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Original research

Effect of Lactobacillus acidophilus on the physicochemical and sensory properties of Aloe vera

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A B S T R A C T

Nowadays, in many developed societies, consumer health awareness towards the role of food in human health and nutrition has been considerably increased. Regarding the biological role of food components on human health, consumers are interested in consuming food products with an improved functionality. Non-dairy probiotic drinks are considered as functional foods. The purpose of this study is to investigate the possibility of development of Aloe vera drink bearing probiotic *Lactobacillus acidophilus*. Therefore, the growth and survival of these bacteria were investigated during 12 days at two temperatures of 4 and 25 °C. The acidity, total sugar and pH of the drink samples were measured at days 1, 6 and 12. The growth of *Lactobacillus acidophilus* in the drinks maintained at 25 °C was considerably better compared to the sample kept at 4 °C. *Lactobacillus acidophilus* increased the acidity during cold storage ($p < 0.05$) and less sugar intake occurred at this condition. Addition of *Lactobacillus acidophilus* to Aloe vera did not have a negative effect on its sensory properties and people who were willing to use probiotic Aloe vera. The results of this study showed that beverage, is a suitable medium for producing a probiotic drink containing an acceptable level of *Lactobacillus acidophilus* and preferred by consumers in terms of sensory evaluation.

Keywords: Aloe vera, Probiotics, Viability, Sensory evaluation

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1. Introduction

Recent economic and social developments have created many problems for human health. Excessive tension and occupation have led to diseases such as heart attack, high blood pressure, intestinal disorders and various types of cancers (Kun et al., 2008). One of the effective ways of preventing or eliminating these diseases is to consider probiotic food products in the daily diet. The consumption of these food products has become prevalent in developed countries such as Europe, the United States and Japan. More than 90 probiotic products containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are produced all over the world (Granato et al., 2010; Sohrabvandi et al., 2010). Probiotic products can be divided into two categories: 1-probiotic dairy products such as yogurt, cheese, sour cream, buttermilk, ice cream, milk, whey drinks and dairy desserts, 2 nondairy probiotic products such as cereals, sweets, beverages, baby food and meat products (Sohrabvandi et al., 2010; Mohammadi et al., 2012).

Fruits and vegetable drinks with nutritional and biological properties are suitable medium bearing probiotic bacteria as some fruits and vegetables contain prebiotics that stimulate the growth of certain probiotics. In addition, It has been shown that the beneficial effects of fruits and vegetables can be improved by biological processes such as lactic fermentation performed by probiotic bacteria. In many studies, probiotics are used in dairy products, but lactose intolerance and cholesterol levels in these products are two main disadvantages, which have led to a limited consumption by some group of people (Moraru et al., 2019). Therefore, the use of probiotics in fruit juice and vegetables can be considered as a good alternative for the group of people with special needs (ie, planters and people with allergic reactions to milk proteins) (Moraru et al., 2019; Krasaekoopt & Kitsawad, 2010).Therefore, fruit juice has the potential for becoming a probiotic product for the following reasons: its use by a wide range of people has acceptable sensory properties of the absence of incompatible compounds (such as lactose) (Nandi et al., 2017).

According to previous studies, the main strains used in non-dairy probiotic drinks are: *Lactobacillus casei, bulgaricus,*

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Lactobacillus acidophilus and *Bifidobacterium* (Granato et al., 2010; Nualkaekul & Charalampopoulos, 2011; Krasaekoopt & Kitsawad, 2010)

Pereira et al. (2011) studied the fermentation of apple juice using *Lactobacillus casei*, which showed suitable survival under refrigerated storage conditions. During fermentation and cold storage condition, the brightness of apple juice increased and its redness decreased.

