# Survey the survival of Lactobacillus paracasei in fermented and non-fermented frozen soy dessert

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# A Survey on the survival of *Lactobacillus paracasei* in fermented and non-fermented frozen soy dessert

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ABSTRACT

Recent developments in functional food have heightened the need for a further investigation about manufacture newest functional products for various consumers. The circumstances of production and preservation of these products need to be surveyed as well. The purpose of this study was to determine the survival rate of *Lactobacillus paracasei* in fermented and non-fermented frozen soy dessert, as well as evaluation of the physicochemical, rheological and sensory properties of the products. Fermented and non-fermented probiotic frozen soy dessert containing *Lactobacillus paracasei ssp. paracasei* was manufactured. Physicochemical, rheological and sensory properties of the products were assessed. The viable cell counts of *L. paracasei* was monitored during 180 days storage at −24 °C. A significant increase ($p < 0.05$) was observed in overrun ($42.57 \pm 8.5$) values in fermented probiotic frozen soy dessert in comparison with other samples. The viscosity of the control sample and non-fermented dessert after 50 min (Respectively 1112 and 1095 cp) was higher than the viscosity of fermented frozen soy dessert (966 cp). The sensory properties of fermented probiotic frozen soy dessert were significantly improved by fermentation. In contrast to non-fermented frozen probiotic soy dessert, there was no significant ($p < 0.05$) decrease in viable cell counts of *L. paracasei* during storage. Both probiotic frozen soy desserts have promising potential for utilization as functional products. But, fermentation could increase the stability of probiotics bacteria especially *L. paracasei* in frozen soy dessert. Also, the physicochemical and sensory properties of frozen soy dessert were improved by fermentation.

## 1. Introduction

With the rise in people awareness about the effect of food on health, the consumption of functional foods is developing. Functional foods such as probiotic, prebiotic and synbiotic foods are considered to provide a health benefit beyond basic nutritional value (Ansari and Pourjafar, 2019; Min et al., 2018; Roy and Kumar, 2018). According to the FAO/WHO (2007), the word probiotic refers to live microorganisms that when consuming in adequate amounts ($10^6$–$10^7$ CFU/g) demonstrate health benefits to the host and have a role in the prevention of many diseases (FAO/WHO, 2007). In 1995, Gibson and Roberfroid introduced prebiotic as components that beneficially affect the host by stimulating the growth and activity of probiotics into the human gastrointestinal tract. Also, they mentioned when both probiotics and prebiotics are used in combination, they are known as "synbiotic" (Ansari and Pourjafar, 2018; Gibson and Roberfroid, 1995).

Soybean is one of the most significant seeds in the world due to availability and wealthy in high-quality proteins, essential amino acids, calcium, phosphorus, iron, vitamins especially A and B, isoflavone aglycones and prebiotic oligosaccharides such as raffinose and stachyose (Endres, 2001; Genta et al., 2002). Soy products can be used as an alternative for the nondairy probiotic carrier. Conversely, because of soy

*Abbreviations:* CFU, Colony Forming Units.

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undesirable beany flavor, soy-based products consumption is limited (Drozen and Harrison, 1998; Novriadi et al., 2019).

According to some studies, frozen products such as ice-creams are appropriate vehicle for delivering of probiotics into the human gut because it has a high total solids level, neutral pH, prebiotic oligosaccharides and transfers these beneficial microorganisms in a frozen state (Homayouni et al., 2018; Homayouni and Norouzi, 2016; Kailasapathy and Chin, 2000). Also, recent studies have provided positive results on the growth and survival rate of *Lactobacillus* species in the ice-cream, especially with regard to cold shock response due to increase in the ability of adaption to the low-temperature (Homayouni and Norouzi, 2016; Sauvageot et al., 2006). Researches have shown promising results on the effectiveness of soy prebiotics components in increasing the survival of probiotics in the unfavorable situation (Champagne et al., 2009; Donkor et al., 2005; Shah et al., 2010). Accordingly, frozen soy dessert could be a suitable vehicle for delivering probiotics into the human gut.

Several *Lactobacillus* are the most widespread types of microbes used as probiotics with many health-promoting effects, and they play an important role by stimulation the immune system to react quickly to infection with pathogens and by bacterial antagonism it inhibits the colonization of the gut by harmful or pathogenic bacteria (Abdolhosseinzadeh et al., 2018; Gaon et al., 2003; Rizzardini et al., 2012). Gaon et al. (2003) found that *L. casei* and *L. acidophilus* strains considerably decreased the number of depositions, shorten the period of diarrhea and duration of symptoms in children with persistent diarrhea.

