

Preparation and evaluation of functional fermented ice cream containing low calorie sugars produced by lactic acid bacteria

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ABSTRACT

This study aimed to produce mannitol and sorbitol by free cells and encapsulated *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *L. Lactobacillus curvatus*, *Leuconostoc mesenteroides* and *Propionibacterium shermanii* in MRS medium containing sucrose, fructose or sorbose as carbon sources, then to prepare and evaluate of functional fermented ice-cream containing high productivity strain. In MRS+ fructose medium, sorbitol concentrations were 911 mg/L and 566 mg/L by encapsulated *Lb. curvatus* and *Leuc. mesenteroides* respectively. The highest productivity of mannitol was produced by encapsulated *Leuc. mesenteroides* at level 400 mg/L and 133.42 mg/L by free cells in MRS +fructose. Encapsulated *Leuc. mesenteroides* was selected to produce functional fermented ice-cream. From our results it can be noticed that rats fed on tested diet showed lower value of body weight gain reached to 21.3% and lower value of serum glucose reached to 21.1% as compared to positive control. Chemical, microbiological, sensory evaluation and nutritional results confirmed the importance of fermented ice – cream with low calorie sugars.

Key words: Low calorie sugars, Encapsulation, Functional fermented, Ice cream Lactic acid bacteria

Introduction

Lactic acid bacteria are a group of microorganism widely used in the industrial food fermentation. They are the cell factors for the production of high-value metabolites involved in flavor, texture or health (De Vos and Hugenholtz, 2004). Mannitol and sorbitol, are low-calorie sugars. Mannitol is assumed to have several beneficial effects, and can therefore be applied to foods leading to health-promoting effects (functional foods) (Le and Mulderrig, 2001). Sorbitol, also used as a sugar substitute. It is referred to as a nutritive sweetener as it provides dietary energy which is used in diet foods, mints, cough syrups (Salminen *et al.* 1993; Barros *et al.*, 2006). As the sweetener is not depended on insulin, it can be applied in diet of diabetic people (Barros and Celligoi, 2006). *Leuconostoc*, *oenococcus* and *Lactobacillus* (heterofermentative lactic acid bacteria), have been reported to produce mannitol effectively (Carvalho *et al.*, 2011; Soetaert, 1990). Many of the researches conducted on production of mannitol with *Leuconostoc mesenteroides* (Ghoreishi and Gholami, 2009; Helanto *et al.*, 2005).

Leuconostoc spp. are natural inhabitants of milk, grapes, and many vegetables, and they are frequently used as starter cultures in fermented milks and vegetables (Hemme and Foucaud, 2004; Choi *et al.*, 2006; Gan Erdene *et al.*, 2011).

Leuconostoc mesenteroides produces high levels of mannitol from fructose or sucrose (Vandamme *et al.*, 1987; Grobber *et al.*, 2001; Wisselink *et al.*, 2002). New food products containing low-calorie sugars and/or fat-replacers are in constant progression on the market in response to the consumer's request (Ladero *et al.*, 2007). Frozen yogurt (FY) is a dessert characterized by having the textural properties of ice cream combined with the acidic taste of yogurt. FY popularity has increased and continues to grow. Frozen yoghurt with live cultures attractiveness to consumers has been increasing worldwide (Alfaro *et al.*, 2015). The aim of this study is to produce low calorie sugars by lactic acid bacteria and using it to prepare functional fermented ice-cream.

Materials and Methods

Bacterial strains

Lactobacillus plantarum was isolated and identified by Dairy Science Dept. (Dairy Microbiology Lab.), National Research Center (Ibrahim *et al.*, 2014) and *Propionibacterium shermanii* was provided by Department of Food Technology, propionibacteria culture collection *Lactobacillus rhamnosus* B-445, *Lactobacillus casei* NRRLB-1922, *Lactobacillus curvatus* NBIMCC4562 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL).

Leuconostoc mesenteroides were isolated and identified by Dairy Science Dept., (Dairy Microbiology Lab.), National Research Center (Ghita *et al.*, 2015)

Materials:

Milk was purchased from Faculty of Agriculture, Cairo University.

Preparation of cells and Encapsulation procedure

Frozen cultures were reactivated 3 times in MRS broth for *Lactobacillus spp.* and sodium lactate broth was used to prepare the cell suspensions of *Pr. shermanii*. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4 °C, and were washed twice with saline and used to prepare capsules, and incubated at 37 °C for 24 h. The cells were harvested by centrifugation at 4000 g for 15 min then the cells washed by sterile saline solution then used for the encapsulation. Encapsulation occurred by extrusion method using sodium alginate sterilized by autoclaving at 121°C for 15 min. The microspheres made by using sterilized syringe through extruding a mixture of cells and sodium alginate (3%) into sterilized 0.1 M calcium chloride solution with continuous stirring at 200 rpm/min till alginate beads were formed, then the beads collected by filtration according to Klinkenberg *et al.* (2001).