Sadaghdar et al. (2012) found that probiotic viability in food samples depends on pH, storage temperature, oxygen content, incubation temperature, refrigerated temperature and storage time, the presence of competitive bacteria and viruses and the deterrent depends. Therefore, the formulation of nutrients is important in the activity and survival of probiotics over a long period of time (Shah 2007; Ding & Shah, 2009). Due to the fact that the pH of most juices is 3.5-3.7 (Buruleanu et al., 2009; Mohan et al., 2013) as well as bacteria sensitive to acidic conditions, strains should be used to survive in. This condition has the health of the product and increase its shelf life (Mousavi et al., 2011; Mohan et al., 2013) . Therefore, the addition of probiotics to fruit and vegetable based drinks is more complex than dairy products due to the need to protect them against acidic conditions. The results of the Kun et al. (2008) showed that if the *Bifidobacterium* the carrot juice is an appropriate growth medium for *Bifidobacteria*. This study was carried out using two different initial bacterial population of 10^6 cfu /mL, and. It was concluded that the inoculation of vegetable juice or fruits with an initial concentration of 10^7 cfu /mL accelerated the process of fermentation. Liu and Lin (2000) found that *Lactobacillus bulgaricus* in Chile slowly grew due to its inability to ferment sucrose and other soya carbohydrates, and produced a little amount of acid.

In a study conducted by Vinderola et al., (2002), it was found that despite of higher level of acids produced by *Lactobacillus casei*, it showed a higher viability than *Lactobacillus acidophilus.*Therefore, it has been concluded that the *Lactobacillus casei* species has suitable resistance to acidic conditions.

Nevertheless, probiotics survival in fruit and vegetable foods is more difficult than dairy products for reasons such as the acidity of the environment, the presence of plant antimicrobials and the lack of nutrient components required for their growth (Mohan et al., 2013; Moraru et al., 2019; Granato et al., 2010; Mousavi et al., 2011). In addition, the probability of persistence and stability of probiotics in dairy products such as yogurt is extensively investigated, but there is not enough information on their survival in non-dairy products (de Almeida et al., 2008). Moreover, the addition of probiotics to fruit juices and vegetables causes an obvious unacceptable taste of the product. Therefore, further studies are needed to increase the probiotic survival capacity of these products and improve their sensory properties.

Aloe vera belongs to the *Liliaceae* family, which includes varieties of plants with different characteristics (Minjares-Fuentes et al., 2018). The most important part of the plant is Aloe vera leaf, because studies have shown that Aloe vera leaves contain essential amino acids, vitamins B_1, A, B_2, B_6, B_{12}, C, E, gum Resins, uronic acid, enzymes such as oxidase and catalase minerals such as calcium, sodium, iron, potassium, chlorine, manganese, copper and zinc, various sugars and various compounds such as alumin, anthraquinone and its derivatives, beryllium glycoside and isobarboleum essential oil, water (Hamman, 2008a; Minjares-Fuentes et al., 2018). According to different researches, the Aloe vera leaf gel have various healing properties, including anti-inflammatory, antiviral, antibacterial and antifungal effects, wound and radiation damage and stimulation of hematopoiesis (Hamman, 2008b; Rahman et al., 2017; Goudarzi et al., 2015) According to literature review performed by the author, no investigation on the probiotication of Aloe vera drink has been performed yet. Therefore, this study was conducted to study the survival of *Lactobacillus acidophilus* in Aloe vera drink and its effect on physicochemical properties and sensory properties of the drink.

2. Material and Methods

2.1. Culture preparation

The bacteria *Lactobacillus acidophilus* was purchased from the Christian Hansen Co. in Denmark. Bacterial culture was stored frozen at -20 ºC in MRS medium (Merck, Germany) containing 20% glycerol. The inoculated drink was incubated at 37 ℃ for 24 h under anaerobic conditions using gas type C in an anaerobic jar. Viable cells were determined at intervals of the first, sixth and twelfth days, by the standard plate count method using MRS agar medium and the results were expressed as colony-forming units per milliliter of sample (CFU/mL).

