A Functional Non-Dairy Beverage Produced from Jujube Extract Using Probiotic Lactic Acid Bacteria

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ABSTRACT

In this research, production of a probiotic drink based on jujube extract by means of fermentation with Lactobacillus plantarum and Lactobacillus delbrueckii as probiotic lactic acid bacteria was studied. The fermentation was performed for 72 hours at 37°C. The changes in microbial population, pH and titratable acidity as well as sugar and organic acid metabolism during the fermentation period were evaluated. In addition, before and after fermentation, the changes in total phenolic compounds and antioxidant activity in the extract were also investigated. Eventually, different drink formulations were developed employing fermented and non-fermented jujube extract, carbonatation, and sour cherry concentrate. Then, sensory properties of the formulated drinks were evaluated. Appropriate growth of L. plantarum and L.delbruckii resulted in an increase in acidity to 1.86 and 1.75, and a decline in pH to 3.4 and 3.56, respectively, after 72 hours. Glucose and fructose were significantly consumed by the strains. Citric acid concentration dropped to 1.12 and 5.8 g L^{-1} in the extract fermented by L. plantarum and L. delbrueckii, respectively, after 72 hours. At the end of fermentation, 23.8 and 11.4 g L^{-1} lactic acid was produced by L. plantarum and L. Delbrueckii, respectively. The contents of phenolic compounds and antioxidant activity in jujube extract after fermentation were significantly increased. The results of sensory evaluation of different drink formulations showed that the carbonated drink containing jujube extract fermented by L.plantarum mixed with sour cherry concentrate obtained the highest score among different drink formulations.

Keywords: Antioxidant activity, Fermentation, Lactobacillus, Ziziphus jujube.

INTRODUCTION

Functional foods are defined as whole foods, enriched, enhanced and fortified foods or dietary compounds that, in addition to traditional nutrients contents, possess healthy and physiological benefits (Bellisle *et al.*, 1998; Kwak and Jukes, 2001; Spence *et al.*, 2006). Food products with probiotics include the majority of functional food market worldwide (Mocanu *et al.*, 2011; Salmerón *et al.*, 2015). Probiotics include live microorganisms and, when consumed sufficiently (at least 10⁶-10⁷ CFU mL⁻¹), provide health benefits to the host (Meira *et al.*, 2015; Reid *et al.*, 2003). Probiotic bacteria affect human health by recovering the intestines microbiota balance (Brown and Valiere Ana, 2004; Kalliomäki *et al.*, 2001).

Dairy based probiotic products are widely commercialized across the world (Salmerón *et al.*, 2015). However, because of the high demand for non-dairy products from vegetarian, lactose intolerant, cholesterol and milk protein allergic consumers, the development of novel non- dairy-based probiotic products has been significant (Behrad *et al.*, 2009; Martins *et al.*, 2013; Reddy *et al.*, 2015b). In recent years, with enhancement of vegetarian consumers around the world, the demand for plantbased probiotic products has increased. On

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the other hand, the risk of high cholesterol and lactose intolerance are two major problems in dairy fermentation products (Prado *et al.*, 2008). For this reason, there is a wide variety of non-dairy fermentation beverages around the world, from which fruit-and-vegetable drinks can be mentioned (Heenan *et al.*, 2004; Mousavi and Mousavi, 2019; Nguyen *et al.*, 2019; White and Hekmat, 2018).

Various researches have reported the suitability of fruit juices, vegetables, and cereals for the production of probiotic functional drink (Di Cagno et al., 2009; Filannino et al., 2014; Mousavi et al., 2011; Prado et al., 2008; Yoon et al., 2004; Yoon et al., 2005). In these researches, lactic acid bacteria have been employed as probiotic bacterial cultures (Reddy et al., 2015a; Salmerón et al., 2015). The ability of these bacteria to consume a broad range of carbohydrates and to metabolize different phenolic compounds has resulted in an appropriate option for the development of new functional plant based drinks (Filannino et al., 2014; Hur et al., 2014).

The jujube (*Ziziphus jujube*), commonly known as red dates, Chinese dates, or Indian dates has been used for thousands of years due to its health benefits (Chen *et al.*, 2017).

