



## Scientific Research

## Production of beneficial synbiotic dairy drink based on milk, extract mushroom Free and encapsulated Ganoderma and Lactobacillus casei

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## ABSTRACT

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Ganoderma mushroom with scientific name *G. clear* It is considered one of the most important herbs in traditional medicine, and for many years It was known as a traditional and effective elixir in the treatment of various diseases. Today, consumers' desire for beneficial food products such as probiotic drinks is increasing, therefore, in this research, the production of a beneficial synbiotic dairy drink based on milk, Ganoderma extract and Lactobacillus casei free and capsule has been done. In the first phase of extraction, Ganoderma mushroom extract It was extracted by percolation method; Then, in the second phase, the encapsulation of probiotic bacteria was done. In the third phase, the ultra-beneficial dairy drink 6 levels (D1 control treatment (without Ganoderma mushroom extract and Lactobacillus casei capsule), D2 Treatment containing Ganoderma mushroom extract, D3 A drink containing free Lactobacillus casei bacteria, D4 A drink containing encapsulated Lactobacillus casei bacteria, D5 A drink containing Ganoderma mushroom extract and Lactobacillus casei Azad bacteria, D6 Drink containing Ganoderma mushroom extract and Lactobacillus casei bacterium (encapsulated) was produced. The parameters examined in this research include: Physicochemical, microbial and sensory characteristics were in the treatments. Statistical analysis using software SPSS V.25 At the probability level ( $0.05 \geq p$ ) was done. The results of this research showed that the average hydrolysis of milk protein increased until 40 hours and then decreased until 120 hours. The sensory evaluation of the aroma score showed a statistically significant difference in some of the drink treatments, so that the drink containing the extract mushroom Ganoderma and Lactobacillus casei capsule had the highest and the drink prepared from the extract alone had the lowest aroma score. review pH showed that lactic acid bacteria at the end of their logarithmic growth period pH reduced to 4.5, which is important from a technological point of view and preventing the growth of other unwanted microorganisms. Also, the results of total bacterial count, Escherichia coli and Staphylococcus aureus bacteria count were negative and no growth was observed. Therefore, it can be said that the ultra-beneficial dairy drink produced in this research is a product with an average shelf life that can In the best case, according to the viability of the bacteria during the period of 30 days, it is very suitable in the refrigerator and at the level of 6.7 logarithms. Offered to have the most health benefits.

## 1- Introduction

For a long time, the microbiological health of food has been one of the important concerns of consumers, producers and control organizations. Microorganisms contaminating food can cause spoilage, reduce the shelf life and lose the organoleptic properties of food, and can even lead to a person's illness [1]. In recent years, more attention has been paid to the third function of foods. The third function in foods is the role of food compounds in preventing diseases by modulating physiological systems. Antioxidant, anticancer, antimutation and antimicrobial activities are examples of the role of these compounds. Due to the increasing concern for a healthy diet, efforts have been made in many countries of the world to develop new foods with third functions. Foods with a third function, which are called super-beneficial foods, have been introduced as an important factor in keeping people healthy [2]. Enriched dairy products can contain bioactive compounds to increase acceptability and improve nutritional and medicinal properties [3]. Also, various compounds can be used to produce these products, including probiotics, prebiotics, synbiotics, unsaturated fatty acids, vegetable fibers, minerals, vitamins, antioxidants, phytochemical compounds, and recently pigments [4].

The use of essential oils and plant extracts are considered as very suitable agents for protecting food. Fewer side effects compared to synthetic drugs, no drug resistance, health and environmental health are among the advantages of using herbal drugs [5,6]. Using plant extracts to produce dairy products can reduce the risk of chronic diseases such as cancer, osteoporosis and heart diseases. Extracts such as glucosinolate, antioxidants, anthocyanins, lycopene and phenolic compounds such as flavonoids are used [7, 4].

mushroom *Ganoderma lucidum* Karst. belonging to the branch Basidiomycota, is [8]. This species has a bean-shaped basidiocarp with a base usually on the side. The upper surface of the basidiocarp has concentric circles and can be seen in orange-brown, red, purple, black-brown colors with a white or yellow to red-brown border [9].

Several reports have been published about the active biological substances found in this mushroom, it has many active substances, the

most important of which are triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins, peptides and many rare substances. He also mentioned [10]. Another substance found in this mushroom is melanin, melanin has antioxidant activity, strengthening the immune system, protecting against radiation and anti-mutation [10].

*Lactobacillus casei* is a gram-positive, non-sporing, facultative anaerobic bacterium, rod-shaped with round ends, single or paired in the form of short chains [11]. *Lactobacillus casei* is one of the fermenting bacteria and most of their products are lactic acid and also have probiotic properties. Therefore, *Lactobacillus casei* is one of the probiotic bacteria that has a special application potential in the production of probiotic milk products such as yogurt. *Lactobacilli* are the best known natural flora of the vagina and their ability to produce pH and maintain an acidic environment. The use of this bacterium in medicinal compounds regulates the natural balance of bacteria and fungi in the digestive system [12]. The activity of this bacterium is more than other *Lactobacillus* species found in fermented milk products and it is able to ferment a wide range of carbohydrates in the environment [13].

