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Scientific Research

Investigating the physicochemical and sensory properties of grape juice containing paraprobiotics*Lactobacillus Gasseri* During shelf life

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Abstract

Paraprobiotics are a new group of functional compounds that result from the inactivation of some strains of probiotics. In the present study, the effect of using paraprobiotic Lactobacillus Gasseri and storage time (30 days) and temperature (4 and 24 degrees Celsius) on the physicochemical and sensory properties of grape juice drink were investigated. Characteristics such as pH, acidity, total dissolved solids, turbidity and sensory characteristics were investigated. The addition of paraprobiotics caused a change in pH and acidity, and the control treatment (pure grape juice) and the paraprobiotic treatment (grape juice containing paraprobiotics) had significant differences in this respect, however, temperature and storage time had no effect on these characteristics. The addition of paraprobiotics increased the turbidity and soluble solid matter in the paraprobiotic drink, and these two characteristics also increased with the increase in the storage time. The paraprobiotic beverage was very similar in sensory properties to pure grape juice and had a similar overall acceptance score as pure grape juice, which changed very little during storage at different temperatures.

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1. Introduction

Today, the demand for the production of practical products is increasing all over the world, and among the 59 practical products, probiotic dairy products have the most consumers in Europe and the Middle East. According to the definition of the World Health Organization[1](WHO), probiotics are living microorganisms that, if used in sufficient quantities, improve the host's condition (1, 2). In order to achieve the health-giving effects of probiotics, it is necessary for a certain number of probiotic cells to survive until they enter the digestive system. The minimum number of living cells needed in the final product to achieve the health benefits should be around 10 cfu/g^7 -10^6 be (3, 4). However, the use of live probiotics in food has caused problems in the industry, including the uncertainty of probiotic viability during processing, storage, transportation, and consumption, the possibility of pathogenic bacteria gene transfer to probiotics, and dysfunction. Their positive points are the possibility of causing allergies in people with weak immune systems or people who have recently undergone surgeries or organ transplants and the production of unpleasant sensory compounds during fermentation in some products such as juice (5). These factors have caused the use of inactive and non-living probiotics known as paraprobiotics to flourish as a new method in the food industry. Scientific research has shown that paraprobiotics also have positive effects on health. The production of paraprobiotics is done in different ways, including heating, applying ultrasound, ultraviolet radiation, infrared radiation, ionizing rays, applying high hydrostatic pressure, and using some chemical compounds (6, 7).Paraprobiotics have various health effects, which include: regulating the immune system, reducing blood cholesterol, reducing respiratory diseases, strengthening fatty liver, preventing cancer growth, reducing stress, treating colitis and preventing tooth decay (8).

It is necessary to create a suitable substrate to preserve the characteristics and transfer paraprobiotics to the human body. According to scientific studies, in general, dairy materials are one of the suitable substrates for the transfer of active and deactivated probiotics. While not all people are able to consume dairy products. Some people are unable to consume these products due to diseases such as lactose intolerance, high cholesterol, and animal milk protein allergy (5). Therefore, fruits and their water can serve as a new platform for transferring these deactivated microorganisms and useful substances to the human body. Due to the high content of nutritious and functional compounds in fruit juice (such as vitamins and antioxidants), these products are a very suitable environment for the transfer of parapsychobiotics and their beneficial properties (9). grape (*Wine vine* L.) is one of the strategic agricultural products in the world. Grapes and its juice, especially red grapes, are a rich source of various polyphenols including catechin, epicatechin, quercetin and anthocyanins. Laboratory and clinical research has shown that red grape flavonoids have various effects such as antioxidant, anti-inflammatory, anti-oxidation of fats and preventing oxidative damage of DNA in living cells (10-12). Different types of grapes are produced in the country, Shahani grape is one of the most popular varieties in Iran; So that it can be used to produce various products including drinks. Due to the high amount of polyphenols in black grapes, they can be used as a medium for the transfer of paraprobiotics.

In the present study, the amount of changes in some physicochemical characteristics of

[1]- World Health Organization

Shahani grape juice due to the addition of paraprobiotics*Lactobacillus Gasseri* Has been studied.

