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Original scientific paper

Viability of *L. casei* during fermentation in soymilk and freeze-dried soymilk; effect of cryoprotectant, rehydration and storage temperature

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Abstract

The aim of the work was to investigate the behaviour of *L. casei* and the effect of sorbitol on its viability during fermentation in soymilk drink. Values for pH, ranging from 6.82 to 3.42 in the soymilk drink without sorbitol and from 6.74 to 3.41 in the drink with sorbitol were noted during 72 h of fermentation at 25^{o}C. The corresponding values for titratable acidity ranged from 0.071% to 0.758% and from 0.073% to 0.761%, respectively. Soymilk was found to support the growth of *L. casei* with improvement in viability for 0.24 log at the end of fermentation when sorbitol was added. Survival of *L. casei* and the effectiveness of sorbitol in improving viability during freeze-drying, subsequent rehydration and during a 5-week period of storage under different temperatures were also investigated. After freeze-drying, *L. casei* exhibited a survival percent of approximately 46%. Sorbitol improved the viability of *L. casei* by 0.51 log immediately after freeze-drying and by 1.30 log and 0.47 log during five weeks of storage at 25^{o}C and 4^{o}C, respectively. Further study revealed that the freeze-dried fermented soymilk rehydrated at 45^{o}C was optimum for the recovery of *L. casei* with improvement in recovery for 0.68 log when sorbitol was added. A higher percent of survival was noted when the dried soymilk was stored at 4^{o}C than at 25^{o}C with improved viability at the end of 5 weeks storage for approximately 6 log for drinks with and without sorbitol. Fermented dried soymilk with sorbitol afforded significant tolerance of *L. casei* to acid stress. Generally, a stable probiotic diary product was prepared in which the concentration of *L. casei* remained above therapeutic level of 10^7 cfu/ml.

Keywords: *L. casei*, fermented soymilk, freeze-drying, sorbitol, viability

Introduction

Soybean is one of the most important oilseeds in the world. Besides the extra protein and fibre, the biggest benefit in soybean are the isoflavones connected to a whole host of health issues, the most important being prevention of many cancers, heart diseases, osteoporosis and more (1-5). In addition, soybean contains powerful antioxidants that help fight disease and aging, thus strengthening the immune system (6-7). Soy fibre, in a healthy diet, may help control diabetes by making the body more sensitive to insulin in the bloodstream, while slowing the release of glucose into the bloodstream to a more manageable rate (8). Also, soybean could be effective in the protection of neuropathy induced by diabetes mellitus (9). Soy's ability to lower LDL cholesterol in the blood (10) also helps to prevent kidney damage (11-12).

However, the oligosaccharide constituents in soybean, such as raffinose and stachyose are not digested by human beings and thus may cause flatulence. This along with the disagreeable beany flavour has limited the consumption of soybean as the raw food material. In order to increase both acceptability and nutritional value, fermentation of soybean products with lactic acid bacteria has been studied extensively to develop more digestible and palatable foods

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such as fermented soybean cheese, sour milk beverage and soybean yogurt (13-15). Soymilk as a water extract of soybean has long been a popular alternative for people who are unable to drink cow's milk (usually due to lactose intolerance). But rather than drink soymilk because you have to, more and more people are deliberately choosing soymilk because of the added health benefits.

Lactic acid has been widely used as an acidulant and many reports have shown the usefulness of lactic acid bacteria as probiotics for humans and animals (16-19). Probiotic culture, known to be (+)-lactic acid producer exhibiting promising therapeutic potential, is *Lactobacillus casei*. The action of this probiotic on intestinal flora results in vital benefits, including prevention and treatment of gastrointestinal disorders, such as colon irritation, constipation, paediatric and travellers' diarrhoea (20-22), ulcerative colitis and Crohn's disease (23-24). Other important properties include liver improvement function (25), prevention of cancer and cardiovascular diseases (anticholesterolaemic effect) (26), antimicrobial activity (27-28) and reduction of food intolerance (29). The beneficial effect is proposed to be due, at least in part, to interference with the innate immune system and possibly the orientation of adaptive immunity (30).

The homofermentative *L. casei* is acidotolerant with an optimum pH of 5.5 and is relatively insensitive to product inhibition by lactic acid (31). In order to exert any beneficial health effect, the concentration in a product that serves as a delivery system needs to be in a therapeutic level ranging from 10^6 to over 10^7 cfu/ml or gram product (32-34). The main obstacle for achieving and maintaining required levels is the strain-dependence and poor survival due to increased acidity and oxygen tension (35).

