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Scientific Research

Investigating the chemical and microbial characteristics of date syrup during the production stages (from the raw material to the final product)

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ABSTRACT

The purpose of this study is to investigate the chemical and microbial properties of date syrup during different stages of production line from the raw material to the final product. The amounts of total soluble solids, pH, acidity, total and reducing sugars, polyphenolic compounds, antioxidant activity and microbial count were measured and the type of microorganisms was determined based on phenotypic characteristics in different stages of production. The obtained results showed that during the stages of juice clarification and concentration, pH gradually decreased and acidity, total sugar, reducing sugar and total solid matter increased and reached their maximum in the syrup. Comparison of traditional syrup with factory produced syrup indicated more pH and acidity, total and reducing sugar, total soluble solid, polyphenolic compounds and lower antioxidant activity for traditional syrup compared to the produced one. In general, the content of polyphenolic compounds and antioxidant activity gradually decreased in different stages of production, so that date syrup (279 mg of gallic acid per 100 grams of sample and 33%) indicated higher content of polyphenolic compounds and antioxidant activity than dates (388 mg of gallic acid per 100 g of sample and 57%). The results of the microbial test revealed the lowest microbial load for the produced syrup compared to dates and different syrups. However, the presence of *B. licheniformis* in the syrup makes the product sour, in this regard finding a suitable solution to increase the shelf life of the product seems necessary. The traditional syrup showed a lower microbial load than the produced syrup, although *B. licheniformis* was observed in the traditional syrup despite the high heat treatment. Based on the obtained results, the date syrup was free of coliforms, which indicates maintaining the hygienic principles of manufacture during the production line.

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1. Introduction

Fruits and their conversion products are one of the most important commercial goods in the world and occupy a part of the economic life of many developing countries [1]. Date (*Phoenix dactylifera* L.) is considered one of the economic items in the export of some Middle Eastern countries, including Iran. The amount of date production in the world in 2007 was about 6 million tons, of which about 940 thousand tons were produced in Iran [2]. According to reportsFAO In 1998, Iran has the first place in date cultivation area and the second place in terms of sales and export of dates in the world. Also, Iran has occupied the second position of date production in the world with its global production of 14.1% in 2012 [3]. Dates contain a significant amount of carbohydrates (73.5%) along with ash (1.5%), protein (2.3%), lipid (0.2%), vitaminsA ،B_1 ،B_2 ،B_6 ،C, niacin, folic acid and pantothenic acid and minerals include chlorine, sodium, potassium, calcium, magnesium, zinc, copper, phosphorus, manganese, cobalt, selenium, barium and iron. Dates contain a high amount of polyphenolic compounds (about 225 mg/100 grams) which prevents cardiovascular diseases and cancer due to its antioxidant properties. Also, having high amounts of dietary fiber (pectic compounds, hemicellulose, cellulose, gums, starch, lignin and xylan) has made dates nutritionally important. The main chemical components of date fruit are carbohydrates, generally reducing sugars such as glucose, fructose and non-reducing sucrose. Since a significant part of date sugars are regenerating sugars, therefore asIt is a suitable substitute for sucrose in the formulation of sweet foods for diabetic patients[4 and 5].

About 60% of the dates produced in the country are consumed or exported, and about 40% of them have declined due to the lack of proper harvesting methods, traditional packaging, transportation, and improper storage methods and due to low marketability. Ripe livestock is converted into date juice or other similar products in transformation industries. In general, dates are consumed in direct (use of fruit) and indirect (industrial dates in transformation industries) methods. Industrial date refers to a date that is lower than the standard and is used in the industry by changing the appearance of its fruit in making sweets, preparing date pulp, date juice, liquid sugar and fermentation industries (alcohol and vinegar fermentation). [6]. Date waste, which is produced in the country due to the lack of attention to by-products and its processing, makes it necessary to research the use of date by-products such as juice, sugar and date pulp. Date juice is usually considered the most product made from dates, which is prepared by two traditional and industrial methods. The traditional method includes extracting and boiling date syrup. The industrial method of the process includes extraction, purification and concentration of date syrup [7]. Concentrating fruit juices while reducing water activity and microbial growth reduces transportation and storage costs and increases product shelf life. [8]. Date juice is a dark brown liquid and sweet product that hasThe presence of sugar compounds including glucose, fructose and sucrose contains other compounds such as protein, fiber and mineral compounds, especially iron, potassium and calcium. Also, due to having high amounts of phosphorus, iron, potassium and calcium, it has a high nutritional value and is considered a nutrient especially for children, the elderly, lactating women and diabetic patients. In addition, the high content of fructose and glucose and the low content of sucrose and economic efficiency have made date juice a suitable food ingredient for the formulation of sweet

foods such as confectionery products, soft drinks and dairy products. Besides the positive nutritional effects, date juice is a rich source of antioxidants. The antioxidant activity of these compounds is related to several mechanisms such as decomposition of peroxides, prevention of chain reactions, property of trapping free radicals and metal ions. Compounds responsible for antioxidant effects includeThey are flavonoids, phenolic acid, ascorbic acid and carotenoids. In this regard, Noshirvani et al [9] investigated the antimicrobial and antioxidant activity of date pulp and extract. Based on the obtained results, the content of polyphenolic and flavonoid compoundsMethanolic extract and pulp Dates were 414.72 and 304.18 (mg gallic acid per 100 g sample) and 28.62 and 20.72 (catechin mg per 100 g sample) respectively. Also, the result of free radical inhibition testDPPH It showed high antioxidant activity of methanolic extract compared to date pulp. Microbial tests and antifungal effects of methanolic extract of date pulp on mold*Aspergillus niger* showed In a study by Abes et al [10] MThe total phenolic content of date juice was reported between 368 and 529 mg of gallic acid per 100 grams. They showed that during different stages of processing, the amount of polyphenolic compounds in the juice decreases, which is attributed to the presence of polyphenol oxidase enzymes in dates, as well as the oxidation of some phenolic compounds by hydrolytic enzymes. In addition, microbial enzymes are also able to break down polyphenolic compounds, so that Sun et al [11] showed the presence of mold*Aspergillus flavus* In asparagus juice, it leads to the oxidation of polyphenolic compounds. Gad et al. [12] showed that adding date juice to yogurt increases total polyphenol capacity and antioxidant properties in yogurt.

Date juice is either consumed directly or used as a raw material in the preparation of many food formulations such as ice cream, beverages, bakery and confectionary industries, the combination of date juice and sesame paste, jam and butter.In this regard, Homayoni Rad et al [13] investigated the glycemic index of diet cherry jam prepared with date juice in healthy people. The results showed that the formulation of jam with date juice had a glycemic index within the acceptable range for diabetics and compared to the control sample, it showed a significantly lower glycemic index. The results of this research introduced date juice as a suitable source of sugar for the formulation of sweet food for diabetics. In another study, Gohari Ardabili et al [14] showed that the useUsing date juice as a substitute for sugar in ice cream, while avoiding the complications caused by sucrose for health, it can also create added value in the product by using low-quality 2nd and 3rd grade dates that are not suitable for fresh eating. Mesbahi et al. [15] studied the effect of replacing sucrose with date juice and liquid sugar on the rheological characteristics of biscuit dough. Based on the obtained results, the dough consistency and its reversibility decreased with the increase in the percentage of sucrose replacement. Decreasing the amount of sucrose decreased the firmness and cohesion of the dough and increased its stickiness. The best results were obtained based on the replacement percentage of 60% for date sugar and 40% for date juice. In another study, Karazayan et al. [16] formulation optimization

They examined the useful cake containing date juice and inulin. The obtained results showed that replacing sugar up to 19% with date juice is suitable. Increasing the consumption of dates and producing valuable products from low quality dates has a great role in increasing the added value of agricultural products. Most of the products made from dates, such as date juice, are prepared in a traditional way, and the product is often inconsistent with the standard. For example, in a research, the amount of mineral salts in 11 samples of grape juice from different regions of Khorasan was investigated. Due to the fact that clay is used to clarify the grape juice product, the examined samples showed more than the allowed amount of nickel, cadmium and cobalt. Also, lack of proper washing of grapes, excessive use of soil and lack of proper purification methods led to a decrease in the quality of the final product [17]. According to the mentioned cases and since there are very limited researches on the quality and characteristics of date by-products in the country.It has been done and considering the importance of using this valuable nutrient in the production of quality products as a suitable alternative to sucrose-containing sweeteners at a low price, it is necessary to conduct more research on date juice. Therefore, in this research, grade 3 dates were used in the preparation of date juice, and the chemical and microbial characteristics of dates, syrup and industrial date juice were investigated at different stages of the date juice production line and compared with the traditional juice available in the local market.

