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Investigation the biopreservative, physicochemical and sensory properties of Masineh drink

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ABSTRACT

During the current research, a traditional nomadic Iranian dairy product named Masineh was produced, which is prepared from heated yogurt drink and herbal additives including turmeric, dill seed powder and cumin. Some physicochemical and microbial characteristics of Masineh samples were investigated during 21 days of storage at 4°C. In addition, the antioxidant properties, antimicrobial effects and organoleptic properties of the product were investigated and compared with the control sample (heated yogurt drink without additives). The results showed that herbal additives significantly reduced the counts of bacteria, mold and yeast in Masineh compared to the control sample ($P<0.05$). In Masineh, the highest inhibitory effect was observed against *Staphylococcus aureus* with 30.91 mm, and the lowest inhibitory effect was observed against *Escherichia coli* with an average of 15.50 mm, followed by *Aspergillus niger* with an average of 18.12 mm. In addition, the phenolic compounds and DPPH and ABTS inhibition percentages were higher in Masineh than control sample. The pH and viscosity of Masineh were higher than that of drinks without additives, and during storage, these values decreased significantly ($P<0.05$). Also, the results of the sensory evaluation showed that the sensory evaluators considered the taste of the masineh to be favorable and did not report a significant difference with the control sample in terms of overall acceptance, although the color of the masineh had a lower score. Therefore, according to the obtained results, Masineh is introduced as a beneficial dairy drink, in which by the use of herbal additives, its antioxidant properties and microbiological properties are strengthed.

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1. Introduction

Nowadays, due to the harms such as the increased possibility of heart and carcinogenic risks and the occurrence of stomach problems that synthetic food additives have for the body, attention to antimicrobial compounds of plant origin is increasing. Medicinal and edible plants due to having antimicrobial compounds, in order to prevent the growth of pathogenic pathogens and delay the growth of spoilage agents in food and also as food additives, have been noticed by the food and packaging industry [1 and 2].Also, since plant extracts have a valuable role in improving the organoleptic characteristics of food, especially dairy products, their use in the processing of these products is very beneficial [3 and 4].

Buttermilk is a product whose production process generally includes milk fermentation with lactic bacteria and then dilution with water. In general, it has been proven that acid milk is more digestible than regular milk [5]. Because large protein molecules are converted into smaller molecules due to acidification. Also, in fermented products such as buttermilk, the majority of lactose in buttermilk is less than milk, and for this reason, buttermilk, like yogurt, is suitable for people who have problems with lactose digestion. In addition to nutritional benefits, buttermilk also contains beneficial bacteria Streptococcus thermophilus and Lactobacillus bulgaricus, which have many effects on the health of the human digestive system [6]. Nowadays, due to the increase in people's awareness of the harms of consuming carbonated soft drinks, the desire to consume natural soft drinks, including buttermilk, is increasing. Masineh drink is one of the traditional nomadic dairy products of Iran. This product is prepared from heated buttermilk, then dry dill, turmeric and other vegetables are added to it and mixed well.

Turmeric from the ginger family with a scientific name*Turmeric is long* And with an English name*Turmeric* Is known. It is a type of spice plant that has anti-inflammatory, antibacterial, anti-nematode and antioxidant properties [7]. dill (*Anethum graveolensl*) is an annual herbaceous and aromatic plant. The leaves of this plant have anti-mutation and cancer properties. This plant lowers blood lipids, stimulates the restoration of liver cells and is an appetite suppressant. The flavonoid in dill has antibacterial properties and cures diarrhea by eliminating microbial infections [8]. Cumin with scientific name*cumin cumin* A medicinal plant of the familyApiaceae Is. This plant has an anti-spasmodic and anti-flatulent effect. In addition, its antimicrobial property has appeared on gram-positive microorganisms and is also effective on microbial infections of the digestive system [9].

Due to the beneficial benefits of natural preservatives, many studies have been done regarding the effect of herbal additives on the physicochemical properties and shelf life of buttermilk.Najafian (2015), during their review stated that dill essential oil against yeast*Kluyuromyces Marcianos*It is effective as one of the polluting and spoilage factors in Iranian buttermilk, and with increasing the storage temperature, the effect of dill essential oil in reducing the number of yeasts was greater, which is probably due to the higher mobility of phenolic compounds and terpenes as antimicrobial agents of essential oils [10]. .Shariatet al. (2020), investigated the effects of coriander extract and watercress seed gum on the physicochemical properties of drinking yogurt (buttermilk). They reported that buttermilk containing 0.05% extract and 0.5% gum had the highest sensory score and the lowest lipid oxidation compared to other samples and the control sample. They suggested the use of coriander extract to enhance the health benefits of dairy products and produce superfoods [11].Representative AndPedram Nia (2018), investigated the effect of plant extracts and essential oils on the characteristics of beneficial dairy ingredients and concluded that adding extracts such as rosemary, chicory, olive leaf, sugar beet, spinach, barberry, burdock and linseed oil to dairy ingredients It leads to the production of dairy products with beneficial properties and affects factors such as the amount of phenolic compounds, the amount of vitamins, product protein, the viability of probiotic bacteria during the storage period and antioxidant properties [12].

Considering that not much research has been done on Masina and since this product has a high nutritional value, it will be beneficial to

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conduct research on this drink. Therefore, the aim of this research is to investigate the physicochemical, microbiological and sensory quality of masina during 21 days of storage at 4°C.

2- Materials and methods

2-1- sample preparation

First, milk (3.2% fat) was pasteurized at 90 degrees Celsius for 5 minutes. Then the temperature was reduced to 42-45 degrees Celsius and starter culture (2%) was inoculated. After that, the process of keeping in a greenhouse until the formation of a suitable clot and reaching the desired pH (maximum 4.6) was performed. Then, according to Table 1, the produced yogurt was mixed with water and heated again at a temperature of 45-50 degrees Celsius for 30 minutes. After heating, additives including salt, turmeric, egg wash powder and cumin powder were added to the product under sterile conditions and packed in sterile bottles. The prepared product was kept at a temperature of 4 degrees Celsius and compared with the control sample (heated buttermilk without additives). It should be noted that the physicochemical and microbial characteristics of the Masina sample were performed during 21 days and at time intervals of 1, 7, 14 and 21 days. In addition, other tests including antioxidant properties, antimicrobial effects and organoleptic properties were conducted only on the first day of production due to possible spoilage and negative effects on the mentioned factors.

