Journal of
Food Biosciences and Technology

ISSN 2228-7086
e-ISSN 2423-8163

Vol. 14, No. 2, 2024

Science and Research Branch, Islamic Azad University

Journal of Food Biosciences and Technology

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CONTENTS

Vol. 14, No. 2, 2024

Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 1-16, 2024

Optimization of the Ultrasound-Assisted Extraction Process of Aspartic Acid from Molasses and its Anti-scaling Capability in Sugar Industry Evaporators

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Received: 17 November 2023 Accepted: 23 April 2024

ABSTRACT: This study aimed to optimize the extraction of aspartic acid from sugar beet molasses using an ultrasound-assisted extraction method and its use as a green anti-scaling agent in the evaporator tubes of the sugar industry. The results of ultrasound-assisted extraction showed that the linear model is the best model to describe the behavior of aspartic acid extraction. It was determined that the optimal conditions for extracting aspartic acid using the ultrasound-assisted method include an extraction temperature of 25.09 °C, pH of 7.00, ultrasound power of 69.99%, and no ethanol. The extracted aspartic acid under optimal conditions was applied with various concentrations (10, 25, and 50 mg/100g) and temperatures (60, 90, and 120 °C) on the scales of the sugar industry evaporators. The highest anti-scaling efficiency for all three processes was related to the treatment performed at 90 °C with a concentration of 50 mg/100 g. Field emission scanning electron microscopy images showed that by increasing temperature up to 90 °C and increasing concentration up to 50 mg/100g, the scales formed on the evaporator tube changed from crystalline and uniform state to porous with fine particles. Energy dispersive spectroscopy analysis showed that by increasing the temperature to 90 °C and increasing the concentration to 50 mg/100g, the calcium and silica content in the scales of the evaporator tubes decreases. Fourier-transform infrared spectroscopy analysis showed that by applying aspartic acid as an anti-scaling of stable crystals, the scales become smaller and more unstable crystals.

Keywords: Aspartic Acid, Green Anti-scaling, Molasses, Optimization, Ultrasound-Assisted Extraction.

Introduction

The sediments deposited on the evaporator tube during the concentration of syrup in the sugar industry act as a limiting factor for further concentration of the syrup. These sediments prevent further concentration of raw syrup by reducing the heat transfer coefficient (Madaeni *et al.*, 2004). Mineral salts that are present in raw syrup and have low solubility are deposited on the evaporator walls as a result of the cooking process of the syrup and will lead to a decrease in the heat transfer coefficient (Djordjević *et al.*, 2018). Silicon, iron, sulfur, phosphorus, and calcium are the most important

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elements in the sediments of the evaporator walls, which reduce the thermal efficiency of the stove bodies and syrup heaters (Smith & Taylor, 1981). However, the most negative effect of these deposits is on the evaporator walls, leading to the formation of hard scales, and their composition depends on the nature of the primary syrup. There are currently several methods (chemical and physical) of descaling that lead to damage to evaporators (Coombs, 1986).

Since the formation of sediments on the evaporator walls is an inevitable thing, sodium hydroxide (caustic soda) is used industrially to solve this problem, which will lead to the softening of the sediments. Therefore, after softening the sediments, water jets are used to remove the resulting sediments (Drennan *et al*., 1995; Phakam *et al*., 2018). However, the main problem related to the physical and chemical removal of sediments from the tubes of the evaporators is to provide conditions for the corrosion of the tubes. Although the corrosion of evaporators and cooking bodies leads to significant economic costs, using anti-scaling chemicals has many negative effects on human health (Ghosh & Balakrishnan, 2003).

Therefore, researchers recommend the use of plant metabolites with a green nature and safe methods of extraction to reduce the adverse effects of chemical compounds. Plant metabolites are biodegradable and non-toxic compounds that can be used as natural sediment inhibitors. The lack of biological accumulation of plant metabolites qualifies their use as natural anti-scaling agents (Chaussemier *et al*., 2015).

By processing sugar beet and extracting sugar from it, molasses is produced as waste, which can be used in various industries as a cheap source of bioactive compounds with unique properties. By extracting these bioactive compounds from molasses, products with high added value can be produced that can be used for different purposes (Varaee *et al*., 2019). In our previous study, it was proved that the aspartic acid extracted from molasses is able to remove scales from the surface of the evaporator tubes. In the last research, aspartic acid was extracted from molasses using a microwave-assisted process and applied to the scales of evaporator tubes under different conditions. The results showed that aspartic acid can transform scales with stable crystals into unstable crystals, which will lead to the formation of porous and fine sediments (Mokhtarian *et al*., 2022).