Preparation of "Functional fermented ice-cream"

Leuconostoc mesenteroides was applied for manufactured ice fermented milk according to method of Dertlia *et al.* (2016) with some modifications. Replaced the sugar that used in ice fermented milk by 10 % fructose which would be converted to low calorie sugar that suitable for diabetes during fermentation of milk.

A-Preparation of fermented milk (80% part of milk):

Whole milk + 10% fructose was pasteurized at 85 °C for 5 min and 1.5% of skim milk powder was added , then cooled to 40°C. Milk was inoculated with 3% *Leuconostoc mesenteroides* and incubated at 25°C/ 3 days.

B-Preparation of milk processing (20% part of milk):

Whole milk was mixed with cream and pasteurized at 85 °C for 5 min. then cooled to 40°C .

Fermented milk " A" (80 %part)whole milk was mixed with processed milk " B" (20% part) to prepare functional fermented ice – cream and ripened at 4 °C for 20 h, then was mixed for 30 min in ice-cream machine . Product was stored at -20°C.

Microbiological analysis

The encapsulated and free cells bacterial counts of *Leuc. mesenteroides* were determined in the mixture of fermented ice cream using modified MRS agar according to Goyal and Katiyar (1996) . Samples of fermented ice cream (25 g) were diluted in 225 ml phosphate buffer (pH 7.5) and homogenized for 5 min. Ten milliliter of this dilution were used to obtain serial dilutions in

physiological solution to determine the count in the capsules according to Haynes and Playne (2002). The plates were incubated at 25 °C for 48h.

Enumeration of molds, yeasts and coliform group

Potato dextrose agar medium was used for counting yeasts and molds. Plates were incubated for 5-7 days at 25°C. Coliform group was determined using solid medium method onto plates of violet red bile agar (Difco) and incubated for 48 h at 37°C.

Determination of sugars

The samples extraction procedure was carried out according to Zielinski *et al.*, (2014). The samples were analysed at Ministry Of Agriculture and reclamation. Center, Food Technology Res. Institute .

Chemical analysis

Acidity, total protein (TP), soluble nitrogen (SN) and fat contents of the mixture were estimated according to AOAC (2007). pH was measured by using a laboratory pH meter with glass electrode (HANNA, Instrument, Portugal).

Sensory properties:

Functional fermented ice-cream samples were scored for sensory properties by a regular taste panel from staff members of Dairy Department, National Research Centre. Sensory evaluated by using a scheme of 10 points for appearance, 10 points for melting quality, 30 points for body & texture and 50 points for flavor according to Magdoub *et al.*(1989).

Textural profile analysis :

Texture profile analysis (TPA) was carried out on mixture of fermented ice cream using the double compression tester (Multi test 1d Memes in, Food Technology Corporation, Slinfold, W.Sussex, UK). Experiments were carried out by a compression test that generated a plot of force (N) versus time (s). A 25- mm-diameter perplex conical-shaped probe was used to perform the TPA analysis at five different points on the sample surface. In the 1st stage, the sample was compressed by 30% of their original depth at a speed of 2 cm/min during the pretest, compression and the relaxation of the sample. From the force–time curve, the following parameters were determined according to the definition given by the International Dairy Federation (IDF,1991):

Hardness (N) = maximum force of the 1st compression

Cohesiveness = area under the 2nd compression/ area under the 1st compression (A2/A1)

Adhesiveness (N.s) = negative area in the curve

Springiness (mm) = length 2nd compression/ length 1st compression (L2/L1)

Gumminess (N) = Hardness × cohesiveness

Chewiness (mJ) = gumminess × springiness

Nutritional Experiment

I. Materials:

The ingredients used for preparation of the diet given to the animals were purchased from the local market. These items were corn starch, sucrose, corn oil. Casein was obtained from Sisco Research Laboratories PVT.LTD India.

Salts and vitamins used for the preparation of the salt and vitamin mixtures were obtained from Merk, Germany (AI N95) and prepared according to Reeves *et al.* (1993). Glucose kits used for the estimation of serum glucose was obtained from Biodiagnostic Company Egypt.

II -Methods:

1-The standard control diet was prepared according to Revees *et al.* (1993) as shown in Table (1).

2-The hyperlipidimic diet was prepared as basal diet with addition of 10% sheep fat and it was supplemented with 1% cholesterol and 0 .25% bile salts (Fukushima *et al.*,1997).

Table 1: Composition of standard, hyperlipidemia and tested formula (g/100g diet)

Ingredient	Group (Negative control)	Group 2 (Positive control)	Group3 (Tested diet)
Casein	15	15	15
Corn oil	10	10	10
Lard	-	10	10
Sucrose	10	10	10
Cellulose	4	4	4
Salt mix	4	4	4
Vit mix	1	1	1
Cholesterol	-	1	1
Bile Salt	-	0.25	0.25
Tested formula	-	-	20
Starch	56	44.75	24.75
Total	100	100	100

Preparation of tested formula :

Fermented Ice cream Product + 0.5 % carboxy methyl cellulose (CMC) Then, 20% of product was added to the hyperlipidemic diet on the expense of starch.