Table 1 Items needed to produce 1 liter of Masineh

Materials	Measure
Yogurt	700 cc
Water	300 cc
Salt	10 gr
Cumin	10 gr
Dill seed	10 gr
Turmeric	10 gr

2-2-Physicochemical tests

2-2-1- pH measurement

The pH of Masina samples was measured at 20 degrees Celsius by a Toledo-Mettler (Switzerland) pH meter according to the national standard No. 2852 during the storage period [13]. First, the device was calibrated and the pH electrode was washed and dried with distilled water according to the instructions of the manufacturer, then the electrode was placed inside the sample and was in contact with the sample for 45 seconds. In the next step, the pH was activated and the pH of the sample was read [14].

2-2-2- Measurement of acidity

The acidity of Masina samples was measured according to the Iranian national standard No. 2852 [14]. 10 milliliters of the sample was poured into a beaker and 0.5 milliliters of phenolphthalein reagent was added to it and titrated with 0.1 normal sodium hydroxide. The titration continued until the appearance of a pale pink color (at least for 5 seconds). The acidity of the samples was calculated in terms of lactic acid and according to equation (1):

relationship (1)

$$A = \frac{V \times 0/009 \times 100}{m}$$

A = total acidity in terms of lactic acid, in grams per 100 grams, V = 0.1 normal milliliters of sodium, m = sample weight in grams. It should be noted that one milliliter of 0.1 normal soda is equivalent to 0.009 grams of lactic acid [14].

2-2-3- Apparent viscosity

Rheological characteristics of the samples using Brookfield viscometer (model*DVII-RV* Made in USA) was checked during maintenance [13]. So that the machine samples were poured into 600 ml cylinders at a room temperature of 25 degrees Celsius and the viscosity of the samples was measured based on the centipoise unit using spindle number 4 at a speed of 6 revolutions per minute [15].

2-3- Antioxidant compounds

2-3-1-Measurement of total phenol

Total phenolic content was measured using Folin Ciocaltio reagent. To 0.5 ml of Masina sample, 2.5 ml of Folin Ciocalto reagent 0.2 normal was added, after 5 minutes 2 ml of 75 g/l sodium carbonate solution was added to it. And after 2 hours, the absorbance of the mixture at a wavelength of 517 nm was read by a

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spectrophotometer in front of the blank, gallic acid was used as a standard to draw a calibration curve, and the amount of total phenolic was based on the amount equivalent to milligrams of gallic acid in 100 milliliters. reported [16, 17].

2-3-2- Measurement of radical inhibitory power

First, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals were produced by dissolving 1 mg of DPPH powder in 17 ml of ethanol. The mixture was uniformly shaken for one minute and placed in a dark environment for 30 minutes at room temperature. The absorbance of the mixture was checked at 517 nm in a spectrophotometer (UV-VIS 1280 Shimadzu, Iran) and 0.2 ml of ethanol was used in the control sample instead of the sample. The DPPH free radical decolorization percentage of the samples was calculated according to equation (2) [16].

$\{1- (A_S-A_0)/A_C\}$ x 100 = radical scavenging activity (%)

relationship (2)

A_S Sample absorption, A_C Shahid attraction, A_0 Absorb a mixture of 5.5 ml of ethanol and 500 µl of the sample.

2-3-3- ABTS radical inhibition power

In this method, ABTS (2,2-azinobis 3-ethyl-benzothiazoline-6-sulfonic acid) solution with a concentration of 7 mM was prepared, potassium persulfate was added to this solution, the resulting solution was left overnight in the dark at room temperature. given. 20 microliters of Masina sample was mixed with 1480 microliters of ABTS radical and absorbance was read at 734 nm wavelength [16].

2-4- Microbial characteristics

The microbial characteristics of the produced buttermilk were evaluated during 3 weeks and compared with the control sample (heated buttermilk without additives). In order to count the total number of microorganisms in the machine and the control sample, first the desired samples were prepared with different dilutions. Then using the mixed culture method[1] Two layers in the culture medium[2]PCA and placing the samples in a greenhouse at 30 degrees Celsius for 24 hours, the number of colonies created was counted [18].

Total count of forms using VRBLA culture medium[3] Done. After mixed culture of two layers of samples, the desired plates were placed in a greenhouse at 30 degrees Celsius for 24 hours under aerobic conditions. After the end of the incubation time, the red cells in each plate were counted [19]. Enumeration of mold and yeast using YGC culture medium[4] Done. For this purpose, the cultured plates were kept in a greenhouse at 25 degrees Celsius for 5 days and after this period, the colonies in each plate were counted [20].

5-2- Antimicrobial property

To analyze the antimicrobial activity of the machine sample prepared on bacteria (*Staphylococcus aureus* ،*Bacillus cereus* ، *Escherichia coli* And*Pseudomonas aeruginosa*), molds (*Aspergillus niger*And*Penicillium digitatum*) and yeast (*candidate Albicans*) well diffusion method was used. Gram-positive and gram-negative bacteria mentioned above, as well as the investigated fungi, are among the most important microorganisms that cause food poisoning and spoilage. Standard strains of bacteria*Bacillus cereus* (PTCC:1984) ، *Pseudomonas aeroginosa* (PTCC:1310) ، *Staphylococcus aureus* (PTCC:1917) and*Escherichia coli* (PTCC:1222) and mushrooms (PTCC:5320)*Aspergillus niger*،(ATCC: 201167) *Fingered pencil* And*Candida albicans* (PTCC:5027) was prepared in lyophilized form from the microbial collection of the Industrial Research Institute of Iran. Microbial samples were activated according to the recommended method. Well diffusion method was used to analyze the antimicrobial activity of plants in Masina. In order to prepare a microbial suspension from a fresh and young culture of multi-clonal bacteria to the nutrient broth culture medium.[5] They were transferred and placed in an incubator with a temperature of 37 degrees Celsius for growth. After incubation, bacterial suspension equivalent to half McFarland standard was prepared in sterile physiological serum solution and after dilution, the amount of CFU/mL was 10^6 It was prepared from bacterial suspension. 100