*Lactobacilli* are also widely used as probiotics and soy products have been examined as a substance for the probiotics, but no information is accessible on the survival of the *Lactobacillus paracasei* (also known as *L. casei* CRL-431) in fermented and non-fermented frozen soy dessert. The main purpose of this study was to comparison survival of the *L. paracasei* in two kinds of fermented and non-fermented frozen soy dessert during 180 days storage at −24 °C. As well as evaluation of the physicochemical, rheological and sensory properties of in both probiotic frozen soy desserts. The research was accomplished through the guidelines by the Declaration of Helsinki. Sensory evaluation of this survey was approved by the ethics committee of Tabriz University of Medical Sciences (No.9160). Written informed consent was obtained from all panelists (the trial was registered in the Iranian Registry of Clinical Trials, available at http://www.irct.ir, identifier, IRCT 201202195554N6).

## 2. Materials and methods

### 2.1. Manufacturing of frozen soy dessert

Soymilk powder was selected as the main ingredient purchased from Shormast Company (Savadkoh, Iran). Cow milk with 3% fat and pasteurized cream containing 30% fat was supplied by Pegah Dairy Industries Co. (Tabriz, Iran). The sugar was selected mainly as a sweetener in order to improve the taste of sweetness (Ahvaz, Iran). Sodium citrate and vanillin that obtained from local suppliers was used as an emulsifier and aroma development respectively. Stabilizer (PROVImel FO 41) was prepared from Provisco Company (Provisco AG, Hauptwil, Switzerland). Pure freeze-dried probiotic culture of *L. paracasei* (CRL-431) was provided from CHR-Hansen (Horsholm, Denmark).

Three batches of frozen soy dessert were formulated based on 38% total solids and 5% fat content (Table 1); the capacity of each batch was 10 kg. For manufacturing frozen soy dessert, after adjusting the dry matter contents of cow milk by soy milk powder and sugar and also after adding emulsifier and stabilizer, the mixture was heated at 72 °C for 30 min. Then mixture cooled to 4 °C and flavored with vanillin and stored for 12 h for ripening. After that mixture was immediately frozen by using a vertical freezing machine of 20 kg capacity (Soft Ice Cream Machine and Hommy Enterprise Co., Jiongmen, China). For production non-fermented probiotic frozen soy dessert, after 12 h ripening, the mixture was inoculated with 1% (W/W) freeze-dried probiotic culture of *L. paracasei* and immediately frozen. In fermented probiotic frozen soy dessert, the mixture was divided into two parts. The first portion flavored with vanillin then stored for 12 h and the second part after cooling to 37–40 °C, was inoculated with *L. paracasei* and incubated at 40 °C until pH was reduced to 5. Afterwards, both parts were mixed and immediately. All three batches of frozen soy dessert were packaged in 100 ml cups and stored at −24 °C. The manufacturing procedure of probiotic frozen soy dessert is schematically presented in Fig. 1.

**Table 1**
The formulation of frozen soy-based dessert.

| Ingredients | Amount (%) |
|---|---|
| Skim milk | 63 |
| Soy powder | 7 |
| Fat | 5 |
| Sugar | 19 |
| Emulsifier/Stabilizer | 0.5 |
| Vanillin | 0.1 |
| Total solid (%) | 38 |

### 2.2. Physicochemical and rheological analysis

The pH values of mixtures were measured by a digital pH-meter (Microprocessor pH-meter RE 357, EDT Instruments, Dover Kent, UK). Specific gravity, overrun and melting resistance of frozen soy dessert were determined according to AOAC (1997).

In this study among all rheological properties, the viscosity of the mixture was measured after aging level at 4 °C by using a Visco Star Plus viscometer (Fungilab Co., Spain) with spindle number 4 during the 50s (Sakurai, 1996). All measurements were done in triplicate.

### 2.3. Sensory evaluation

Sensory properties including flavor, color, mouth-feel, and overall acceptance are considered to be the most important characteristics for customer preferences. The sensory evaluation test was performed with 8 trained and 32 panelists. Panelists were tested on the basis of sensory acuity and consistency by testing their accuracy to recognize four principle tastes. Each judge received randomly coded samples and used mineral water for rinsing the mouth between samples. A five-point hedonic scale was used to verify the sensory acceptance of the product (Homayouni et al., 2018; Lawless and Heymann, 2010).