Animals:

Animals used in this experiment were Sprague Dawley albino rats obtained from the animal house of the National Research Center; their body weight ranged between 140-150 g. and comprised both sexes.

Design of Animal Experiment

The animal experiment comprised 3 groups each 8 rats

- The 1st group was fed on basal diet and served as negative control
- The 2nd group was fed on hyperlipidimic diet and served as positive control
- The 3rd group was fed on hyperlipidimic + 20% tested product.

Animals were kept individually in stainless steel cages; deionized water was allowed ad-Libitum. The room temperature was adjusted at 25°C. The feeding period continued for 6 weeks. During the experimental period the food consumption and body weight of the animals were followed.

At the end of the experimental period (after 6weeks) rats was recorded.

Blood samples were obtained from the orbital vein and were receives into clean dry centrifuge tubes. Left and right inguinal adipose pads were removed and weighted. The sum of pads to body weight multiplied by 100, yielded adiposity index (Jeyakumar *et al.*, 2006). The experimental procedure was carried out according to the institutional Animals Ethics Community of the N R C, Egypt.

Serum glucose Analysis:

Serum glucose was determined according to the method described by Trinder (1969).

Results and Discussion

Production of sorbitol and mannitol by lactic acid bacteria and propionic acid bacteria

This study included the effect of the type of low calorie sugars producing bacteria and the source of carbon in the growth media with the use of a temperatures and pH values suitable for the growth of

individual species . Six strains of lactic acid bacteria and propionic acid bacteria were investigated to produce sorbitol from fructose , sucrose and sorbose as carbon source in growth medium.