2- Materials and method

1-2- Materials

Introduction to FolinCiocalto and 2 and 2 diphenylpicrylhydrazyl[1] They were purchased from Merck and Sigma-Aldrich respectively. All culture mediums used were obtained from Merck, Germany. Other materials used such as sodium carbonate,Monopotassium phosphate etc. were prepared in laboratory grade from reputable companies. The traditional date juice was prepared from the local market and was used to compare with the industrial sample.

2-2-Date juice preparation method

To prepare date juice from grade 3 industrial datesCabbage from Khuzestan and Bushehr provinces were used and dates used in the factory of Shirin Toshe Baharan Company (Baharan Industrial Town, Hamadan, Iran) by national standard No. 803 [18] by measuring moisture content, total solids, pH, acidity, total sugar And regeneration and ash were subjected to quality control. In order to reduce the microbial load, dates were first washed by water and then sterilized by steam. After that, dates were boiled with 3 times their weight of water inside the tanks and stirring was done in order to facilitate the release of sugar from the fruit and accelerate the process of diffusion (syrup 1). Then the cap and core are separated by a pulper and the resulting pulp is filtered through a cloth filter and the extract obtained, which is called syrup 2 at this stage and has a total solid content of 13-14 degrees Brix, is directed to the filter press and filtered (syrup 3)). Finally, syrup 3 was concentrated in the evaporator under vacuum until reaching a total solid of 72 degrees Brix (date juice). The production steps are shown in Figure 1.

[1]- 2,2-diphenyl-1-picrylhydrazyl (DPPH)

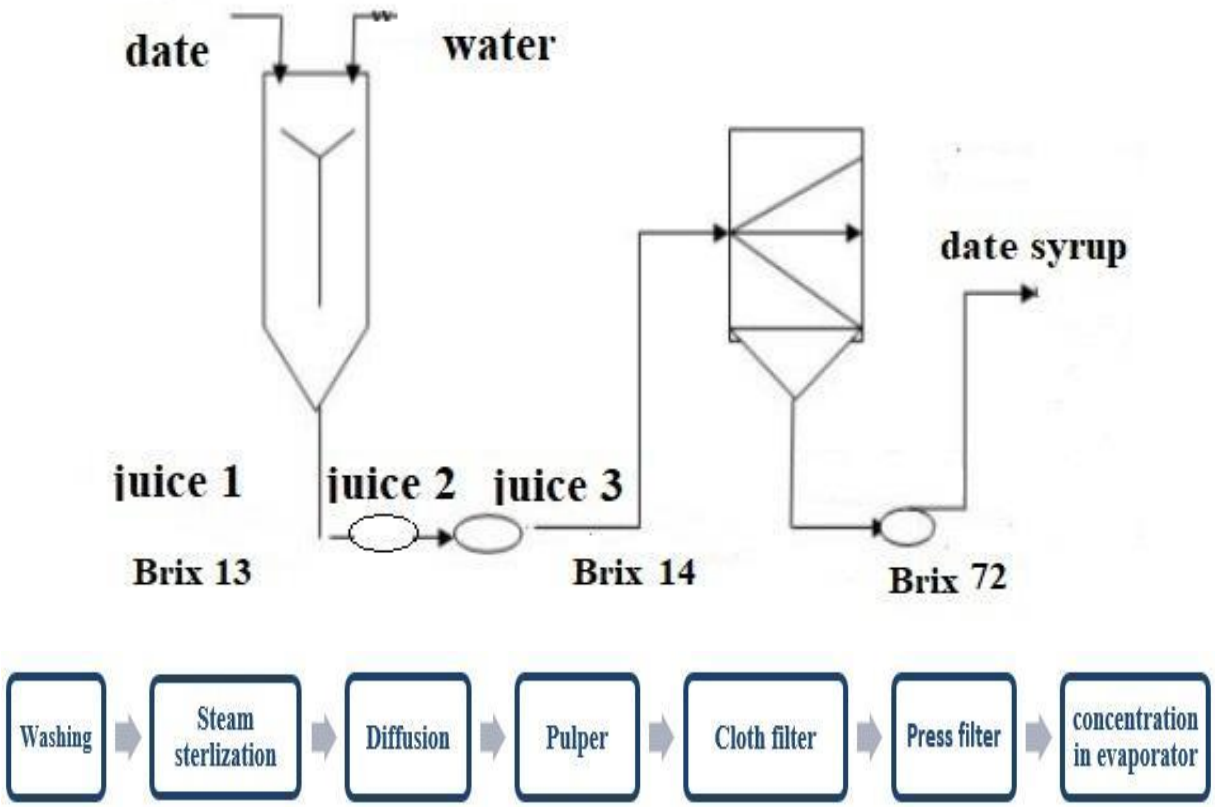

Fig 1 Flow diagram of date syrup production in the factor

2-3- Sampling

Dates, syrups 1, 2 and 3 and date juice were sampled.

4-2-Exams

1-4-2- Ash measurement

Ash measurement based on standardAOAC [19] took place.

2-4-2-Protein measurement

Measurement of protein by microkjeldahl method based on standardAOAC [19] took place.

3-4-2- Measurement of materialsTotal dissolved solids

The amount of dissolved solid matter of all samples based on the standardAOAC [19] using a refractometerRomiziAtagoJapan modelMaster-4ain temperature°C20 was measured.

4-4-2- Humidity measurement

Size Moisture measurement based on standardAOAC [19] took place. For this purpose, the amount of 2 grams of the sample was weighed in the petri dish and until it reached a constant weight inside the oven with temperature°C 105 was placed. The moisture percentage was calculated from equation 1:

Relationship 1: Humidity (%)

$$=\frac{Initial\ sample\ weight-weighed\ dry\ sample\ weight}{Initial\ sample\ weight-empty\ weight\ container}$$

2-4-5-MeasurementpH

MeterYpH BypH meter modelSelectlab in temperature°C20 and according to the national standard of Iran No. 5075 [20].

6-4-2- Acidity measurement

Acidity measurement according to malic acidIran's national standard No. 5075 [20] was carried out.

2-4-7-determination of sugar percentage

The measurement of the percentage of total sugar and reducing sugar was carried out according to the Iranian national standard No. 5075 [20] and the results were reported based on the percentage.

8-4-2-Measurement of polyphenol compounds

The content of polyphenolic compounds in date sap was determined by Folin-Ciocalto method. For this purpose, first, 0.5 ml of date juice was diluted with 12.5 ml of water. Then, 0.2 ml of diluted date juice was mixed with 1 ml of 10% v/v Fulin Ciocalto reagent and kept at room temperature for 5 minutes. After that, 0.8 ml of 7.5% w/v sodium carbonate was added to the mixture and kept in the dark for 1 hour. In the followingThe absorbance of the solution was measured by a spectrophotometer at a wavelength of 725 nm. To calculate the amount of phenolic compounds in terms of milligrams of gallic acid in 100 grams of sample, the standard curve drawn at concentrations from 0 to 200 ppm was used [21].

