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Production of Synbiotic Raspberry Jam with Inulin Content and Lactic Acid Bacteria

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ABSTRACT

Inulin consists of unabsorbed fructans that increase absorption of calcium in addition to probiotic nature. pH, acidity, reducing sugars, brix and viability (survival) of probiotic bacteria in Synbiotic Raspberry jam produced by inulin content (0.5%, 1%, and 1.5%) and two bacteria of *Lactobacillus* (*L.*) *casei* and *L. acidophilus* (1.5×10^8 cfu/ml) have been examined during storage of treatments for four weeks. During four weeks of storage, pH level was reduced in synbiotic raspberry jam; rate of acidity was increased; and quantities of reducing sugars and brix were decreased. Due to consuming sugar and existing nutrients in fruit, number of probiotic bacteria was reduced during period of storage of treatments. The level of viability of probiotics varied within range (10^6 – 10^7 cfu/ml) for all treatments during four storage weeks. N3M1 treatment (1.5% of inulin and 10^8 cfu/ml *L. casei*) comprised of the highest viability rate in probiotics and it was introduced as premier treatment. Taste sensitivity assessment during first and fourth weeks of storage, N1M1 treatment (0.5% inulin with incubation of 10^8 cfu/ml of *L. casei*) possessed the highest score and N3M2 treatment (1.5% of inulin with incubation of 10^8 cfu/ml of *L. acidophilus*) obtained the lowest score among all of treatments.

What is "already known":	• Inulin consists of unabsorbed fructans that enhance Ca^{2+}absorption and possess probiotic properties • pH, acidity, reducing sugars, brix, and the viability of probiotics may change during storage of synbiotic Raspberry jam • Raspberry jam produced with inulin (0.5, 1, and 1.5%) and probiotic (*L. casei* and *L. acidophilus* at 1.5×10^8 cfu/ml
What this article adds:	• Over 4 weeks of storage, pH decreased, acidity increased, while reducing sugars and brix decreased • Probiotic count decreased due to sugar consumption and nutrient availability in the fruit • Probiotic viability ranged between 10^6 and 10^7 cfu/ml across all samples over 4 weeks • The highest viability was achieved at 1.5% inulin and 10^8 cfu/ml *L. casei*. • Sample contains of 10^8 cfu/ml of *L. case* and 0.5% inulin received the highest score while *L. casei* and 1.5% inulin led to the lowest score among all treatments

1. Introduction

Attractiveness of healthy diets has been increased during recent years where this may contribute to prevention from diseases. Following to this trend, study and advancement of new synbiotic foods is crucially important. Some issues such as allergy to dairy products and intolerance to lactose and level of cholesterol in dairies are assumed as two main defects relating to synbiotic dairy products [1].

Unlike many nutrients humans consume them every day to provide his/ her energy, prebiotics are some of very special types of nutrients that are impermeable inside body and at the same time they will include healthy properties. Prebiotics are unabsorbed nutritional compounds which selectively stimulate growth and/ or lead to activity of one or more limited numbers of large intestinal bacteria and they can improve health in host by their useful effects [2].

With respect to way of activity of probiotics in body as well as effect of prebiotics in nutrition of probiotics, term of synbiotics may be proposed. Synbiotics are composed of probiotics plus prebiotics that possess equally prebiotic and probiotic properties [3].

Selection of appropriate probiotic type at suitable dosage is deemed as one of the most essential factor for developing of probiotic nutritional product. Survival during nutritional processing, storage, transference through intestine, and effect on health of consumers are considered as the most basic factors for selection of probiotic bacterium species [4].

Lactobacilli are stronger than Bifidobacteria in terms of strength. Lactobacilli are more resistant against pH reduction and their potential is higher for adaptation to milk and other nutrients. Probiotic Lactobacillus species are more suitable than Bifidobacteria technologically [4].