1 .For flat

2. Plate Count Agar

3. Violet Red Bile Lactose Agar

4. Yeast extract glucose chloramphenicol agar

5. Nutrient Broth

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microliters of bacterial suspension prepared on the surface of nutrient agar plates.[6] It was transferred and cultured with a sterile swap. After creating the wells, 60 microliters of the solution containing the filter was transferred to the wells in a completely sterile manner, and the plates were kept in a greenhouse at a temperature of 37 degrees Celsius for 24 hours. After this period of time, the bacterial kills in terms of the formation or non-formation of the no-growth halo were checked and the diameter of the no-growth halo in millimeters was measured by a caliper [21 and 22].

Regarding the investigation of the antimicrobial activity of the studied yeast, the same preparation method as the bacteria was carried out. After diluting and preparing the desired suspension, culture on Sabro dextrose agar culture medium[7] done. To prepare the suspension of the studied molds, the desired mold on the MEA solid culture medium[8] which is an environment for mushroom spore production, was cultured in a slant in a tube. Then it was kept at 30 °C for 4 to 7 days. After finishing the incubation, a suspension of the fungus was prepared with sterile distilled water and vortexed for 5 minutes until the spores were separated. To count the spores, 0.1 ml of the suspension was taken and a hemocytometer slide was used to count mold spores. Next, on Sabro dextrose agar culture medium, 100 microliters of the desired mold spore suspension at the rate of 10 spore/mL^{7} 1/5 poured and completely spread. After creating the wells, 60 microliters of the solution containing the filter was transferred to the wells in a completely sterile manner, and the plates were kept in a greenhouse at 30 degrees Celsius under aerobic conditions for 3-7 days (when the mycelium of the fungi appeared). After this time, the size of the inhibitory halo was measured using a caliper and expressed in millimeters [23].

6-2- Sensory evaluation

Machine and control samples were evaluated on the first day. After the preliminary training, 12 people (male and female) were selected as evaluators using the 5-point hedonic method, in the order of unacceptable, relatively satisfactory, good, very good, and excellent, and the attributes of taste, color, smell, and acceptability were evaluated. All were reviewed by the judges. Then the qualitative (non-parametric) data was converted into quantitative (parametric) data, in such a way that the terms unacceptable to excellent were given a score of 1 to 5 respectively. In this test, each evaluator was given a coded sample along with a glass of water and a scoring form. The evaluators evaluated all the samples randomly [24].

2-7-Statistical analysis

In this research, data analysis was done using spss and excel software. All experiments were done based on a completely randomized block design and in three replications. To check the test results, use Duncan's non-parametric test[9] used. All tests in*significance level 95%*(05/0>*P*) *Was considered.*

3. Results and Discussion

3-1- pH and acidity of Masina samples

Tables 2 and 3 show the changes in pH and acidity of Masina and control samples during 21 days of storage in the refrigerator.

6. Nutrient Agar
7. Sabouraud Dextrose Agar: SDA
8. Malt Extract Agar
9. Duncan Test

Table 2 pH values of Masineh samples during the storage of 21 days storage at refrigerator

Samples	Day 1	Day 7	Day 14	Day 21
Machine	4.17 ± 0.007^{Aa}	4.01 ± 0.000^{Not}	$3.77\ \ 0.014^{That}$	3.54 ± 0.007^{And}
Control	3.87 ± 0.035^{Ab}	3.74 ± 0.021^{Bb}	3.58 ± 0.021^{Cb}	3.30 ± 0.000^{Db}

The results are reported as mean ± standard deviation. Different uppercase letters indicate a significant difference between the values of each row at $P < 0.05$. Different lower-case letters indicate a significant difference between the values of each column at $P < 0.05$. Control sample: heated yogurt drink without additives

pH and total acidity are important quality parameters whose changes should be monitored during the storage period. Changes in the pH of macine significantly affect the structural features and chemical composition of macine, and its decrease causes an increase in the solubility of minerals and changes in the structure of casein micelles. Both phenomena affect the nature and intensity of protein interactions. The results of Table 2 showed that the average pH of Masina had a decreasing trend over time, and this decrease was observed in both treatment and control groups (P<0.05). The findings showed that the average pH of the treated sample was (4.17) on day 1 and it had a decreasing trend in the following days and reached 3.54 on day 21. This decreasing pattern was also observed in the control sample. In addition, the results showed that the average pH of Masina sample was higher than the control sample (P<0.05). A significant difference between the treatments was observed in the acidity examination (P<0.05). The results of Table 3 showed that the average acidity of masina had an increasing trend over time, and this increase was observed in both treatment and control groups (P<0.05), the highest amount on day 21 in the control sample (51.0 ± 0.014). 1) and the lowest amount was shown on day 1 in Masina sample (1.00 ± 0.014). Mortazavian and Sohrabvandi (2004), reported that bacteria*Lactobacillus bulgaricus* which is the factor of post-acidification or over-acidification in yogurt, acts in the same way in buttermilk product, so that unlike bacteria*Streptococcus thermophilus*which stops its activity at a pH lower than 4.5, even up to a pH lower than this value (3.5-3), it can continue to produce acid [25]. For this reason, in Masina's fermented product, bacteria are even able to reduce the pH to less than 3.8. According to the standard, the pH of buttermilk should be a maximum of 4.5, and based on the results obtained during this study, it was found that all the produced samples have a pH lower than 4.5 and they are all within the standard range [26].