It grows mainly in Europe, southern and eastern Asia, and Australia (Gao *et al.*, 2013). The fruit is rich in minerals including potassium, phosphorus, manganese and calcium as the major minerals as well as sodium, zinc, iron and copper. Furthermore, flavonoid, polysaccharide, and triterpenic acid are the main active ingredients within jujube, contributing to immune-modulating, anti-inflammatory functions, anticancer activities and cardiovascular health (Gao *et al.*, 2013; Gao *et al.*, 2012; Pawlowska *et al.*, 2009; Tiwari and Banafar 1995).

Despite the wide studies on jujube fruit, which generally have summarized the fruit composition and its health benefits, few limited studies have been performed on its application in food products (Gao *et al.*, 2013). Therefore, in this study, we have tried to investigate the possibility of producing a probiotic drink using fermented jujube extract as the basis of the formulation. We aimed to conduct extract fermentation by *L. plantarum and L. delbruec*kii and evaluate cell growth and metabolism, functional and sensory properties of the formulated drink based on jujube extract.

MATERIALS AND METHODS

Preparation of Jujube Extract

Initially, the jujube fruit was purchased in dried form from a local grocery store in Birjand city located in Khorasan province, Iran. The samples were stored at -20°C until use. The fruits were soaked overnight in distilled water for subsequent extraction; the extraction was performed using distilled water for 20 minutes at 80°C. The Brix of recorded with the extract was a refractometer (Belingham, UK) and subsequently was adjusted to Brix 18° with distilled water. The prepared extract was sterilized for 15 minutes at 121°C and was kept in -20°C until use.

Bacterial Strains

Lactobacillus plantarum (DSMZ 20174) and Lactobacillus delbrueckii (DSMZ 20006) were supplied by DSMZ (German Collection of Microorganisms and Cell Cultures, Germany). All bacterial cultures were stored at -20 °C in 2 mL MRS (De Man, Rogosa and Sharpe agar) medium (Merck, Germany) containing 20% glycerol. The strains were reactivated utilizing double passage on MRS when needed.

Fermentation of Probiotic Jujube Extract

The overnight culture of the probiotic strains was prepared by transferring a few colonies of cells into MRS broth followed by incubation at 37 °C for 24 hours. Then, the jujube extract was inoculated with 10% volume/volume of inoculated overnight culture. The inoculated extract was incubated at 37°C for 72 hours and sampling was carried out every 24 hours for microbiological and chemical analyses. Viable cells were determined by standard plate count method using MRS agar medium and expressed as colony-forming units per mL of the sample (CFU mL⁻¹).

Chemical Analysis

pH and Acidity

A digital pH meter (Metrohm 744, Netherland) was used for the pН measurements. Total titratable acidity, expressed as citric acid percentage, was determined by the sample with titrazol 0.1N (Merck, Germany) to pH 8.2. Eventually, the titratable acidity was reported as g citric acid 100 g⁻¹ sample using following equation:

$$A = \frac{m \times 0.064 \times 100}{100} \times 100 \tag{1}$$

Where, A =Titratable acidity (g citric acid100 g⁻¹ of sample); m= mL used titrazol 0.1N,

w= Weight of sample.

HPLC measurement of sugars and organic acids

Fructose and glucose were determined by HPLC (Knauer, Germany) attached to a K-2310 Refractive Index (RI) detector. Separation conditions were as follow: The column Eurokat H 250×30 mm, 20 µL injection volume, sulphuric acid (2.25 mM) as the mobile phase with 0.4 Ml min⁻¹ flow rate at 45°C. All samples were diluted with distilled water (1:10 ratio) and filtered through a cellulose acetate syringe filter (VWR, 0.2 µm, USA) before injection. Samples were studied in triplicate, and the findings are reported as

the average of these three independent measurements.

Quantitative analysis of organic acids (lactic and citric acid) was also performed using HPLC (Knauer, Germany) apparatus attached to a K-2600UV-visible detector. A separation column (Ultrasep ES-FS special 250×30 mm) set at room temperature with 2.25 mM sulfuric acid as the mobile phase and injection volume of 20 μ L was utilized at a flow rate of 0.2 mL min⁻¹. All samples were diluted with distilled water (1:10 ratio) and filtered using a cellulose acetate syringe filter (VWR, 0.2 μ m, USA) before injection. Organic acids content was reported using external standards.