9-4-2-Antioxidant activity

ActivityAntioxidant was done by DPPH free radical scavenging method. For this purpose, 0.1 ml of diluted sample (2:1) was mixed with 3.9 ml of DPPH reagent (0.1 mM solution). Then the sample was placed in the dark for 1 hour and the absorbance at 517 nm wavelength was read by a spectrophotometer. The control sample was prepared by adding methanol instead of juice and similar to the original sample. The percentage of antioxidant activity was calculated based on equation 2 [9].

Relationship 2:

Antioxidant activity (%) $=100 \times \frac{Absorption\ witness - absorption\ sample}{Watch\ Attraction}$

10-4-2- Microbial tests

For microbial sampling, sampling of the primary dates in the production line was done randomly. Other samples include syrup 1, syrup 2, syrup 3 and cooked juiceIt was placed in sterile glass containers and kept in the refrigerator until the microbial test. To prepare samples before testing,In sterile conditions, 10 ml of each sample with 90 ml of saline buffer (Monopotassium phosphate) mixed sterile and different dilutions up to[3]-10 was prepared and used for microbial analysis.Referring to the national standard of Iran for date juice No. 5075 [20] and its test methods and according to the acidity of the juice samples that provide special conditions in terms of microbial activities, different killing environments were used. Table 1Microbial test factors in date standard, syrup and date juiceis showing. In addition to general counting, the species of dominant microorganisms in each production stage, including spore bacteria, molds and yeasts, were identified.Kant agar plate culture medium[2] (PCA) for total count of microorganisms in aerobic conditions at temperature∘C30 was used. Dichloran culture medium 18% glycerol agar[3] (DG18) to isolate and count smophilic molds and yeasts at temperature˚C25 was used. Orange serum agar culture medium[4] (PART), for diagnosis Mesophilic acidophilic bacteria at temperature˚C30 to 35 and to identify gram-negative bacilli from the family*Enterobacteriaceae*(general form) at temp∘C30 to 37 from culture mediumViolet Red Bile Salt Agar (VRBL)[5]‘ used, and in case of colony growth, confirmatory test with lactose fermentation and gas production in culture mediumBrilliant Green Lactose Bile Broth[6] (BGB) usebecame. To check the type of bacteria isolated from the samples from the instructions in Appendix 1 during severalstage using catalase tests[7] (Using 3% hydrogen peroxide solution), Voges-Proskauer (VP) test by methyl red Voges Proskauer Broth culture medium[8]MR-VP Broth)) at temp∘C37 and growth under anaerobic conditions, growth at temp∘C50 and growth in basic culture medium containing 7% salt (nutrient agar[9]+ NaCl 7%) was tested [22].

Table 1 Microbial test factors in date, date syrup and date juice standards

	Control limit (CFU/g)	Test method
Total mesophilic bacteria	5×10^2	ISRI 5272-1 [23]
Yeast and molds	10 <	ISRI 10899-2 [24]
Acidophilic bacteria	Negative	ISRI 2326 [25]
Coliforms	Negative	ISRI 9263 [26]

[2]- Plate Count Agar
[3]- Dichloran 18% mass fraction glycerol agar
[4]Orange serum agar

[5]-Crystal violet neutral red bile lactose agar (VRBL)
[6]- Brilliant green lactose bile broth
[7]- Catalase test
[8]- Methyl Red - Voges Proskauer Broth
[9]- Nutrient agar

5-2-Statistical analysis

In this research, a completely random design with factorial arrangement was used. Experiments were performed in four replicates. In order to decompose the varianceResults and comparison of averages from the softwareSPSS 16 used. Means were compared at the 5% statistical level.

3. Results and Discussion

1-3-Chemical compounds

The amount of compoundsThe chemistry of dates and date juice is listed in Table 2. The amounts of protein, ash, total sugar and regenerating sugar in dates are 1.86, 2.12, 55.54, 50.62% and in date juice are 0.95, 1.75, 70.01, and 86% respectively. It is 66%, which is consistent with the study of El Hoti et al. [27]. Based on the resultsThe obtained regenerating sugars form the main composition of date juice. Fructose and glucose are the main part of the sugars that make up juice, and sucrose accounts for a small percentage of juice sugar. Compared to sucrose, reducing sugars have high nutritional effects and are sweeter than sucrose due to their higher solubility and reduce the possibility of sap crystallization [28]. The amount of protein in dates is higher than that of juice, which is due to the removal of impurities, including protein compounds, during the clarification stage of the syrup by the filter. Although dates are not a rich source of protein, studies have shown that dates contain a high amount of essential amino acids [27]. As can be seenpH The juice is in the acidic range, which is related to the presence of acidsIt can be titled in it [29]. Also sap compared to datespH is less, which is due to the removal of suspended impurities or insoluble compounds with amphoteric properties or structural changes in some acidic compounds during the physical treatments of syrup processing [30].

2-3- Checking the chemical characteristics during the production line

1-2-3-pH and acidity

ResultspH And the acidity of date samples, syrups 1, 2 and 3, juice produced by the factory and traditional juice prepared from the market are shown in Figure 2.T. LevelpH Dates were 5.58, which increased to 6.03 after preparation of syrup, which is due to diluting the date sample with water and approachingpH it topH It is neutral. However, after this stage, during the various stages of processing date juicepH Decreases and increases acidity. So that date juice has significantly at the 5% probability levelpH It is less than dates and syrup 1. This is probably related to the increase in juice concentration by removing water during the syrup concentration stage in the evaporator, as well as the separation of suspended materials in the date pulp including pectin, starch and cellulose during the clarification process, as a result of the separation of these compounds,pH Decreases and increases acidity. Also, the removal of proteins in the syrup clarification stage due to the presence of amino groupspH Alkalinity causes reductionpH In the clarifying stage, the syrup becomes after removing the protein compounds. Comparison of factory-produced juice with traditional juice.pH Higher for traditional sap showed that the reason is probably related to the use of sap soilIn order to clarify syrup in the production of traditional juice and the presence of alkaline compounds in it.

Acidity changes of different samples during different stages of production with changespH Correspond. So that syrup samples showed less acidity compared to dates, which is due to dilution of date

samples after preparation of syrup. The juice showed the highest level of acidity among other samples, which is the result of observing the lowestpH It corresponds to the juice sample. The comparison of the juice produced by the factory with the traditional juice shows a big difference between the acidity, the reason of which, as mentioned, is related to the use of sap soil in order to clarify the traditional juice and in contrast to the removal of protein compounds from the industrial juice during the filtration steps, which as a result causes DecreasepH Industrial juice is compared to traditional juice.