According to the obtained results, it was found that the addition of plant powders and the storage time significantly affect the pH and acidity of the masina samples. In other words, the addition of turmeric, dill and cumin powders increased the pH and decreased the acidity of the treatments, while increasing the storage time of the samples significantly decreased the pH and increased the acidity of the treatments. The reason for these behaviors is that with the increase of time, the activity of the microorganisms in the machine increased and more lactose was converted into lactic acid, and the amount of hydrogen ions in the environment increased, which in turn led to an increase in acidity and a decrease in pH. The machine and the witness are kept during the course. On the other hand, due to the antimicrobial effects of the added plant powders, the use of these plants in the formulation of Masina reduces the activity of microorganisms and then reduces acid production [27]. Dinpajhooh et al. (2019), in their study, observed that by adding dill and garlic extracts to heat-treated buttermilk without gas, pH increased and acidity decreased, which is consistent with the results of this research [4]. Also, Amirdivani and Baba (2011), stated that by increasing the percentage of aqueous extracts of dill, mint, and basil in yogurt, its acidity decreased compared to the control sample, but with the passage of storage time, acidity increased significantly [27]. .

Table 3 Acidity (% lactic acid) of Masineh samples during the 21 days storage at refrigerator

Samples	Day 1	Day 7	Day 14	Day 21
Machine	1.00 ± 0.014^{Db}	1.12 ± 0.021^{Cb}	1.20 ± 0.014^{Bb}	1.38 ± 0.021^{Ab}
Control	1.08 ± 0.000^{And}	1.22 ± 0.000^{That}	1.38 ± 0.007^{Not}	1.51 ± 0.014^{Aa}

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The results are reported as mean ± standard deviation. Different uppercase letters indicate a significant difference between the values of each row at $P < 0.05$. Different lower-case letters indicate a significant difference between the values of each column at $P < 0.05$. Control sample: heated yogurt drink without additives

3-2- Checking the apparent viscosity of the sand

In this study, the viscosity of Masineh and control samples was measured during the 21-day storage period. The results of the average viscosity analysis indicate that there is a significant difference between the viscosity of Masina samples on different days (P<0.05). The control sample on the 21st day has the lowest viscosity (37.85 mPa.s) and the machine sample on the first day has the highest viscosity (74.66 mPa.s).

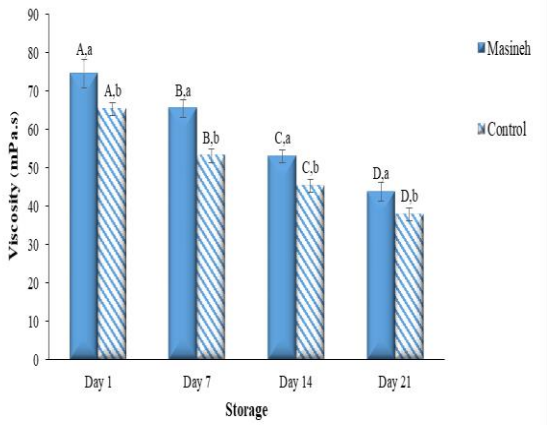

Fig 1 Changes in viscosity (mPa.s) of masineh during 3 weeks storage at refrigerator

The same lowercase letters are not significantly different between different treatments for each day at $P > 0.05$. Values of the same treatment, followed by the same uppercase letter, are not statistically different at $P > 0.05$. Control sample: heated yogurt drink without additives

According to the figure, the apparent viscosity of Masina has decreased over time, and this decrease was observed in both treatment and control groups (P<0.05). One of the important indicators of fermented dairy products is its viscosity. Drinks made from yogurt can be considered as mixed yogurts with low viscosity. The machine sample containing the additive and the control had a diluting behavior with the passage of time, which actually indicates a non-Newtonian (diluting) behavior. With the passage of time, the proteins are broken, the amount of water binding decreases, and as the acidity increases and the pH reaches the isoelectric pH of each of the milk proteins, the viscosity decreases. In addition, this decrease may be due to the action of microbial enzymes on the casein micelle matrix during the storage period [28]. Shariati et al.*Lactobacillus plantarum* and coriander leaf extract observed that it is consistent with the results of the present study [11]. Of course, it should be noted that various factors such as percentage of dry matter, amount of fat and mechanical factors such as homogenization also affect the stability and rheological properties of fermented milk products. In normal conditions, instability and reduction of viscosity in dairy fermented products does not reduce its nutritional value, but makes its natural appearance undesirable [29].

According to the figure, the viscosity of the macine sample was significantly (P<0.05) higher than the control sample, which can be attributed to the absorption of water by plant powders, as well as the higher pH of the macine and preservation of the casein micelle matrix. Shiravani and Ansari (2021), in their review, have mentioned the increase in viscosity by adding walnut leaf extract to yogurt. But on the other hand, Lotfizadeh Dehkordi et al. (2013), in the study of Sheng plant extract on the viscosity of yogurt, stated that this extract had no significant effect on the viscosity of yogurt [31].

3-3- Measurement of total phenol

The results of measuring the amount of total phenolic compounds related to the Masina sample and the control sample are shown in Figure 2. The results of total phenol showed that the amount of total phenol in the prepared masina is significantly (P<0.05) higher than the control sample. More amounts of phenol lead to an increase in the amount of antioxidant property in Masina.