Probiotics usually have limited shelf-life in conventional yoghurt as well as in the product itself. Freeze-drying is a process that not only preserves the yoghurt, but also helps to maintain a sufficient quantity of viable probiotics. Previous research (36) has found that certain *L. casei* strains are able to survive the freeze drying process with the percent of survival ranging from 41% to 75% depending significantly on the bacterial strain. During the processing and storage of freeze-dried yoghurt, oxygen content, high temperature, low pH, water activity and elevated solution concentration may all affect the viability of probiotic organisms (36-37). Compatible cryoprotectants may be added to media or into the yoghurt mix prior to fermentation to assists in the adaptation of probiotic to the environment and maintain the viability during freeze-drying (38-40). As compatible cryoprotectants accumulate within the cells, the osmotic difference with their external environment is reduced. The extent to which cryoprotection is provided by any given cryoprotectant varies between cultures (39; 41-45).

Considering the above-mentioned, the behaviour of *L. casei* in soymilk drink during fermentation was studied and the effect of sorbitol as a sugar derivative present in the growth medium was evaluated. In this study, an attempt was made to dehydrate fermented soymilk with freeze-drying, which is commonly employed in food industry. Survival of *L. casei* during the drying process and the effect of sorbitol in preserving probiotic's viability were investigated. In addition, chemical properties and the viability of *L. casei* in the dried soymilk after rehydration, acid challenge and storage under different temperature conditions during 5 weeks were compared.

Materials and methods

2.1. Microorganism and chemicals

L. casei (FD-DVE/L. casei-01-nu-trish was kindly provided by Chr. Hansen (Copenhagen, Denmark). For fermented milk preparation whole sterile pasteurized soymilk (SoVita, Vitalia, Skopje, Macedonia) was used. As a cryoprotectant, sorbitol was purchased from Merck KGaA (Darmstadt, Germany). For bacterial enumeration, MRS Agar (CM0361, de man, Rogosa, Sharpe, Oxoid LTD, Basingstoke, Hampshire, England) and peptone water (Oxoid LTD, Basingstoke, Hampshire, England) were used.

2.2. Preparation of fermented probiotic soymilk

In this study, a strain of *L. casei* was used as a single culture for production of fermented soymilk. For the preparation, whole sterile soymilk inoculated with *L. casei* (0.05 g/L) was used. Samples with and without cryoprotectant were prepared. As a cryoprotectant, sorbitol was used in concentration of 25 g/L. Sorbitol was added to the soymilk during phase of fermentation. According to the preliminary results related to the microbial and chemical characterisation, which were in accordance with those of probiotic producer, inoculated soymilk fermented for 3 days without shaking at temperature of 25ºC until a pH of 3.42 was reached in both samples with and without sorbitol. The initial population of L. casei was 2.98×10^8 cfu/ml and at the end of fermentation the maximum population of 9.7×10^{19} cfu/ml was obtained in the samples without sorbitol, while in the samples with sorbitol, the corresponding values were 2.7×10^8 and 1.68×10^{20} cfu/ml, respectively.

2.3. Drying of fermented probiotic soymilk

In this study, the fermented probiotic soymilk was subjected to drying with freeze-drying. The samples were stored in tightly sealed sterile bottles and frozen at -20ºC. Then, frozen samples were than lyophilized with a freeze-dryer (Freeze Dry System, Labconco, USA) at a condenser temperature of -45ºC and 0.180 mBar vacuum for about 50 h (13-14).

2.4. Storage of the dried fermented probiotic soymilk

Each of the freeze-dried products of fermented probiotic soymilk (10 g) was placed in a 100 ml glass bottle and vacuum sealed before storage. Then, were stored at either 25ºC or 4ºC for a period of 5 weeks. The chemical characterization, viability of probiotic organism and moisture content of the samples were measured at predetermined time intervals. Moisture content of the samples was determined according to the AOAC method (46).

2.5. Rehydration study

When the rehydration phase was performed, the dried fermented samples were mixed with peptone water (0.1 g/ml; Peptone water, Oxoid LTD, Hampshire, England), which was pretempered at 25ºC, 45ºC and/or 90ºC. The constituted samples were then serially diluted with peptone water and spread-plated on the appropriate medium to enumerate the test organisms.