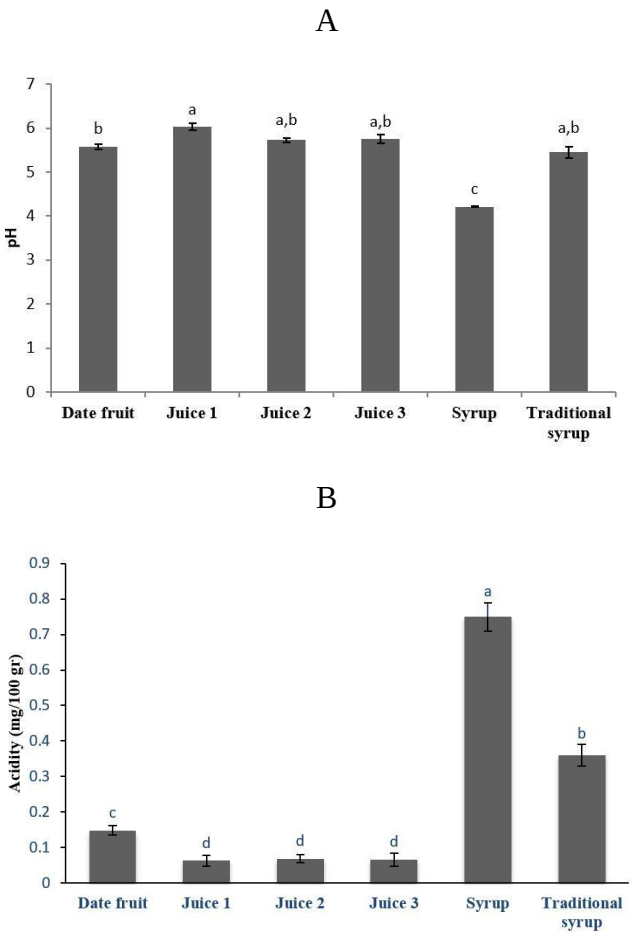

Fig 2 pH (A) and acidity (B) in different stages of date syrup production (different letters indicate significant differences (P < 0.05))

2-2-3-Solved solid materials

ChangesThe dissolved solids of date samples, syrups 1, 2 and 3, juice produced by the factory and traditional juice prepared from the market are shown in Figure 3. AsIt can be seen that the amount of solids in date solution is 52.56 degrees Brix, which decreases after diluting with water and making syrup, and then gradually increases during the filtration process. So that the amount of solids in the syrup 1 solution before the cloth filter is 13.25 degrees Brix, which increases to 13.87 and 14.25 degrees Brix by passing through the cloth filter (syrup 2) and filter press (syrup 3) respectively. . The juice prepared significantly showed the highest amount of dissolved solids (72.33 Brix) at the probability level of 5%, which is due to the evaporation of water as a result of syrup concentration in the evaporator and the increase in the content of soluble solids in the juice. The amount of dissolved solids in traditional juice is 71 degrees Brix, which statistically, at the 5% probability level, was not different from industrially produced juice.

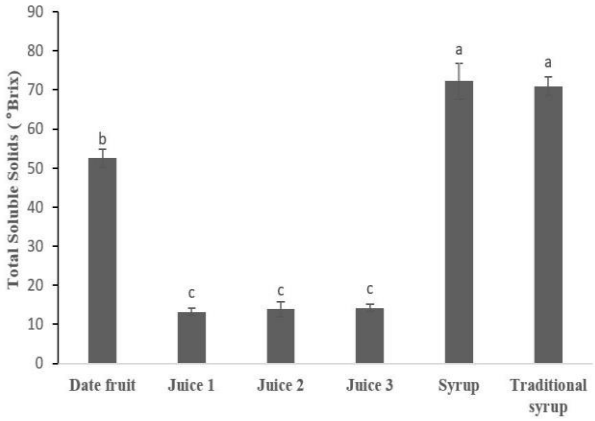

Fig 3 Total soluble solids in different stages of date syrup production (different letters indicate significant differences (P < 0.05)).

3-2-3-Total sugar and reducing sugar

Dates are considered a valuable product due to their high regenerating sugars such as glucose and fructose and low percentage of sucrose. Fructose does not need insulin for absorption, and therefore it is considered a suitable sugar for food formulation for people with diabetes. Also, the high percentage of reducing sugars, especially fructose, in dates compared to sucrose due to solubilityOn top of that, it reduces the amount of sap crystallization during the storage period. The amount of total sugar and reducing sugar of date samples, syrups 1, 2 and 3, juice produced by the factory and traditional juice prepared from the market are shown in Figure 4. By checking the amount of total sugarAnd the rejuvenating sugar present in different samples of the production line can be seen that the amount of sugar in the juice is significantly ($P<0.05$) higher than that of dates and the resulting syrup.is. The reason for this can be attributed to the separation of impurities in the flesh of the fruit, which increases the amount of soluble solid matter and thus sugar in the product during different stages of purification by reducing these compounds. By comparing the sugar content of the samplesSyrup can be obtained, the amount of sugar increases during different stages of syrup purification, although this increase is not significant ($P > 0.5$). During syrup concentration in the evaporator by removing water, the amount of total sugar and reducing sugar increases to 70.34% and 66.86%, respectively. As it was said, during the different stages of purification by removing the cellulose and pectin compounds, the purity of the syrup increases, which can be easily seen by observing the solid matter of the different samples of the syrup. The sample of traditional date juice has less total sugar (65.23%) and regenerating agent (61.72%) than factory juice, although this difference is not significant at the 5% probability level.

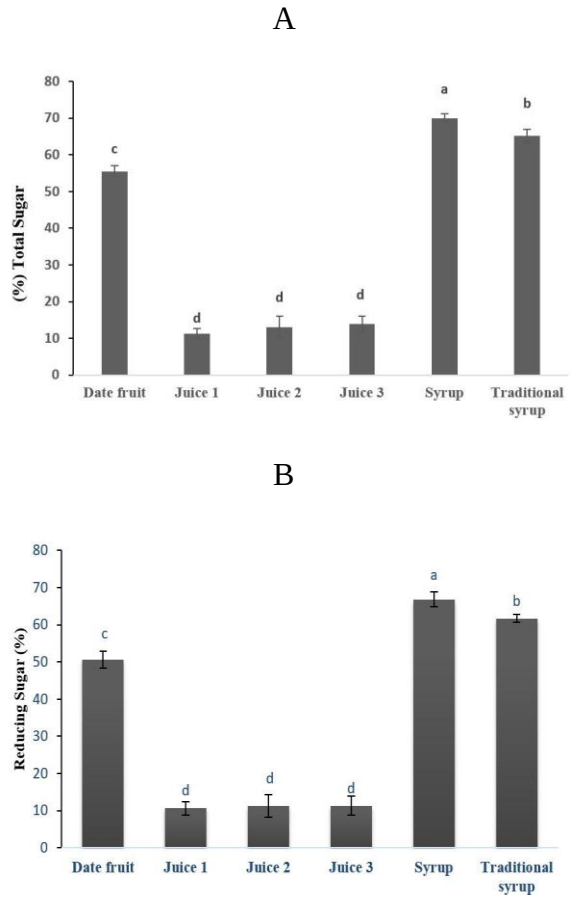

Fig 4 Total sugar (A) and reducing sugar (B) content) in different stages of date syrup production (different letters indicate significant differences ($P < 0.05$))

4-2-3-polyphenol compounds

The amount of polyphenolic compounds related to date samples, syrups 1, 2 and 3, juice produced by the factory and traditional juice prepared from the market is shown in Figure 5. The results showed that the ratePhenolic compounds in dates are significantly higher than all samples (388 mg of gallic acid per 100 grams of sample). After preparing syrup 1 and becauseDiluting the sample, the content of polyphenolic compounds decreased significantly at the 5% probability level (97 mg/100 g of sample). Also, during the steps of