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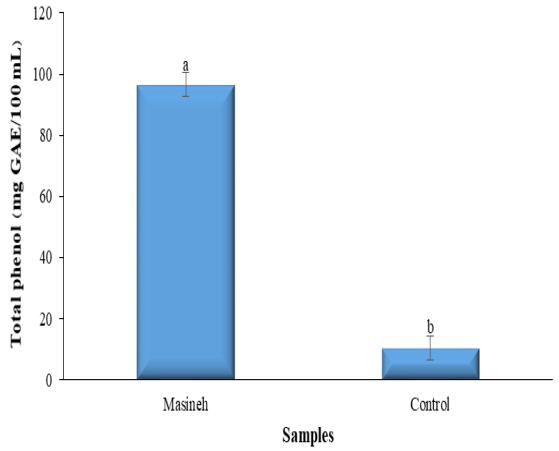

Fig 2 Measurement of Total phenol (mg GAE/100 mL) in Masineh sample containing additives
Different lowercase letters indicate the existence of a significant difference between the examined samples at the 5% probability level. Control sample: heated yogurt drink without additives

Secondary metabolites derived from plants such as phenol and total flavonoid have a strong potential to clear free radicals that exist in all different parts of plants such as leaves, fruits, seeds, roots and skin [32]. Dill, turmeric and cumin have beneficial nutritional compounds and nutritious compounds including various vitamins, magnesium, potassium, phosphorus, iron and antioxidant compounds [7-9]. Therefore, it is expected that the addition of these plants to the machine will lead to an increase in the nutritional and health-giving properties of the product. Hasani et al. (2015), during a study related to the addition of barberry extract to yogurt during storage, investigated the amount of total phenolic compounds and stated that the phenolic compounds in flavored yogurts are significantly higher than the control sample [33]. In the study of Elhamirad et al. (2012), on the effect of pomegranate extract on the amount of phenolic properties of yogurt, it was also observed that the addition of pomegranate extract increased the phenolic properties and the highest amount of total phenolic content was related to the sample containing the highest amount of extract [34]. Due to the increase in the number of hydroxyl groups in reactions, phenolic compounds can lead to hydrogen donation to free radicals, and as a result, the inhibitory power increases [32].

3-4- Antioxidant property

3-4-1- DPPH free radical inhibitory power

Since free radicals are responsible for the destruction of nutrients, the use of natural antioxidant compounds in food increases the ability to preserve food. The antioxidant effect was evaluated by measuring the reduction of the free radical capacity with the help of 2-diphenyl-1-picrylhydrazyl DPPH radical. DPPH is a purple compound that easily becomes a radical due to the presence of a phenyl group in its structure and is actually a source of free radicals. This compound changes color from purple to yellow by taking a free electron of an antioxidant compound. The free radicals in DPPH have maximum light absorption at 517 nm, which follow the Beer-Lambert law, and the reduction of its absorption has a linear relationship with the amount of antioxidant. The more the antioxidant substance is added, the more radicals are consumed and the purple color changes more to yellow.

The results of the DPPH test showed that there is a significant difference between the machine prepared sample and the control sample ($P<0.05$). According to Figure 3, the average DPPH in Masina sample was equal to 8.27 mg TE/100 mL and in the control sample was equal to 1.72 mg TE/100 mL, which showed that there is a significant difference between the two samples. Studies show that with the increase in the amount of essential oils and plant extracts, the amount of phenolic compounds increases and the absorption amount of the desired light spectrum increases, and the results of the present study were consistent with this. The obtained results can be justified with the results obtained from the measurement of phenolic compounds. Phenolic compounds as natural antioxidants have the property of absorbing free radicals and therefore it is expected that with the increase of these compounds, the antioxidant property will also increase. In general, increasing the concentration of phenolic compounds directly increases the ability of plant extracts and essential oils to inhibit free radicals. In higher amounts of phenolic compounds, due to the increase in the number of hydroxyl groups in the reaction environment, the possibility of

hydrogen donation to free radicals and, subsequently, the inhibitory power of the plant compound in question increases [35].

The antioxidant properties of turmeric have been known for a long time. Chemically, turmeric includes volatile and non-volatile compounds [36]. The escape part contains Turmeron[10], free acids, Corlon[11] and gingerbread[12] It is that they create the aroma of turmeric. The non-volatile part, which is mostly composed of phenolic compounds, causes the yellow color of turmeric. These phenolic compounds are called curcuminoids and include curcumin[13], dimethoxy curcumin[14]and bis dimethoxy curcumin[15] are. Ferulic acid and protocatechuic acid are also phenolic compounds of turmeric that have antioxidant properties [36]. In a research, the most important compounds in dill plant have been identified as alpha-flandrene, dil ether, alpha-pinene and anthracosan, which played a role in the antioxidant properties of this plant [37]. The results obtained from the test of the antioxidant properties of cumin have shown that this plant has good antioxidant properties. In a study of the composition of cumin essential oil, it was reported that most of it consists of paraceemol, alpha and beta-pinene, comic alcohol, comic aldehyde, alpha and beta phlandrene, eugenol, perylaldehyde, alpha-terpineol and myrcene [9]. Ashrafi Yorghanloo and Gheybi (2018), observed that by adding dill extract to molded yogurt, the antioxidant property increased significantly [38]. Rafiei et al. (2017), in a review of the main compounds of cumin essential oil, including cumin aldehyde, alpha-terpinene-7-L and gamma-terpinene, and observed that the most effective treatment against cheese fat oxidation was at a concentration of 2% of cumin. is [39]. Several factors affect the amount of phenolic compounds present in plant tissues and then their antioxidant properties, which include genetic factors, species and variety, amount of sunlight, soil conditions, environmental conditions and weather. [40].

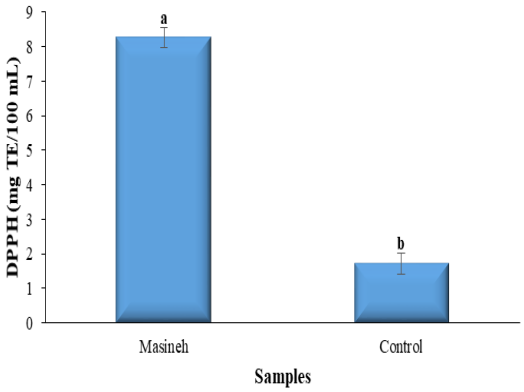

Fig 3 Measurement of DPPH (mg TE/100 mL) in Masineh sample containing additives

Different lowercase letters indicate the existence of a significant difference between the examined samples at the 5% probability level. Control sample: heated yogurt drink without additives

3-4-2- ABTS free radical inhibition power

Another indicator of the antioxidant activity of the Masina sample is ABTS cationic radical inhibition and evaluation of free radical inhibition capacity equivalent to Trolox. The results of this research indicated that the average ABTS ($P<0.05$) in the treated sample (Masina) was equal to 34.36 mg TE/100 mL compared to the control sample (3.32 mg TE/100 mL). (Figure 4).