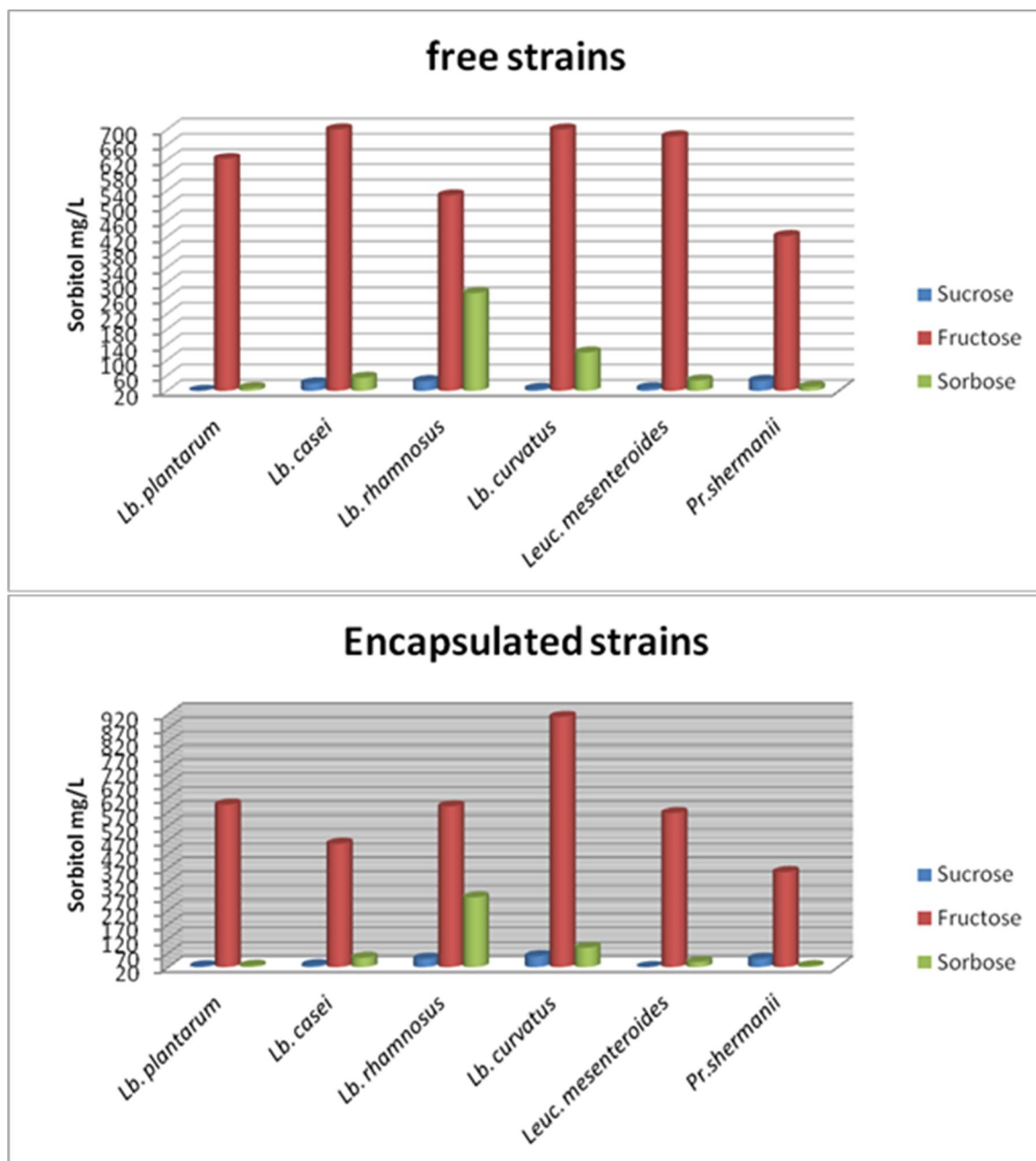
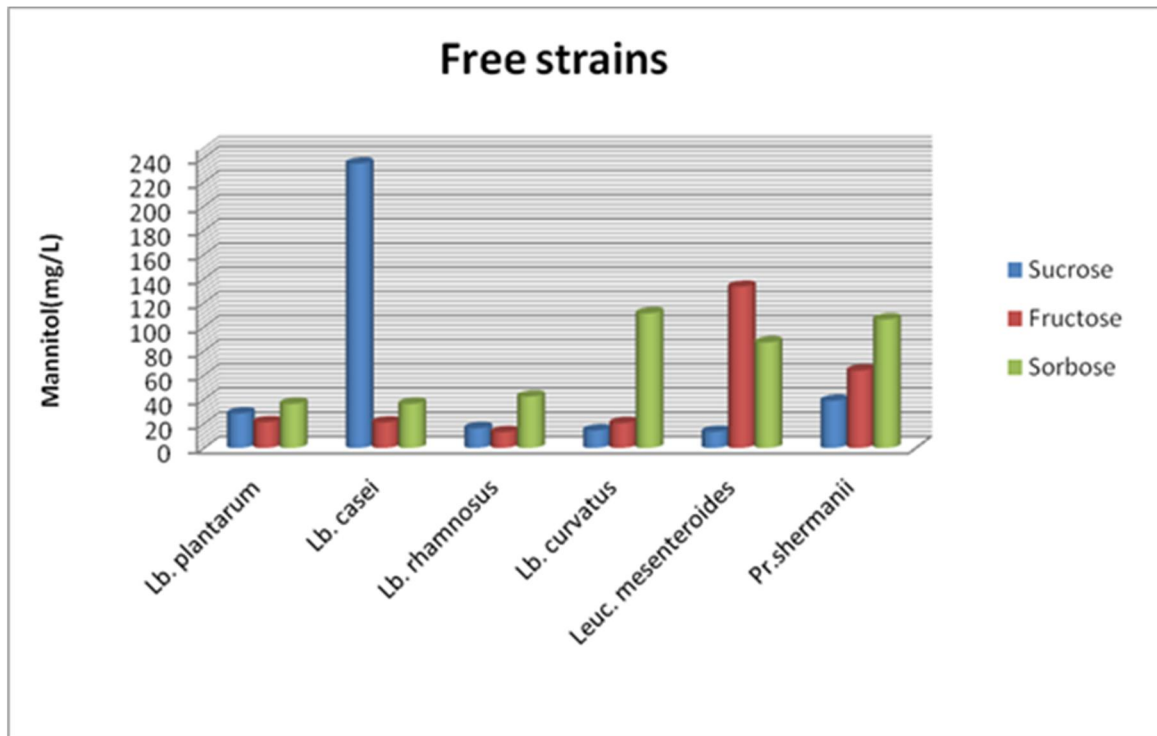