On the other hand, as a synbiotic product, production of Synbiotic Raspberry Jam has been less noticed by experts and researchers of processing industries. With respect to extraordinary properties of raspberry fruit and daily consumption of this jam on breakfast tablecloth, the embedded information in this study may be noticed by the interested parties throughout the world.

2. Materials and Methods

2.1. Materials

The varieties of *L. casei* 1608 and *L. acidophilus* 1643 were prepared as lyophilized vial from Institute of Standard and Industrial Research of Iran (ISIRI) to produce probiotic product. Selection of basic medium and suitable platform for growth to enter useful probiotic microorganisms is related to raspberry jam that was prepared from Darya Confectionary Shop (at Chaloos City). Some of chemical specifications of raspberry jam are listed in Table (1).

Table 1. Specifications of chemical analysis on raspberry jam

Property	Quantity
Brix	68.5%
pH	4.2
Acidity	0.68g/100g
Reducing sugar	52.07g/100ml

The needed inulin was prepared as package from α-LAB Company. The specifications of inulin are mentioned in Table (2).

Table 2. Chemical- microbial specifications of consumed inulin

Substance	Quantity	Substance	Quantity
Glucose + fructose+ sucrose	Less than 0.5%	Carbohydrate	99.5%
Dry material	% 97±1.5	Mean inulin DP	More than 23
Ash	% 0.2	Heavy metallic elements (lead)	Less than 0.02 mg/kg
pH	5-7	Heavy metallic elements (arsenic)	Less than 0.03 mg/kg
Mesophyll bacteria- total count	Maximally 1000 per gram	Heavy metallic elements (cadmium and mercury)	Less than 0.01 mg/kg
Yeast and mold	Each of them maximally 20 per gram	Salmonella	Negative in 250g
Coagulase positive staphylococcus	Negative in 0.1g	Listeria	Negative in 25g
Enterobacteracea and *Escherichia coli* and clostridium	Each of which negative per 1g	*Bacillus cereus*	Maximally 100 per gram

2.2. Methods

2.2.1. Pasteurization of samples before incubation

100 mL of Raspberry jam were prepared including 10 mL of inulin at different percent comprise of 0.5%, 1%, and 1.5% (according to table of studied treatments). In order to pasteurize raspberry jam, sample was treated at temperature 85°C for 10 min. After reaching to the given temperature and spending the needed period in samples, they were

cooled immediately by cold water to complete pasteurization operation [5, 6].

2.2.2. Incubation of microorganisms to sample

McFarland technique was utilized for microbial incubation to determine quantity of bacterium that was at level 10^8 *cfu/ml* [7]. The turbidity (10^8 *cfu/ml*) was created by variety of given bacterium to prepare both types of suspensions of used bacteria in this research (*L. acidophilus* and *L. casei*) and at the same time rate of incubation was reduced to 10^8 *cfu/ml* for any suspension to this jam so that to incubate totally the same turbidity (10^8 *cfu/ml*) by bacteria in samples.

Table 3. Research treatments

Treatments	Type of bacteria	Inulin content (%)
A_1B_1	*L. acidophilus* 10^8 *cfu/ml*	0.5
A_2B_1	*L. casei* 10^8 *cfu/ml*	0.5
$A_{2,1}B_1$	*L. acidophilus* 10^4 *cfu/ml* and *L. casei* 10^4 *cfu/ml*	0.5
A_1B_2	L. acidophilus 10^8 *cfu/ml*	1
A_2B_2	*L. casei* 10^8 *cfu/ml*	1
$A_{2,1}B_2$	*L. acidophilus* 10^4 *cfu/ml* and *L. casei* 10^4 *cfu/ml*	1
A_1B_3	*L. acidophilus* 10^8 *cfu/ml*	1.5
A_2B_3	*L. casei* 10^8 *cfu/ml*	1.5
$A_{2,1}B_3$	*L. acidophilus* 10^4 *cfu/ml* and *L. casei* 10^4 *cfu/ml*	1.5
Control	-	-

2.2.3. Methods

A. Count of L. acidophilus and L. casei

MRS agar medium was utilized to count probiotic bacteria and impregnated samples were counted after 72h storage in warm house at temperature 37 Celsius degree and under anaerobic conditions. Number of colonies was calculated according Formula (1) by multiplication to reciprocal of dilution factor and the final product was expressed as number of colony per milliliter (*cfu/ml*). [8].