purification and clarification of the syrup, the content of phenolic compounds showed a further decrease (76 and 82 mg/100 grams of sample in syrup samples 2 and 3, respectively), although this decrease was not significant at the 5% probability level. The reason for the decrease in the content of phenolic compounds during syrup clarification is attributed to the activity of the polyphenol oxidase enzyme and the oxidation of polyphenolic compounds by it. These results with the studyIt is consistent with Abs et al. [10]. After syrup concentration and juice production, the content of polyphenolic compoundsSignificantly ($P < 0.05$) increased (279 mg/100 grams of sample), which is attributed to the syrup concentration in the evaporator. Comparison of produced juice with traditional juice (252 mg/100 grams of sample) showed a lower content of polyphenolic compounds in the traditional sample.(05/0 < P) The reason for this is attributed to severe heat treatment in traditional samples and the destruction of the structure of polyphenolic compounds during heat treatment. Date juice is a tonic food with high energy and rich in carbohydrates, minerals, as well as a complex combination of saccharides, amino acids, polyphenols and carotenoids. In addition to the nutritious ingredients, date juice is rich in antioxidants. The compounds responsible for antioxidant activity include flavonoids, phenolic acids, ascorbic acid and carotenoids [10]. Flavonoids and phenolic compounds such as cinnamic acid and its derivatives are responsible for the antioxidant activity of date fruit. Cinnamic acid is easily capable of losing hydrogen and inhibits free radicals to achieve resonance stability. According to the study of Abes et al [10], catechin, vanillic acid, caffeic acid, synergic acid, pcoumaric acid, ferulic acid and cinnamic acid are phenolic compounds derived from date sap. In the meantime, pcoumaric acid was found to be the dominant compound of the juice. Chaira et al. [31] examined the amount of phenolic acid compounds, flavonoids and antioxidant activity of dates and showed that the content of polyphenolic compounds varies from 15.73 to 54.66 mg per 100 grams of wet weight in several date varieties. . Date varieties with the highest content of polyphenolic compounds had the highest anti-radical activity. Taleb et al. [32] showed for the first time that date juice and its phenolic compounds have antimicrobial activity against bacteria.*Staphylococcus aureus* And*Escherichia coli*are attributed to the production of hydrogen peroxide. In another study, Mehdi et al. [29] reported the content of total phenolic compounds in different date varieties between 58 and 143 mg/g of sample. The reason for the difference in the values obtained in this study with other studies depends on the difference of date varieties, fruit ripening rate, climatic conditions of the cultivation area, date storage conditions, etc. The structure of phenolic compounds is affected by the process conditions. In order to maintain or improve the primary activity of dates when preparing date juice, it is necessary to determine the effect of the preparation steps on active components of the juice such as phenolic compounds and carotenoids. For this purpose, the content of polyphenol compounds in different stages of the date juice production line was investigated.

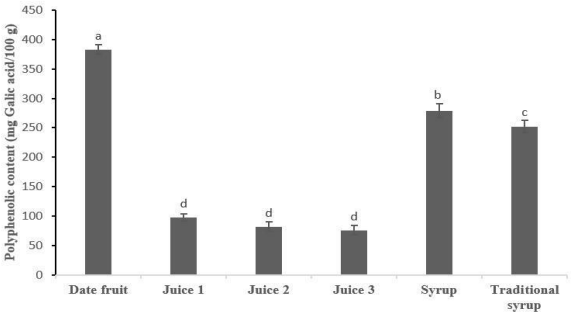

Fig 5 The content of phenolic compounds in different stages of date syrup production (different letters indicate significant differences ($P < 0.05$))

5-2-3-Antioxidant activity

Date by-products are a good source of antioxidant compounds and can be used as an additive in the formulation of practical foods. The results of the antioxidant activity test of different samples along the production line with the free radical scavenging testDPPH It is shown in figure 6. As it can be seen, date significantly ($P < 0.05$) has the highest antioxidant activity (57%) among other samples. After preparing the syrup and diluting dates, the amount of antioxidant activity decreased significantly ($P < 0.05$) and again increased to 33% by concentrating the sample in the evaporator. The traditional juice showed a lower antioxidant activity ($P < 0.05$) compared to the juice produced by the factory (25%), which is attributed to the intense thermal process during the syrup concentration process in this sample. The results of this test are consistent with the results of the content of polyphenolic compounds. In general, studies have shown that there is a strong correlation between antioxidant activity and polyphenol content. Many phenolic compounds such as polyphenols and flavonoids have properties due to their reducing activityThey are antioxidants. Due to the property of trapping free radicals, these compounds have positive effects on the characteristics of the manufactured product, and hence date juice is introduced as a practical food product [32].

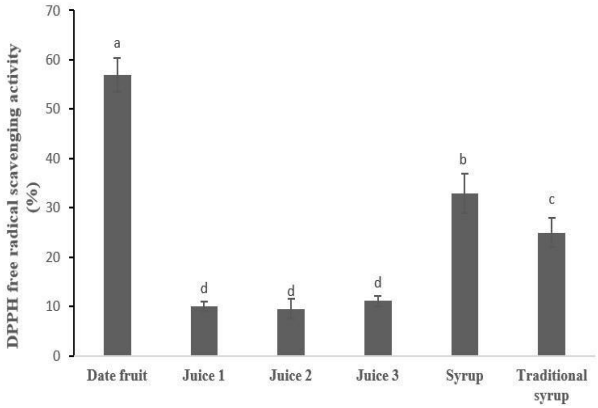

Fig 6 DPPH free radical scavenging activity (antioxidant activity) in different stages of date syrup production (different letters indicate significant differences ($P < 0.05$))

3-3- Microbial characteristics

1-3-3- Microbial count

In order to investigate the effect of process steps on the characteristicsMicrobiality of date juice, sampling was done from different stages of the production line and the results of microbial tests are shown in Figure 7. In the process of preparing date juice, as can be seen, the microbial load decreases with the production of syrup 1 from dates. During the different stages of syrup purification, and when the syrup passes through the fabric filter, the microbial load decreases more (Syrup 2). However, a significant increase in the microbial load was observed after passing the syrup through the filter press (sorbet 3).It is possible that the reason is related to not washing dailyThe filter cloths are press, as a result of which conditions are provided for the growth of all kinds of microorganisms, especially yeast, and when the syrup passes through this part, the microbial load shows a significant increase. In the next step, after cooking the syrup in the evaporator, the microbial load decreased again, which is probably related to the concentration of the syrup in the vacuum evaporator.and unsuitable conditions for the growth of microorganisms. Also, the amount of abundance

Different microorganisms, including total count, total form, mold, osmophilic and bacillus yeasts in different stages of juice preparation are shown in Figure 7. The amount of bacillus in dates is high, the reason of which is probably related to the contamination of the sample with soil. Before preparing the syrup, due to washing the dates with water and then diluting the dates with water and preparing the syrup, the microbial load is reduced. The juice sample is free of mold and yeast, which may be due to the destruction of mold and yeast during heat treatment, because most of the vegetative forms of mold and yeast grow at temperatures slightly higher than°C 50 are deactivated or destroyed [1]. This result is consistent with the results of Mehdi et al.'s study [29]. However, the total number of bacteria and bacillus bacteria in the juice does not reach zero, which is probably due to the low temperature of the evaporator and the inadequacy of the thermal process to destroy all spore-forming microorganisms.. Since the evaporator is under vacuum, the cooking temperature is approx°C60 and therefore will not have a significant effect on the reduction of spore bearing bacteria. In the prepared juice sample, the total number of micro-organisms, mold and yeast and spore-bearing bacteria were 10 respectively.^{3}x3, 0 and 10^{2}×6/ g CFU It was found that the total count and the number of spore-bearing bacteria are higher than the limits set in the national standard. Therefore, it seems necessary to find a suitable method for sanitizing the sample. In none of the stages of juice production, except for syrup 1, the total form was not observed, which is due to the lack of use of contaminated water, the lack of involvement of the workers' hands with the product, the high concentration of sugar, and the creation of unfavorable conditions for the growth of the total form and the use of heat in time. It is syrup concentration. In the sample of traditional juice, like the juice produced by the factory, mold and yeast and overall form were not observed, while the traditional juice showed a significantly lower microbial load than the produced juice, which is due to the use of a high temperature for cooking the syrup around◦100 degrees C is given when boiling and condensing. Also, spore-bearing bacteria were also observed in traditional juice, although its amount was significantly compared to factory juice.(05/0 < P) It was less.

