Journal of Biological Studies
ISSN 2209-2560

Research Full-Text Paper

The influence of inulin on the survival of *Lactobacillus casei* in a synbiotic squash jam

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Received January 16,2022; Accepted February 12,2022; Published online April 10,2022

Abstract

Inulin is a non-digestible linear polysaccharide. It cannot be digested in small intestine, but can be fermented in the colon by lactic acid bacteria and stimulates the growth of healthy bacteria. In this study, the effect of inulin addition on the survival of the probiotic strain *Lactobacillus casei* was assessed within the production of the symbiotic jam. *Lactobacillus casei* was inoculated to the produced squash jam in a density of 10^8 and 10^7*CFU/ml*. Then inulin was added at three different amounts (0.5, 1 and 1.5 percent). The jam inoculated with the probiotic strain and without inulin was considered as the control. Eight treatments and a control (totally 9 treatments) were analyzed in triplicate. The mean values were compared by Duncan's multiple range test at 95% confidence level. The samples were stored for four weeks at 4℃. Then pH, reducing sugar, acidity in terms of lactic acid, Brix and microbial counts were evaluated. The effect of the inulin percent, bacterial density and shelf life of the jam showed significant differences on microbial count ($p<0.01$). The effect of inulin percentages showed significant differences on the jam sugar ($p<0.01$). The sugar content of the synbiotic squash jam had a significant increase with the increase of inulin ($p\leq0.05$). The highest amount of sugar belonged to the treatment T2N4 (150 g jam + 1.5 ×10^8CFU/ml *Lactobacillus casei* + 1.5% inulin) was ($p<0.01$). With the increase of inulin percentage and shelf life, the highest growth and survival of *L. casei*,equal to 8.968 Log *CFU/ml*, was observed in the treatment T1N3 (containing 150 g jam + 1.5 ×10^7*CFU/ml Lactobacillus casei* + 1% inulin) as the superior treatment. The results showed that both squash and inulin are suitable substrates for the growth of *Lactobacillus casei*.

Keywords: Squash jam, Synbiotic, Inulin, Lactobacillus casei

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1 Introduction

Probiotics reduce lactose, cholesterol and blood pressure. These bacteria are able to stimulate the immune system and strengthen the body's immune response and help intestinal absorption of minerals and vitamins. Probiotics cause anti-mutagenic and anti-carcinogenic properties by inhibiting harmful intestinal bacteria. One of the main challenges in the field of producing and processing probiotic foods are low viability of probiotic bacteria because of the sensitivity to the difficult conditions of the food and also digestive tract. As reported by FAO, a standard probiotic product is such a product containing at least 10^6-10^7*CFU/ml* of the alive and active microorganism at the moment of consumption. In this regard, it seems necessary to use prebiotic ingredients which can stimulate the growth of probiotics in intestine and also help better stability of the product during storage (Salminen et al., 2021). Among these compounds, inulin can be noted. Inulin is composed of fructose polymers with a polymerization degree of 2 to 60 linked by fructosyl β-(1-2) bonds. Inulin is found in nature as a storage carbohydrate in plants and extracellular polysaccharides in some microorganisms. Inulin enters the colon without any changes by the enzymes of the upper gastrointestinal tract in where it is fermented by beta-fructosidase (inulinase) produced by the probiotics. As inulin is mixed with water or any other aqueous fluid, fine crystals of inulin form three-dimensional gel matrices, therefore a creamy structure with a texture of low capability of rubbing is created. Moreover, inulin can be used as an organoleptic improver in the food industry (Petrova and Petrov, 2017).

Table 1. Chemical composition and physical properties of inulin

The constituents of inulin	Amount
moisture	less than 3.5%
dry matter	96%±1
ash	maximum 0.3%
carbohydrate	minimum 99.7%
dietary fiber	minimum 90%
sugars (glucose, fructose, sucrose)	maximum 10%
color	white
taste	slightly sweet
pH	6
degree of polymerization	10
grading	less than 500 microns
Stability	heat resistant

2 Materials and Methods

2. 1. Material

"Lactobacillus casei 1608" was provided from the Iranian Research Organization for Science and Technology in the form of lyophilized vials. The culture media including MRS Broth and MRS Agar were prepared by Merck Company.