Formula (1): Number of colony per one milliliter (*cfu/ml*) = Quantity of colony × reciprocal of dilution factor

B. Measurement of pH

pH rate of samples was measured by digital pH-meter at lab temperature [9].

C. Acidity

Acidity was calculated according to lactic acid and by titration technique and based on Formula (2).

Formula (2): $$A = \frac{v \times 0.009008 \times 100}{M}$$

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

M = Weight of tested sample per gram

V = Volume of consumed NaOH.

D. Reducing sugar

The quantity of reducing sugar was measured by means of Lane- Eynon Method and amount of reducing sugar was calculated according to Formula (3) [10].

Formula (3): T×W×10 = Percent of reducing sugar

T = Volume of sugar solution before hydrolysis

W = Weight of uniform (homogeneous) sample

E. Taste sensitivity assessment

The effect of taste sensitivity of 9 treatments was exerted by 9-point hedonic scale on properties of produced synbiotic raspberry jam including odor and scent, sweetness, acidity, stability of taste, natural sense, concentration, color, and appearance by 7 trained raters and compared with each other at level 5% [9].

3. Results and discussion

3.1. Assessment of survival results in probiotics of Synbiotic Raspberry Jam

With respect to the given results, microbial count of synbiotic Raspberry jam was reduced by increase in percent of inulin from 0.5 to 1%. However, survival (viability) of probiotics was increased in the jam in those samples with 1.5% of inulin content.

Likewise, type of impregnated microorganisms was effective on microbial count of synbiotic raspberry jam so that the maximum microbial count belonged to the impregnated jams by *L. casei* while the minimum quantity of bacteria belonged to the incubated treatments by *L. acidophilus*. With respect to Figure (1) for effect of treatments on microbial count during storage period, it was characterized that number of microorganisms of synbiotic Raspberry jam has been reduced by increase in period of storage.

Akin *et al* (2008) indicated that due to the prebiotic effect inulin could improve survival of *L. acidophilus* and bacterium lactis so that rate of loss in number of probiotic cells in treated samples with inulin was less than control sample during 90days of storage [11]. Inulin may act as a stabilizer and through improvement of tissue during storage period, it may prevent from damage of cellular wall in probiotics and thus avoid from noticeable loss.

Randazzo *et al* (2013) examined survival of probiotic bacterium of *L. rhamnosus* in peach jam samples during storage period at different temperatures [12]. Almost all of wild species in the stored samples survived at temperature 25°C and higher than critical level 10^6 *cfu/g* up to 45 days while *L. rhamnosus* (GG species) that were employed as control samples destroyed after 15 days. In vitro, except species H12, other species showed better survival rate in incubated samples at temperature 25°C and they survived up to higher than critical level until 45 days. Compared to the conducted studies, the similar results were observed in experiments done by Randazzo et al (2013) that suggested the possible growth of *L. casei* and *L. acidophilus* in synbiotic raspberry jam [12].

3.2. Assessment of pH results

With respect to the given results, it was identified that pH rate was reduced in synbiotic raspberry jam following to increase in percentage of inulin. It should be mentioned that there was no significant difference in the jams with 0.5% and 1% inulin in terms of pH ($p \geq 0.05$). Similarly, the lowest to highest rates of pH in these jams belonged to *L. acidophilus*, the jams with

equal contents of *L. acidophilus* and *L. casei*, and the jams with content of *L.* casei. With respect to Figure (2), regarding treatment on pH rate during storage period, it was specified that storage time impacted on pH in synbiotic Raspberry jam and pH rate was reduced in synbiotic Raspberry jam by increase in storage time.