Milk, which is considered as a complete food, has many products that are consumed by the general public, and since synbiotics are a type of nutritional supplement containing a combination of probiotic bacteria and prebiotic food components, and prebiotics are indigestible or poorly digestible food components, against the digestive enzymes of the human body, which selectively stimulate the growth and activity of probiotic microorganisms. and improve the health of the host. Therefore, the beneficial synbiotic dairy drinks are of particular importance as one of the important milk products. And based on this, in this research, the investigation and production of a synbiotic super-beneficial dairy drink based on milk, *Ganoderma* extract and *Lactobacillus casei* free and capsule has been done.

## 2-Test materials and methods

In this research, cultivation environments MRS Agar, MRS Broth, and glycerol, DPPH, sodium alginate and other consumable laboratory materials were purchased from Merck, Germany, bacteria *Lactobacillus casei* with no

PTCC 1608 From the microbial collection of the Scientific and Industrial Research Organization of Iran, and low-fat sterilized milk was purchased from Kale Amol Company. This research was conducted in the first half and fall of 1402 in the laboratory unit of Islamic Azad University, Noor Branch.

### **1-2- Preparing Ganoderma mushroom and preparing the plant for extraction**

Ganoderma mushroom with scientific name *Ganoderma lucidum* It was purchased from Pozohan Amard Mehrzoist Commercial Research Company and after checking and removing inappropriate parts, it was prepared for extracting.

### **2-2- Extraction of Ganoderma mushroom by percolation method**

In this research, 80% ethanol and percolation method was used for extraction. 80% ethanol solvent has been suitable as a polar solvent in maximum extraction of Ganoderma active ingredients. In this way, 50 grams of the desired plant sample powder was poured into the decanter, and then 80% ethanol was added to it step by step. Continue adding ethanol until the entire volume of the plant inside the decanter is soaked and the ethanol is completely absorbed by the sample and some ethanol is on the surface of the sample inside the decanter. After 24 hours, the obtained extract was filtered with filter paper and with the help of a rotary machine, the ethanol solvent in the extract was removed.

### **2-3-Preparation of storage cultivation and production of beneficial dairy drink**

From bacteria grown in the environment MRS<sup>1</sup> To prepare a freezer sample, use bacteria in the freezer and in the presence of glycerol v/v 30% and nonfat dry milk W/V10% was maintained (Ah et al., 2000). To prepare the pre-culture sample, 1 ml of the frozen sample was added to 10 ml of sterile medium and placed in a greenhouse at a temperature of 37 degrees. After the growth reaches the end of the logarithmic phase based on the growth curve, kill in the round g 5000 for 10 minutes and the cells are centrifuged. The inoculum population was considered using the standard McFarland half sample and the inoculation ratio of 1

volume of bacterial suspension to 10 volumes of milk, and the concentration of Ganoderma extract was 400 µg/ml. The studied treatments are according to the list below, the inoculated milks were kept for 72 hours at a temperature of 37 degrees Celsius and with an interval of 24 hours, sampling was done and according to pH, proteolysis and antioxidant activity were investigated (Solimanzadeh et al., 2016).

### **2-4- Different treatments of the beneficial dairy drink**

Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1)

Dairy drink containing Ganoderma extract (D2)

Dairy drink containing free lactobacillus casei (D3)

Dairy drink containing Lactobacillus casei capsules (D4)

Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5)

Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)

### **1-5-2- Size<sup>2</sup>Measuring the amount of hydrolysis of milk proteins**

Intensity of proteolysis using ortho-phthalaldehyde photometric method OPA(O-phthalaldehyde) It was measured according to the method of Charach et al. For this purpose, fresh OPA solution containing 25 mL of sodium tetrahydroborate mM100, mL 5/2 solution SDS 20 % and mg40 OPAdissolved in 1 ml of methanol and 100 µl of mercaptoethanol with distilled water to a final volume of 50 mL and mL1 from that to µL 25 samples were added and after 2 minutes of storage at room temperature, the amount of light absorption in the wavelength nm340 It was measured. of L-leucine solution with concentration mg/ML4 – 1/0 It is used to draw a standard curve and determine the amount of free amines [14].

### **2-5-2- Antioxidant activity of milk**

Antioxidant activity of milk fermentation product by the researched bacterium through two radical inhibition mechanisms. ABTSand DPPH<sup>3</sup> It was checked [15].