2- Materials and methods

1-2- Materials

Grape juice concentrate was obtained from Iran's Behnos company and active bacterial culture*Lactobacillus Gasseri* It was obtained from the Center of Genetic Resources of Iran (Jihad Davanghi). All the chemicals used were of laboratory grade.

2-2- Methods

1-2-2- Production of grape drink containing paraprobiotics

Heat-inactivated cells were produced according to the method described below. At first, to increase the bacterial population, probiotic cells were cultured on MRS liquid medium in anaerobic conditions (in the presence of a gas pack) at 37 degrees Celsius to reach a population of 10 cfu/mL.$^{9}\times$ 2 Receipt. Then, using centrifugation and multi-step washing, bacterial cells were separated from the liquid culture medium. Inactivated bacterial cells were produced using heat (75°C for 20 minutes). In order to produce the final drink, Shahani grape juice concentrate and water were mixed in a certain proportion (final Brix 14) and then the resulting juice was pasteurized in a bain-marie at a temperature of 90 degrees Celsius, and then inactivated bacteria cells were added to the juice in completely hygienic conditions; So that the amount of population $10^{9}\times1$ bacterial cell was present in the drink. The control drink consisted of reconstituted pasteurized juice from Shahani red grape concentrate. The glass containers containing the samples were kept at two temperatures of 4 and 24 degrees Celsius, and the changes made during one month were checked at time intervals every 10 days (13, 14).

2-2-2- Measurement of dissolved solids

Dissolved solids were measured using a digital refractometer (Optech, Germany) (15).

3-2-2- pH measurement

Using a pH meter (Inolab, Germany), the pH of each sample was measured during storage (15).

4-2-2- Measurement of acidity

According to the 1634 fruit juice standard, 10 ml of each sample was titrated with 0.1 normal NaOH solution until pH = 8.2 (16). The final acidity is based on the amount of profit consumed in terms of tartaric acid according to the equation1 Calculated.

Equation (1)
$$Acidity = \frac{N \times 0.0075}{S} \times 100$$

where: N = amount of profit and S = sample amount

5-2-2- Turbidity

The turbidity of beverages during production and storage was measured using a spectrophotometer (Epoch, USA). According to the 1634 standard, the samples were diluted with distilled water and then the absorbance of each sample was measured at the limit of visible light at a wavelength of 440 nm (16, 17).

6-2-2- sensory characteristics

20 semi-trained evaluators in the age group of 20 to 30 years were selected to participate in the sensory evaluation test. Each of the evaluators examined the main sensory characteristics of the drink, including color and taste, and overall acceptance on days 0, 10, 20, and 30 days after production based on a 5-point hedonic test; A score of 5 indicates the complete desirability of a feature and the number 1 indicates the lack of desirability of a sensory feature (18).

7-2-2- Statistical analysis

The samples were produced in three replicates and tested. The experimental design was complete factorial. In order to

check the statistically significant difference of the average data from the ANOVA test, Duncan's test was performed using SPSS version 21 software at a significance level of 0.05%.