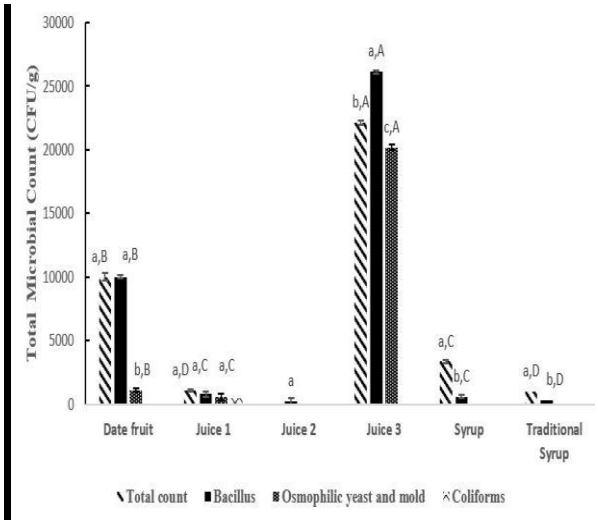

Fig 7 The abundance of each of the dominant microorganisms in different stages of date syrup production.

2-3-3- Morphological examination of the type of microorganisms

Macroscopic and microscopic images related toMicroorganisms observed in the juice production line are given in Figure 8. Molds generally by sex*Aspergillus* And*Penicillium* belonged Morphological studies conducted on cultured samples showed that the dominant microbial flora in dates probably includes types of mold, yeast and bacillus; There is bacillus in the mold and yeast syrup and in the juice in the majority of samples. Moldy dates*Aspergillus*And*Penicillium* were observed; In some cases, yeast and bacillus

were also isolated. Examining the syrup samples during the purification process showed that the dominant microflora of the raw syrup is before the mold cloth filter. Molds observed may include*Aspergillus* And*Penicillium* Are. Aspergillus are often sugar-loving or at least sugar-tolerant and cause spoilage of those food products that have a high concentration of sugar. In addition, osmophilic yeasts are among the most problematic microorganisms in concentrated sugar solutions, which in these tests*Saccharomyces*was observed. Also, due to contamination of dates with soil, there is a possibility of the presence of spore bearing bacteriaAs*Basilosaha* And*Clostridium*It is very abundant in dates. Among the non-sporing bacteria, bacteria*Lukonostok* And*Lactobacilli* cause corruption of this product [33] which was not observed in this test. Ahmed and Robinson [34] observed that mold*Aspergillus parasiticus*It is able to grow on the surface of date extract and therefore causes the synthesis of aflatoxin. The amount of aflatoxin synthesized depends on the content of sugar (glucose and fructose). Therefore, due care should be taken while processing dates to produce juice and similar products, because dates are usually produced and stored in warm conditions, which provide the necessary conditions for the growth of mold and the production of aflatoxin.

3-3-3-Biochemical tests to determine Bacillus species

First, bacteria grown in PCA environments andOSA was examined in terms of gram and spore staining, and the results showed that the samples were gram-positive and spore-bearing bacilli. Based on the biochemical tests of the indicator bacteria isolated from the date juice sample*Bacillus lychniformis*[10] was detected and its results are given in Table 3.

[10] -*Bacillus Licheniformis*

Table 3 Confirmatory biochemical tests of *B. Licheniformis*

Test type	Catalase	VP	Growth in anaerobic agar	Growth at 50◦C	Growth in 7% NaCl
B.licheniformis	+	+	+	+	+

After passing syrup 1 through a fabric filter and producing syrup2, the microbial load decreased significantly, while the microbial load of syrup 2 increased significantly after passing through the filter press (sorbet 3), and the dominant microflora at this stage is related to yeast, the majority of which are probably yeast.*Saccharomyces* Forms. As it was said, the increase in the microbial load after the filter press stage can be attributed to the contamination of the filter press cloths with the syrup from the previous production shifts and the remaining yeast in it, as a result of which the syrup entering this part is contaminated with yeast and after exiting Yeast increases from the filter press. Examples ofThe sap has a significant amount of bacillus, which is probably based on biochemical testsThis type of bacillus*Bacillus Lychni formis* is shown in Table 3. In general, dates and its derived products are mostly fermented by osmophilic yeasts (*Saccharomyces cerevisiae*), spoilage molds like*Aspergillus niger* and bacillus bacteria in particular

Bacillus Lychni formis They get infected. bacteria*Bacillus Lychni formis* It is found abundantly in the soil and is able to form colonies in all parts of the body such as the skin and intestines. Although this microorganism is not pathogenic for humans, its presence in the product causes it to sour and reduce the quality of the juice [35]. Since the use of heat treatment is limited due to the darkening of the juice and the negative effects on its sensory properties to make the product healthy, the production of a healthy and quality product has become a serious problem for the juice producers. Based on the obtained results, date juice showed the lowest microbial load, which is much lower than dates. Considering that Bacillus bacteria is not pathogenic and only leads to product spoilage and based on its significant reduction in the process of turning dates into juice, producing juice seems to be a suitable method for preserving dates and using dates in the production and formulation of foods. It is beneficial to use non-thermal methods to increase the shelf life of the product.