### **3-5-2- Radical inhibitory activity DPPH**

To evaluate free radical inhibitory activity DPPH, µl 80, from serum samples of fermented milk or water, to µl 720 solution of 0.002%

1-M de Man, Rogosa, and Sharpe

2- 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

3 -2,2-diphenyl-1-picrylhydrazyl

(w/v) DPPH They were added in ethanol, mixed for 10 seconds and kept in the dark for 30 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm and the percentage of free radical inhibitory activity was calculated using the following formula.[15]:

$$100 \times \frac{\text{Absorption control} - \text{absorption sample}}{\text{Absorption control}} = \text{percentage of inhibitory activity}$$

From the Trolox standard curve  $\mu\text{M}$  0-250 to express the antioxidant activity in terms of  $\mu\text{MTE}/\text{mg}$  protein was used

#### 2-5-4- ABTS radical inhibitory activity

In this test, by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate, and after 12 to 16 hours of storage in the dark, the prepared solution was diluted using 5 mM phosphate buffer with  $\text{pH} = 7.4$  until the absorbance at 734 nm reached  $0.7 \pm 0.02$ . The tested sample was added to 1 ml of ABTS solution in the amount of 25 microliters and after 6 minutes, the absorbance of the sample was measured at the wavelength of 734 nm. The percentage of ABTS radical inhibitory activity was calculated using the following formula [15]:

$$100 \times \frac{\text{Absorption control} - \text{absorption sample}}{\text{Absorption control}} = \text{percentage of inhibitory activity}$$

The standard curve of Trolox  $\mu\text{M}$  0-4000 was used to express the antioxidant activity in terms of  $\mu\text{MTE}/\text{mg}$  protein.

#### 5-5-2- Amount of dissolved solids (Brix)

First, the device was calibrated to zero with distilled water. A few drops of the drink solution were placed on a refractometer (Cruz, Germany), spread evenly and then its concentration was read at  $20^\circ\text{C}$  and the result was expressed in Brix degrees (grams of dissolved solids per hundred grams of solution). [16].

#### 6-5-2- Measurement of dry matter

The amount of 2 grams of the sample was weighed in plates that had already reached a constant weight and heated in a Bain-Marie for 30 minutes. Then the plates were heated in an oven at 105 degrees Celsius for 2 hours. The samples were weighed after cooling in a desiccator [17].

#### 7-5-2- Measurement pH

In order to perform this test, using Iran's national standard method number 2852 PH meter was measured. Weigh 5 grams of syrup, pour it into Erlenmeyer flask and calibrate it pHmeters, pHThe samples were read [17].

#### 8-5-2- Total count of bacteria

In order to perform the testMicrobials 100 microliters of each of the dilutions prepared from the drink to the culture medium of Kant agar plate (PCA) prepared in advance with a temperature of about 42-44 degrees Celsius was added. Then the plates were placed in an incubator at  $30^\circ\text{C}$  for 24 to 48 hours [18].

#### 9-5-2- Counting Staphylococcus aureus bacteria

0.1 ml of the suspension prepared from the dilution prepared from the desired treatment was placed on the surface of Parker Agar culture medium.<sup>4</sup> In the form of surface cultivation<sup>5</sup> Cultivated, and spread by glass spreading rod. The plates were kept in a greenhouse at  $37^\circ\text{C}$  for 30 to 48 hours. Possible shiny black colonies with a thin white edge (transparent and colorless halo) around are Staphylococcus aureus bacteria. [19].

#### 10-5-2- Counting the number of Escherichia coli bacteria

To count Escherichia coli bacteria from the culture medium WILLOW<sup>6</sup> He benefitedIt was to So that 0.1 ml of the aqueous phase of the study suspension prepared with sterile peptone solution was added to the plate containing violet red bile agar culture medium. After mixing, they were kept in a greenhouse at 37 degrees Celsius for 24 hours [20].

#### 11-5-2- Sensory evaluation

15 non-professional evaluators and 5-point hedonic method (very good=5, good=4, average=3, bad=2, and very bad=1) were used to evaluate the sensory characteristics of the product. In this way, a quiet room with proper lighting and ventilation and several chairs were prepared for sensory evaluation, before each test, each evaluator was given the necessary instructions on how to perform the test and to consume drinks and lukewarm water after consuming each sample [21].

4-Baird parker Agar

5- Surface plate count method

6-Violet Red Bile Agar

### 12-5-2- Statistical analysis

In this research, the production of a beneficial synbiotic dairy drink based on milk, Ganoderma extract and Lactobacillus casei free and capsule using a completely random design, using the method of repeated measurements at the level of probability ( $p < 0.05$ ) has been done. The treatments were examined in three replications and the results were obtained using one-way analysis of variance (One-Way – ANOVA (and univariate analysis of variance) GLM Univariate) at the probability level ( $p < 0.05$ ) has been examined and the averages have been compared using Duncan's multi-range test method at the probability level ( $p < 0.05$ ) has been subjected to statistical analysis. Statistical analyzes using software SPSS V.25 Done and graphs using the software EXCEL has been drawn.