For preparing jam, squashes were washed and peeled and coarsely grated. Sugar was dissolved in water and then heated. Squashes were added and heated for 20 minutes to cook (Isah, 2017). The amounts of 0.5, 1 and 1.5 g of inulin were weighed and dissolved in 100 ml of distilled water for preparing different percentages of inulin. Then 1 ml of inulin was added to 10 g of the squash jam followed by pasteurizing at 80℃ for 5 minutes (Ravani and Joshi, 2013). In order to conduct physicochemical tests, 15 grams of inulin were added to 150 grams of jam.

2. 2. Methods

2. 2. 1. Pasteurization Before the bacteria inoculation

The mixture of the jam and inulin was pasteurized by heating at80℃ for 5 minutes. The samples were poured into the vials and closed, then were placed in the water bath set at the desired time and temperature. To complete the pasteurization process, the samples were immediately cooled with cold water (Liu et al., 2021).

2. 2. 2. Inoculation of samples with the bacteria

For microbial inoculation, Mc Farland method was used in order to determine microbial count at the level of$1.5 \times 10^8 CFU/ml$ (Sousi et al., 2020). For preparing the bacterial density of $1.5 \times 10^7 CFU/ml$, 1 ml of the created opacity which is equivalent to $1.5 \times 10^8 CFU$ of the bacteria strain per ml (suspension 1) was transferred into a falcon containing 9 ml sterile distilled water by a sterile pipet (suspension 2). After this step, the strains were ready to inoculate. The inoculation with the densities of$10^7$and 10^8 *CFU/ml* were inoculated to the jam and then incubatedat 30℃ for 72 hours for fermentation (Maciel and de Souza, 2020).

2. 2. 3. Chemical tests

2. 2. 3. 1. pH determination

For measuring pH, a pH meter (model WTW) was used according to the National Standard of Iran No. 214 (Frankær et al., 2018).

2. 2. 3. 2. Determination of total acidity based on lactic acid

Total acidity was measured by titration and lactic acid acidity was calculated by eq. 1 according to the National Standard of Iran No. 214 (Lorusso et al., 2018).

eq. (1): $A= (V\times0/009008\times100)/M$

A= Total acidity based on lactic acid per 100 g of sample

M=sample weight in grams

V=consumed volume of NaOH 0.1 N

Note: 1 ml of NaOH 0.1 N is equivalent to 0.009008 g of lactic acid.

2. 2. 3. 3. Measurement of water-soluble solids (Brix at 20 °C)

Brix is soluble solids in grams per hundred grams of sample. A digital refractometer (RX-7000 α) was used for Brix measurement according to the method presented in the National Standard of Iran No. 214 (Bai et al., 2019).

2. 2. 3. 4. Measurement of reducing sugars

The reducing sugar content was measured by Lane and Eynon titration method and calculated by the equation (2) according to the National Standard of Iran No. 3684 (Shao and Lin, 2018).

eq. (2): $M=(F\times100\times100)/(V\times25)$

M= Amount of reducing sugars (sugar before hydrolysis) gr per 100 ml.

F = fehling factor

V = Consumed volume of the neutralized solution A.

3 Results and Discussions

The inulin effect on sugar content during fermentation was evaluated and table 2 shows the results of the comparison of the mean values of inulin effect on sugar content (and analysis of the data by Duncan's multiple range test). We have shown that the synbiotic squash jam sugar content had a significant increase by increasing the percentage of inulin ($p\leq0.05$); howevere, Licciardello and Muratore (2011) by examining the effect of temperature and some added compounds on blood orange marmalade stability at 35˚C and 20˚C found that the sugar content is decreased by increasing the temperature during storage (Licciardello and Muratore, 2011). This finding disagreed with the results of the present study indicating that the amount of reducing sugar increases during fermentation due to the breakdown of inulin molecules into smaller ones.