2.6. Acid challenge

One gram of fermented whole and freeze-dried probiotic soymilk was homogenized in 9.0 ml 0.025 N HCl, leading to final pH 2.0 prior to a 60 min incubation. After 60 min of acid challenge, samples were quickly diluted in peptone water and surviving cells counted (47). Acid challenge test was applied to samples with and without sorbitol, and for the freeze dried samples the test was applied after 1 week storage at 4ºC and 25ºC, respectively.

2.7. Chemical analyses

2.7.1. Titratable acidity (TA) and pH

TA was determined using the method (number 16.023) of AOAC (46) by titration with 0.01 N NaOH solution and expressed as percent lactic acid, while the pH of the samples was measured using a pH meter (Jenway ltd., Felsted, UK).

2.8. Microbial analyses

2.8.1. Enumeration of probiotic L. casei

For the enumeration of *L. casei*, de man Rogosa Sharpe (MRS) agar, as described by Otieno et al. (2), Minelli et al. (15) and Ha et al. (48) was used.

When the enumeration of bacteria was performed, 1 g of the dried fermented soymilk or 1 ml of the fermented soymilk sample was mixed with 9.0 ml of peptone water, vortexed for 15 s and serially diluted with peptone water. Serially diluted sample (0.1 ml) was spread-plated onto the appropriate medium mentioned above. After 72 h of incubation at 37ºC, the colonies that appeared on the plates were counted (cell counter, Esko, Yugo Lek, Belgrade, Serbia) and the cfu per ml was calculated.

2.9. Statistical analysis

The mean values and the standard deviation were calculated from the data obtained with triplicate trials. These data were compared with the Student,s *t*-test and two-factorial analysis of variance with a confidence interval of 95% (49).

Results and discussion

3.1. Moisture content of the fermented soymilk after drying

Water content is an important parameter for the stability of dried cultures. In general, microorganisms survive better in low-water activity. However, over drying may diminish the viability and stability of microorganisms (50). Besides, it is reported that the optimum residual moisture content varies with the composition of the fluid in which the microorganisms are dried, with the storage atmosphere and with the species of organisms (51). The moisture content in the samples of freeze-dried fermented probiotic soymilk, with and without sorbitol, was determined immediately after freeze-drying and it was approximately 5%. No significant difference in moisture content was observed among the samples with and without sorbitol. According to literature data (51), freeze-dried samples may be considered as stable.

3.2. Changes in pH and titratable acidity in soymilk during the fermentation and storage of freeze-dried fermented soymilk with L. casei

In general, pH value determines the processes, such as milk fermentation, enzyme activity, bacterial growth and it is an indicator for the taste of the product. The growth of lactobacilli is usually associated with production of acids, such as lactic and acetic acid. In order to detect measurable increase in acidity, the number of microorganism has to increase for several millions per millilitre. During fermentation in the food product, the content of acids increases, that of carbohydrates decreases and consequently pH value too. This process of acidification is one of the most desirable side effects of bacterial growth. pH value may decrease below 4.0, which is low enough to inhibit the bacterial growth of many other microorganisms, including most of the human pathogens, by which the storage time of the product is prolonged. Acidity may change the texture of the food product due to the protein precipitation, while biochemical changes resulting from the bacterial growth may change the odour and taste of the product. Fermentation is self-limited due to the sensitivity of lactobacilli to low pH.

During the study, changes in acidity have been followed in non-freeze-dried probiotic samples of whole soymilk with and without sorbitol and in freeze-dried samples stored at 25ºC and 4ºC during 5 weeks. pH value for the whole soymilk in the beginning of the study (without probiotic

and cryoprotectant) was 6.95, while for titratable acidity, 0.061% of lactic acid was determined. During the fermentation (3 days), pH values in non-freeze-dried samples without sorbitol decreased from 6.82 to 3.42 in milk stored at 25ºC and to 6.28 in milk stored at 4ºC (fig. 1). In the samples in which sorbitol was added during the fermentation phase, the pH values ranged from 6.74 to 3.41 and to 5.74 (Fig. 1), accordingly. Non-significant difference in the acidity among samples with and without sorbitol at the beginning of the study can be explained by the presence of sorbitol. Namely, the pH of whole soymilk without sorbitol was 6.95, and for samples with sorbitol pH of 6.87 was measured.