3. Results and Discussion

1-3- Solution solids

Figure 1 shows the changes of total dissolved solids in each of the samples during 30 days of storage at two temperatures of 4 and 24 degrees Celsius. as seen in the figure. The obtained results show the dependence of changes in the amount of dissolved solids on the presence of paraprobiotics. As shown in Figure 1, the addition of paraprobiotics caused a significant increase in the amount of soluble solids in the drink. The findings showed that the amount of dissolved solid matter significantly increased from 15.01 in the control sample to 15.65 in the drink sample containing inactivated cells.*Lactobacillus Gasseri* Increased. Applying heat to bacteria causes some intracellular compounds to leak out to the outside environment; As a result, the addition of the bacterial carcass suspension has increased the amount of soluble solids in the drink, which is due to the dissolution of these intracellular substances and the increase of the total soluble solids in the drink. Storage factors, including temperature and storage time, also caused changes in this parameter in drinks. As shown in Figure 1, in the control treatment, with the increase of the retention time at 4 degrees Celsius, at the end of the retention time, the amount of dissolved solids decreased significantly, although this decrease in the amount is not numerically significant. However, increasing the storage time at 24 degrees Celsius caused a decrease in soluble solids after 10 days ($P<0.05$) and after that there was no change until thirty days of storage. The reason for this may be the effect of temperature on increasing the speed of some chemical reactions. The results of the analysis of soluble solids in paraprobiotic drinks showed that increasing the storage time at each temperature had an increasing effect on the amount of total soluble solids ($P<0.05$). According to the research of Tim et al. (2020), time is one of the necessary and effective factors for the leakage of intracellular materials of damaged bacteria to the surrounding environment (19). Based on the results of this research, with the increase in storage time, especially at 24 degrees Celsius, there is enough time for the intracellular solution to leak into the grape juice, and as a result, it has increased the total soluble solids of the drink. This increases the bioavailability of the health-giving compounds of probiotics. By comparing the probiotic drinks that were produced in the past with the current drink, it can be concluded that due to the presence of live bacteria in probiotic drinks, many soluble solids (such as sugars) were consumed by the bacteria, and the soluble solids were reduced and the solids The insoluble content increased, which increased the turbidity in this type of drinks (14), while the soluble solids did not change due to the inability of paraprobiotics and their inactivity.

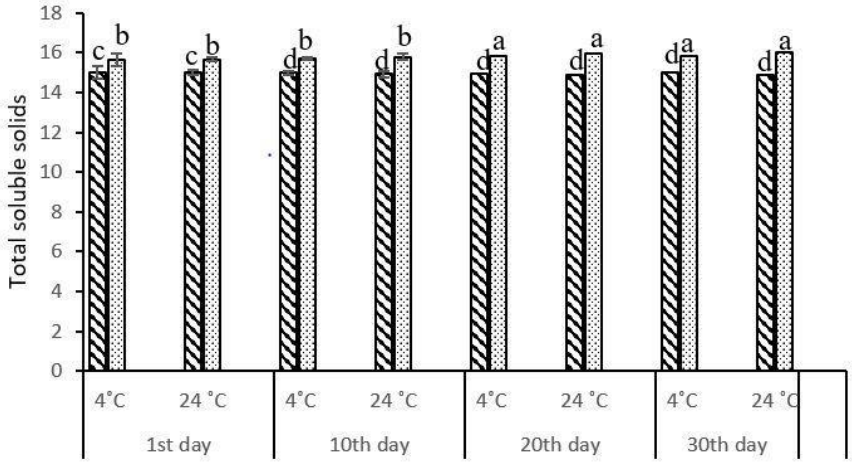

Figure 1. Total soluble solids of control and paraprobiotic grape drinks stored at 4 ˚C and 24 ˚C for 30 days.

2-3- pH

Figure 2 shows the pH changes of the control and paraprobiotic drinks during the 30-day storage period at 4 and 24 degrees Celsius. As shown in Figure 2, the storage time and temperature had no significant effect on the pH of the drinks. But the addition of paraprobiotics caused a slight significant difference between control treatment and paraprobiotic treatment. The reason for the slight increase in the pH of the sample containing paraprobiotics may be the entry of buffer materials into the bacterial cells during storage. In general, adding active probiotics to drinks causes the consumption of sugars and the production of lactic acid, which ultimately leads to a decrease in the pH of dairy and non-dairy drinks. In such a way that in non-dairy drinks such as juices, it increases sourness and creates an acidic taste of dairy, which reduces the acceptance of this type of drinks to some extent (14, 20). Therefore, this can be prevented by adding inactivated probiotics bacteria, in addition to using their effective compounds and turning the drinks into useful drinks without significant changes in chemical properties.