In a survey on addition of inulin to probiotic yogurt, Rezaei *et al* (2013) found results similar to the present research and expressed that addition of inulin to the formulation has reduced (pH) [13]. Probably, addition of inulin to sample that is followed by formation of gelatinous state plays role in inactivation of probiotic bacteria during storage in fridge and thus it is led to rise of acidity and reduced pH rate.

Randazzo *et al* (2013) conducted study on survival of six wild species of bacterium of *L.* rhamnosus in peach jams at different temperatures during storage period [12]. Metabolism of lactobacilli changed pH where in comparison with conducted studies; the similar results were derived in present research to experiments done by Randazzo et al (2013) and based on which pH rate was reduced over the time [12].

3.3. Assessment of acidity results

With respect to the given results, it was identified that as inulin percent was added, acidity of synbiotic raspberry jam has been increased. Likewise, there was no significant difference between impregnated jams by *L.* casei and the jams with equal composition of *L. acidophilus* and *L. casei* in terms of acidity level ($p \geq 0.05$) and the jams with content of *L. acidophilus* possessed the maximum acidity rate.

With respect to Figure (3) about effect of treatments on acidity during storage period, it was specified that acidity of synbiotic Raspberry jam was increased following to rise of storage period.

According to national Iranian standard No 214, the rate of acidity of citric acid is at allowed level (0.1-1.5) in fruit jam and marmalade [14] in which acidity rate was at permitted level in all of treatments (since moment of production and during four storage weeks).

Amini Nia *et al* (2016) explored production of synbiotic celery extract drinking using Lactic acid bacteria of *L. acidophilus* and *L. delberucci [15]*. The findings were similar to the present research in which both of serotypes can grow and act well in celery extract and the maximum effect in rise of acidity as well as production of organic acids was seen in *L.* acidophilus bacterium.

Mashayekh *et al* (2015) examined feasibility for production of fermented probiotic drinking based on mixture of extracts of pineapple, apple, and mango by means of *L. casei [16]*. Fermentation was done at temperature 37°C for 72h and sample was stored under temperature 4°C for 4 weeks. During storage period, acidity level was increased in all of treatments so that with respect to the given results from present research that suggested rise of acidity during storage of Synbiotic Raspberry jam, the findings were consistent.

3.4. Assessment of results from reducing sugars

With respect to the given results, it was characterized that sugar was increased in synbiotic Raspberry jam following to rise of percentage of inulin. Similarly, type of impregnated microorganism has been effective on sugar of synbiotic Raspberry jam and the jams with content of *L. acidophilus* included the highest amount of sugar. Given Figure (4) regarding effect of treatments on brix during storage period, it was identified that sugar was reduced in synbiotic Raspberry jam, as storage time was increased.

In study on effect of temperature and some of added compounds for stabilization of Thompson Orange Marmalade at two temperatures 20°C and 35°C, Licciardello and Muratore (2011) implied that amount of sugar was reduced more by rise of temperature during storage period and this finding was consistent with results came from the current research that

suggested decrease in quantity of reducing sugars during fermentation [17].

Randazzo *et al* (2013) conducted study on survival of bacterium *L. rhamnosus* in peach jam samples at different temperatures during storage period [12]. Samples were compared at temperatures 25 and 5°C during 78 days of storage. Metabolism of *L.* changed in sugar compound where this finding was consistent with the results of conducted investigations in present research that suggested decreased rate of reducing sugars during 28 days of storage.

3.5. Assessment of taste sensitivity results

With respect to the given results from taste sensitivity assessment on synbiotic raspberry jam, it was determined that inulin percent, type of incubated microorganisms, and storage period affected on taste sensitivity assessment in such a way that after storage period, 7 studied properties (scent and odor, sweetness, acidity, fixed taste, natural state, concentration, color and appearance) were reduced in Synbiotic Raspberry jam.