2.2. Drink preparation

Aloe vera leaves were bought from the local market in Tehran. Weighted, rinsed with tap water and disinfectant. Then, the skin was peeled off and the gel was extracted. The extracted gel (40 mL) was homogenized by a mixer, then, pasteurized at 70 °C for 30 min. After cooling the gel to 5 °C under vacuum condition provided by a rotary evaporator (Büchi, Switzerland), 60 mL of previously prepared syrup (30 g sugar in 70 mL: distilled water pasturized at 80 °C for 30 min) was added to it. Then 0.5% w/v Citric Acid (Merck, Germany), 0.3 %w/v Ascorbic acid, were added to the mixture.

2.3. Chemical analysis

2.3.1. pH measurement

The pH of the samples was measured by a digital pH meter (Jenway, Germany), previously calibrated with Buffer 4 and 7.

2.3.2. Acidity measurements

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

$$A = \frac{m \times 0.064 \times 100}{w} \quad (1)$$

where, *A* was Titratable acidity (g citric acid/100 g of sample), *m* was mL used titrazol 0.1 N and *w* was weight of sample.

2.3.3. Sugar measurement

Measurement of sugars was carried out by Lyon Weinon method (Chandraju et al., 2013).

2.4. Sensory evaluation

In this study, sensory properties of Aloe Vera drink samples fermented under different conditions were studied. Some sensory properties of the samples, including color, aroma, taste, and overall acceptance were evaluated by 10 non-trained panelists using a 5-point hedonic scale (INSO, 2008). For this purpose, a questionnaire was prepared and given to the panelists along with the samples. The panelists were provided with the coded drink samples and asked to rate the products from 1 to 5. Score 1 and 5 represented the samples with worst and best quality, respectively.

2.5. Statistical Analysis

Experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean ± SD (standard deviation). The two way analysis of variance (ANOVA) was used to analyze the experimental data (SAS 9.1 software Institute Inc., Cary, NC, USA). Mean analysis using Duncan's multiple range tests was carried out if needed.

3. Results and Discussion

3.1. pH and acidity changes during fermentation

Fig. 1 and 2 shows the effect of *Lactobacillus acidophilus* inoculum percentage, storage time and temperature on pH and acidity of Aloe Vera drink. According to the figure, the pH of all samples has decreased over time.

Average, in the first day of storage, no significant difference was observed between the samples. However, at Day 6 of storage the highest pH and the lowest acidity belonged to the control sample (without bacteria and kept at 4 °C) and the sample with 1% inoculum maintained in 25 °C, and no significant difference was observed between the remaining samples. On the sixth day, the lowest acidity related to the control sample was 3.39 and the lowest pH related to the 1% sample stored at 25 °C, 3.34 and the highest acidity related to the 2% sample stored at 25 °C. According to the results on the day 6 of storage, the pH of the samples kept at 25 °C was lower than those stored at 4 °C. On the 12th day, the highest pH and the lowest acidity related to the control sample (no bacteria stored at 4 °C) (pH 3.39) and (0.39% by weight) then the samples containing 1% bacteria (pH 3.31), and the lowest pH and The highest acidity was related to the sample containing 2% bacteria and kept at 25 °C, (pH 3.26 and acidity 0.43% by weight). On day 12, the pH of the samples containing 2% bacteria (3.28 and 3.26) was less than the pH of the samples containing 1% bacteria (3.31).

The main reason of the increased acidity in free-form free probiotic treatment may be due to the activity of *Lactobacillus acidophilus* in Aloe Vera, caused by the consumption of sugars, and the production of lactic acid and acetic acid, which can increase the acidity and decrease the pH of the samples. These bacteria produce organic acids with consumption of the sugar in the beverage, which increases the acidity and decreases the pH during storage (Yoon et al., 2006).

Also, samples stored at a refrigerated temperature, had a lower growth rate and therefore, less sugar was utilized by the strains, and consequently, lower organic acids were produced. However, samples kept at 25 °C had a higher metabolism rate and consumed more sugar as a main substrate for the growth of the strains.