## 3-Results and discussion

### 1-3- Measurement of hydrolysis of milk proteins

One of the common methods to check the amount of production of bioactive peptides is the fermentation process, and evaluating the degree of hydrolysis based on the amount of free amine groups is an important factor for checking the peptides produced during the fermentation process. The results of the variance analysis of the data obtained from the amount of protein hydrolysis of fermented milk in the beneficial dairy drink containing the treatments of Ganoderma extract, Lactobacillus casei azad and capsules compared to the control sample during 120 hours of fermentation in graph (1) show that with the passage of time up to the fortieth hour, the amount of protein hydrolysis in the treatments increased and then decreased until the 120th hour. Therefore, in the 40th hour of the treatment containing Ganoderma extract and Lactobacillus casei capsules with an average of 4.06 and the control sample with an average of 1.01, they had the highest and lowest values, respectively. Each treatment had the highest and lowest amount of protein hydrolysis at 40 and 0 hours, respectively.

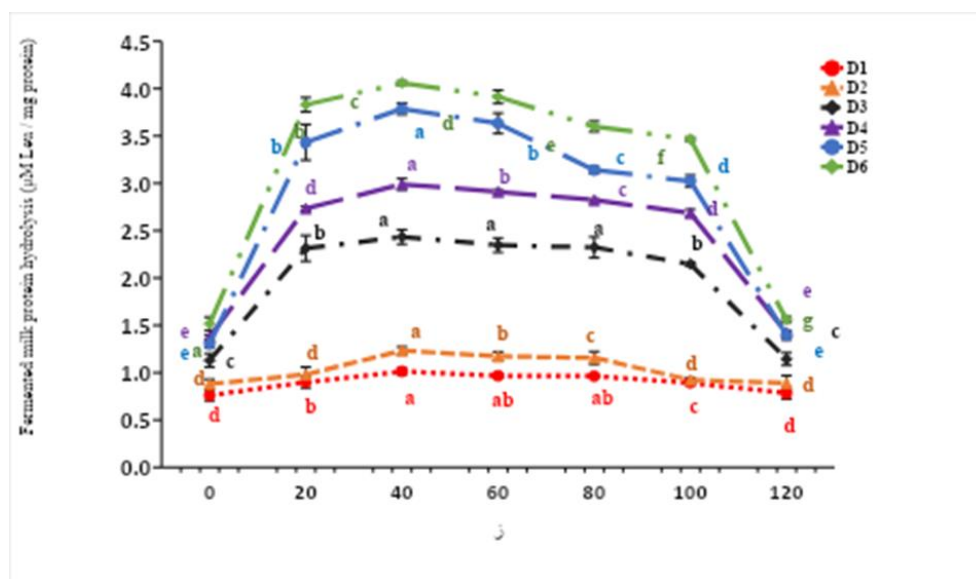


Chart 1- Comparison of the average hydrolysis of milk proteins in the investigated treatments during 120 hours of fermentation (Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

In this regard, Perez-Scalante et al. (2018) in a study evaluating the antithrombotic activity of milk protein hydrolysates by lactic acid bacteria isolated from commercial fermented milks, reported that after 30 hours of fermentation, the amount of free amine groups increased

significantly. They stated the reason for this is the bacteria's need to obtain nitrogen from oligopeptides and amino acids from the main pathway of protein hydrolysis. In addition, Lactobacillus casei showed higher proteolytic activity at 1 and 8 hours compared to

*Lactobacillus genoni*. This increase can be related to the activation of dipeptidases and tripeptidases. It has also been found that *Lactobacillus casei* species have a higher capacity to hydrolyze small peptides and produce free amine groups.

### 2-3- Antioxidant activity based on free radical inhibition DPPH and ABTS

Radical scavenging activity results DPPH Compared to the control sample during 120 hours of fermentation, graph (2) shows that in the control sample, the treatment containing the extract and the treatment containing

*Lactobacillus casei* Azad, up to the twentieth hour, and in the other treatments up to the fortieth hour, radical inhibitory activity DPPH It increased, and it continued to decrease from 40 to 120 hours. During the treatment period, *Lactobacillus casei* capsule and control sample had the highest and lowest values, respectively. in radical scavenging activity ABTS (Chart 3) In most of the treatments up to the twentieth hour, and only in the treatment containing the extract and *Lactobacillus casei* capsules at the fortieth hour, radical inhibitory activity ABTS has increased, and continued to decrease until 120 hours.