Determination of Antioxidant activity using DPPH inhibition assay

In this research, the antioxidant activity of the samples was assessed using the method of Brand-Williams et al. (1995) with a slight methanolic modification. The DPPH solution (0.1 mM) was freshly provided daily, kept in a flask covered with aluminum foil, and stored in the dark at 4°C until utilization. Two mL of the extract was mixed with 2 mL methanolic DPPH and the mixture was vigorously shaken and placed in the dark for 30 minutes to achieve a stable absorption. Afterwards, the absorbance of the samples was read by a Cecil spectrophotometer (CE2502, Instruments, U3) at a wavelength of 517 nm. radical scavenging activity The was measured by the following formula:

$$Radical scavenging activity.\% = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where, $Abs_{control}$ is the Absorbance of the control (1 methanolic DPPH solution+3 mL methanol), and Abs_{sample} is the Absorbance of the extract or standard.

Total phenolic compounds

In order to determine the amount of total phenolic compounds present in the samples,

Folin-Ciocalteu method used by Brand-Williams et al. (1995) was applied with a slight modification. A volume of 1,000 micromolar of each diluted extract with methanol was mixed to 1,000 µL of Folin-Ciocalteu reagent and 1,000 µL of saturated sodium carbonate (0.75 g L^{-1}). Then, 2 mL distilled water was added to the mixture and incubated at 25°C for 30 minutes. Finally, the absorbance of the mixture was determined against the blank sample at 750 nm. The total phenolic content of each extract was estimated by comparison with a standard curve generated from analysis of gallic acid solutions and reported as mean µg of gallic acid equivalents per mL extract.

Drink formulations preparation

Different drink formulations were developed in the research and development sector at ZAMZAM Company. The final accepted formulations for sensory evaluation were as follows:

- Sour cherry+jujube extract fermented by *L. plantarum* (carbonated),
- Sour cherry+unfermented jujube extract,
- Sour cherry+jujube extract fermented by *L.delbrueckii* (carbonated),
- Sour cherry+jujube extract fermented by *L.plantarum* (non-carbonated),
- Sour cherry+jujube extract fermented by *L.delbrueckii* (non-carbonated),
- · Sour cherry drink.

Sensory evaluation testing

In this study, the sensory properties of different drinks formulated based on fermented and non-fermented jujube extracts were evaluated by 14 trained panellists recruited in ZamZam company (7 Women/7 Men) aged 25-45 using a 5-point hedonic scale. The sensory attributes included the aroma, mouthfeel, sweetness, sourness, brightness, aftertaste, color, and smell. For this purpose, each panellist was given one sample placed in plastic cups encoded with a random 3-digit code on the cup, an evaluation form, a pen, and a glass of water to rinse their mouth between tasting. The panellists were requested to rate the samples from one (extremely dislike) to five (extremely like).

Statistical analyses

Samples were analyzed in triplicate and Analysis Of Variance (ANOVA) was done utilizing SPSS software (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL, USA). Mean analysis by Duncan's multiple range tests at significance level of P< 0.05 was carried out, if needed.

RESULTS AND DISCUSSION

Growth Kinetics, pH and Acidity Changes during Fermentation of Jujube Extract

The growth of L. plantarum and L. delbrueckii in jujube extract for 72 hours is presented in Figure 1. In the first 12 hours of the fermentation process, the population of the bacteria grew slowly. Thereafter, the bacteria entered the logarithmic phase of growth, and population of *L.plantarum* and *L*. the *delbrueckii* increased from 2.48×10^7 and $3.2 \times$ 10^7 to 3.56×10^8 and 3.3×10^8 CFU mL⁻¹ respectively, after 24 hours. The microbial growth of both bacteria ceased to increase after 24 hours and the cells remained in a stationary state until 40 hours of fermentation process, and began to drop in number after this fermentation time.