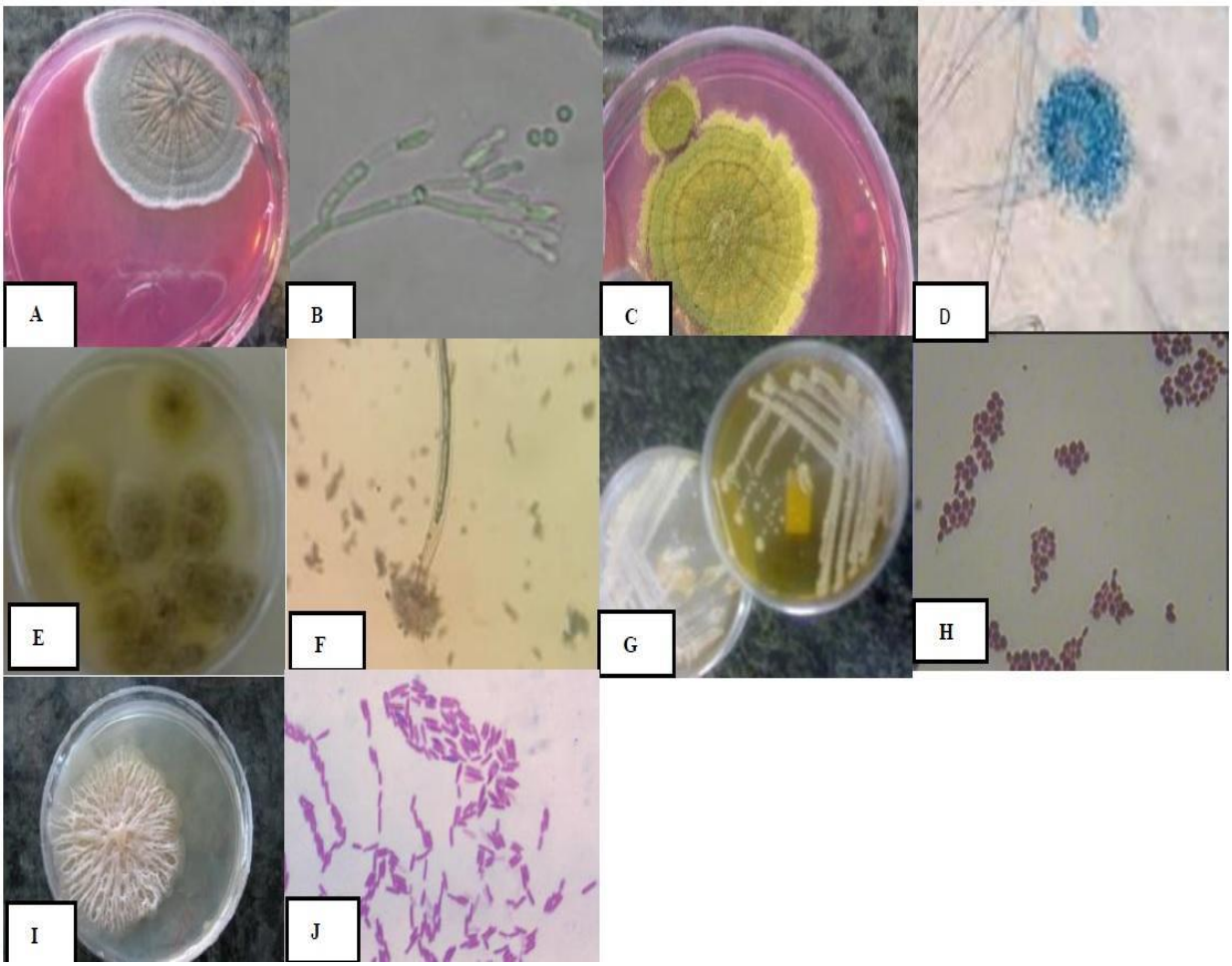

Fig 8 A: Macroscopic, and B: microscopic images of *Penicillium*, C & E: macroscopic, and D & F: microscopic images of *Aspergillus* , G subculture and H: microscopic images of *Saccharomyces*, I**:** macroscopic, and J: Gram stain images of *B. licheniformis*.

4 - Conclusion

usingDue to the high content of rejuvenating sugars and the low amount of sucrose instead of sugar, dates and its products help to maintain the health of society and the formulation of sweet foods for diabetic patients. In this study, the chemical and microbial characteristics of the date juice product were investigated from the beginning of the date entering the production line until its conversion into juice. Date polyphenol compounds are very active in terms of antioxidant and anti-radical properties and these propertiesIt is directly related to the content of phenolic compounds. Comparing two samples of industrial juice with traditional juice, higher content of polyphenolic compounds and higher antioxidant activity for produced juice compared to traditional juice showed that the reason for the better preservation of bioactive compounds in industrial juice is attributed to applying a milder heat process in it. The results of the microbial test showed a decrease in the amount of microbial load in different stages of production from the raw material to the final

product, so that date juice has very little microbial load inComparison with other samples of dates and syrups 1, 2 and 3 showed. The results of morphological investigation showed that the dominant microbial flora in dates probablyincluding types of mold, yeast and bacillus bacteria; In the syrup, mold and yeast and in the juice in the majority Examples are Bacillus bacteria. However, due to the possibility of the presence of spore-bearing bacteria, especially bacteria*Bacillus Lychni formis* In the final product, it seems necessary to find a suitable solution to make the final product healthy without affecting the sensory characteristics of the product. In the end, it can be concluded that the production of date juice from Grade 3 dates are a suitable solution for using low quality dates and increase the added value of agricultural products. Based on the findings of ShireDue to its high regenerative sugar content, dates are sweeter than other sources, while being within the acceptable range of the glycemic index for the formulation of practical foods, especially for diabetic patients, due to the content of polyphenolic compounds and antioxidant activity and high fiber. It is considered a suitable option. However, in order to use it in the formulation of beneficial foods, other non-thermal sanitization methods can be usedand in this way caused the least effect on the sensory properties of the product.

5- Thanks and appreciation

The authors would like to thank Shirin Toshe Baharan Company (Bahar, Hamadan) for providing them with sufficient facilities to carry out this research.

6- Attachment

Table S1. Simplified table of biochemical tests to identify *Bacillus* species:

	Test type	Result	Test path
1	Catalase	positive	2
		negative	17
2	Vosges-Proskauer	positive	3
		negative	10
3	Growth in anaerobic agar	positive	4
		negative	9
4	Growth at 50°C	positive	5
		negative	6
5	Growth in 7% NaCl	positive	*B. licheniformis*
		negative	*B. coagulans*
6	Acid and gas from glucose	positive	*B. polymyxa*
		negative	7
7	Reduction of NO_3 to NO_2	positive	8
		negative	*B. riverbeds*
8	Parasporal body in sporangium	positive	*B. thuringiensis*
		negative	*B. cereus*
9	Hydrolysis of starch	positive	*B. subtilis*
		negative	*B. pumilus*
10	Growth at 65°C	positive	*B. stearothermophilus*
		negative	11
11	Hydrolysis of starch	positive	12
		negative	15
12	Acid and gas from glucose (inorganic N)	positive	*B. macerans*
		negative	13
13	Width of rod 1.0µm or greater	positive	*B. megaterium*
		negative	14
14	pH in V-P broth <6.0	positive	*B. circulans*
		negative	*B. firm*
15	Growth in anaerobic agar	positive	*B. laterosporus*
		negative	16
16	Acid from glucose (inorganic N)	positive	*B. brevis*
		negative	*B. spherical*
17	Growth at 65°C	positive	*B. stearothermophilus*
		negative	18
18	Decomposition of casein	positive	*B. larvae*
		negative	19
19	Parasporal body in sporangium	positive	*B. nipples*
		negative	*B. lentimorbus*

7- References

[1] Ramard Ghadiri, Gh., & Kalbasi Ashtari, A. (2012). Investigating the chemical and microbial characteristics of apple Lavashak.*Iranian Journal of Nutrition Sciences & Food Technology*, 7(4), *324-329.*

[2] Vaezi zadeh, M., Ghazanfari moghadam, A., & Fooladi, M. H. (2011). An Investigation and Modeling of Sugar Reduction, Bioethanol and Carbon Dioxide Production during Fermentation of Date Fruits. *Iranian Journal of Biosystem Engineering*, 41(2), 121-246.

[3] FAO. 2012. Available at: http: // www.Fao.org.[4] Al-Rawahi AS, Kasapis S, & Al-Bulushi I. M. (2005). Development of a date confectionary: Part 1 relating formulation to instrumental texture. *International J of food properties*, 8, 457–468.

[4] Al-Rawahi AS, Kasapis S, & Al-Bulushi I. M. (2005). Development of a date confectionary: Part 1 relating formulation to instrumental texture. *International J of food properties*, 8, 457–468.

[5] Sanchez Zapata E, Fernandez Lopez J, Penaranda M, Fuentes Zaragoza E, Sendra E, Sayas E, & Perez Alvarez J. A. (2011). Technological properties of date paste obtained from date by-products and its effect on the quality of a cooked meat product. *Food Research International*, 44, 7, 2401-2407.

[6] Abarc, M. L, Bragulat, M. R, Castella, G., & Cabanes F. J. (1994). Mycoflora and aflatoxin producing strains in animal mixed feeds. *Journal of food portection*, 57, 256-258.

[7] Al Farsi, M. (2003). Clarification of date juice. *International Journal of Food Science*, 38, 241–245.

[8] Tavakolipour, H., & Kalbasi Ashtari, A. (2013). Determination of rheological properties of grape molasses. *Journal of Food Science and Technology*, 40 (10), 129-137.

[9] Noshirvani, N., Fasihi, H., Nourmohammadi, E., & Moradipayam, A. (2017). Study on the antioxidant and antifungal activities of extract and pulp of date (*Phoenix dactylifera* L.) by-products. *Iranian Journal of Nutrition Sciences & Food Technology*, *12*(3), 77-88.

[10] Abbes, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G.)2013(. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Industrial Crops and Products*, 44. 634-642.

[11] Sun, T., Powers, J. R., & Tang, J. (2007). Loss of rutin and antioxidant activity of asparagus juice caused by pectolytic enzyme preparation from *Aspergillus niger*. Food Chemistry, 105, 173–178.

[12] Gad, A. S., Kholif, A. M., & Sayed, A. F. (2010). Evaluation of nutritional value of functional yogurt resulting from combination of date syrup and skim milk. *American Journal of Food Technology*, 5(4), 250-259.