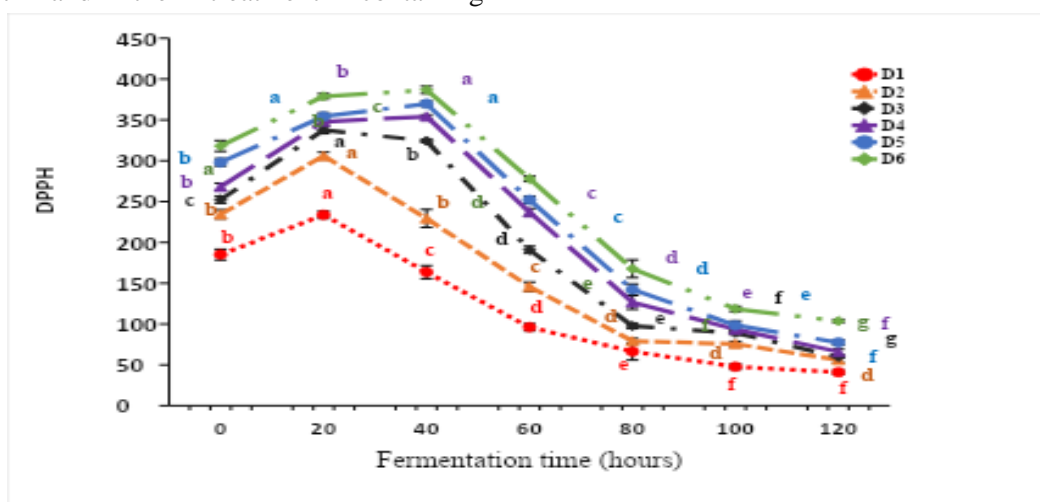


Chart 2 Comparison of the average DPPH radical inhibitory activity in different treatments of the investigated ultra-beneficial drinks (in a period of 120 hours) (Dairy drink without adding Ganoderma and *Lactobacillus casei* extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free *Lactobacillus casei* (D3), Dairy drink containing *Lactobacillus casei* capsules (D4), Dairy drink containing Ganoderma extract and *Lactobacillus casei* Azad (D5), Dairy drink containing Ganoderma extract and *Lactobacillus casei* capsule (D6)).

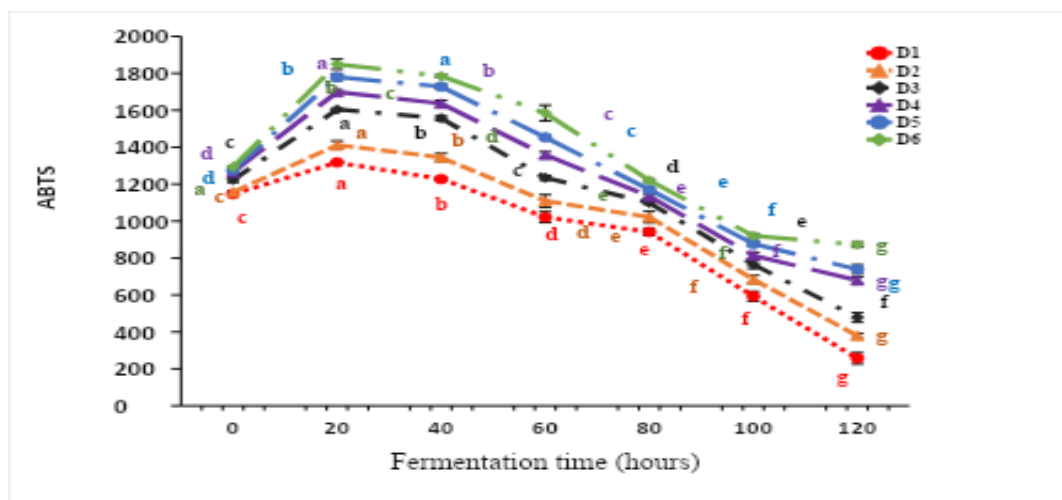


Chart 3- Comparison of the average ABTS radical inhibitory activity in different treatments of the studied ultra-beneficial drinks (in a period of 120 hours) (Dairy drink without adding Ganoderma and *Lactobacillus casei*



extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

In this regard, Suleimanzadeh et al. (2016) evaluated the antioxidant activity of camel and cow milk fermented by lactic acid bacteria isolated from traditional fermented camel milk and reported an increase in the antioxidant activity of cow milk fermented by lactic acid bacteria strains during 24 hours of fermentation time. They also in 2019 in a research entitled antioxidants derived from b- Casein and inhibitory active peptide ACE which is fermented by camel milk *Leuconostoc lactis* PTCC1899. They reported that the antioxidant activity observed during 24 hours of fermentation, at the same time as the amount of free amine groups increases, indicates the relationship between the proteolytic activity of bacteria and their ability to produce antioxidant peptides. Although a set of metabolites and antioxidant peptides during fermentation can increase the antioxidant activity, but in many studies this activity has been related to the presence of antioxidant peptides [22].

Virtanen et al. (2007) in a research titled development of antioxidant activity in milk whey during fermentation with lactic acid bacteria, increase in activity antioxidant during fermentation of milk serum by lactic acid bacteria strains and direct relationship between the progress of proteolysis by *Lactobacillus lactis*, *Leuconostoc. Cremoris* and *Lactobacillus gensenii* and reported radical scavenging activity. Therefore, it can be said

that the decrease or no change observed after 40 hours in both antioxidant mechanisms DPPH and ABTS. Despite the increased proteolysis during this stage, it indicates that probably due to the proteolysis activity of bacteria, peptides with antioxidant activity have changed their structure or hydrolyzed more and therefore their activity has decreased. In this regard, Jafari et al. (2017) investigated the antioxidant activity of raw milk and common dairy products in Fars province.