In the next step of the study, the acidity of the samples stored at 25ºC did not change significantly (pH 3.49 in the samples with probiotic only and pH 3.16 in the samples with probiotic and sorbitol after 5 weeks; Fig. 1). These data, along with the most significant changes in pH during 3 days, sensory properties and microbial growth (presented bellow) have pointed to the optimal period of fermentation and freeze-drying by that, also. It is important to emphasize that at the time of freezing, the samples possessed acceptable sensory properties and for freeze-drying, samples fermented at 25ºC were used due to the faster and quantitatively more significant bacterial growth (presented bellow).

Acidity, expressed with pH values was determined in freeze-dried samples also immediately after freeze-drying and during the examined period of 5 weeks. It ranged from 3.25 to 3.44 in samples with probiotic stored at 25ºC (Fig. 2) and from 3.25 to 2.74 in samples stored at 4ºC (Fig. 3). In the samples with sorbitol, these values ranged from 3.21 to 3.32 at 25ºC (Fig. 2) and from 3.21 to 2.68 at 4ºC (Fig. 3). These data, besides water content, havered point also to the product with acceptable stability. In all freeze-dried samples sensory properties of the fermented soymilk were preserved, including light yellow colour and pleasant odour and taste.

Considering the changes in pH value in freeze-dried soymilk samples with *L. casei*, with and without sorbitol, stored at 25ºC, decrease in pH was observed during the first 2 weeks from 3.25 to 3.03 in samples without sorbitol and from 3.21 to 3.02 in samples with sorbitol (Fig. 2). Afterwards, the pH values began to increase to 3.44 and 3.32, respectively, showing decrease in survival rate, which was further confirmed by microbial studies (presented bellow). In all freeze-dried samples, with and without sorbitol, stored at 4ºC, the acidity was continuously decreasing during the whole period of the study, indicating higher survival rate of *L. casei* when product was stored at 4ºC, which was also confirmed by the microbial studies (Fig. 3). In addition, comparison of pH values between the samples with and without sorbitol has shown higher viability when sorbitol is added during the fermentation, which was more pronounced in the samples stored at 4ºC.

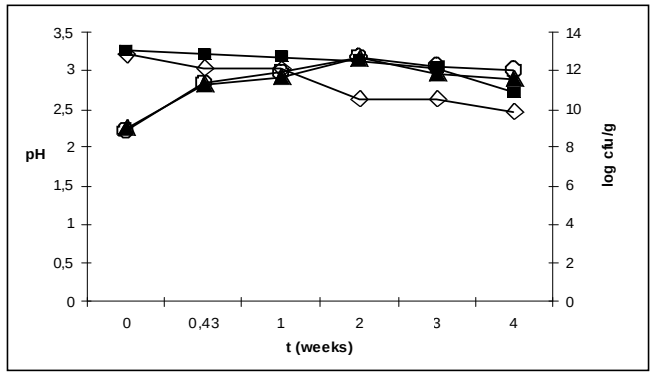

Fig. 1. Changes of pH in soymilk during the growth of *L. casei* in wohole fermented soymilk; samples without (■) and with (◊) sorbiol stored at 25^0C; samples without (▲) and with (○) sorbiol stored at 4^0 C.

As was previously mentioned, the acidity was determined by the values of TA, also. In general, decrease in pH was followed by increase in TA. TA in non-freeze-dried soymilk without sorbitol stored at 25ºC during 5 weeks ranged from 0.071 to 0.857% *vs.* TA of the same sample stored at 4ºC, where TA ranged from 0.071 to 0.504% (Fig. 4). Considering the samples with sorbitol, the values for TA ranged from 0.073 to 0.945% and to 0.738%, respectively (Fig. 4). In addition, the whole soymilk without probiotic and sorbitol had TA of 0.061%, while for samples with sorbitol only, TA of 0.070% was determined. During the fermentation period, TA values increased to 0.758% and 0.137% in the samples without sorbitol stored at 25ºC and 4ºC, respectively, while for the samples with sorbitol, the corresponding values were 0.761% and 0.246%, respectively.