### 2.4. Bacteriological analysis

The colony counts of *L. paracasei* was enumerated using the pour plate technique in selective medium MRS Agar (Merck, Darmstadt, Germany) before freezing, immediately after freezing as well as at the end of every month till 180 days storage at −24 °C and incubated at 37 ± 1 °C for 72 h. The averages of all results were expressed as colony-forming units per gram of each sample (CFU/g) (Haynes and Playne, 2002; Pourjafar et al., 2018).

### 2.5. Statistical analysis

All experiments were performed in three separate batches. Results for each analysis were expressed as mean ± standard deviation. The statistical analysis was performed using SPSS statistical software, Version 13. Independent *t*-test was used for paired comparison between groups. Sensory evaluation results were analyzed by Kruskal–Wallis test. The criterion for statistical significance was $p < 0.05$.

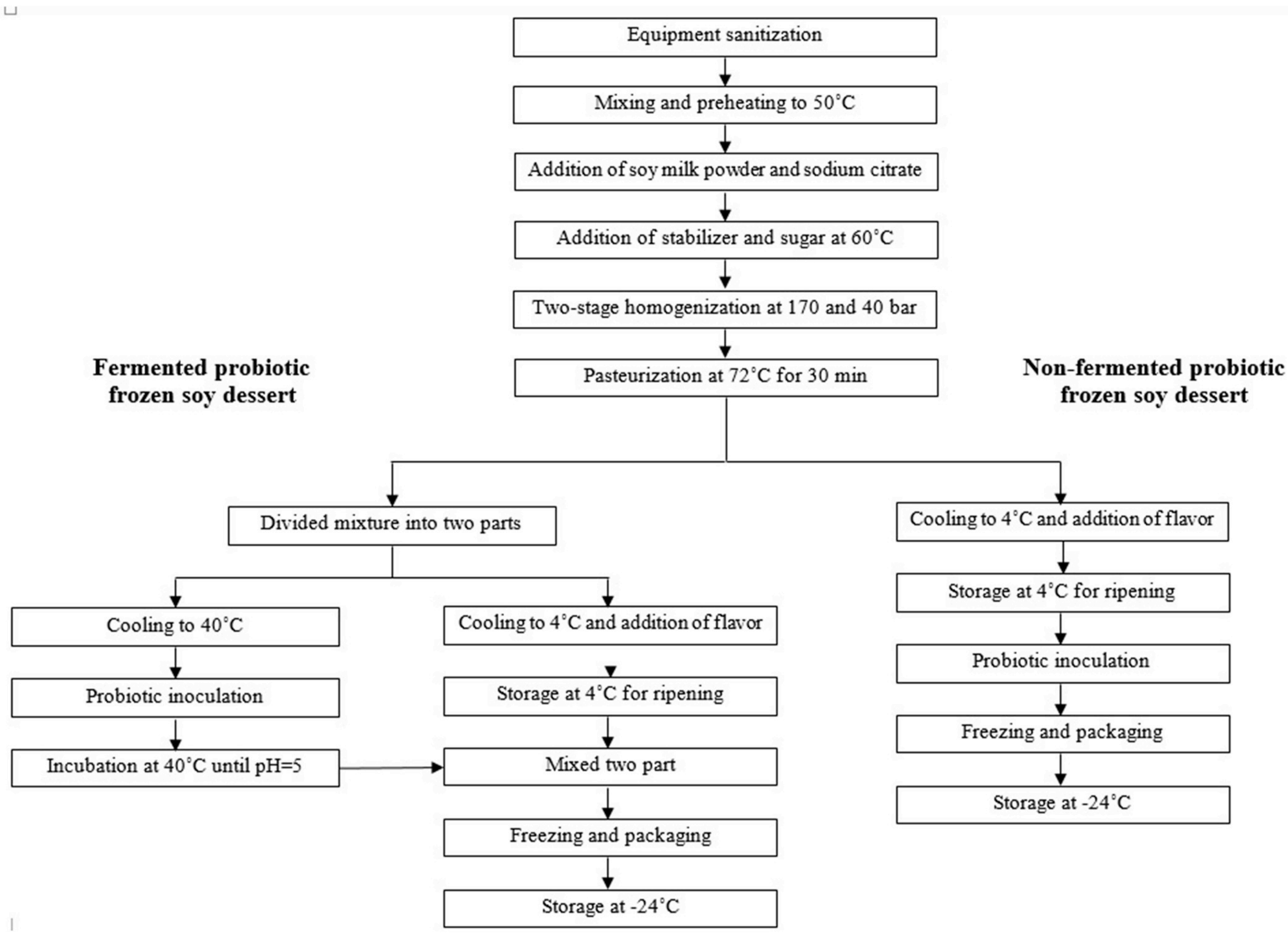


**Fig. 1.** Schematic representation of the manufacturing procedure for probiotic frozen soy dessert.

## 3. Results and discussion

### 3.1. Physicochemical and rheological characteristics

Table 2 provides the results obtained from the analysis of the physicochemical properties of three samples. As table shows, no significant differences were found in melting resistance of samples ($P < 0.05$); but, there is a significant difference in pH and overrun property of probiotic frozen soy dessert in comparison with other samples ($P < 0.05$). The specific gravity of control sample is completely different from fermented and non-fermented probiotic frozen soy dessert ($P < 0.05$). Viscosity changes in all samples during the 50 s at 4 °C are present in Fig. 2. According to the figure significant difference was found in viscosity property of fermented probiotic frozen soy dessert mixture in comparison with other samples ($P < 0.05$).