### 3-3- Amount of dissolved solids (Brix)

The results of the statistical analysis of the average Brix percentage in the ultra-beneficial dairy drink containing treatments of Ganoderma extract, Lactobacillus casei Azad and capsules compared to the control sample during 30 days of storage at 4 degrees Celsius in the refrigerator in graph (4) show that the control sample had a lower Brix percentage than other treatments. At the end of the storage period, the treatment containing Ganoderma extract with an average of 13.13 and the treatment containing free lactobacillus casei with an average of 8.39 had the highest and lowest Brix percentages, respectively. By increasing the storage time, Brix has decreased in the drink samples

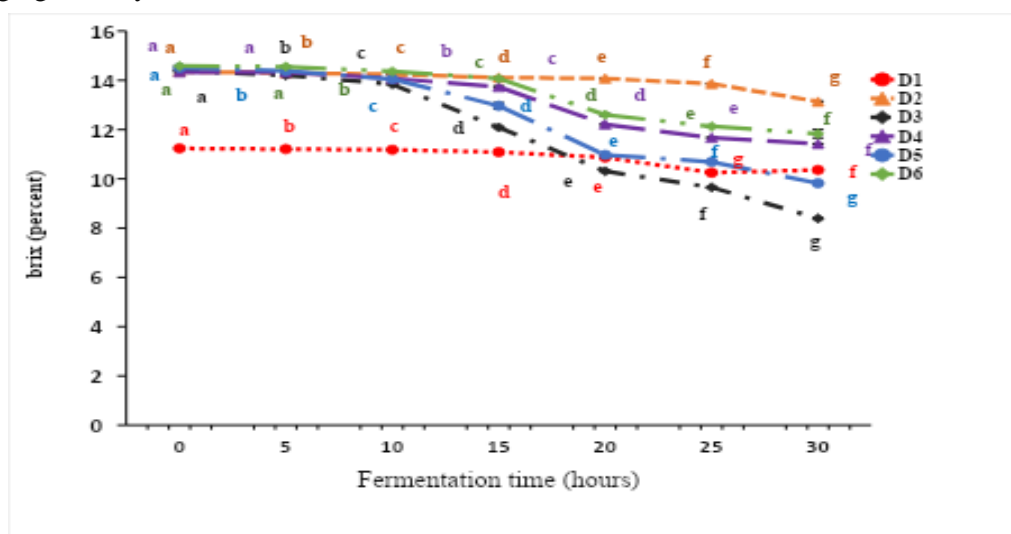


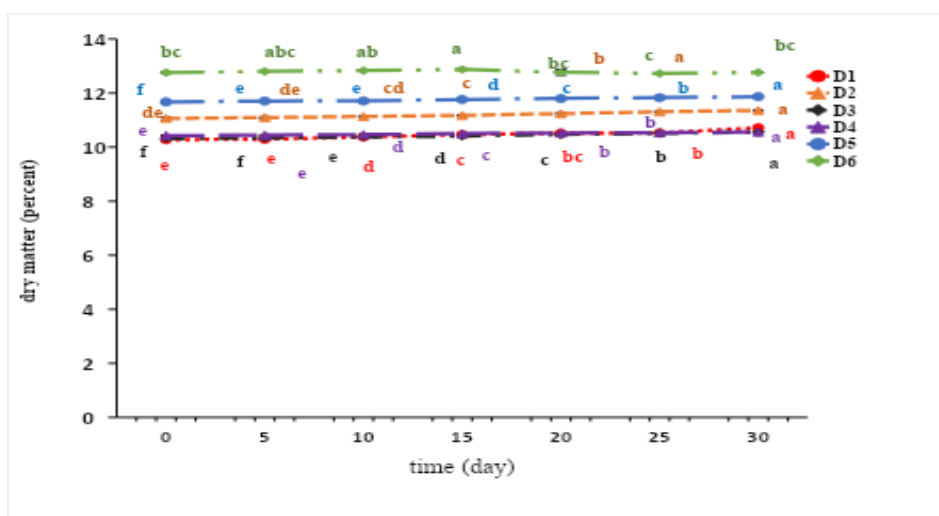
Chart 4- Comparison of the average Brix percentage in different treatments of the investigated ultra-beneficial drinks (in a period of 30 hours). (Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1),

Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

The results of the present study are consistent with the results of other researchers who investigated the brix of probiotic fermented drink based on a mixture of pineapple, apple and mango juice and stated that the brix values of the samples decreased due to the consumption of sugars and the production of organic acids [23].