Table 2. The effect of inulin on reducing sugar

gr/100gr (reducing sugar)	Inulin %
74.38 ± 2.46^{d}	0
76.91 ± 4.05^{c}	0.5
80.04 ± 3.25^{b}	1
85.17 ± 3.19^{a}	1.5

Similar letters in each column are not significantly different ($p>0.05$).
a, b, c and d indicate significant difference between groups.

Measurement of reducing sugars during the storage of synbiotic squash jam revealed that the reducing sugar in the synbiotic squash jam significantly increased by increasing inulin amount ($p\leq0.05$), while bacterial density and storage time did not show significant change ($p>0.05$) (Figure 1).

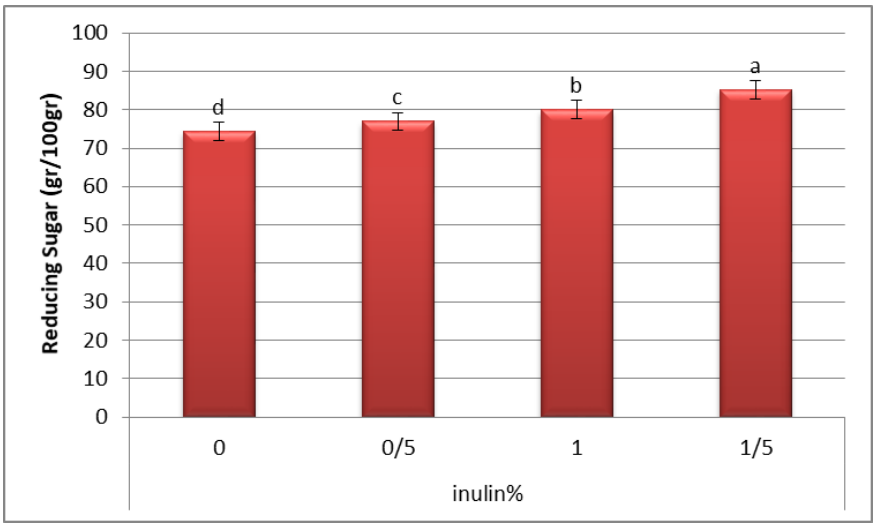

Figure 1. Reducing sugar mean values and the kinetics of reducing sugar during the storage of synbiotic squash jam. a, b, c and d indicate significant difference between groups.

Table 3 shows the effects of inulin percentage and density of *Lactobacillus casei* on the jam microbial counts during 4 weeks. The results show the amount of inulin and density of bacteria both had significant differences between treatments ($p<0.01$). However, trilateral interaction (amount of inulin, bacterial density and storage time) did not show significant effect on the jam microbial counts ($p>0.05$).

Table 3. Reducing sugar mean values (gr / 100gr) in control and treatment groups.

4th week	3rd week	2nd week	1st week	72 hr. after inoculation	0 (beginning)	T
72.25±2.47^{Ac}	75.00±2.12^{Ab}	74.00±5.65^{Ac}	75.00±5.65^{Abc}	73.00±1.41^{Ad}	72.75±1.06^{Ae}	T2N1
78.00±8.48^{Abc}	79.00±5.65^{Aab}	80.00±7.07^{Aabc}	75.00±7.07^{Abc}	77.00±2.82^{Abcd}	74.50±0.70^{Ade}	T2N2
75.75±0.35^{Abc}	74.50±0.70^{Bb}	75.75±0.35^{Abc}	76.25±0.35^{Abc}	76.00±.0.00^{Abcd}	75.50±0.70^{ABcde}	C
77.00±4.24^{Abc}	76.25±6.01^{Aab}	78.50±4.94^{Abc}	75.00±4.24^{Abc}	76.75±1.76^{Abcd}	76.00±1.41^{Acde}	T1N2
81.75±2.47A^{abc}	82.75±3.18^{Aab}	83.25±3.18^{Aabc}	81.00±1.41^{Aabc}	79.25±1.06^{Aabcd}	79.75±0.35^{Abc}	T2N3
78.25±5.30A^{abc}	77.00±7.07^{Aab}	79.25±4.59^{Aabc}	78.25±0.35^{Abc}	81.00±5.65^{Aabc}	79.00±1.41^{Abcd}	T1N3
82.50±1.41A^{ab}	85.50±3.53^{Aab}	85.25±.0.35^{Aab}	82.75±3.18^{Aab}	84.75±6.01^{Aa}	82.50±3.53^{Aab}	T1N4
87.75±1.06A^{a}	87.25±0.35^{Aa}	88.65±.0.21^{Aa}	88.75±0.35^{Aa}	81.50±1.41^{Bab}	85.00±4.24^{ABa}	T2N4
73.25±2.47A$_{bc}$	75.25±6.71^{Ab}	74.75±3.18^{Ac}	73.00±4.24^{Ac}	73.50±0.70^{Acd}	73.50±0.70^{Ae}	T1N1