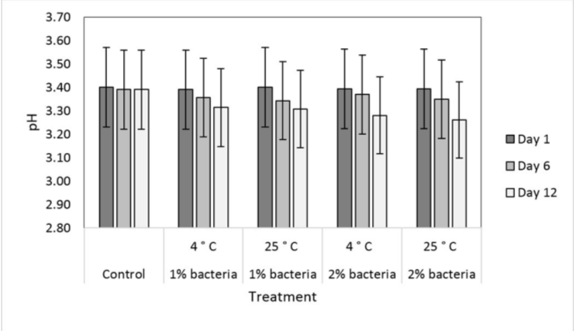

Fig. 1. The change of pH in Aloe vera drink samples under different conditions (Day, Temperature and inoculum percentage).

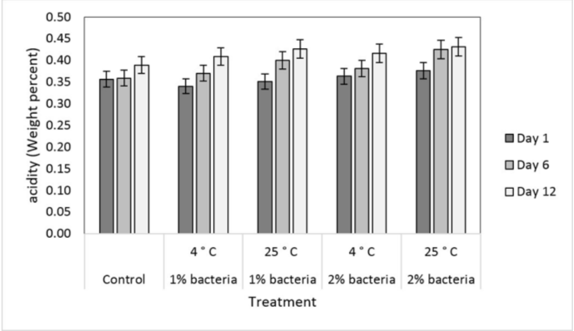

Fig. 2. The changes in the acidity of Aloe vera drink samples during 12 days of storage under different conditions (Temperature and inoculum percentage).

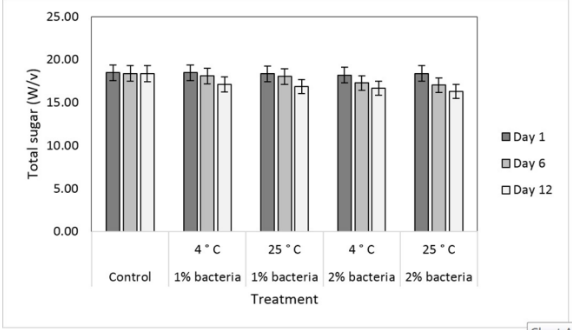

Fig. 3. The changes in total sugar content (%w/v) changes of Aloe vera drink samples during 12 days of storage under different conditions.

3.2. Changes in sugar concentration of Aloe vera drink during fermentation

Fig. 3 shows sugar percentage (w/v) in Aloe vera drink in different conditions over time. As observed in the figure, the total sugar concentration decreased in all samples during fermentation. It is obvious that that during the storage, the highest decrease in sugar content was in 6th and 12th days in the samples containing 2%. In addition, sugar level in the samples stored at 25 °C was lower than those stored at 4 °C.

Based on the results, over time, the weight-volume percentage of sugar in the beverage samples decreased over a period of 12 days (from 0.5% in control sample to 12% in 2% bacteria samples). In samples that were stored at 25 °C, sharper decrease in sugar content (8-12%) was observed. This decrease in sugar content over time is probably due to the higher growth rate and metabolic activity of *Lactobacillus acidophilus* and in this condition. However, in the refrigerated samples, the metabolic activity is considerably lower.