Traditional common methods for extracting and separating products have disadvantages. In centrifugation and sedimentation, the size and density of the material are involved. Therefore, optimal separability is not achieved. The energy cost is very high, especially for viscous solutions. Chromatographic methods such as column chromatography or high-pressure liquid chromatography, although specific, can transfer only a small part of the feed (Aguilar & Rito-Palomares, 2010). In recent years, the use of ultrasound as a new method with its advantages, high efficiency, convenience, and low maintenance costs has gained an important place in the food industry.

The term ultrasound refers to sound waves whose frequency is higher than the range of human hearing (about 20 Hz to 20 kHz). When the liquid medium is affected by ultrasonic radiation, microbubbles form, grow, and oscillate rapidly and finally explode with full force (if the sound pressure is high enough). The bubbles created inside the media are the cavitation phenomenon, which is responsible for the effect of ultrasound. When the size of the bubbles reaches a

critical point, they explode during the compression cycle, releasing a large amount of energy. The temperature and pressure at the moment of explosion are estimated to be around 5000 K and 5000 atmospheres. The creation of these hot spots can significantly accelerate the speed of chemical reactions in the desired media (Kumar *et al*., 2021). Therefore, this study not only aims to optimize aspartic acid extraction from sugar beet molasses using the ultrasound-assisted method but also to explore its potential use as a natural anti-scaling agent in the sugar industry evaporators.

Materials and Methods

Aspartic acid standard, methanol, acetic acid, and HPLC grade acetonitrile were purchased from Merck Co. Darmstadt, Germany. Molasses was obtained from Hegmatane Sugar Company, Hamadan, Iran.

- Ultrasound-assisted extraction

In order to extract aspartic acid from sugar beet molasses according to the method proposed by Patil *et al*. (2021) with some modifications in the ultrasound-assisted extraction process. Sugar beet molasses was extracted by the response surface method and the Box-Benken design. The variables include the concentration of ethanol (0, 25, and 50 % v/v), temperature (25, 40, and 55 °C), pH (3, 5, and 7), and ultrasonic power (30, 50, and 70 % of 20 kHz) (Table 1). In order to carry out the object, an ultrasound machine (Bandelin - AMMM - M.P.I. Germany) consisting of a 20 kHz ultrasound generator and a cylindrical probe was used for 15 min. Molasses samples were mixed with water and water/ethanol at a ratio of 1:30, and the desired pHs were adjusted by 0.1 N HCl and 0.1 N NaOH. After adjusting the pH of the solution, the probe of the ultrasonic device was placed inside the samples, and the extraction operation was performed using different ultrasonic ranges. It should be noted that the thermal jacket system connected to the device was used to adjust the extraction temperature (Kumar *et al*., 2021).

- Determination of aspartic acid content

High performance liquid chromatography (HPLC) was used to determine the aspartic acid content in the samples extracted from sugar beet molasses. To determine the inhibition time of aspartic acid, the aspartic acid standard was injected into the device separately. Then the samples extracted from sugar beet molasses were injected into the HPLC device. During this research, an HPLC device equipped with a fluorescence detector was used. HPLC analysis was performed with the SGE Hypersil ODS C18 column on a laboratory scale (250 × 4.6 mm i.d.) and a particle size of 5 µm. HPLC conditions were as follows: mobile phase A, ammonium phosphate (pH 6.5) 30 mM in methanol with 85:15 ratio v/v; water; mobile phase B, 15: 85 v/v methanol; water; mobile phase C, 90: 10 v/v acetonitrile; water. The washing gradient (min/A%/B%/C%) was as follows: 13:63:0.24, 46:43:11.32, 46:43:11.34, 100:0:0.05 /34, 100: 0: 0/5/36, 13: 63: 24/55/36, 13: 63: 24/50. The flow rate was constant and equal to 1.1 ml/min and the ambient temperature was maintained at 38 °C. Finally, the extraction yield was calculated using the following equation (Sánchez-Machado *et al*., 2010).