When freeze-dried samples analyzed, the values for TA in the samples without sorbitol ranged from 0.612% immediately after freeze-drying to 0.630% when stored at 25ºC (Fig. 5) and to 0.759 when stored at 4ºC (Fig. 6). From these data, one can notice that when product was stored at 4ºC, higher percent of lactic acid is obtained, pointing to the higher viability of *L. casei* at this storage temperature, which was confirmed by the microbial growth also (Fig. 5-6). These differences can be explained by the phases of microbial growth i.e. the factors influencing the microbial growth, including the medium and the temperature, for which the comment is given further in this paper. The same observations were confirmed for the values of TA obtained in the samples with sorbitol in which TA ranged from 0.846% to 0.792 and to 1.094% when stored at 25ºC (Fig. 5) and 4ºC (Fig. 6), respectively.

When comparing the values for TA between samples with and without sorbitol at constant storage temperature, one can observe that at 25ºC, the values for TA were continuously increasing and they were higher in samples with sorbitol during the entire examined period. The difference was more significant ($p<0.05$) when samples were stored at 4ºC.

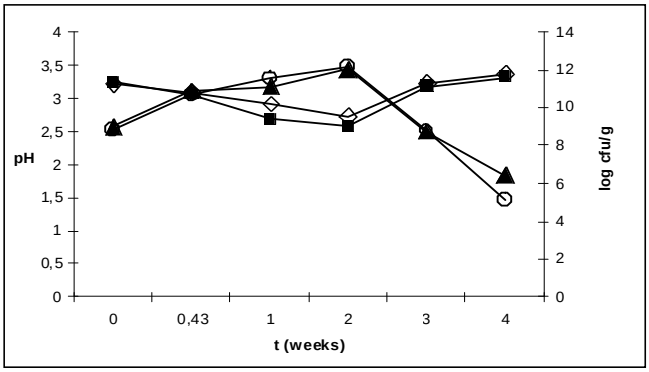

Fig. 2. pH (■, ◊) and growth (▲, ○) of L. casei in freeze-dried fermented soymilk stored at 25^{0}C; (■, ▲) samples without sorbitol; (◊, ○) samples with sorbitol.

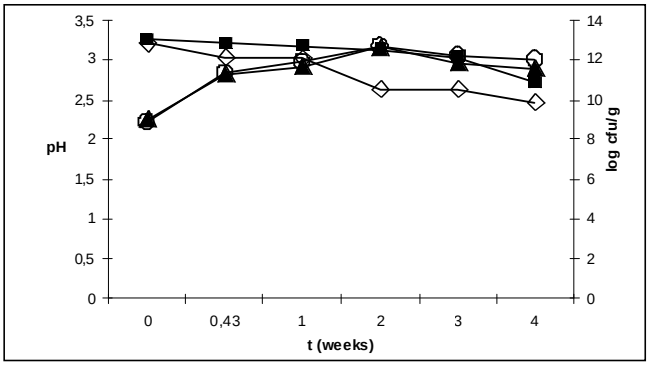

Fig. 3. pH (■, ◊) and growth (▲, ○) of L. casei in freeze-dried fermented soymilk stored at 4^{0}C; (■, ▲) samples without sorbitol; (◊, ○) samples with sorbitol.

3.3. Microbial analyses

3.3.1. Survival of L. casei after freeze-drying and during storage in the fermented dried soymilk

The growth and survival of *L. casei* in the fermented soymilk was monitored during the fermentation, freeze-drying and storage at different temperatures. The presence of *L. casei* in the medium was confirmed microscopically (Nikon, E-800, Japan) and by culture preparation (Fig. 7).

Initially, the population of *L. casei* in the soymilk after 1 h of inoculation and during the three-day fermentation in the samples with and without sorbitol was determined. Also, the initial population of *L. casei* immediately after freeze-drying was determined in the samples with and without sorbitol. In the samples without sorbitol stored at 25ºC, the number of cfu/ml was 2.98 x 10^8 in the beginning of the fermentation and it increased up to 9.7 x 10^{19} cfu/ml by the end of the fermentation (the 3rd day). In the samples with sorbitol fermented at the same temperature, the number of cfu/ml was 2.7 x 10^8 and 1.68 x 10^{20} cfu/ml, respectively. The number of cfu/ml determined immediately after freeze-drying did not differ significantly when comparing freeze-dried samples with and without sorbitol. Thus, in the samples without sorbitol, the population level was 9.7 x 10^8 cfu /ml (≈ 45% of survival), while in the samples with sorbitol, it was 3.1 x 10^9 cfu/ml (≈ 47% of survival).