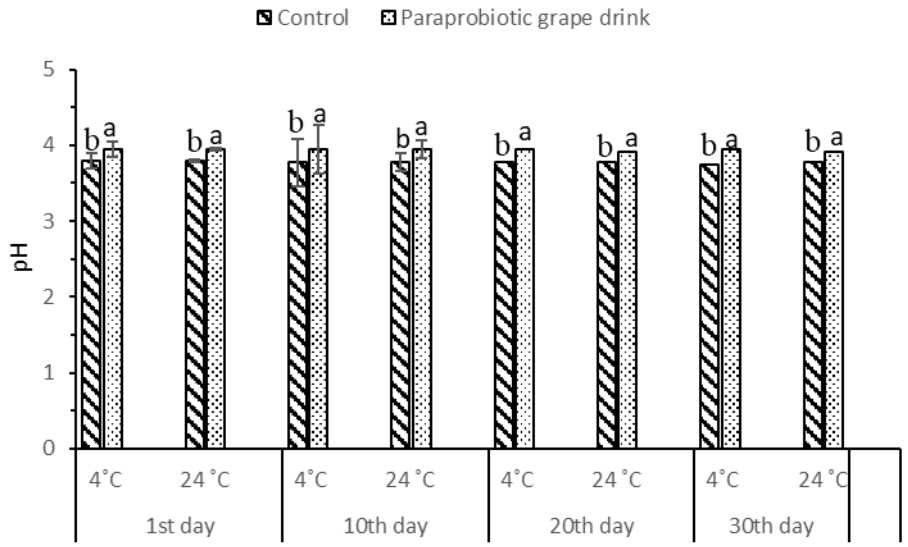

Figure 2. pH of control and paraprobiotic grape drinks stored at 4 ˚C and 24 ˚C for 30 days.

3-3- Drink acidity changes

Organic acids are effective in creating aromatic characteristics, which play an important role in quality indicators such as stability, color, and flavor. Grape juice obtained from ripe grapes contains the highest amount of organic acid compounds. Malic acid and tartaric acid are the main acids in grape juice, which comprise more than 90% of the acidity of grape juice. Among other organic acids found in grapes, we can mention acetic acid, citric acid, fumaric acid and gallic acid, which are found in small amounts in grape products (21). Figure 3 shows the amount of changes in the acidity of the control treatment and the treatment containing paraprobiotics. As can be seen in the picture, the changes in acidity are not noticeable, and the acidity of none of the samples is different, and the acidity data is in line with the pH data, and it shows that the duration of storage and the storage temperature had no effect on acidity ($P>0.05$). According to the results of this test, it can be said that by producing fruit drinks containing paraprobiotics, it is possible to eliminate the disadvantages of using live varieties (such as increasing the acidity and sourness of the final product) and to produce a stable drink with health properties that Its characteristics are similar to pure juice. The

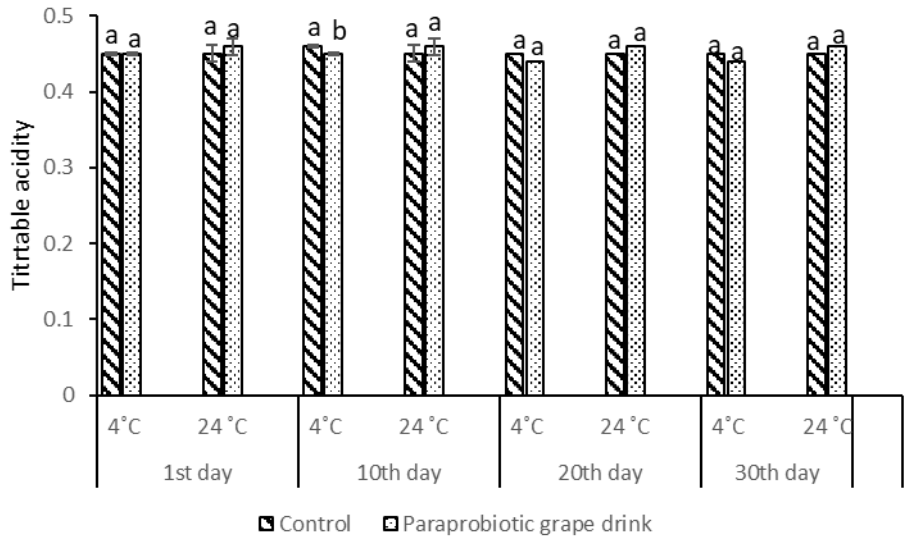

Figure 3. Titratable acidity of control and paraprobiotic grape drinks stored at 4 ˚C and 24 ˚C for 30 days.