Reduction in pH of fermented frozen soy dessert related to the growth of the *L. paracasei* and production of organic acids (Millette et al., 2007). The observed increase in the overrun value of fermented probiotic frozen soy dessert could be attributed to the ability of *Lactobacillus spp.* to product bio-surfactant and bio-emulsifiers that they increased the rate of overrun in ice-creams (Gudiña et al., 2011; Reid et al., 1999). Increased in Specific gravity value of probiotic frozen soy desserts in this study might be attributed to enhancement in *L. paracasei* biomass.

This significant decrease in viscosity of fermented probiotic frozen soy dessert mixture might be explained by increasing acidity during fermentation as well as consumption of protein and carbohydrate contents of soy powder by *L. paracasei*. This decrease in viscosity in our study seems to be consistent with other research which found an increase in acidity of yogurt can decrease the viscosity of product (Akdeniz and Akalın, 2019).

### 3.2. Sensory properties

Table 3 compares the results obtained from the sensory analysis of probiotic frozen soy dessert samples. It is apparent from this table that there are no differences in sensory properties of non-fermented probiotic frozen soy dessert and control sample. Although, a positive acceptance was found in mouth-feel, color and overall acceptance of fermented

**Table 2**
Physicochemical properties of fermented and non-fermented probiotic frozen soy dessert and frozen soy dessert (control sample).

| | Fermented probiotic frozen soy dessert | Non-fermented probiotic frozen soy dessert | Frozen soy dessert (control sample) |
|---|---|---|---|
| pH | $5.81 \pm 0.01^{a}$ | $7.12 \pm 0.01^{ab}$ | $7.18 \pm 0.01^{ab}$ |
| Specific gravity | $1.1319 \pm 0.0002^{a}$ | $1.1318 \pm 0.0002^{a}$ | $1.1239 \pm 0.0001^{ab}$ |
| Overrun (%) | $42.57 \pm 8.5^{a}$ | $15.69 \pm 3.74^{ab}$ | $13.38 \pm 1.86^{ab}$ |
| Melting resistance (%) | $90.39 \pm 0.42^{a}$ | $87.98 \pm 1.52^{a}$ | $88.42 \pm 0.81^{a}$ |

[a,b] means in the same row followed by different letters were significantly different ($P < 0.05$).