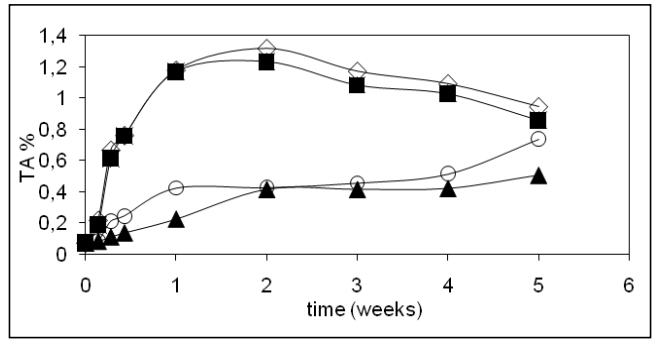

Fig. 4. Changes of TA in soymilk during the growth of L. casei in whole fermented soymilk; samples without (■) and with (◊) sorbitol stored at 25^{0}C; samples without (▲) and with (○) sorbitol stored at 4^{0}C.

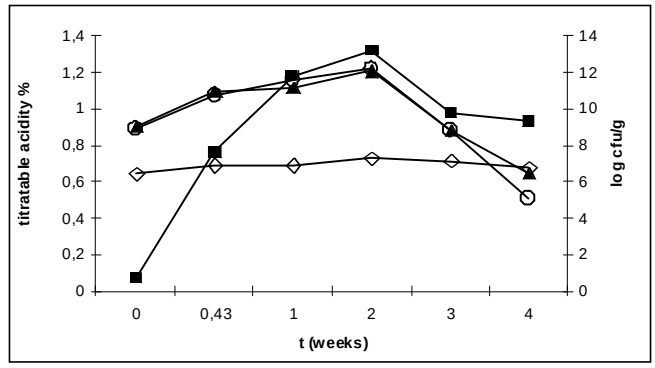

Fig. 5. TA (■, ◊) and growth (▲, ○) of L. casei in freeze-dried fermented soymilk stored at 25^{0}C; (■, ▲) samples without sorbitol; (◊, ○) samples with sorbitol.

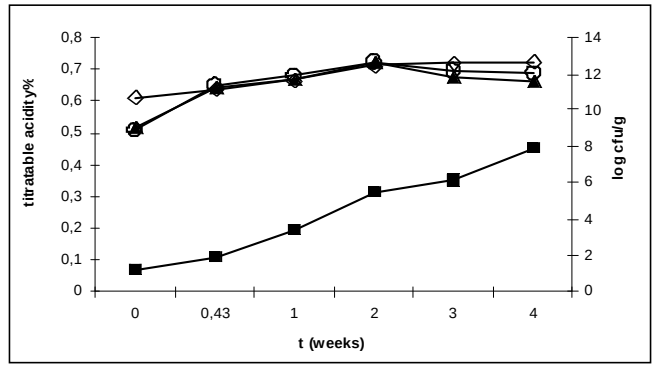

Fig. 6. TA (■, ◊) and growth (▲, ○) of L. casei in freeze-dried fermented soymilk stored at 4^{0}C; (■, ▲) samples without sorbitol; (◊, ○) samples with sorbitol.

Literature data (37) point out the factors affecting bacterial growth, including intrinsic factors, drying medium, storage and rehydration conditions, emphasizing the storage and rehydration temperature the most important. At low temperatures, bacterial metabolism is expected to decrease, including toxins' synthesis and resulting in increased viability. In order to determine the influence of drying medium, including sor-

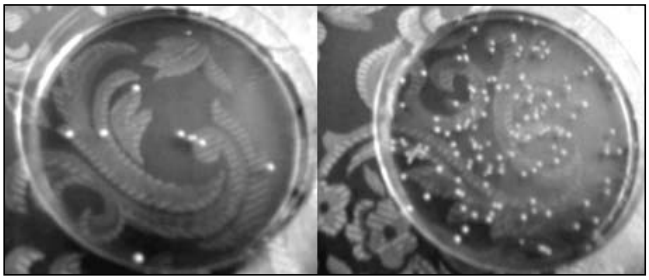

Fig. 7. Cell populations of *L. casei* in fermented dried soymilk estimated by the plate dilution method using MRS agar; (a) stored at 25^0C and (b) stored at 4^0C.

bitol and storage temperature on probiotic growth, bacterial enumeration was performed in regular time intervals (the 1st and the 3rd day and once weekly during 5 weeks).