Evaluation of ABTS water-soluble radical inhibition is another indicator for determining the strength of antioxidant compounds. ABTS radical is an artificial stable radical that is used to evaluate the antioxidant activity of various compounds. This method is based on the regeneration of radical cation ABTS ($ABTS^{+}$) which has a high absorption at 734 nm. This assay method requires the production of ABTS chromophore by oxidation of ABTS (Azino-bis-3-ethylbenzothiazoline) in the presence of an oxidant (usually potassium persulfate).

10. Turmerone
11. Curlone
12. Zingiberene
13. Curcumin
14. Dimethoxy Curcumin
15. Bisdemethoxy Curcumin

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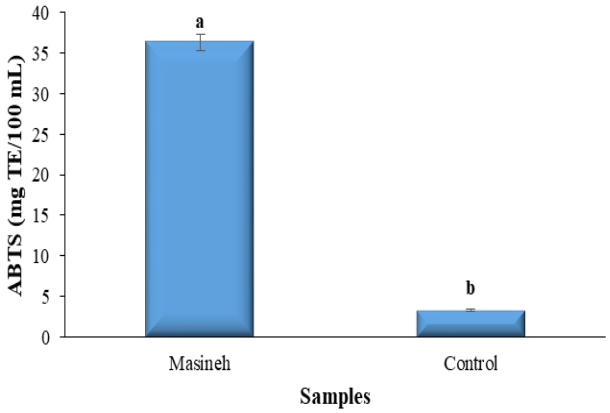

Fig 4 Measurement of ABTS (mg TE/100 mL) in Masineh sample containing additives
Different lowercase letters indicate the existence of a significant difference between the examined samples at the 5% probability level. Control sample: heated yogurt drink without additives

In this method, the amount of color reduction when the antioxidant is added to the ABTS solution^{+o} is added and evaluated. This method is widely used to evaluate the antioxidant power of plant extracts [41]. As mentioned in the discussion related to DPPH radical inhibition, plant powders added in the machine can be effective in inhibiting free radicals compared to the control sample. The effective compounds in these plants stop or slow down the oxidation process by donating hydrogen atoms to free radicals. It seems that according to the research results, phenolic compounds that are widely found in plants have high antioxidant power. It should be noted that phenolic compounds act effectively as hydrogen donors, therefore they act as an effective antioxidant [9]. Regarding the free radical inhibitory property, as seen, the control sample had a lower antioxidant percentage than Masina. Similarly, Oshaghi et al. (2015) reported a high inhibition percentage of dill extract during their research [42]. Also, Amirdivani and Baba (2011), by adding dill to probiotic yogurt, stated that the antioxidant property of samples containing dill is higher than normal yogurt [27]. Similar results have been reported to increase the antioxidant properties by adding peppermint [27], barberry [33] and water extract of marjoram [43] to yogurt. In a research, Yousefi et al. (2018), stated that the essential oil of angelica plant can be used efficiently in buttermilk formulation as a preservative [44].

5-3- Microbial characteristics

Total microbial, mold and yeast and coliform counts were investigated during 3 weeks of storage of Masina in the refrigerator. According to Table 4, the data obtained in each stage of total microbial, mold and yeast count shows that in Masina and control samples, the growth rate of bacteria, mold and yeast increases significantly ($P<0.05$) with the passage of time, but the rate The growth of bacteria, mold and yeast in the Masina sample was significantly ($P<0.05$) less than the control sample. The results indicate that the machine containing plants has an antimicrobial effect and the microbial count is lower than the control sample. Masina and Shahid samples were reported to be negative for the growth of coliform bacteria during a period of 21 days. According to the national standard guidelines of Iran, the number of coliforms in simple industrial buttermilk is maximum 10 CFU/mL and the number of mold and yeast is maximum 100 CFU/mL [26]. By comparing the results obtained from the present research with the standard, it was found that the microbial count was within the standard range and in terms of consumption, the machine sample produced containing dill, turmeric and cumin plants had no problems and has high health quality. The presence of curcuminoids in turmeric, linalool in dill, and cumin aldehyde in cumin can cause the number of aerobic mesophilic bacteria, mold, and yeast in the Masina sample to be lower compared to the control sample [45, 46, 47]. The phenolic compounds in plants destroy the outer membrane of microorganisms and cause the release of liposaccharides and increase the permeability of the cytoplasmic membrane to ATP. The release of ATP leads to the depletion of cell energy reserves and cell death [48 and 49]. Dinpajhooh et al. (2019), while investigating the effect of dill extract and garlic on the shelf life of heat-treated buttermilk without gas, stated that no mold and yeast were observed in the highest percentage of dill and garlic extract. They attributed this to the compounds of carotenoids, limonenes and flavonoids in dill extract, which have anti-mold

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effects, and allicin in garlic extract, which prevents the growth of yeasts, which causes a decrease in microbial activity and, as a result, shortens the shelf life of buttermilk. they increase During a study, Razzaghi et al. (2019) investigated the effect of Melissa plant extract and powder on the microbial characteristics of buttermilk and observed that the coliform results were negative and the extract was more effective than the powder in inhibiting microbial growth. They expressed this difference in the growth of mold and yeast in the extract and powder as the powder can be a suitable substrate for the growth of microorganisms, but because the extract acts earlier, the growth of mold and yeast is delayed [50].