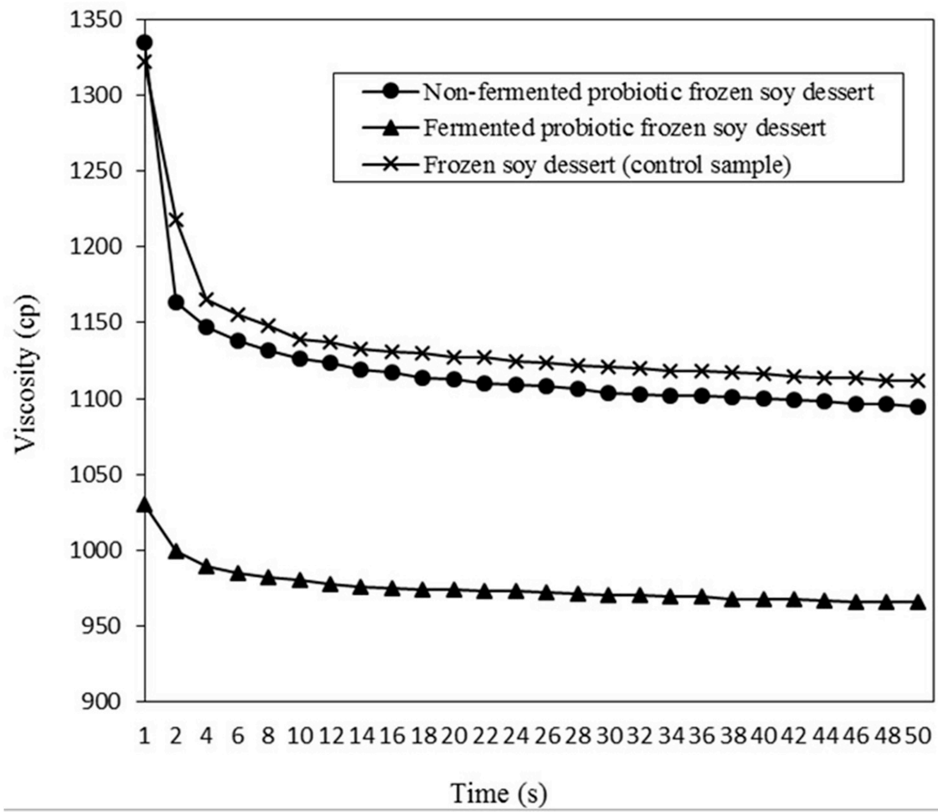


**Fig. 2.** Changes of viscosity in fermented and non-fermented probiotic frozen soy dessert mix and frozen soy dessert mix (control sample) at 4 °C.

**Table 3**
Sensory properties of fermented and non-fermented probiotic frozen soy dessert and frozen soy dessert (control sample).

| | Flavor | Color | mouth feel | Overall acceptance |
|---|---|---|---|---|
| Fermented probiotic frozen soy dessert | $3.65 \pm 0.95^{a}$ | $4.22 \pm 0.92^{a}$ | $4.32 \pm 1^{a}$ | $4.1 \pm 0.8^{a}$ |
| Non-fermented probiotic frozen soy dessert | $3.92 \pm 0.89^{a}$ | $3.87 \pm 0.99^{ab}$ | $4 \pm 0.93^{ab}$ | $3.92 \pm 0.76^{ab}$ |
| Frozen soy dessert (control sample) | $3.77 \pm 0.97^{a}$ | $3.5 \pm 1.06^{ab}$ | $3.87 \pm 1.02^{ab}$ | $3.72 \pm 0.89^{ab}$ |

[a,b] means in the same column followed by different letters were significantly different ($P < 0.05$).

probiotic frozen soy dessert. These results are significant at the $P < 0.05$.

No significant difference in the flavor of desserts did not indicate that this sensory property of samples is exactly the same, it demonstrated that panelists approbated flavor of all kind of frozen soy desserts. Fermentation improved color, mouth-feel and overall acceptance of fermented probiotic frozen soy dessert (Homayouni and Norouzi, 2016; Liu, 2012).

### *3.3. Survival of L. paracasei in probiotic frozen soy desserts*

The results obtained from the analysis of survival of *L. paracasei* in probiotic frozen soy desserts are presented in Fig. 3. According to the figure, there is no significant ($P < 0.05$) decrease in viable cell number of probiotics in fermented probiotic frozen soy dessert after freezing and during 180 days storage at −24 °C. However, it is apparent from this figure that there is a significant decline in viable cell count of *L. paracasei* in non-fermented probiotic frozen soy dessert during the same storage conditions ($P < 0.05$). The *L. paracasei* count showed 0.49 log decreases for fermented probiotic frozen soy dessert after 180 days, while the non-fermented probiotic frozen soy dessert showed a decline of 1.83 log.

According to various studies, the minimum necessary concentration of probiotics bacteria to cause a beneficial result is about $10^6$ viable cell/g-ml of product at the moment of consumption (Ghasemnezhad et al., 2017; Hekmat and McMAHON, 1992; Parvez et al., 2006). So, despite significant decrease in viable cell number of *L. paracasei* in non-fermented probiotic frozen soy dessert, this dessert, as well as fermented probiotic frozen soy dessert, achieve this target.