The presence of sorbitol increased viability of *L. casei* during freeze-drying and storage at both storage temperatures applied in the study. The maximum population was reached the second week, which can be considered as a period of exponential bacterial growth (52). During this period, the number of cfu/ml in the freeze-dried fermented soymilk with sorbitol increased to 1.57×10^{12} cfu/ml when stored at 25ºC and to 4.83×10^{12} cfu/ml when stored at 4ºC, while in the samples without sorbitol the population number increased to 1.08×10^{12} cfu/ml and 4.5×10^{12} cfu/ml, respectively (Fig. 2-3; 5-6). In the next step of the study, no stationary phase of probiotic growth was observed in the samples stored at 25ºC with and without sorbitol. Namely, constant decrease in the population level was observed; thus, at the end of the study the population level was 1.35×10^5 and 2.7×10^6 cfu/ml in the samples without and with sorbitol, respectively. Considering the probiotic growth in the samples stored at 4ºC, a prolonged stationary phase was observed by the end of the study when the population number was 3.82×10^{11} cfu/ml and 1.12×10^{12} cfu/ml in the samples without and with sorbitol, respectively.

In order to determine the dominant factor affecting probiotic growth, two-factorial analysis of variance was applied to analyse data obtained from the viability examination studies. The statistical analysis confirmed that storage temperature dominantly influenced on the viability of *L. casei* in both samples with and without sorbitol ($p < 0,05$). The critical value of F for testing the dominant influence was 4.35, while the test statistic values of the variance ratio were 5.76, 0.81 and 0.10, respectively for the temperature, sorbitol and the interaction between process variables.

3.3.2. Rehydration studies

Rehydration is an important step in the recovery of probiotic from dried diary products. An organism which survives various steps, such as freezing, drying and storage may lose its viability during rehydration. Poor recovery of cells may be attributed to inadequate rehydration procedure. Yuksekdag et al. (53) indicated that rehydration temperatures between 15ºC and 50ºC were the best for *L. casei*, with the temperature of 37ºC as the most convenient, while in the study of Prasad et al. (54), temperature of 45ºC and exposure of 30 min was evaluated as the best for *L. rhamnosus* HN001 (subtype of *L. casei*). On the other hand, several investigators have reported that rehydration at the refrigeration temperature may cause leakage of intracellular substances from the cells, thereby resulting in low viability (51).

Table 1. shows the effect of rehydration temperature on the recovery of *L. casei* from the dried fermented soymilk. The studies were performed in the second week when maximal probiotic growth was observed in the samples stored at 25ºC.

According to the results obtained one can conclude that there was higher recovery rate in the samples rehydrated at 45ºC, which can be considered as optimum rehydration temperature. These results confirm the positive effect of sorbitol on bacterial growth. Namely, at all rehydration temperatures studied, recovery was greater in the samples with sorbitol. The possible mechanisms underlying sorbitol protection of probiotic cells would be prevention of damage to the membrane via interaction therewith, prevention of lipid oxidation owing to its antioxidant properties, stabilisation of the protein structure, and hence preservation of functionality associated with formation of sorbitol-protein complexes (37).

3.3.3. Acid challenge

Results obtained for acid tolerance of *L. casei* in fermented soymilk are presented in Table 2. As one can see, the survival of *L. casei* was between 43.52 and 47.53% and it was higher in the samples with the sugar substrate in the growth medium with improvement in viability of approximately 1 log.

Results obtained for acid tolerance of *L. casei* in the freeze-dried soymilk after 1 week storage at 25ºC and 4ºC (Fig. 8) have revealed higher percents of survival in comparison with those in non-freeze dried soymilk samples with and without sorbitol. Namely, more than 65% of them survived this challenge with improvement in viability when sorbitol was added between 0.3 and 0.5 log depending on the storage temperature.

Conclusion

In conclusion, a stable probiotic diary product of dried fermented soymilk with *L. casei* was prepared in which the concentration of *L. casei* remained above therapeutic level of 10^7 during the investigated storage period of 5 weeks and after acid challenge. Adding sorbitol in the growth medium

Table 1. Effect of rehydration temperature on the recovery of *L. casei* from dried fermented soymilk.