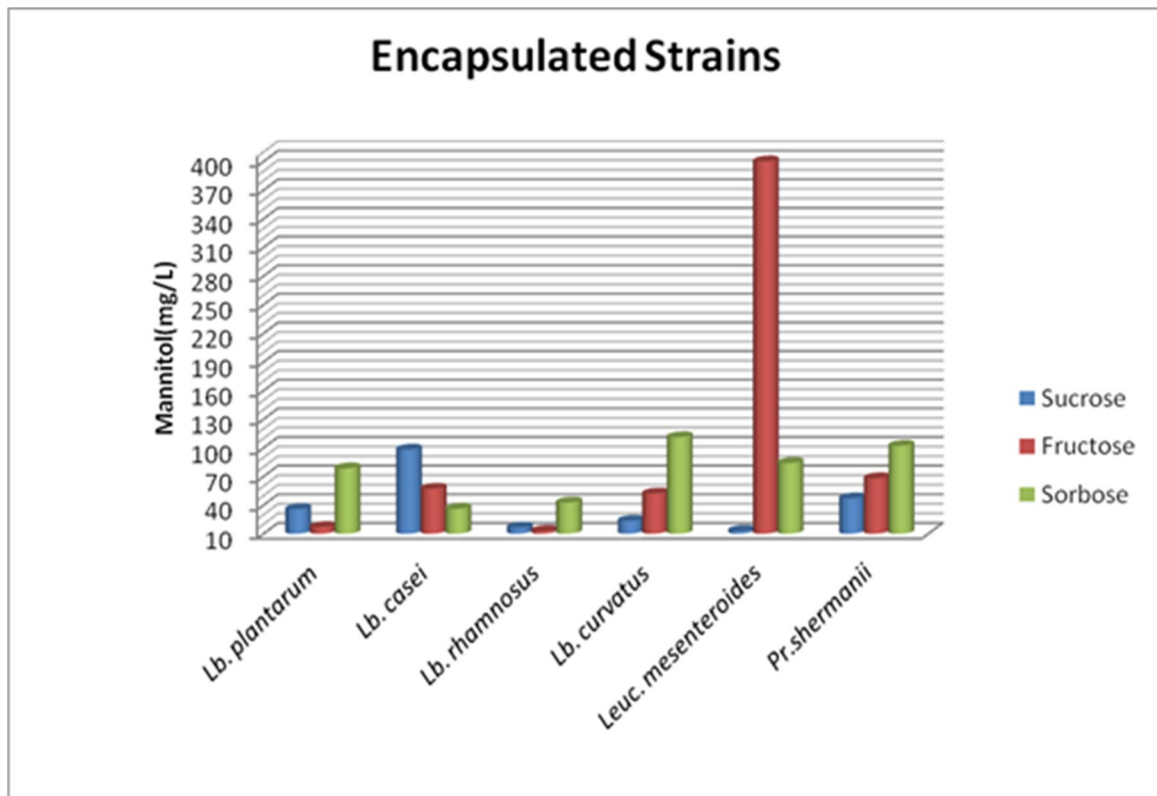
Fig. 1: Production of Sorbitol (mg/L)

Results in Fig.1 (A and B) revealed that in MRS+ fructose medium, higher sorbitol concentrations 911 mg/L, 566 mg/L and 596 mg/L were achieved by encapsulated *Lb. curvatus* ,*Leuc.mesenteroides* and *Lb. plantarum* respectively . Ladero *et al.* (2007) produced high sorbitol concentrations by metabolic engineering of *Lb. plantarum* and recommended the future use of *Lb. plantarum* for in situ sorbitol production in fermented food products.

The results of the mannitol production in the tested strains in MRS medium are presented in Fig. 2(A and B).



A



B

Fig. 2: Production of Mannitol(mg/L)

A variety of lactic acid bacteria were screened for their ability to produce mannitol. All strains produced mannitol. Large differences in mannitol production for different strains. The highest productivity of mannitol was produced by *encapsulated Leuc. mesenteroides* at level 400 mg/L and 133.42 mg/L by free cells in MRS +fructose and *Lb. casei* at level 235.30 mg/L in MRS +sucrose medium, but strain *Lb. curvatus* produced mannitol 111.36mg/L in MRS +sorbose, also, *Lb. plantarum*

produced 94.002 mg/L in the same medium. For microbial production of mannitol, yeast, filamentous fungi, and bacteria have been used for the advantage of cofactor regeneration. However, yeast and fungi produce mannitol in too low volumetric productivities and it makes the process less useful. Meanwhile, bacteria, namely lactic acid bacteria, seem to be efficient producers of mannitol and the genera *Lactobacillus* and *Leuconostoc* have led the attention (Von Weymarn, 2002a). It has been reported that many factors play an important role in mannitol production; *i.e.*, the strains, cultural conditions, type and concentration of substrates. Studies using different *Leuconostoc* species report various mannitol yields: 65% by *Leuconostoc* sp. Y-002 (Yun and Kim, 1998), 97% by *Leuc. mesenteroides* 9135 (von Weymarn *et al.*, 2002b) and 91% by *Leuc. mesenteroides* 9135 (Soetaert *et al.*, 1995). Gan-Erdene *et al.* (2011) found in MRS medium, high mannitol concentrations, 30.4, 27.3, and 29.4 g/l, were achieved by *Leuc. citreum* KACC 91348P, *Leuc. mesenteroides* D1, and *Leuc. mesenteroides* B-742C, respectively. In simplified medium, high mannitol concentrations, 26.1, 18.9, and 18.9 g/l, were obtained by *Leuc. citreum* KACC 91348P, *Leuc. mesenteroides* B-512F, and *Leuc. mesenteroides* MU3, respectively. Erten (1998) found that two *Leuc. mesenteroides* strains under anaerobic conditions produced 0.26 mol mannitol per mol fructose at 25°C. *Leuc. mesenteroides* immobilized on polyurethane foam increased continuous mannitol production in a plug-flow reactor compared with batch fermentations (Soetaert *et al.*, 1999). Screening, half of the population of 105 isolates of *Leuconostoc* spp. exhibited ability to produce mannitol to a variable extent. High mannitol production was favored by high temperature and high pH (Patra *et al.*, 2011). Different *Leuconostoc* species showed various growth rates and mannitol yields depending on strains and culture conditions (Carvalho *et al.*, 2011). The final mannitol concentrations produced by the *Lactobacillus* and *Leuconostoc* strains under the optimal culture conditions were 73 and 26 g/l from 100 g/l fructose respectively (Yun and Kim, 1998). *Leuc. mesenteroides* ATCC 9135 produced high amounts of mannitol, using high cell-density membrane cell-recycle cultures. In addition increasing the initial fructose concentration from 100 to 120 g/l and further to 140 g/l resulted in decreased productivities (Von Weymarn *et al.*, 2002b). *Leuconostoc mesenteroides* NRRL B-1149 was used in batch culture fermentation contained 5% or 10% fructose the yield of mannitol was 78% or 59.6%, respectively of expected theoretically (Kim *et al.*, 2002).