[13] Homayouni Rad, A., Vaghefmehrabany, L., Vaghefmehrabany, E., & Javadi, M. (2013). Glycaemic index and load of dietetic sour cherry jam prepared with date syrup in healthy persons. *Journal of Kerman University of Medical Sciences*, 20(2), 193.

[14] Gouhari Ardebili, A., Habibi Najafi, M. B., & Hadad Khodaparast, M. (2005). Effect of date syrup as a substitute for sugar on the physicochemical and sensory properties of soft ice cream. *Iranian Food Science and Technology Research Journal*, 1(2), 23-32.

[15] Mesbahi Gh, Mansour H, Majzoobi M, & Farahnaky A. (2016). Effect of sucrose replacement with date syrup and date liquid sugar on rheological properties of biscuit dough. *Journal of Food Science and Technology*, 58 (13), 45-55.

[16] Karazahyan R, Ehtiati A, Nazari Z, & Mehraban Sanghatash, M. (2021). Optimizing the formulation of functional cake with date syrup and inulin. *Journal of Food Science and Technology*, 111 (18), 147-157.

[17] Ehteshami Moein Abadi, M. J., Khodaparast, M., & Habibi Najafi, M. B. (2003). Investigation of the level of contaminants in traditionally grape syrup. *Agriculture*, 5(1), 1-6.

[18] Kabkab date-Specifications and test methods, Iranian National Standardization Organization. INSO 803, 4st. Edition, 2022.

[19] AOAC. (1990). Official methods of analysis. Association of official analytical chemists, 15thed. University of Alabama. Washington DC

[20] Date syrup-specifications and tests methods, Iranian National Standardization Organization. INSO 5075, 1st. Edition, Nov. (2013).

[21] Noshirvani, N., Fasihi, H., & Moradipayam, A. (2015). Study on the antioxidant effects of extract and powder of green walnut hulls on the oxidation of sunflower oil. *Iranian Journal of Nutrition Sciences & Food Technology*, *10*(3), 79-90.

[22] Dworkin, M. (2006). The prokaryotes: a handbook on the biology of bacteria (Vol. 7). S. Falkow, E. Rosenberg, E. Stackebrandt, & K. H. Schleifer (Eds.). New York, NY: Springer.

[23] Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique, Iranian National Standardization Organization. INSO 5272-1, 1st. Edition, (2015).

[24] Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 2: Colony count technique in products with water activity less than or equal to 0.95, Iranian National Standardization Organization. ISIRI 10899-2, 1st. Edition, (2008).

[25] Microbiology of Canned foods- Commercial sterility- Specifications and test methods, Iranian National Standardization Organization, INSO 2326, 4st. Edition, (2016).

[26] Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique, National Standardization Organization, ISIRI 9263, 1st. Edition, (2007).

[27] Al-Hooti, S. N., Sidhu, J. S., Al-Saqer, J. M., & Al-Othman, A. (2002). Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. *Food chemistry*, 79(2), 215-220.

[28] Raiesi Ardali., F., Rahimi, E., Tahery, S., & Shariati, M. A. (2014). Production of a new drink by using date syrup and milk. *Journal of Food Biosciences and Technology*, 4(2), 67-72.

[29] Mahdi, Z. I., El-Sharnouby, G. A., & Sharoba, A. (2022). Physicochemical Properties and Microbiological Quality of Dates Syrup Prepared from some Egyptian and Iraqi Dates Palm (*Phoenix dactylifera* L.) Fruits.*Egyptian Journal of Chemistry*, 65(13), 175-184.

[30] Farahnaky, A., Mardani, M., Mesbahi, G., Majzoobi, M., & Golmakani M. T. (2018). Some physicochemical properties of date syrup, concentrate, and liquid sugar in comparison with sucrose solutions. *Journal of Agricultural Science and Technology*, 18, 657-668.

[31] Charia, N., Smaali, M. I., Martinez Tome, M., Mrabet, A., Murcia, M. A., & Ferchichi, A. (2009). Simple phenolic composition, flavonoid contents and antioxidant capacities in water-methanol extracts of Tunisian common date cultivars (Phoenixdactylifera L.). *International Journal of Food Sciences and Nutrition*, 60, s7, 316-329.

[32] Taleb, H., Maddocks, S. E., Morris, R. K., & Kanekanian, A. D. (2016). The antibacterial activity of date syrup polyphenols against *S. aureus* and *E. coli*. *Frontiers in microbiology*, 7, 198.

[33] Kim, Se., & Rajapakse, N. (2005). Enzyme production and biological activities of chitosan oligosaccharides (COS): A review. *Carbohydrate Polymers Science direct*, 62, 357-368.

[34] Ahmed, I. A., & Robinson, R. K. (1999). The ability of date extracts to support the production of aflatoxins. *Food Chemistry*, 66, 3, 307-312.

[35] Heshmati, A., Azizi, M., Ghadimi, S. (2017). Influence of Ethanol and Methanol Extracts of Walnut Leaf and Green Hull on *Saccharomyces cerevisiae*, *Bacillus Licheniformis* and *Aspergillus niger* in Date Syrup. *Iranian Journal of Nutrition Sciences & Food Technology*, 11 (4) ,81-88.

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چکیده

هدف از این پژوهش بررسی ویژگی‌های شیمیایی و میکروبی شیره خرما طی مراحل مختلف تولید از ماده اولیه تا محصول نهایی می‌باشد. مقادیر ماده جامد محلول، pH، اسیدیته، قند کل و احیاء کننده، محتوای ترکیبات پلی فنولی، فعالیت آنتی اکسیدانی و شمارش میکروبی اندازه گیری شده و تعیین نوع میکروارگانیسم‌ها بر اساس ویژگی‌های فنوتیپی در مراحل مختلف تولید انجام شد. نتایج بدست آمده نشان داد طی مراحل شفاف سازی و تغلیظ شربت به تدریج pH کاهش و اسیدیته، قند کل، قند احیاء کننده و ماده جامد کل افزایش یافت و در شیره به حداکثر خود رسید. مقایسه شیره سنتی با شیره تولیدی کارخانه pH بیشتر و اسیدیته، قند کل و احیاء کننده، ماده جامد کل، ترکیبات پلی فنولی و فعالیت آنتی اکسیدانی کمتری برای شیره سنتی نشان داد. به طور کلی محتوای ترکیبات پلی فنولی و فعالیت آنتی اکسیدانی در مراحل مختلف تولید به تدریج کاهش یافت به طوری که شیره خرما به طور معنی داری محتوای ترکیبات پلی فنولی و فعالیت آنتی اکسیدانی کمتری (۲۷۹ میلی گرم اسید گالیک بر ۱۰۰ گرم نمونه و ۳۳٪) نسبت به خرما (۳۸۸ میلی گرم اسید گالیک بر ۱۰۰ گرم نمونه و ۵۷٪) نشان داد. نتایج آزمون میکروبی کمترین بار میکروبی برای شیره تولیدی در مقایسه با خرما و شربت‌های مختلف را نشان داد. هر چند حضور *باسیلوس لیچنی فورمیس* در شیره احتمال ترش شدن فرآورده را فراهم می آورد و یافتن راهکاری مناسب برای افزایش مدت زمان ماندگاری محصول در این خصوص ضروری به نظر می‌رسد. شیره سنتی بار میکروبی کمتری نسبت به شیره تولیدی نشان داد هر چند باکتری باسیلوس *لیچنی فورمیس* در شیره سنتی با وجود تیمار حرارتی بالا نیز مشاهده شد. بر اساس نتایج بدست آمده شیره خرما عاری از کلی فرم بود که بیانگر حفظ اصول بهداشتی حین تولید می باشد.