Rehydration (°C)	Population of *L. casei* in dried fermented soymilk without sorbitol (cfu/ml)	Population of *L. casei* in dried fermented soymilk with sorbitol (cfu/ml)
25°C	1.08×10^{12}	1.57×10^{12}
45°C	6.07×10^{12}	7.56×10^{12}
90°C	9.00×10^{5}	1.73×10^{6}

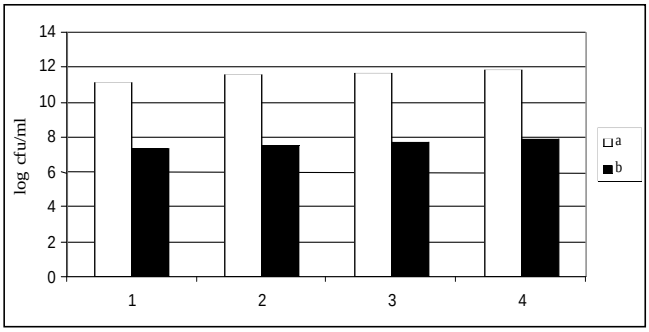

Fig. 8. Viability of L. casei in fermented freeze-dried soymilk after 1 week storage at 25^0C (1: without and 2: with sorbitol) and 4^0C (3: without and 4: with sorbitol); a: no acid challenged and b: acid challenged samples.

increased survival rate of *L. casei* during fermentation, freeze-drying, rehydration and storage, and it significantly improved the protection of bacterial cells from acid stress injury. A higher percent of survival was also noted for *L. casei* when the dried fermented soymilk was stored at 4°C than at 25°C. In order to administer higher therapeutic level of probiotic cells to, a rehydration temperature of 45°C is recommended.

Table 2. Acid tolerance of *L. casei* in fermented soymilk.

Sample	Viability of *L. casei* in acid non-challenged sample (cfu/ml)	Viability of *L. casei* in acid challenged sample (cfu/ml)
Fermented soymilk	9.7×10^{19}	5.0×10^{8}
Fermented soymilk with sorbitol	1.7×10^{20}	4.1×10^{9}

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Резиме

Виталност на *L. casei* при ферментација во млеко од соја и во млеко од соја во прав; влијание на крипротектантот, рехидратацијата и температурата на чување

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***Клучни зборови**: L. casei*, ферментирано соја млеко, лиофилизација, сорбитол, виталност

Подготвен е стабилен пробиотски прехрамбен производ на соја млеко во прав со *L. casei* во кој терапевтската концентрација на пробиотикот опстојува над терапевтското ниво од 10^7 cfu/ml. Испитувана е виталноста на *L. casei* и ефектот на сорбитол при ферментација на млекото од соја. На крајот од ферментацијата која се одвиваше 72 часа на температура од 25°C одредени се pH вредноста и титрациската киселост на млекото од соја и тие изнесуваа приближно 3.41 и 0.760%. Млекото од соја го поддржува растот на *L. casei* со подобрување на виталноста од 0.24 log на крајот од ферментацијата кога во медиумот е додаден сорбитол. Испитуван е и ефектот на сорбитол врз подобрувањето на виталноста во текот на сушењето со лиофилизација, рехидратацијата и при чувањето во период од 5 недели под различни температурни услови. По сушењето, процентот на преживување на *L. casei* изнесува 46%. Сорбитолот ја подобрува виталноста на *L. casei* за 0.51 log веднаш по сушењето и за 1.30 log, односно 0.47 log на крајот од 5-неделниот период кога се чува на 25°C, односно 4°C. Млекото од соја во прав, рехидрирано на 45°C, е оптимално за размножување на *L. casei* со подобрување во виталноста од 0.68 log кога во млекото е додаден сорбитол. Забележан е повисок процент на преживување на *L. casei* кога млекото од соја во прав се чува на температура од 4°C во однос на 25°C, со подобрување на виталноста од 6 log на крајот од 5 неделниот период на чување на примероците со и без сорбитол. Ферментираното млеко од соја во прав со сорбитол обезбедува висока толерантност на *L. casei* во симулирани услови на желудник со одржување на терапевтската концентрација над 10^7 cfu/ml.