The changes in pH and acidity of jujube extracts before and after fermentation are shown in Figure 2. The recorded value for pH and acidity in the jujube extract at the beginning of fermentation was 4.9 and 1.3, respectively. Fermentation of the extract by *L. plantarum* and *L.delbruckii* resulted in an increase in acidity to 1.86 and 1.75, and a decline in pH to 3.4 and 3.56, respectively, at the end of fermentation process. The increase in acidity and decline in the pH of fermentation medium were also observed in

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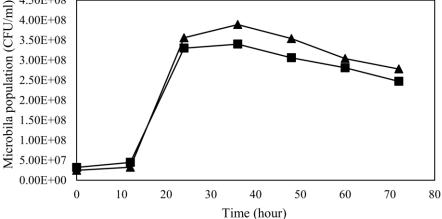


Figure 1. Growth kinetic of *L. plantarum and L. delbrueckii* during fermentation at 37 C in jujube extract. Filled triangle= *L. plantarum*, and Filled square= *L. delbrueckii*. (All values are obtained with three replications).

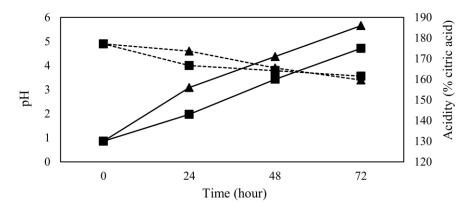


Figure 2. pH and titratable acidity changes in jujube extract during fermentation. Dash lines indicate pH changes during fermentation at 37 C. (All values are averages of three replications).

similar investigations that studied the effect of fermentation on fruit beverages, tomato juice and Doogh, respectively (Castro-López *et al.*, 2016; Di Cagno *et al.*, 2009; Hashemi *et al.*, 2016).

Change in Organic Acids Concentration in Jujube Extract through Fermentation Process

The contents of citric acid as the dominant organic acid found in the tested jujube extract, and lactic acid as the main produced organic acid through fermentation are shown in Figure 3. The initial

concentration of citric acid (21 g L^{-1}) in the extract consumed was unfermented considerably through fermentation by both bacteria, such that after 72 hours of fermentation, its concentration in the extract fermented by L. plantarum and L. delbrueckii dropped to 1.12 and 5.8 g L⁻¹, respectively. These results revealed the high capacity of citric acid metabolism by the selected strains. Meanwhile, growth and substrate consumption of the bacteria resulted in the production of 23.8 and 11.4 g L^{-1} lactic acid by L. plantarum and L. delbruckii, respectively. The main increase in the lactic acid concentration was observed in the first 24 hours. In similar studies, L.

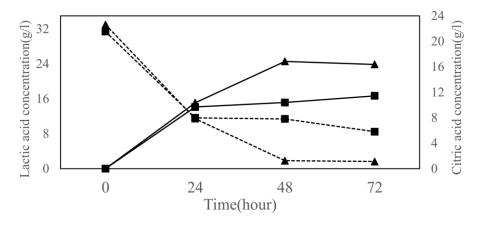


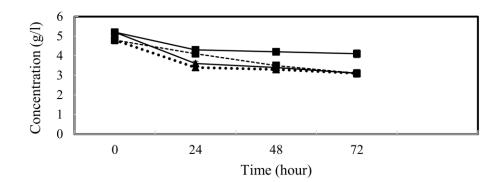
Figure 3. Kinetics of organic acid consumption and production in jujube extract during fermentation at 37°C. Filled triangle= *L. plantarum*, and Filled square= *L. delbrueckii*. (Dash lines indicate citric acid concentration changes).

plantarum showed higher capacity in the consumption of citric acid and production of lactic acid (Mousavi *et al.*, 2011; Palles *et al.*, 1998).