Values with similar lowercase or uppercase letters in each column or rows indicate non-significant difference between groups ($p>0.05$).

According to the results of comparison of mean values of inulin effect on microbial counts and kinetics of microbial count during the storage period of the synbiotic squash jam (Figure 2), it was found that by increasing the density of bacteria, microbial count of the synbiotic jam had a significant increase ($p\leq0.05$). Microbial count of the synbiotic squash jam was increased by increasing the storage time from the moment of zero to four weeks. The treatment groups were:

Treatment T1N1: 150 g jam + 1.5 × 10^7*CFU/ml Lactobacillus casei*
Treatment T1N2: 150 g jam + 1.5 × 10^7 CFU/*ml Lactobacillus casei*+ 0.5% inulin
Treatment T1N3: 150 g jam + 1.5 × 10^7*CFU/ml Lactobacillus casei* + 1% inulin
Treatment T1N4: 150 g jam + 1.5 × 10^7*CFU/ml Lactobacillus casei* + 1.5% inulin
Treatment T2N1: 150 g jam + 1.5 × 10^8*CFU/ml Lactobacillus casei*
Treatment T2N2: 150 g jam + 1.5 × 10^8*CFU/ml Lactobacillus casei* + 0.5% inulin
Treatment T2N3: 150 g jam + 1.5 × 10^8*CFU/ml Lactobacillus casei* + 1% inulin
Treatment T2N4: 150 g jam + 1.5 × 10^8*CFU/ml Lactobacillus casei* + 1.5% inulin
Control treatment (C): without inulin and bacteria

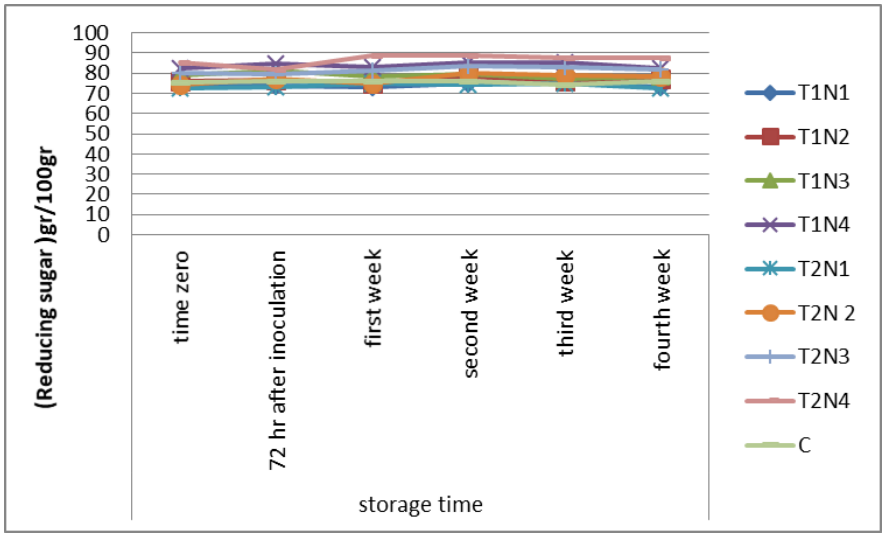

Figure 2. Linear graph of sugar kinetics during storage time.