Ghaemi *et al* (201) explored production of synbiotic ultra-refined white cheese by means of probiotic species of *L. acidophilus* and inulin [18]. Likewise, during taste sensitivity assessment, treatment with content of *L. acidophilus* with 2% inulin possessed the best taste and tissue. The final results indicated that one could use probiotic bacterium of *L. acidophilus* along with inulin as prebiotic compound successfully for production of ultra- refined cheese so this finding was consistent with the results came from present research that signified possible production of synbiotic Raspberry jam by means of *L. acidophilus* and inulin (1.5%).

In a survey, Rezaei *et al* (2013) examined effect of various quantities of inulin (1 and 2%) on taste properties and survival of *L. acidophilus* and *Bifidobacterium lactis* in frozen yogurt [13]. The results of taste sensitivity assessment indicated that the sample including 2% of inulin was accepted by consumers at the highest level and this finding was consistent with the results of current research that suggested rise of total approval for R aspberry jam including probiotics and inulin during 28 days of storage.

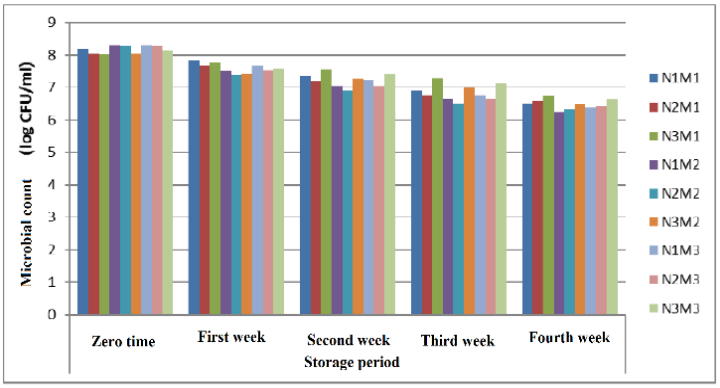

Figure 1. Effect of treatments on microbial count during storage period

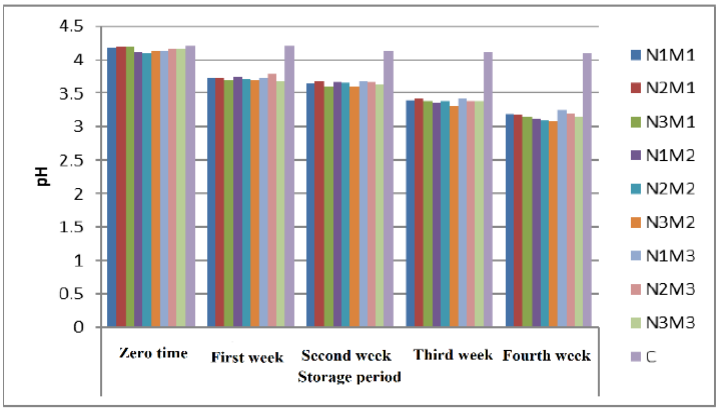

Figure 2. Effect of treatments on pH during storage period

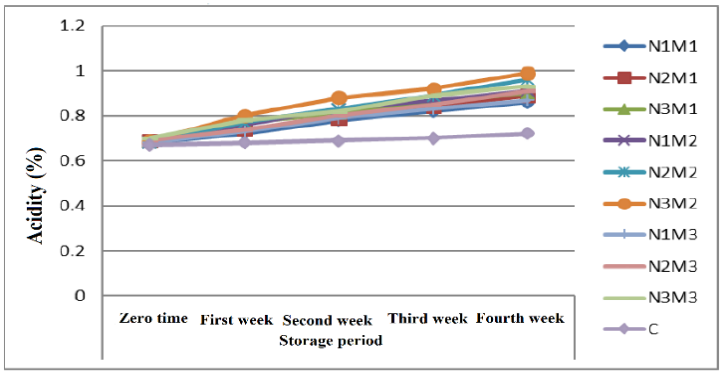

Figure 3. Effect of treatments on acidity during storage period

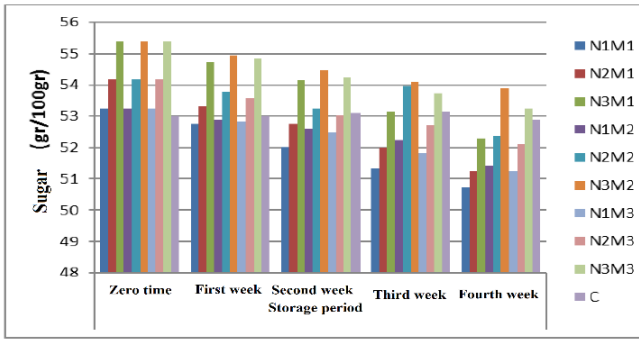

Figure 4. Effect of treatments on sugar during storage period

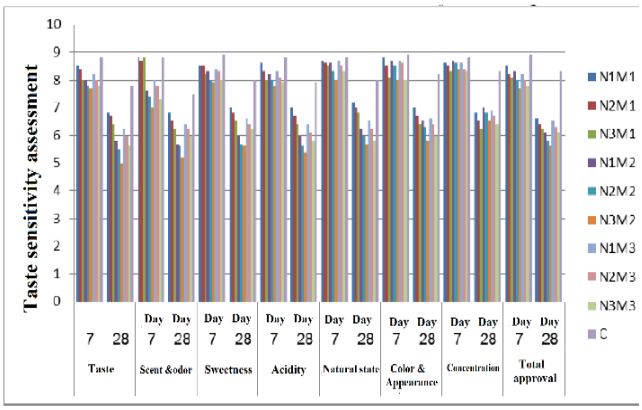

Figure 5. Effect of treatments on taste sensitivity assessment at 7th and 28th days of storage

N1M1 treatment: 0.5% inulin + 10^8 *cfu/ml L. casei*

N2M1 treatment: 1% inulin + 10^8 *cfu/ml L. casei*

N3M1 treatment: 1.5% inulin + 10^8 *cfu/ml L. casei*

N1M2 treatment: 0.5% inulin + 10^8 *cfu/ml L. acidophilus*