3.3. The growth of Lactobacillus acidophilus in the Aloe vera drink samples

In all probiotic food products, the number of 10^6 CFU/g per gram or milliliter of product is needed at the time of consumption to make the food product effective on consumer's health (Maetins et al., 2016). According to Fig. 4, the population of probiotic cells did not decreased below the mentioned value during the storage period, which approved that Aloe could be considered as suitable environment for probiotics growth. After one day of storage, the highest bacterial content was found in the sample containing 2% bacteria (8.3 Log) kept at 25 °C and there was no significant difference between the other samples. At the day 6, no significant difference was observed between treatments containing 1% bacteria and stored at 25 °C and treatments containing 2% bacteria. The lowest bacterial viable cells was for the 1% stored sample at 4 °C (7.33 Log). On the 12^{th} day, the highest bacterial population was stored in the sample containing 2% bacteria and stored at 25 °C (8.23 Log) and 4 °C (8.0 Log). The lowest bacterial content was found in samples containing 1% bacteria (log 7.31) at 4 °C. After 12 days of storage, the number of live cells declined below the recommended value. *Lactobacillus acidophilus* showed a decrease in bacterial population after 12 days of cold storage, the viable cell counts dropped below the acceptable value (10^6 CFU/mL). Temperature is an important factor of the survival of probiotics and is also effective in reducing the suppressive effect of organic acids in the growth medium on the deterioration of viable cells (Moussavi et al., 2011). The results indicated that the cell growth at 25 °C is better than 4 °C, and by increasing the inoculum percentage in the drink, the bacterial population increased considerably. Yoon and colleagues conducted a study to evaluate the viability of probiotics during storage in the refrigerator. The results show that *L. Plantarum* and *L.delbruekii* could maintain their bioavailability at 4 °C for several weeks.

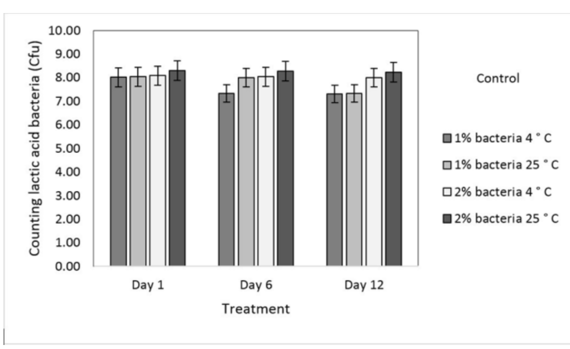

Fig. 4. Comparison of the effect of *Lactobacillus acidophilus* inoculation volume on the bacterial growth in Aloe Vera drink during 12 days of storage at 25 and 4 °C.

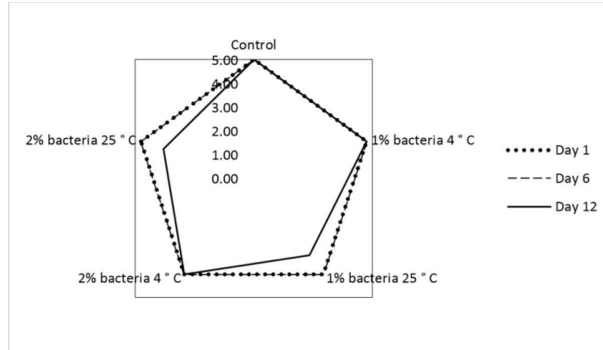

Fig. 5. Effect of different conditions (temperature: 4 ° and 25 °C, inoculum percentage 1 and 2%, time: 1, 6 and 12 days) on the color score of fermented Aloe vera given by the panelists.

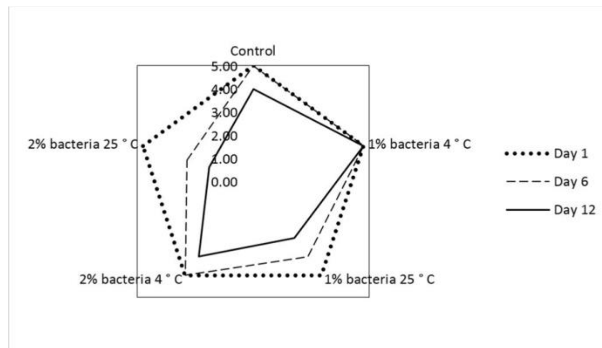

Fig. 6. Effect of different conditions (temperature: 4 ° and 25 °C, inoculum percentage 1 and 2%, time: 1, 6 and 12 days) on the aroma score of fermented Aloe vera given by the panelists.