Table 4 Total bacterial count, mold and yeast and coliform in the Masineh during the 21 days storage at refrigerator

Samples	Day 1	Day 7	Day 14	Day 21
	Total Count(log CFU/mL)			
Machine	1.49 ± 0.05^{And}	2.43 ± 0.06^{Cb}	3.39 ± 0.10^{Bb}	4.71 ± 0.03^{Ab}
Control	1.42 ± 0.01^{And}	3.14 ± 0.03^{That}	4.66 ± 0.15^{Not}	5.91 ± 0.11^{Aa}
	Mold & Yeast(CFU/mL)			
Machine	0.00 ± 0.00^{That}	0.00 ± 0.00^{Cb}	18.00 ± 1.41^{Bb}	35.50 ± 3.54^{Ab}
Control	0.00 ± 0.00^{And}	29.00 ± 2.83^{That}	46.00 ± 1.41^{Not}	66.50 ± 4.24^{Aa}
	Coliforms(CFU/mL)			
Machine	ND	ND	ND	ND
Control	ND	ND	ND	ND

The results are reported as mean ± standard deviation. Different uppercase letters indicate a significant difference between the values of each row at $P < 0.05$. Different lower-case letters indicate a significant difference between the values of each column at $P < 0.05$. ND: Not Detected. Control sample: heated yogurt drink without additives.

6-3- Antimicrobial property

The results of the antimicrobial test were analyzed based on the halo diameter obtained. The results of the well diffusion test showed that there is a significant difference between the Masina sample and the control sample in the effect on bacteria and fungi.

The results of Table 5 showed that Masina has the most inhibitory or antimicrobial properties against*Staphylococcus aureus* with a value of 30.91 mm and the least inhibitory effect against*Escherichia coli*It was observed with an average of 15.50 mm. The most sensitive bacteria among gram positive bacteria*Staphylococcus aureus* And among Gram-negative bacteria,*Pseudomonas aeruginosa* It was obtained and according to the results, gram-positive bacteria are more sensitive to the samples compared to gram-negative bacteria. In fact, gram-negative bacteria show less sensitivity to antimicrobial agents due to the type of wall they have, which can be caused by the presence of lipopolysaccharides in the cell wall of gram-negative bacteria, which prevents large and hydrophobic molecules from entering the cell and Because the effective compounds in plants are hydrophobic, it can be concluded that the type of wall present in Gram-negative bacteria prevents the penetration of these compounds into the cell and as a result, the resistance of Gram-negative bacteria to plant extracts [51 and 23]. In addition, the Masina sample had inhibitory properties against the studied fungi in such a way that the average diameter of the halo against*Aspergillus niger* 12.18 mm,*Penicillium digitatum* 19.88 mm and vice versa*Candida albicans* 21.44 mm was obtained. In a research on the antibacterial activity of aqueous dill extract against*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri and Salmonella typhi murium.* was investigated and the results showed that the extract of this plant has a high antimicrobial activity against all bacterial strains investigated [52]. In addition, studies have shown that flandrene in dill essential oil has an inhibitory effect on*Bacillus cereus* ،*Candida albicans* And*Aspergillus niger* is [53]. The high presence of cumin aldehyde in cumin can explain its antibacterial activity. Also, the secondary components of cumin essential oil and extract, such as alpha pinene and sabinene, also have antimicrobial activity. On the other hand, the act of stopping the growth of fungi by

essential oils and plant extracts is done due to the reaction of the aldehyde group with sulfhydryl groups effective in the growth of fungi [54]. Studies have shown that turmeric has a compound called curcuminoid against*Bacillus cereus, Escherichia coli*And *Bacillus subtilis*it is affect. Essential oils, alkaloids, curcumin, tourmerol and valeric acid are responsible for the antimicrobial activity of turmeric [45].

By comparing the average antimicrobial effects of two samples of Masina and the control, it was found that the sample of Masina has a significantly higher inhibitory effect than the control sample ($P<0.05$), so that the control sample is only against two bacteria.*Staphylococcus aureus* And*Bacillus cereus* It had an inhibitory effect and it did not have an inhibitory effect on the other investigated microorganisms. According to published reports, a group of plants have the ability to synthesize aromatic compounds and some of these compounds are phenolic derivatives. Flavonoids are one of the most important sub-branches of phenolic compounds that are made in plants to fight against microbial infections. The research conducted in recent years shows that the inhibitory properties of flavonoids are effective on a wide range of Gram-positive and Gram-negative microorganisms. This property can be due to binding to the external proteins of the cell, binding to the bacterial cell wall, or due to disintegrating the bacterial membrane. Also, polyphenolic compounds by affecting the cell membrane and the enzyme system cause disruption in cell function and eventually lead to the death of microorganisms [17 and 55].

Table 5 Antimicrobial activity of Masineh against some food borne bacteria, molds and yeasts measured as the diameter of growth inhibition zones (mm)

Microorganism	Machine	Control
Shigella sonnei	22.37 ± 0.11^{D}	ND
Pseudomonas aeruginosa	23.18 ± 0.10^{C}	ND
Escherichia coli	15.50 ± 0.12^{G}	ND
Staphylococcus aureus	30.91 ± 0.18^{Aa}	9.65 ± 0.15^{Ab}
Bacillus cereus	27.09 ± 0.35^{Not}	4.15 ± 0.35^{Bb}
Aspergillus niger	18.12 ± 0.14^{F}	ND
Fingered pencil	19.88 ± 0.61^{AND}	ND
Candida albicans	21.44 ± 0.19^{AND}	ND

The results are reported as mean ± standard deviation. Different lower-case letters indicate a significant difference between the values of each row at $P < 0.05$. Different uppercase letters indicate a significant difference between the values of each column at $P < 0.05$. ND: Not Detected. Control sample: heated yogurt drink without additives.