The results of the study supported the previous surveys which found soybean oligosaccharides such as raffinose and stachyose as well as other growth factors like peptides and free $NH_3^-$ groups could improve survival of probiotics. Moreover, *Lactobacillus* strains have the highest resistance to simulated frozen conditions (Champagne et al., 2009; Donkor et al., 2005; Homayouni et al., 2012). When compared survival of *L. paracasei* in both probiotic frozen soy dessert, it is clear that the viable cell count of *L. paracasei* in fermented probiotic frozen soy dessert is more than other samples. The most possible reason for this increase in enumeration of probiotics might be due to fermentation because fermentation by probiotics increased the resistance of this beneficial bacteria in the production and storage condition of products (Kailasapathy and Chin, 2000; Moslemi et al., 2016).

## 4. Conclusions

The most important conclusion of this study is that frozen soy dessert could improve the viable cell number of *L. paracasei* more than $10^6$ CFU/g at the end of 180 days storage at −24 °C. So, both probiotic frozen soy desserts have promising potential for utilization as functional products.

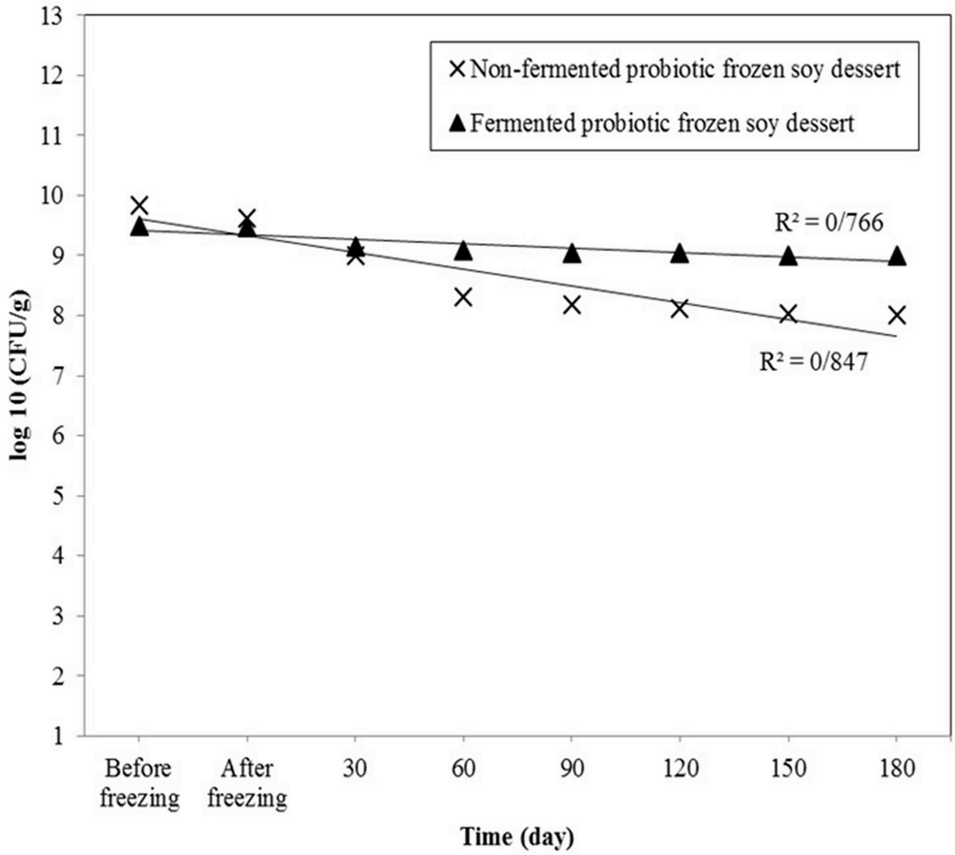


**Fig. 3.** Survival of *L. casei* cells in fermented and non-fermented probiotic frozen soy dessert after freezing and during 180 days storage at −24 °C.

But, fermentation could increase the stability of probiotics bacteria especially *L. paracasei* in frozen soy dessert. Also, the physicochemical and sensory properties of frozen soy dessert were improved by fermentation. In general, therefore, it seems that probiotic frozen soy dessert could be an appropriate vehicle for delivering of *L. paracasei* into the human gastrointestinal.

This research has thrown up some question in need of more investigation; such as the effect of production and preservation circumstances of fermentation on the isoflavones. As well as survey survival of different strains of probiotics in other products of soybean.

## Conflicts of interest

The authors have declared that they have no conflict of interest.

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