Evaluation of functional fermented ice cream

Microbiological counts and mannitol quantities

Encapsulated *Leuconostoc mesenteroides* counts in fermented ice cream reached to 9.7 log cfu/g and free count to 7.9 log cfu/g. Encapsulated *Leuc. mesenteroides* produced mannitol at level 422.4 mg/100 ml (Table 2).

Table 2: *Leuc. mesenteroides* counts (log cfu/g) and mannitol (mg/100ml) in functional fermented ice cream

Treatments	Fresh fermented ice cream (log cfu/g)	Mannitol (mg/100ml)
Free cells of <i>Leuc. mesenteroides</i>	7.9	156.8
Encapsulated <i>Leuc. mesenteroides</i>	9.7	422.4

The use of encapsulated bacteria to produce economical quantities of low calorie sugars needs to numerous experiments. Review the results for the free cells and the encapsulated bacteria, show that there are many variations as some bacteria produce larger amounts when microencapsulated. Our results revealed the protective effect of microencapsulation on the viability of *Leuconostoc* in fermented ice cream. The results are in agreement with those obtained by Karthikeyan *et al.* (2014); Godward and Kailasapathy (2003); Jayalalitha *et al.* (2011). They reported that bacterial encapsulation improved the viability of probiotic strains and microencapsulated cells survived in freezing and ice cream containing probiotic bacterial cultures better than free cells. Moreover, *Leuconostoc* spp. have great importance in the production of many fermented foods such as sauerkraut, pickles, meat products, and kimchi, where it gives a refreshing soft sweet taste by production of mannitol, whereas converted fructose to

mannitol more slowly and its yield (40% at 20 h) was much lower than that of *Lactobacillus* (Wisselink *et al.*, 2002; Yun and Kim, 1998) Coliform group, yeasts and molds .

No growth of coliform group, yeasts and molds were detected in all samples. This indicates that proper care was taken to avoid contamination throughout the process and the product has good quality .There was no post processing contamination

Chemical composition contents:

The pH values and acidity were illustrated in Fig. (3) The mixture of functional fermented ice cream was obvious to tend to acidity due to the fermentation process. Acidity had taken the reversed direction of pH values. All of total solids, total protein, total nitrogen and fat were within in range of fermented ice cream.

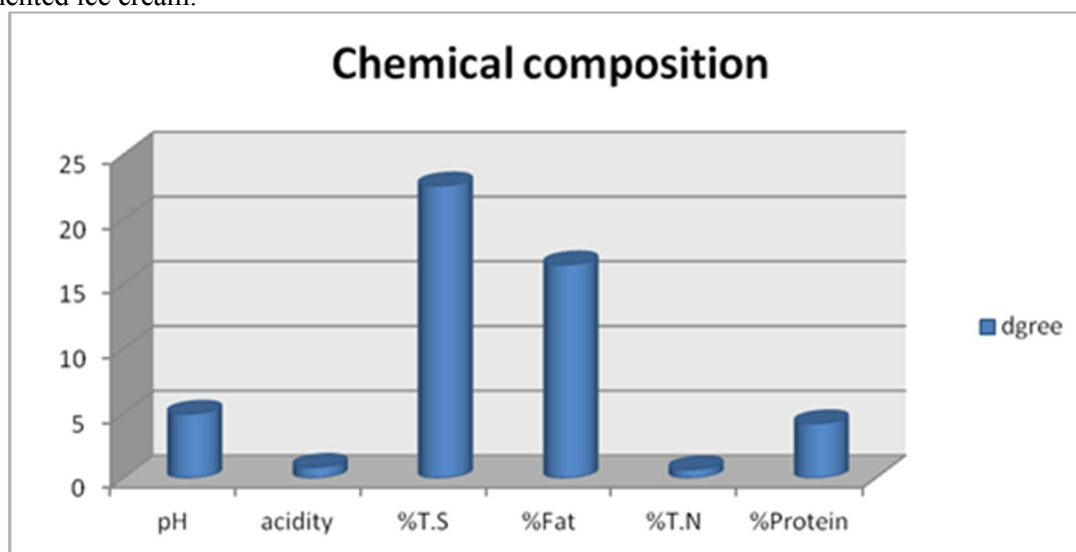


Fig. 3: Chemical composition of functional fermented ice cream

Sensory properties:

The consumer's perspective is one of the most important items for success of any new product. Thus, sensory evaluation of ice fermented milk sample containing 2% *Leuc.mesenteroides* was shown in Fig. (4). In general, addition of 2% *Leuc. mesenteroides* which converts fructose to mannitol led to give sweetness to the product without any addition of sugar to the sample. This may be more suitable for people who suffer from diabetes or obesity. Appearance had taken the best record of ice fermented milk, while melting quality and body & texture were moderate and in range. On the other side, the result was satisfactory for flavor of the final product of fermented ice cream .

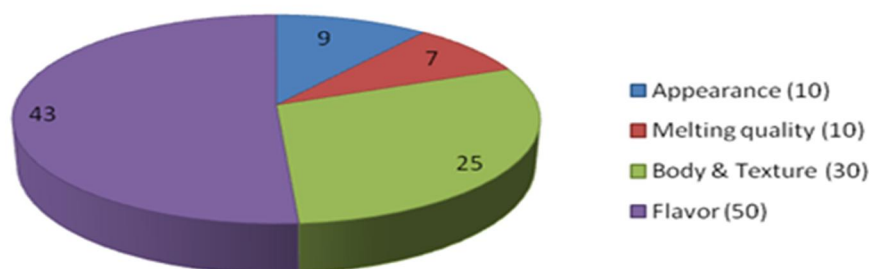


Fig. 4: Sensory properties of functional fermented ice cream

Textural profile analysis:

Texture profile analysis (TPA) imitates the conditions in the mouth by compressing a product twice. Fig (5) showed the parameters of TPA which evaluated in the mixture of fermented ice cream.

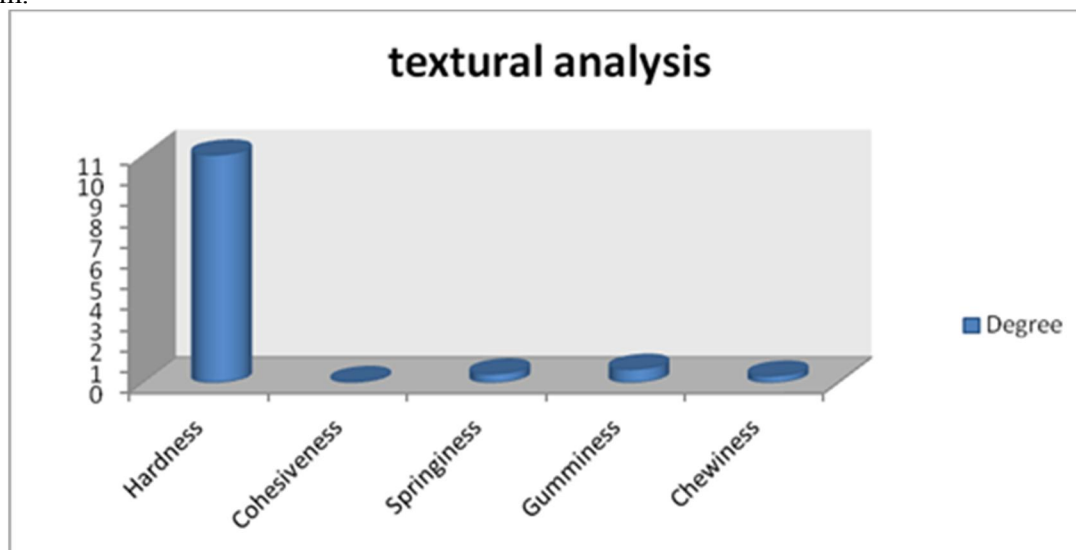


Fig. 5: Textural profile analysis of fermented ice cream sample.

The texture of fermented ice cream mixture is dependent on the composition (mainly protein and sugar content) and the effectiveness of the freezing process (e.g. overrun % and freezing time) . Consequently, the high instrumental hardness of the frozen fermented milk can be attributed to their lower overruns (%). Alfaro *et al.*(2015) reported an inverse correlation between overrun and hardness of ice cream.

Nutritional results

The mean values of food intake of rats fed on negative control, positive control and tested diet were 444.25, 471.7 and 559.5g. respectively as shown in Table (3).

Table 3: Food intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER)of the different experimental group.