It has been previously shown that addition of prebiotics compounds such as inulin can help viability of microorganisms in inappropriate conditions and meanwhile improve the texture and sensory properties (Granato et al., 2010).

In this study, according to the comparison of the mean values of inulin effect on microbial count and analysis of the data by Duncan's multiple range test, it was found that by increasing the percentage of inulin from 0 to 1%, microbial count of the synbiotic squash jam significantly increased, though it significantly decreased in the jams containing 1.5% inulin (Table 4).

Table 4. Inulin effect on microbial counts.

Microbial count (log *CFU/ml*)	Inulin %
8.482±0.620^{c}	0
8.734±0.737^{ab}	0.5
8.913±0.842^{a}	1
8.622±0.715^{bc}	1.5

Similar letters indicate not significant difference (p>0.05)

The data of the present study have shown a significant increase in microbial count of the synbiotic squash jam by increasing the density of bacteria (p≤0.05) (Figure 3).

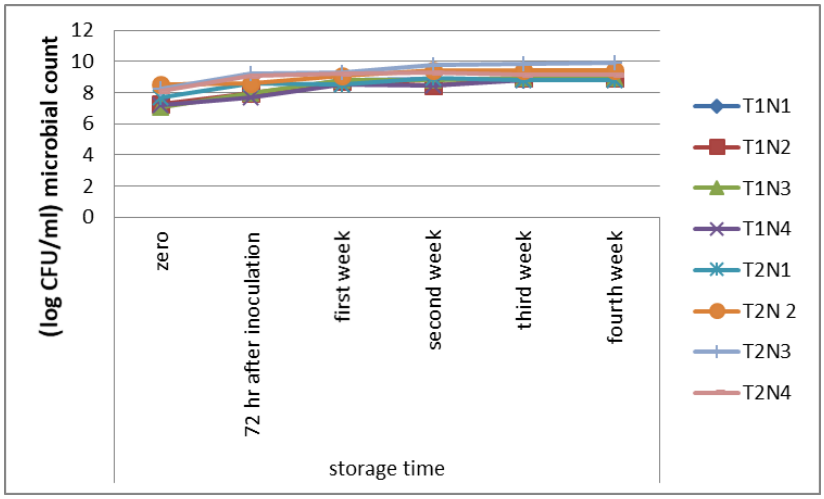

Figure 3. Linear graph of kinetics of microbial count during the storage period

Consistent with our findings, Granato et al. (2010) investigated symbiotic UF white cheese production using the probiotic strain *Lactobacillus acidophilus* and inulin. By examining UF White Feta Cheese as a probiotic carrier, it was found that although the bacteria numbers decreased in all the samples by increasing storage time, this product could maintain acceptable numbers of *Lactobacillus acidophilus* to the end of 45-day storage period (Granato et al., 2010). In another study, Rad et al. (2013) examined physicochemical and microbial characteristics of milk chocolate matrix as a carrier of novel *Lactobacillus acidophilus*. In this study, two types of chocolate, control and probiotic, were produced. They reported that there was no significant difference between the number of bacteria counted at temperatures 22°C and 4°C in probiotic samples. Logarithm of the number of bacteria counted in the chocolate stored at 4°C was 66.8 *CFU/ml*, so it was known as a probiotic product (Rad et al., 2013), indicating the possibility of producing synbiotic squash jam containing inulin and *Lactobacillus casei*.

