Blood parameters are presented in **Table 7**. There was No significant difference in the value of Haematocrit (Hct); Haemoglobin content; Red blood cells count and the value of the WBC count in the remaining treated groups compared to the positive and negative control groups. These results also are in agreement with the reported data investigated that oral injection of the probiotic strains in humans did not lead to cytokine changes beyond normal values [45]. This finding provides evidence for the safety of the probiotic cultures.

3.8. Blood Sugar, Total Cholesterol and T. Lipids

Lactic acid bacteria are normal components of the intestinal microflora in both humans and animals and have

Table 6. Body weight and weight gain of Feeding mice with normal yoghurt and fermented milk with probiotic microorganisms and starter yoghurt.

	Weeks	A	B	C	D	E
Body weight (g)	1	25.9 ± 0.2	26.1 ± 0.3	25.8 ± 0.1	26.6 ± 0.2	27.9 ± 0.2
	2	30.2 ± 0.2	31.2 ± 0.1	31.9 ± 0.1	31.3 ± 0.1	33.5 ± 0.1
	3	35.9 ± 0.1	36.7 ± 0.2	36.9 ± 0.2	37.1 ± 0.4	38.5 ± 0.1
	4	44.2 ± 0.2	45.4 ± 0.6	45.2 ± 0.3	45.8 ± 0.6	46.1 ± 0.2
Body weight gain (g)	2 - 1	4.3 ± 0.1	5.1 ± 0.1	6.1 ± 0.2	4.7 ± 0.1	5.6 ± 0.2
	3 - 2	5.7 ± 0.2	5.5 ± 0.3	5.0 ± 0.4	5.8 ± 0.3	5.0 ± 0.2
	4 - 3	8.3 ± 0.2	8.7 ± 0.2	8.3 ± 0.2	8.7 ± 0.4	7.6 ± 0.1
	4 - 1	18.3 ± 0.1	19.3 ± 0.3	19.4 ± 0.1	19.2 ± 0.1	18.2 ± 0.3

Data are presented as mean ± SD. All probiotic strains were added at (10^7 - 10^8 CFU mL^{-1}). Group A: were fed by the normal yoghurt (as control); Group B: diabetic mice (were fed by normal yoghurt plus 1.0% (w/w) cholesterol; 0.2% (w/w) oxgal); Group C: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); Group D: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 2.5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal and Group E: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal).

Table 7. Blood analysis of mice after feeding with yoghurtand probiotic microorganisms.

Mice groups[a]	Hct value		Hb content		RBC		WBC	
	%	% of control	g/100 mL^{-1}	% of control	X 10^6 uL^{-1}	% of control	X 10^3 uL^{-1}	% of control
A	43 ± 1	100	12.8 ± 3	100	5.9 ± 1	100	6.4 ± 1	100
B	40 ± 2	100	11.5 ± 1	100	5.4 ±2	100	9.5 ± 1	100
C	41 ± 2	103	13.3 ± 2	113.0	5.8 ± 2	107	6.9 ± 2	73
D	42 ± 3	105	13.5 ± 3	117.4	5.9 ± 3	109	6.8 ± 1	72
E	42 ± 1	105	13.6 ± 3	118.3	5.9 ± 1	109	6.5 ± 1	69

Data are presented as mean ± SD. All probiotic strains were added at (10^7 - 10^8 CFU mL^{-1}). Group A: were fed by the normal yoghurt (as control); Group B: diabetic mice (were fed by normal yoghurt plus 1.0% (w/w) cholesterol; 0.2% (w/w) oxgal); Group C: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); Group D: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 2.5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal and Group E: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal).

Table 8. Sugar blood, Total Cholesterol (TC) and T. lipids in mice after feeding with yoghurt and probiotic microorganisms.

Mice groups[a]	Blood Sugar	Total Cholesterol (TC) mg/g	T. lipids Picogram
A	74 ± 0.1	145.2 ± 0.1	25.1 ± 1.4
B	89 ± 0.3	188.1 ± 0.4	30.8 ± 1.1
C	83 ± 0.1	149.2 ± 0.1	27.1 ± 1.0
D	76 ± 0.2	146.2 ± 0.2	25.0 ± 1.1
E	75 ± 0.1	145.9 ± 0.3	24.1 ± 1.0

Data are presented as mean ± SD. All probiotic strains were added at (10^7 - 10^8 CFU mL^{-1}). Group A: were fed by the normal yoghurt (as control); Group B: diabetic mice (were fed by normal yoghurt plus 1.0% (w/w) cholesterol; 0.2% (w/w) oxgal); Group C: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); Group D: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 2.5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal and Group E: diabetic mice (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 5% (w/w) *Jerusalem artichoke* extraction plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal).

been associated with various health-promoting properties. One beneficial effect is a reduction in serum cholesterol levels.

Data presented in **Table 8** showed the mean value of blood sugar; total cholesterol TC and T. lipids for deferent groups after using different levels of *Jerusalem artichoke* 2.5% and 5% on mice. As shown the mean values of serum glucose levels for groups D & E were 76 and 75, respectively while the mean value of control negative group (A) and control positive group (B) were 74 and 89, respectively. Our results are in agreement with those of Alles *et al.* (1999) [46] who recorded that, inulin and oligofructose play an active role in reducing the caloric value and they do not lead to arise in serum glucose or stimulate insulin secretion. However, Molis *et al.* [47] reported that mentioned action to the possible beneficial effects of inulin on blood glucose.

Serum cholesterol increased in the group B compared to the other treated groups especially group D&E (**Table 8**). The use of probiotic bacteria with prebiotic reduce serum cholesterol levels has attracted much attention. Various studies have shown that some lactobacilli could lower total cholesterol [48] [49]. Data in this table indicated that total lipids were decreased after received different levels of *Jerusalem artichoke* by the 2.5% and 5% in comparing to positive mice group.

Pushparaj *et al.* [50] reported that, administration of inulin extract of *Cichorium intybus* produced a significant reduction in serum glucose, triglycerides and total cholesterol in diabetic rats.

4. Conclusion

In this study, we had shown that *Lb. acidophilus* P106 had no adverse effects on the hematological parameters and gave best results on cholesterol and diabetic and total lipids in the serum of mice fed with bio-yoghurt fermented by *Lb. acidophilus* P106 with *Jerusalem artichoke*. These effects may be due in part to the deconjugation of bile salts by strains of bacteria that produce the enzyme bile salt hydrolase (BSH). We recommend use *Lb. acidophilus* P106 with *Jerusalem artichoke* for production of new fermented milk with high biological value and health benefits of functional food on diabetic and hyperlipidemic.

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