The concentrations of glucose and fructose throughout the fermentation process are shown in Figure 4. The initial amount of glucose and fructose in the unfermented extract were 5.2 and 4.8 g L⁻¹, respectively. Sugar consumption by both bacteria took place mainly in the first 24 hours of fermentation. However, further decrease in sugar contents occurred after 24 hours and remained constant after 48 hours. L. plantarum utilized more glucose compared to L. delbrueckii, and its concentration dropped to 3.1 g L⁻¹ at 72 hours. However, the intake of fructose in both bacteria was similar and their content was 3.1 g L⁻¹ at the end of fermentation. Similar work performed by the author also showed that L. plantarum utilized sugars more efficiently than other probiotic Lactobacillus strains in non-dairy medium. In general, the metabolism of carbohydrates by Lactobacillus varies from strain to strain and relies on the substrate and also on the fermentation time (Hou et al., 2000).

The Antioxidant Activity and Total Phenolic Changes during Fermentation

The changes in the antioxidant activity and the total phenolic contents of the samples before and after fermentation are shown in Figures 5 and 6. The results revealed that fermentation could effectively increase antioxidant activity and total phenolic content in the jujube extract. The antioxidant activity in the unfermented extract was 0.77% while its level significantly increased to 0.94 and 0.89% in the fermented extracts by L. plantarum and L. delbrueckii, respectively, after 72 hours fermentation. Hashemi et al. (2017) also observed that antioxidant activity of lemon juice drink increased significantly by means of lactic acid fermentation. These results are also in concordance with findings of Mousavi et al. (2013), who reported that during the fermentation of pomegranate by lactic acid bacteria, could improve the antioxidant capacity of the juice. Dueñas et al. (2005) found that fermentation of Koupee by lactic acid bacteria could increase its antioxidant capacity. The total phenolic compounds in jujube extract fermented by L. plantarum and L. delbrueckii increased from initial level of 52.45 μ g mL⁻¹ gallic acid to 94.84 and 87.20 μg mL⁻¹ gallic acid, respectively. According to statistical analysis, the increase in total phenolic compounds in the extract fermented by L. plantarum was more significant. Increase in phenolic contents through fermentation by lactobacilli in various drinks has been reported by several



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Figure 4. Glucose and fructose consumption during fermentation of jujube extract by *L. delbruekii* and *L. plantarum* at 37 C. Filled triangle= *L. plantarum*, and Filled square= *L. delbrueckii*. (Dash lines indicate fructose concentration).

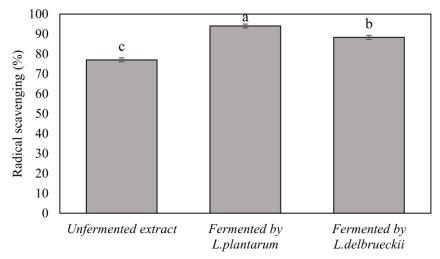


Figure 5. Antioxidant activity of jujube extract before and after fermentation by *L. plantarum* and *L. delbrueckii*. Values with different superscripts (a-b) are significantly different (P < 0.05).

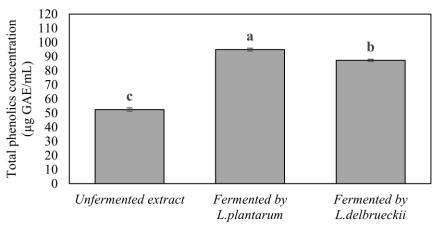


Figure 6. Total phenolic concentrations of jujube extract before and after fermentation by *L. plantarum* and *L. delbrueckii*. Values with different superscripts (a-b) are significantly different (P < 0.05).

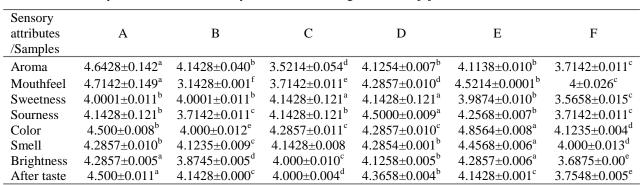


Table 1. Sensory evaluation of differently formulated beverages based on jujube extract.^a

^{*a*} (A-F) A= Sour cheery concentrate+Fermented Jujube extract by *L. plantarum* (carbonated); B= Sour cherry concentrate+Unfermented Jujube extract (non carbonated); C= Sour cherry concentrate+Fermented Jujube extract by *L. delbruekii* (non carbonated); D= Sour cherry concentrate+Fermented Jujube extract by *L. plantarum* (non carbonated); E=Sour cherry juice+Fermented Jujube extract by *L. delbruekii* (non carbonated), F= Sour cherry concentrate.