Extraction Yield (% w/w) = [Weight of extracted aspartic acid (mg) ÷ Weight of molasses (g)] × 100

Table 1. RSM design related to different levels of ultrasound-assisted extraction process variables and response (extraction yield)

Run	Ethanol (%)	Temperature (°C)	pH	Power (%)	Extraction Yield (mg/100 g sample)
1	25	55	7	70	52.33
2	25	25	5	50	56.61
3	50	40	3	30	48.41
4	25	55	5	30	55.68
5	25	25	5	70	55.67
6	25	40	3	50	61.57
7	25	25	3	50	45.2
8	0	55	5	50	64.02
9	50	40	7	70	45.95
10	0	40	5	50	49.61
11	50	25	5	50	42.03
12	25	40	5	50	59.34
13	0	40	7	30	52.76
14	25	40	7	30	61.09
15	50	40	5	50	43.38
16	25	40	5	50	45.38
17	25	25	7	70	62.61
18	25	40	7	30	61.16
19	0	40	5	70	44.06
20	25	55	5	50	57.92
21	0	40	3	70	52.6
22	50	40	5	50	62.5
23	0	25	5	50	57.73
24	50	55	5	30	44.74
25	25	40	3	50	44.57
26	25	55	3	50	40.02
27	25	40	5	50	59.54

- **Anti-scaling activity of extracted aspartic acid**

To investigate the anti-scaling activity of aspartic acid extracted from sugar beet molasses under optimal conditions of three different concentrations of aspartic acid solution (10, 25, and 50 mg/l) with three different temperatures of 60, 90, and 120 °C at pH equal to 8 was applied on the evaporator tube of the sugar industry for 6 h.

- **Anti-scaling efficiency**

To check the efficiency of anti-scaling, the difference in the weight of scales on the evaporator tubes before and after anti-scaling was evaluated. For this purpose, sugar industry evaporator tubes were cut with the same dimensions (length 1.5 cm) and the scales deposited on the primary pipes were gently scraped and completely collected. The weight of the scraped scales before anti-scaling was recorded as the initial weight. Then the intact pipes were subjected to different treatments of anti-scaling operations and the weight of the scales after descaling was recorded as secondary weight. Finally, the anti-scaling efficiency is calculated with the obtained weight difference (Jampílek *et al*., 2019).

- **Morphological characteristics and Energy dispersive spectroscopy analysis (EDS) analysis**

Field emission electron microscope (FESEM; MIRA3, TESCAN, Czech Republic) is used to investigate the microstructural characteristics of scales

before and after applying aspartic acid with different concentrations and different temperatures (Rezaeinia, Ghorani, *et al*., 2020). Before analysis, the scales were coated with a thin layer of gold by a sputter coater (K-450X, EMITECH, England) for 150 s at 20 mA. Also, for elemental analysis and ensuring the change of composition of elements after applying aspartic acid as an anti-scaling agent, FESEM equipped with EDS was used (Xie *et al*., 2020).

- **Fourier transform infrared spectroscopic analysis**

FTIR device (Bruker Alpha FTIR, US) was used to investigate the possibility of reaction between different compounds of scales and aspartic acid as well as change the type of bonds. The FTIR spectrum of each of the samples was obtained in the wave number range of 4000 to 400 cm^{-1} (Rezaeinia, Emadzadeh, *et al*., 2020).

- **Design of experiments and statistical analysis**

To investigate the effect of each of the variables of the ultrasound-assisted extraction process, i.e., concentration of ethanol, temperature, pH, and ultrasonic power, on the extraction efficiency, Box-Benken and response surface methodology were used by Design Expert 10.0 software. In addition, the comparison of average data was made by one-way ANOVA using Duncan's multiple range test and by IBM SPSS statistics software (version 22.0, IBM Corp., USA) at a probability level of 5%.

Results and Discussion

- **Ultrasound-assisted extraction**

The ultrasound-assisted extraction process was used to extract aspartic acid from molasses. For this purpose, the parameters of ethanol concentration (0, 25, and 50% v/v), temperature (25, 40, and 55 °C), pH (3, 5, and 7), and ultrasound power (30, 50, and 70%) were used. Aspartic acid extracted from each sample was analyzed using HPLC and its amount was determined (Table 1). The obtained results showed that the linear model (Eq. 1) between the data is the best model to describe the behavior of aspartic acid extraction from sugar beet molasses.

$$Y_i = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 \quad \text{Eq.1}$$

Where, Y_i is the dependent variable (Y_1: aspartic acid extraction efficiency), A_0 is the response index fitted at the center point of the plot which is equivalent to the point (0, 0, 0), A_1, A_2, and A_3 are linear coefficients, and X are independent variables (X_1: the concentration of ethanol, X_2: extraction temperature, X_3: solvent pH, and X_4: ultrasound power). The significance test was performed based on the total error at the 95% confidence level. The efficiency of the model was calculated and evaluated by R^2 and adjusted R^2.