#### 4-3-Measurement of dry matter

The statistical analysis of the average percentage of dry matter in the ultra-beneficial dairy drink containing treatments of Ganoderma extract, free Lactobacillus casei and capsule compared to the control sample during 30 days of storage at 4 degrees Celsius in the refrigerator in graph (5) shows that the treatment containing extract and Lactobacillus casei capsule had the highest percentage of dry matter during the period, which was the highest on day 15 with an average of 12.87% has been



**Chart 5- Comparison of the average percentage of dry matter in different treatments of the investigated super-beneficial drink (within 30 days)** (Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

#### 5-3- Measurement pH

pH It is part of the index parameters for fermentation by microbial culture. The ability of lactic acid bacteria to produce acid during the milk fermentation process, with measurements pH was investigated. Therefore, the results of the analysis of variance of the data obtained from the average pH in the beneficial dairy drink containing the treatments of Ganoderma

extract, Lactobacillus casei free and capsules compared to the control sample during 30 days of storage at 4 degrees Celsius in the refrigerator, graph (6) showed that with the passage of time, the amount pH decreases, that this decrease was much higher in the treatments containing Lactobacillus casei bacteria, which this amount pH in treatments containing free Lactobacillus casei, it was less than encapsulated Lactobacillus casei.



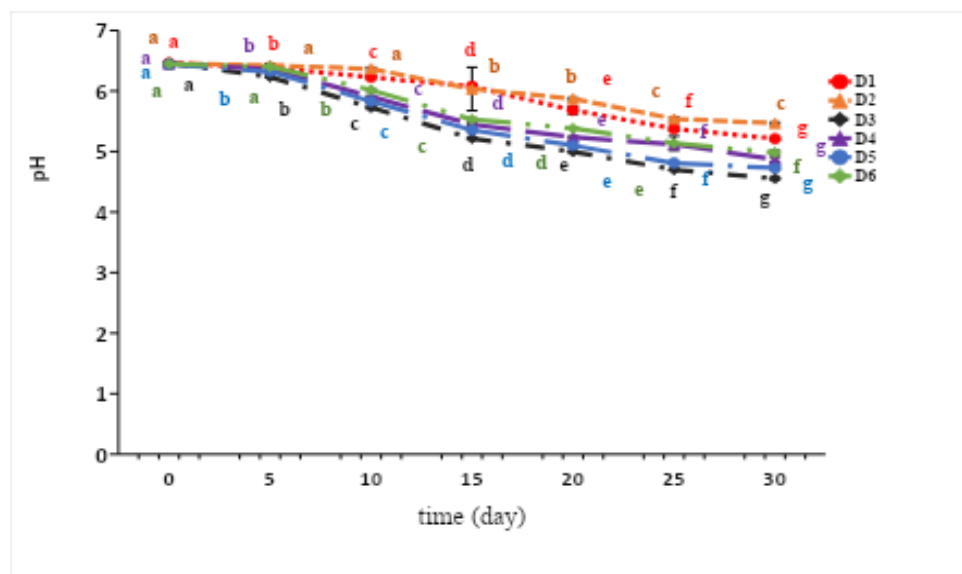


Chart 6-Comparison of the average pH level in different treatments of the examined ultra-beneficial drinks (in 30 days). (Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

In this regard, Bagheri et al. (2018) in a research titled production of beneficial fermented milk by lactobacilli isolated from traditional Iranian dairy products, reduced pH reported values from 6.46 to 3.72 in fermented milk after 12 hours of fermentation by *Lactobacillus fermentum* and *Lactobacillus heloticus* strains. The results of their research showed that the difference in values pH measured after 24 hours for bacterial strains may be due to the difference in growth rate and different metabolic pathway for each bacterium. So that *Lactobacillus heloticus* has a homogeneous lactic fermentation pathway and has delayed growth due to higher growth needs and higher acid production power, while *Lactobacillus fermentum* has a heterogeneous fermentation pathway, a gentler speed of acid production and faster growth, which can also be true for *Lactobacillus casei* bacteria.

### 3-6- Total count of bacteria

The results of total bacterial count, *Escherichia coli* and *Staphylococcus aureus* bacteria count were negative and no growth was observed.

### 3-7- Sensory evaluation

The results of the analysis of variance of the data obtained from the sensory evaluation of the aroma score in some of the drink treatments had statistically significant differences, so that the drink containing Ganoderma extract and Lactobacillus casei capsules had the highest and the drink prepared from the extract alone had the lowest aroma score, while the sensory evaluation indicators of taste, color, texture and overall acceptance in the different treatments of the multipurpose dairy drink prepared had no significant statistical differences with each other.



chart 7- The results of sensory evaluation in different treatments of ultra-beneficial dairy drink (Dairy drink without adding Ganoderma and Lactobacillus casei extract (D1), Dairy drink containing Ganoderma extract (D2), Dairy drink containing free lactobacillus casei (D3), Dairy drink containing Lactobacillus casei capsules (D4), Dairy drink containing Ganoderma extract and Lactobacillus casei Azad (D5), Dairy drink containing Ganoderma extract and Lactobacillus casei capsule (D6)).