3-7- Sensory evaluation

In the sensory evaluation, the factors of taste, color, taste and overall acceptance (acceptability) of Masina and Shahid samples were evaluated on the first day of production. This choice was due to the freshness of the samples produced at the beginning of the storage period to avoid possible spoilage as well as negative effects on the investigated sensory factors. The sensory evaluation results of Masina sample containing herbal additive and the control sample (heated buttermilk without additive) are given in Figure 5. In terms of taste and overall acceptance, no significant difference was observed between the Masina sample and the control sample ($P>0.05$), in terms of smell, the Masina sample scored higher and in terms of color, it scored lower than the control sample ($P<0.05$). Probably, because of the aromatic additives, the Masina sample scored more points in terms of smell. In terms of color, getting a lower score than the control sample can be due to the yellow color of turmeric, which was less desirable in the eyes of sensory evaluators. In terms of taste, no significant difference was observed between the two samples, which could be related to the taste of the sensory evaluators, some people gave a higher score to Masina's sample and some found the control sample to be favorable. In terms of overall acceptance, according to the points given, it was found that both samples were favorable and had no statistically significant difference ($P<0.05$). Dinpajhooh et al. (2019), observed that by

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adding dill and garlic extract to heated buttermilk, the scores of most of its sensory characteristics (taste, smell, mouthfeel, and overall acceptance) increased, and the control sample mainly had the lowest scores of sensory characteristics (except for color) is [4]. Ardalanian and Fadaei (2018), in their research, showed that the addition of ginseng extract does not have a negative effect on the sensory properties of probiotic buttermilk enriched with mint powder, but as the percentage of the extract increases, its score decreases slightly, mainly due to the presence of saponin compounds and bitter tastes. And there is soil in it [56]. It should be noted that herbal additives, despite having favorable nutritional properties, should be used in such quantities that the final product remains acceptable to consumers.

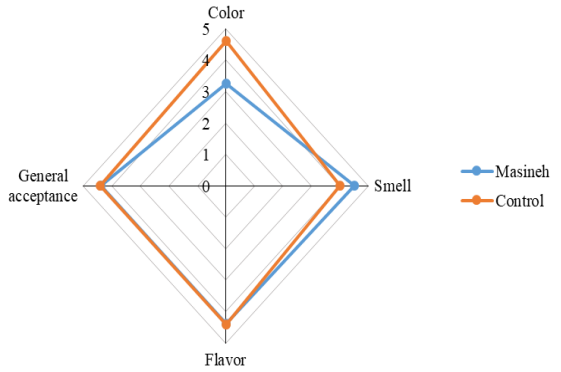

Fig 5 Sensory evaluation of Masineh sample containing herbal additives.
Control: heated yogurt drink without additives

4 - Conclusion

Considering the nutritional importance and health-giving effects as well as the antimicrobial properties of turmeric, dill and cumin plants, as well as the desire of today's consumers for natural products and avoiding artificial preservatives, the need to study and expand in this field is obvious. The present research showed that the use of herbal additives in buttermilk formulation leads to an increasepH, viscosity and acidity decrease compared to the sample. Also, in the Masina sample, the total phenol content and antioxidant properties were significantly higher than the control sample. The results of counting molds and yeasts as well as total counting of microorganisms showed that the use of herbal additives in buttermilk formulation led to a significant decrease in the number of molds, yeasts and the total number of microorganisms in buttermilk. In addition, the prepared machine has a significant inhibitory effect on the growth of the studied bacteria (*Staphylococcus aureus* ،*Bacillus cereus* ، *Escherichia coli* And*Pseudomonas aeruginosa*), molds and yeasts (*Aspergillus niger*And*Penicillium digitatum and Candida Albicans*) compared to the control sample. The results of the organoleptic properties showed that there was no significant difference between the Masina sample and the control sample in terms of overall taste and acceptability. From the point of view of sensory evaluators, the color of the produced Masina sample was not favorable compared to the control sample, but it had a better taste compared to heated buttermilk without additives. Finally, the results of this research showed that by adding herbal powders including turmeric, cumin and dill seeds to heated buttermilk, a useful food can be produced and a new choice can be provided for the consumers of dairy products, which in addition to the good taste, also has good nutritional properties. verify it.

5- Resources

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بررسی ویژگی‌های نگهدارندگی، فیزیکوشیمیایی و حسی نوشیدنی ماسینه

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چکیده

در طی تحقیق حاضر، یک محصول لبنی سنتی عشایری ایران با نام ماسینه تولید شد که از دوغ حرارت دیده تهیه شده و به آن افزودنی‌های گیاهی شامل زردچوبه، پودر تخم شوید و زیره اضافه می‌شود. برخی ویژگی‌های فیزیکوشیمیایی و میکروبی نمونه ماسینه در طول ۲۱ روز نگهداری در دمای ۴ °C مورد بررسی قرار گرفت. به علاوه، خواص آنتی اکسیدانی، اثرات ضد میکروبی و خصوصیات ارگانولپتیک محصول تولیدی در روز اول تولید، بررسی و با نمونه شاهد (دوغ حرارت دیده بدون افزودنی) مقایسه شد. نتایج نشان داد که افزودنی‌های گیاهی به طور معنی‌داری باعث کاهش شمارش باکتریایی و شمارش کپک و مخمر در محصول ماسینه نسبت به نمونه شاهد شد (P<۰/۰۵). در ماسینه بیشترین خاصیت بازدارندگی بر علیه *استافیلوکوکوس اورئوس* با ۳۰/۹۱ mm مشاهده شد و کمترین اثر بازدارندگی بر علیه *اشریشیاکلی* با میانگین ۱۵/۵۰ mm و بعد از آن *آسپرژیلوس نایجر* با میانگین ۱۸/۱۲ mm مشاهده شد. به علاوه، ترکیبات فنولی و درصد بازدارندگی DPPH و ABTS در ماسینه بالاتر از نمونه شاهد بدست آمد. pH و ویسکوزیته ماسینه بیشتر از دوغ بدون افزودنی بود که در طول نگهداری، این مقادیر به طور معنی‌داری کاهش پیدا کرد (P<۰/۰۵). همچنین نتایج ارزیابی حسی نشان داد که ارزیابان حسی طعم ماسینه تولیدی را مطلوب دانستند و از نظر پذیرش کلی تفاوت معنی‌داری با نمونه شاهد گزارش نکردند، اگرچه رنگ ماسینه از امتیاز پایین‌تری برخوردار بود. بنابراین با توجه به نتایج بدست آمده محصول ماسینه به عنوان یک نوشیدنی لبنی سودمند معرفی می‌شود که با کاربرد افزودنی‌های گیاهی، خصوصیات آنتی‌اکسیدانی و ویژگی‌های میکروبیولوژیک آن تقویت می‌شود.