3.4. Effect of fermentation by Lactobacillus acidophilus on the sensory properties of Aloe vera drinks fermented under different conditions

The results of sensory evaluation based on 5 points hedonic scale are reported in Fig. 5-8. Based on the given scores, the panelists did not find any significant difference between the color of the samples maintained one and 6 days at 4 °C. On the 12^{th} day, the samples containing 1 and 2% inoculum kept at 25 °C had lower scores.

According to the Fig. 6 and 7, the panelist gave the highest aroma and taste score to the samples kept in one day of storage, However by increasing storage time to day 6, the samples stored at 25 °C were given lower scores compared to the control sample (without bacteria stored at 4 °C). Eventually, the scores of all samples decreased dramatically from day 6 to day 12 of storage.

The overall acceptance score given by the panelists differed among the samples. The control sample, sample with 2% kept at 25 °C, samples with 1% kept at 4 °C received higher score compared to the other samples. On the sixth day, according to the scores given by the panelists, the overall acceptance of the control sample and samples containing 1 and 2% bacteria stored at 4 °C did not changed. The lowest score was given to the drinks kept at 25 °C. Over time, from the sixth to the twelfth day, the overall acceptance of all samples decreased dramatically. On the twelfth day, the drink containing 1% inoculum kept at 4 °C had the highest score.

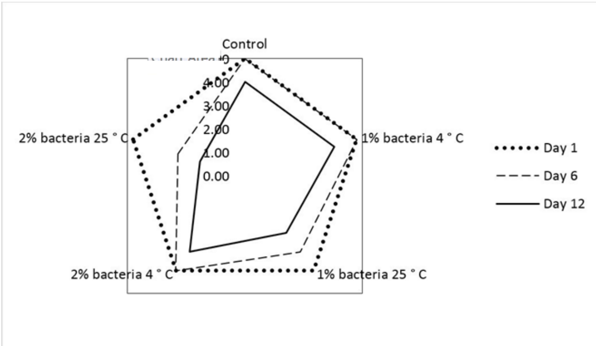

Fig. 7. Effect of different conditions (temperature: 4 ° and 25 °C, inoculum percentage 1 and 2%, time: 1, 6 and 12 days) on the taste score of fermented Aloe vera given by the panelists.

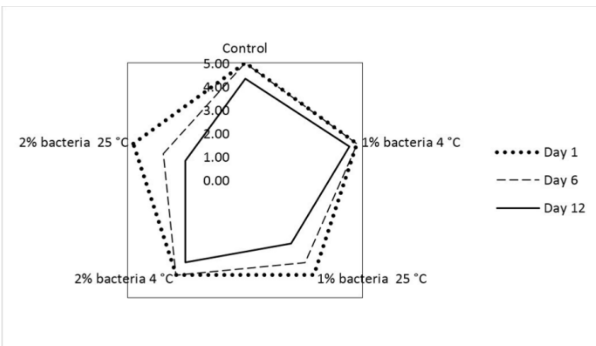

Fig. 8. Effect of different conditions (temperature: 4 ° and 25 °C, inoculum percentage 1 and 2%, time: 1, 6 and 12 days) on the overall acceptance score of fermented Aloe Vera given by the panelists.

4. Conclusion

A healthy probiotic drink based on Aloe vera was produced in this study. Based on the results obtained in this study the probiotic strain of *L. acidophilus* grew in this medium and reduced the pH and increased the acidity of the drink over time. The growth rate of the bacteria was higher at 25 °C than 4 °C and following this, the sugar in the samples is consumed more and then the acidity of the product increases and its pH decreases. The transparency of the probiotic Aloe vera drink was reduced during storage, due to increased turbidity due to increased *lactobacillus* cell mass inoculated with fruit juice. The increase in the inoculum percentage resulted in higher bacterial population at the end of fermentation. In terms of sensory properties, sample kept at cold storage condition gained higher score. It is suggested to formulate the Aloe vera drink with other fruits to improve its taste. In addition, providing information about the healthy properties of the probiotic drink could definitely increase consumer acceptability toward this product.

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