### 8-3- The final conclusion

The durability of probiotics in food systems depends on many factors such as the selected species, the reaction between the microbial species in the environment and the final acidity of the product. The aim of this project was to produce and determine the characteristics of a synbiotic super-beneficial dairy drink based on milk, Ganoderma extract and Lactobacillus casei free and capsule. In terms of health-promoting properties, such as increased antioxidant activity, the fermentation process led to a significant improvement in these properties, the results showed that the average hydrolysis of milk protein increased until 40 hours and then decreased until 120 hours. The sensory evaluation of the aroma score in some of the drink treatments showed a statistically significant difference, so that the drink containing Ganoderma extract and Lactobacillus casei capsules had the highest and the drink prepared from the extract alone had the lowest aroma score. pH showed that lactic acid bacteria at the end of their logarithmic growth period pH reduced to 4.5, which is important from a technological point of view and preventing the growth of other

unwanted microorganisms. Also, the results of total bacterial count, *Escherichia coli* and *Staphylococcus aureus* bacteria count were negative and no growth was observed. The results obtained from the survival of the bacteria during 30 days of shelf life in the refrigerator were very good and at the level of 6.7 logarithms in the treatment containing Lactobacillus casei capsule extract.. Therefore, it can be said that the ultra-beneficial dairy drink produced in this research should be offered as a product with an average shelf life of 30 days in order to have the most health benefits.

### 4-Resources

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### مقاله علمی-پژوهشی

تولید نوشیدنی لبنی فراسودمند سین بیوتیک بر پایه شیر، عصاره قارچ گانودرما و لاکتوباسیلوس کازئی آزاد و کپسوله

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### چکیده

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قارچ گانودرما با نام علمی *G.lucidum* جزء یکی از مهمترین رستنی ها در طب سنتی محسوب می شود، و در طی سالیان متمادی به عنوان اکسیری سنتی و مؤثر در درمان انواع بیماری ها شناخته شده بود. امروزه تمایل مصرف کنندگان به محصولات غذایی فراسودمند مانند انواع نوشیدنی های پروبیوتیک رو به افزایش است، لذا در این پژوهش به تولید نوشیدنی لبنی فراسودمند سین بیوتیک بر پایه شیر، عصاره گانودرما و لاکتوباسیلوس کازئی آزاد و کپسوله پرداخته شده است. در فاز اول عصاره گیری، عصاره قارچ گانودرما به روش پرکولاسیون استخراج شد؛ سپس در فاز دوم کپسولاسیون باکتری های پروبیوتیک انجام گرفت. در فاز سوم نوشیدنی لبنی فراسودمند در ۶ سطح (D1 تیمار شاهد (فاقد عصاره قارچ گانودرما و کپسول لاکتوباسیلوس کازئی)، D2 تیمار حاوی عصاره قارچ گانودرما، D3 نوشیدنی حاوی باکتری لاکتوباسیلوس کازئی آزاد، D4 نوشیدنی حاوی باکتری لاکتوباسیلوس کازئی کپسوله شده، D5 نوشیدنی حاوی عصاره قارچ گانودرما و باکتری لاکتوباسیلوس کازئی آزاد، D6 نوشیدنی حاوی عصاره قارچ گانودرما و باکتری لاکتوباسیلوس کازئی کپسوله شده) تولید گردید. پارامترهای مورد بررسی در این تحقیق شامل: ویژگی های فیزیکی و شیمیایی، میکروبی و حسی در تیمارها بود. آنالیز آماری با استفاده از نرم افزار SPSS V.25 در سطح احتمال (  $p \leq 0.05$  ) انجام گرفت. نتایج این پژوهش نشان داد که میانگین هیدرولیز پروتئین شیر تا ساعت ۴۰ روند افزایشی و در ادامه تا ساعت ۱۲۰ روند کاهشی داشته است. ارزیابی حسی امتیاز عطر در برخی از تیمارهای نوشیدنی اختلاف آماری معنی داری نشان داد، به طوری که نوشیدنی حاوی عصاره قارچ گانودرما و لاکتوباسیل کازئی کپسوله از بیشترین و نوشیدنی تهیه شده از عصاره تنها، از کمترین امتیاز عطر برخوردار بوده اند. بررسی pH نشان داد که باکتری لاکتیکی در پایان دوره رشد لگاریتمی خود pH را به ۴/۵ کاهش داده که از نظر تکنولوژیکی و ممانعت از رشد سایر میکروارگانیسم های ناخواسته مورد توجه است. همچنین نتایج حاصل از شمارش کلی باکتری ها، و شمارش باکتری های اشریشیاکلی و استافیلوکوکوس اورئوس منفی بوده است و رشدی مشاهده نشده است. لذا می توان گفت نوشیدنی لبنی فراسودمند تولید شده در این پژوهش به عنوان محصولی با مدت زمان ماندگاری متوسط که می تواند در بهترین حالت با توجه به زنده ماندن باکتری در طی مدت زمان ۳۰ روز ماندگاری در یخچال بسیار مناسب و در حد ۶/۷ لگاریتم عرضه شود تا بیشترین فواید سلامتی بخش را داشته باشد.