4-3- Turbidity

Turbidity is a key feature in product acceptance by consumers, because turbidity is considered a defect in clear beverages such as grape juice (22). In fruit drinks, it is one of the factors in creating the turbidity of minerals in these types of products. Grape juice contains 2 to 6 percent of minerals such as potassium, sodium, iron, phosphate, sulfate, and chloride (14). As shown in Figure 4, the addition of paraprobiotics caused a noticeable increase in the turbidity of the drink ($P<0.05$). With increasing storage time, especially at high temperature, the amount of turbidity increased. The reason for this is that at the beginning of the production of the drink, the cells of the paraprobiotics are dispersed separately in the drink and do not precipitate, but they are still capable of refracting light, and at the same time as the shelf life of the cell particles increases, they are interconnected and the larger the particles are. light refraction happens more often (22). In general, in beverages containing bacterial cells, with the increase in the retention time, the deactivated cells join together and create large particles that cause light refraction (14). Also, the turbidity data were consistent with the data of dissolved solids of the drinks. According to past research, there is a strong relationship between dissolved solids and turbidity, which often increases with the increase of dissolved solids in Kurt, which indicates the phenomenon that the leakage of substances from the bacterial cell into the drink increases the refraction of light in the drink and increases the numerical turbidity. (14, 22).

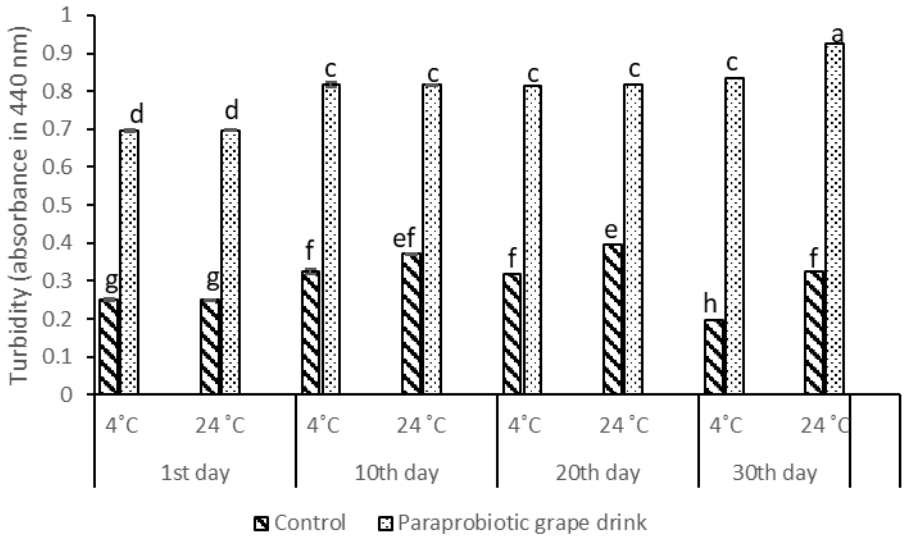

Figure 4. Turbidity (absorbance in 440 nm) of control and paraprobiotic grape drinks stored at 4 ˚C and 24 ˚C for 30 days.

5-3- Sensory characteristics

The average scores related to each of the features in two different treatments were statistically analyzed and the results are shown in Figure 5. According to the obtained results, the control and paraprobiotic drinks were different in terms of color so that the color difference was recognized by the evaluators ($P<0.05$). Storage temperature and storage time also had a significant effect on this feature ($P<0.05$). The difference in color may be due to the increase in turbidity due to the addition of inactivated cells to the grape juice. In previous studies and researches, the addition of live probiotics has also caused changes in the color characteristics. According to Mokhtari et al. (14), the addition of probiotics decreased the acceptance of color by the sensory evaluator. Of course, in the research conducted by Molaeiparvari et al. (13) on yogurt, yogurt containing paraprobiotics was not different from yogurt containing fermented with probiotics in terms of color characteristics. In yogurt, the bacterial cells are the same color as the white background of yogurt, and the color changes are not noticeable; Meanwhile, in colored and transparent products such as grape juice, the presence of bacterial cells may affect the appearance and color of the drink.