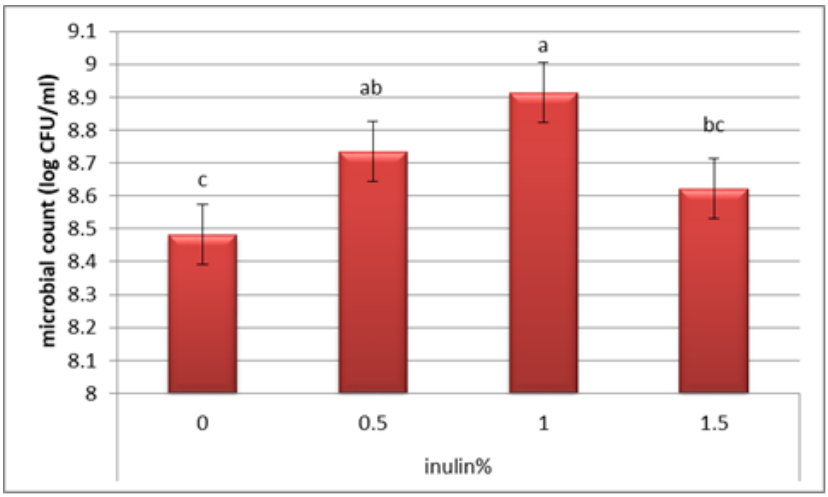

Figure 4. Comparison of the mean values of inulin effect on microbial count (analysis of the data by Duncan's multiple range test). c, ab, a and bc indicate significant difference between groups ($p>0.05$).

According to the results of the analysis of Pearson correlation between sugar and microbial count of symbiotic squash jam there was a significant positive correlation at 95% confidence level with maximum determination coefficient (R^2) of 0.06 (Table 5).

Table 5. Pearson correlation between reducing sugars and microbial count of synbiotic squash jam

Microbial count (CFU/ml)	Reducing sugar	Studied characteristics
0.257*	1	sugar
1	0.257*	Microbial count

*: significant correlation at the 0.05 level

The results of linear regression indicated that microbial count is significantly increased by increasing the amount of sugar (Figure5). The linear regression equation:
Relation (1): microbial count=5.894 + 0.0354 × sugar amount

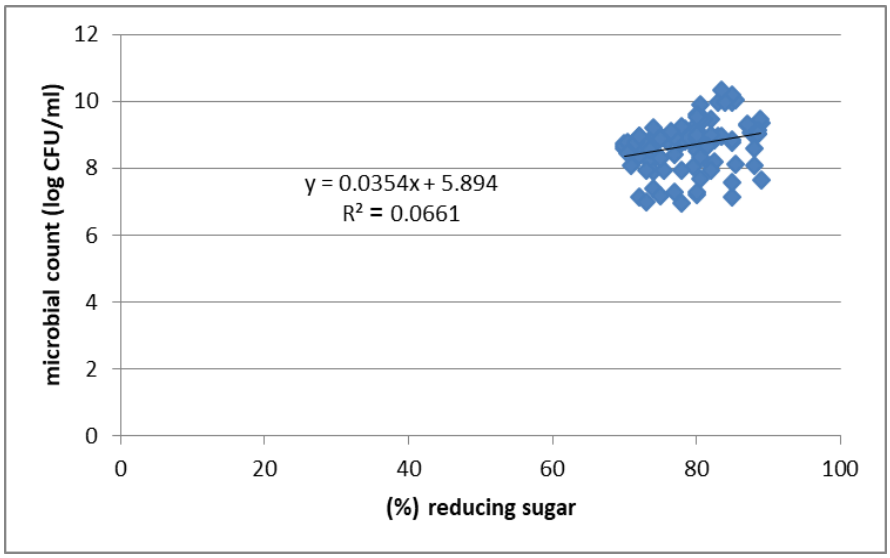

Figure 5. The correlation between reducing sugar and microbial count of synbiotic squash jam.

4 Conclusion

The sugar amount increased by rising of bacterial density indicating that the breakdown of inulin molecule into smaller ones by the probiotic strain *Lactobacillus casei*. During fermentation, the viability of probiotic bacteria *Lactobacillus casei* increased by sugar and jam nutrients consumption. By increasing the density of bacteria, microbial count of the synbiotic jam showed a significant increase. Reducing sugar levels increased by increasing inulin percentage and the highest microbial count belonged to the treatment T1N3 (150 g jam + 1.5 × 10^7*CFU/ml L. casei* + 1% inulin).

Acknowledgments

I would like to appreciate Global Research, Education, and Event Network (GREEN) for their help and support.

Conflict of interests

The author declares that there are no competing interests.

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