Groups test	Food intake (g)	Body weight gain (g)	FER
Negative control (normal rats)	444.25	92.8	0.203
Positive control (hyperlipidimic rats)	471.7	141.3	0.299
Hyperlipidimic + 20% formula	559.5	111.5	0.199

From the data, it can be noticed that rats fed on tested diet showed higher value of food intake reached to 25.9% as compared to negative control, while positive control showed lower value reached to 18.6% as compared to tested diet. On the other hand, rats fed on positive control showed higher value of food intake reached to 6.0% as compared to negative control.

Meanwhile, the mean values of body weight gain of rats fed on negative control, positive control and tested diet were 92.8, 141.3 and 111.5g.respectively as shown in Table (3).It can be noticed that rats fed on tested diet showed higher value of body weight gain reached to 20.0% as compared to negative control, while positive control showed higher value reached to 21.0% as compared to tested diet. On the other hand, rats fed on positive control showed higher value of body weight gain reached to 52.2% as compared to negative control.

With regard to food efficiency ratio (FER), the mean values of (FER) of rats fed on negative control, positive control and tested diet were 0.203, 0,299 and 0.199 respectively as shown in the same table. It can be noticed that rats fed on tested diet showed lower value of (FER) reached to 33.4% as

compared to positive control. Food efficiency ratio of tested diet was near to negative control. On the other hand, rats fed on (positive) Control showed higher value of (FER) reached to 47.3% as compared to negative control.

The mean values of liver weight of rats fed on negative control, positive control and tested diet were 2.755, 4.195 and 3.760 g. respectively as shown in the Table (4).

Table 4: Liver weight percentage and adiposity of rats fed tested diet

Groups test	Liver %	Adiposity index
Negative control (Normal rats)	2.755	1.06
Positive control (Hyperlipidimic rats)	4.195	1.83
Hyperlipidimic + 20% formula	3.760	1.40

From the data, it can be noticed that rats fed on tested diet showed higher value of liver weight reached to 3.6% as compared to negative control, while positive control showed higher value reached to 10.3% as compared to tested diet. On the other hand, rats fed on positive control showed higher value of liver weight reached to 52.2% as compared to negative control. From the same table, it can be noticed that adiposity index of rats fed tested diet showed lower value than positive control but around negative control.

Serum glucose level of rats fed tested diet

The mean values of serum glucose of rats fed on negative control, positive control and tested diet were 64.6, 109.75 and 86.8g. respectively (Table 5).

Table 5: Serum glucose level of the different experimental groups

Groups test	Glucose
Negative control (Normal rats)	64.6
Positive control (Hyperlipidimic rats)	109.75
Hyperlipidimic + 20% formula	86.8

From the data, it can be noticed that rats fed on tested diet showed lower value of serum glucose reached to 21.1% as compared to positive control, while negative control showed lower value reached to 25% as compared to tested diet. On the other hand, rats fed on positive control showed higher value of serum glucose reached to 41.3% as compared to negative control.

Sugar alcohols like lactitol, maltitol and mannitol are one type of reduced-calorie sweetener. It can be found in ice creams and cookies. Sugar alcohols provide fewer calories than sugar and have less of an effect on blood glucose (blood sugar) than other carbohydrates. They provide 800 kilocalories or less per day (American Diabetes Association, 2004).

Several studies have shown that the use of low calorie sweeteners can help people with type 2 diabetes to control their body weight (Mann *et al.*, 2004). From our results it can be noticed that rats fed on tested diet showed lower value of serum glucose reached to 21.1% as compared to positive control.

Also, successful weight reduction depends on creating an energy deficit within a healthy, balanced diet by combining lower energy intake and higher energy expenditure. Also, results revealed lower value of body weight gain reached to 21.3% as compared to positive control. de Ruyter *et al.* (2012) showed that substitution of added sugar by sweeteners in carbonated soft drinks has beneficial effects on body mass index (BMI).

Finally, our present nutritional results confirmed the previous researches (Wisselink *et al.*, 2002; Schiweck *et al.*, 1994; Mozzi *et al.*, 2010). They found that the reduced caloric values are due to the fact that sugar alcohols are only partially absorbed in the upper intestine. Thus, a large part of the ingested sugar alcohols reaches the large intestine, where bacteria degrade it. Besides that, several health-promoting properties have been attributed to lactic acid bacteria themselves. The utilization of mannitol-producing probiotics might therefore be of considerable interest.

Conclusion

In conclusion, the highest yield of low calorie sugars (mannitol) was reported by encapsulated *Leuc.mesenteroides* using sodium alginate at 25°C, pH 6 for 24 hr, in modified MRS medium. The results of sensory evaluation of manufactured fermented ice cream were satisfactory for acceptability of the final product. The results also revealed that viable cells of *Leuc.mesenteroides* reached a beneficial level as probiotic, therefore it can be used as a potential production of active ingredients (low calorie sugars) in food, and incorporated into dairy products as therapeutic products.. However, further numerous experiments are required in order to use of encapsulated bacteria to produce high quantities of mannitol .

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