From the point of view of the taste characteristics of the control drink and the paraprobiotic drink, there was no statistically significant difference ($P>0.05$), and both were favorable in terms of pleasant acidic taste. Storage temperature conditions and storage time did not have much effect on this characteristic. Meanwhile, the use of live bacteria causes noticeable changes in the acidic taste of fruit drinks. By fermenting environmental sugars, living bacteria produce acid as the last product of this process, which increases the acidity of the product and gives the sour taste of dairy products to the juice, which is considered an undesirable phenomenon (23).

Considering the overall acceptance at the end of the shelf life, there was no significant difference between the control drink and the paraprobiotic drink, however, the control drink had the highest overall favorability. One of the reasons for reducing the desirability of paraprobiotic drink is the

increase in turbidity and decrease in the color characteristics of the product, which has a greater effect on the drink with increasing time. In order to reduce the effect of turbidity on the general characteristics, this defect can be solved by optimizing the inactivation processes and also by choosing pulpy and cloudy drinks such as citrus juice.

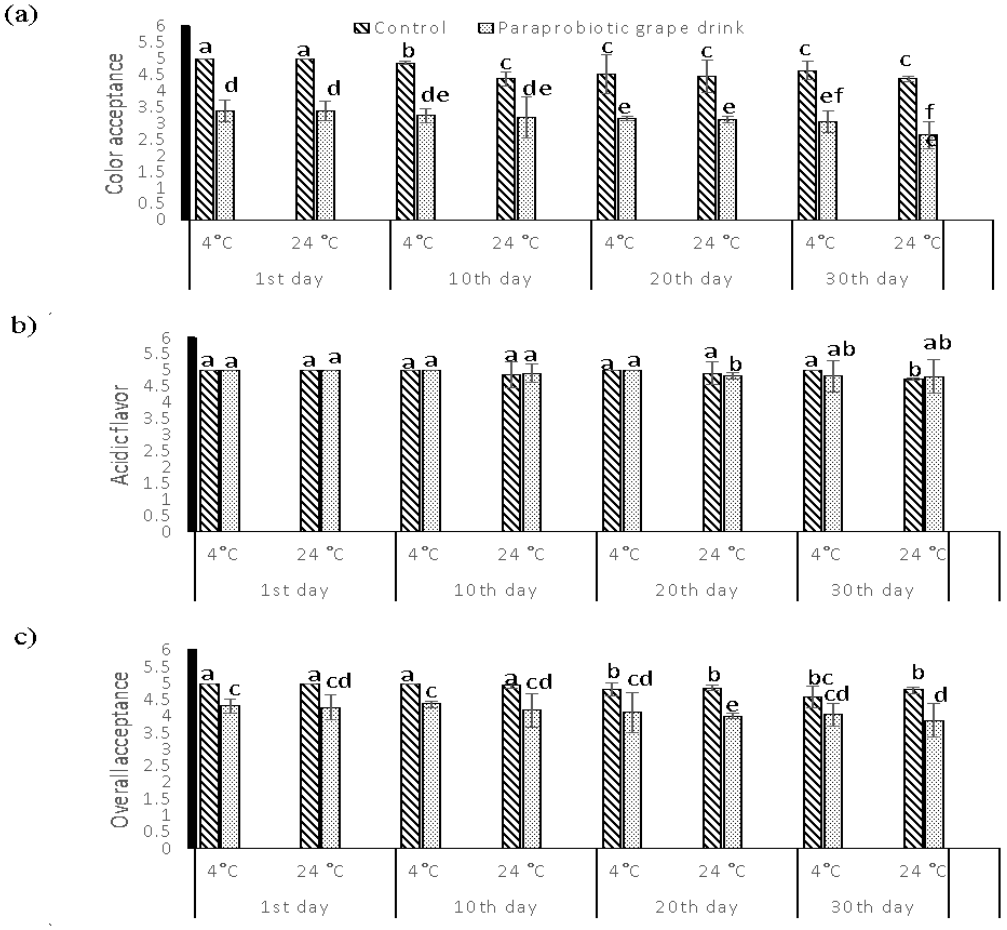

Figure 5. Sensory evaluation of different treatments of grape juice during 30 days at stored at 4 ˚C and 24 ˚C: control and paraprobiotic grape. Color (a), acidic flavor (b), and overall acceptance (c).

4 - Conclusion

Paraprobiotic is an inactivated probiotic cell that has many beneficial health effects on the human body. The use of paraprobiotics in products such as juices is preferable to the use of their living cells, which is due to the absence of noticeable negative effects of these inactive cells on the characteristics of drinks. The purpose of this research was to introduce paraprobiotics to the juice industry and to introduce products containing them as practical products. The results of the present study showed that the drink containing paraprobiotics was almost similar to pure grape juice from the point of view of acidity and pH, and temperature and storage time had a significant effect on two characteristics. The soluble solid matter of the paraprobiotic drink was more than that