N2M2 treatment: 1% inulin + 10^8 *cfu/ml L. acidophilus*

N3M2 treatment: 1.5% inulin + 10^8 *cfu/ml L. acidophilus*

N1M3 treatment: 0.5% inulin + 10^4 *cfu/ml L. casei* +10^4 *cfu/ml L. acidophilus*

N2M3 treatment: 1% inulin + 10^4 *cfu/ml L. casei* +10^4 *cfu/ml L. acidophilus*

N3M3 treatment: 1.5% inulin + 10^4 *cfu/ml L. casei* +10^4 *cfu/ml L. acidophilus*

C- Treatment (Control): Lack of inulin and bacterium

4. General Conclusion

pH rate was reduced in synbiotic Raspberry jam during four storage weeks while level of acidity was increased. Due to consuming of sugar and the existing nutrients in fruit, number of probiotics was reduced during storage time. Similarly, amount of reducing sugars was decreased. The rate of survival for probiotics was within range (10^6 – 10^7 *cfu/ml*) for all of treatments during four storage weeks. In N3M1treatment in which Raspberry jam includes 1.5% inulin with 10^8 *cfu/ml L. casei,* it possessed the maximum survival of probiotics and introduced as the premier treatment. In terms of taste sensitivity assessment during first and fourth storage weeks, N1M1 treatment (jam with 0.5 inulin content by incubation of 10^8 *cfu/ml L. casei*) possessed the highest score while N3M2 treatment (the jam with content of 1.5% inulin with impregnation of 10^8 *cfu/ml L. acidophilus*) had the lowest score.

5. Declarations

5.1. Acknowledgments

None.

5.2. Authors' Contributions

All authors equally contributed to this study.

5.3. Declaration of Interest

The authors of this article declared no conflict of interest.

5.4. Ethical Considerations

All ethical principles were adheried in conducting and writing this article.

5.5. Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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5.7. Using Artificial Intelligent chatbots

Please declare whether Artificial Intelligent chatbots has been used in any section of work.

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