researchers (Álvarez-Fernández et al., 2014; Moktan et al., 2008; Puértolas et al., 2010). In a comprehensive study on phenolic compounds and lactic bacteria conducted by Rodríguez et al. (2009), it was concluded that L. plantarum was able to break down some complex phenolic compounds to their free form. The effect of Lactobacillus growth on the colour and degradation of compounds olive phenolic in was investigated by Lamia and Moktar (2003). They reported that the growth of lactic acid bacteria resulted in the depolymerisation of high molecular weight phenolic compounds. Therefore, complex polyphenols are hydrolyzed to simpler and more biologically active compounds during fermentation.

Sensory Evaluation

Sensory properties of drinks have a significant effect on consumer behavior in food choice and acceptability. The results of the sensory evaluation test of different drinks containing jujube extracts are shown in Table 1. By examining the results, it was concluded that fermentation of jujube extract could result in the production of new compounds affecting the sensory attributes of the formulated drinks. The carbonated drink containing jujube extract fermented by

L. plantarum obtained the highest score for aroma, mouthfeel, brightness, after taste by mouthfeel in the the referees. The formulated drink containing unfermented jujube extract received the least score. The sweetness of noncarbonated drink containing jujube extract fermented by L. plantarum and L. delbrueckii obtained the highest scores among evaluated drinks. According to Sternini (2013), carbonation could reduce sweetness perception by the brain. This justifies the lower score for sweetness in carbonated drinks compared to non-carbonated drinks. The sourness of the formula containing sour cherry+the jujube extract fermented by L. plantarum and L. delbrueckii obtained higher score by the panelists. The sourness could be attributed to the potential organic acids produced by the selected strains through the fermentation of iuiube extract. In addition, the inclusion of sour cherry concentrate could assist in higher sourness perception in relevant formulations. Meanwhile, formula the containing the jujube extract fermented by L. delbrueckii received a lower score. The highest score for aroma was recorded for the carbonated drink containing jujube extract fermented by L. plantarum. The preference of the formula containing jujube extract fermented by Lactobacillus plantarum can be attributed to the ability of this bacterium

to significantly change pH and acidity and as well as metabolizing phenolic compounds to new compounds that influence the sensory properties of the extract. Daneshi et al. (2013) also performed a sensory assessment of a probiotic beverage based on milk-carrot concluded juice. They that. after fermentation, sensory parameters including color, consistency, taste, and aroma could change significantly. In addition, they found that the sensory features of orange juice changed significantly after fermentation and during storage.

CONCLUSIONS

Probiotic lactic acid bacteria were studied for the production of a healthy drink based on jujube extract. The selected probiotic strains showed a suitable growth in this medium. They were able to consume sugars and organic acids. The antioxidant activity and phenolic acid contents were improved after fermentation. The sensory evaluation showed that consumers preferred the drink formula containing jujube extract fermented by L. plantarum. The overall results of this study showed that the incorporation of lactic acid probiotic bacteria to jujube extract could potentially improve its healthy properties, which can be utilized as the base of functional non-dairy beverages.

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تولید نوشیدنی غیر لبنی عملگرا بر پایه عصاره عناب با استفاده از باکتری های اسید لاکتیک پروبیوتیک

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چکیدہ

در این تحقیق ، تولید یک نوشیدنی پروبیوتیک بر اسا س عصاره عناب با استفاده از تخمیر لاکتوباسیلوس پلانتاروم و لاکتوباسیلوس دلبروکی به عنوان باکتری های اسید لاکتیک پروبیوتیک بررسی شده است. تخمیر به مدت ۷۲ ساعت در دمای ۳۷ درجه سانتیگراد انجام شد. تغییرات در جمعیت میکروبی ، pH و اسیدیته قابل تیتراسیون و همچنین متابولیسم قند و اسید آلی در طول دوره تخمیر مورد بررسی قرار گرفت. علاوه بر این ، تغییرات ترکیبات فنلی کل و فعالیت آنتی اکسیدانی