of pure grape juice, which indicates the release of substances inside the bacterial cell and the increase in biological access to bioactive and useful compounds inside the bacterial cell, and with the increase in shelf life, it had a positive effect on the increase of the solid matter of the paraprobiotic drink solution. Addition of the inactivated bacterial cell increased the turbidity due to the refraction of light by the bacterial particles. From the point of view of sensory characteristics, the main sensory characteristics of the drink were very close to pure grape juice and had a high overall acceptance, so that this characteristic did not decrease much with the increase in temperature and storage time, and the stored product had an acceptable sensory characteristic. However, research on the effect of choosing the type of juice as a carrier of paraprobiotics and the type of inactivation process and the type of bacterial strain can be a way to produce high quality products. The use of paraprobiotics in food, especially juices, is in the beginning, and there is a great need for research in the field of clinical effects and their effectiveness on health and increasing the quality of human life.

5- Resources

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بررسی خصوصیات فیزیکوشیمیایی و حسی آب انگور حاوی پاراپروبیوتیک *لاکتوباسیلوس گاسری* در زمان ماندگاری

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چکیده

پاراپروبیوتیک گروه جدیدی از ترکیبات عملگرا هستند که در اثر غیرفعالسازی برخی سویه‌های پروبیوتیک‌ها حاصل می‌شود. در پژوهش حاضر اثر استفاده از پاراپروبیوتیک لاکتوباسیلوس گاسری و زمان (۳۰ روز) و دمای (۴ و ۲۴ درجه سلسیوس) نگهداری بر خصوصیات فیزیکوشیمیایی و حسی نوشیدنی آب انگور بررسی شد. ویژگی‌هایی نظیر pH، اسیدیته، ماده جامد محلول کل، کدورت و ویژگی‌های حسی مورد بررسی قرار گرفت. افزودن پاراپروبیوتیک تغییر در pH و اسیدیته ایجاد کرد و تیمار شاهد (آب انگور خالص) و تیمار پاراپروبیوتیک (آب انگور حاوی پاراپروبیوتیک) از این نظر تفاوت معنادار داشتند، با این حال دما و زمان نگهداری اثری بر این خصوصیات نداشت. افزون پاراپروبیوتیک‌ها موجب افزایش کدورت و ماده جامد محلول در نوشیدنی پاراپروبیوتیک شد و با افزایش مدت زمان نگهداری این دو ویژگی نیز افزایش یافت. نوشیدنی پاراپروبیوتیک از نظر خصوصیات حسی بسیار مشابه با آب انگور خالص بود و دارای امتیاز پذیرش کلی مشابهی با آب انگور خالص بود که در طول زمان نگهداری در دماهای مختلف تغییر بسیار کمی کرد.