The results of this study to investigate the effect of extraction process conditions (ethanol concentration, temperature, pH, and ultrasonic range) on the efficiency of aspartic acid extraction from sugar beet molasses are presented in the form of two-dimensional images (Figure 1). The results of analyzing the variance of the data (ANOA) related to each answer as well as the coefficients of the regression model of the first order equation for the extraction efficiency of aspartic acid are shown in Tables 2 and 3, respectively.

Based on the results obtained from data variance analysis and ANOA table, it was found that the extraction efficiency of aspartic acid is significantly (p<0.05) influenced by the ultrasound-assisted extraction (ethanol concentration, temperature, pH, and ultrasound power).

Therefore, as shown in Table 2, the extraction efficiency of aspartic acid is significantly (p<0.05) influenced by the concentration of ethanol used as a solvent, pH and ultrasonic power, but the extraction efficiency is significantly (p<0.05) was not affected by process temperature (Table 2). Also, the data dispersion and their normality compared to the proposed model are shown in Figure 2, which shows the appropriate data dispersion and their normality. Figure 1 shows the effect of ultrasound-assisted extraction process variables on the recovery of aspartic acid from molasses in two dimensions plan. As shown in Figure 1, with the increase of extraction temperature from 25 to 55 °C, the extraction efficiency of aspartic acid from molasses decreases, but this decrease is not statistically significant (p<0.05). However, increasing the concentration of ethanol from 0 to 50% v/v significantly (p<0.05) led to a decrease in the extraction efficiency of aspartic acid from molasses. It was also found that increasing the pH from 3 to 7 and increasing the ultrasonic power from 30 to 70% significantly (p<0.05) increases the recovery of aspartic acid from molasses by the ultrasound-assisted method. The increase in the extraction efficiency of aspartic acid from molasses using the ultrasound-assisted extraction process as a result of increasing the pH from 3 to 7 and also increasing the ultrasound power from 30 to 70% can be attributed to the increase in the solubility of aspartic acid as a result of the ultrasound-assisted extraction process. Based on the studies, it has been determined that amino acids and proteins have higher solubility in the pH range higher or lower than their isoelectric pH. Because aspartic acid has an isoelectric pH of about 3, therefore, by increasing the pH of the solvent from 3 to 7, because the pH of the solvent moves away from the isoelectric point of aspartic acid, therefore, the solubility of aspartic acid in the media increases and the efficiency of extracting aspartic acid from molasses using the ultrasonic extraction process is enhanced. It has also been found that increasing the ultrasonic power, due to the high energy it creates, improves the mass transfer process and causes more aspartic acid to penetrate the solvent from the molasses matrix, and as a result, the extraction efficiency will increase. In addition, studies have shown that aspartic acid has very little or insoluble solubility in alcohol (ethanol) and alcohol-based solvents. Therefore, it can be expected that by increasing the percentage of ethanol in the solvent to extract aspartic acid from molasses, the recovery efficiency of aspartic acid will decrease. Carrera *et al*. (2015), studied the optimization of amino acid extraction from grapes. Based on the results obtained by these researchers, it was found that by increasing the ultrasonic power and moving away from the isoelectric point of amino acids, the extraction efficiency of amino acids from grapes increases (Carrera *et al*., 2015).

Table 2. ANOA table related to the effect of ultrasound-assisted extraction process variables on the extraction efficiency of aspartic acid from sugar beet molasses

Source	Extraction Yield (mg/100 g)
Model	< 0.0041
A (Ethanol)	< 0.0478
B (Temperature)	0.3331
C (pH)	< 0.0032
D (Power)	0.0439
R^2	0.92
Adj-R^2	0.86
CV	10.75
Lack of Fit	0.9350 (not significant)

Table 3. Regression coefficients of linear equation for aspartic acid extraction by ultrasound-assisted extraction

Source	Yield (mg/100 g)
Intercept	52.38
A_1 (Ethanol)	-2.81
A_2 (Temperature)	-0.43
A_3 (pH)	3.63
A_4 (Power)	3.74

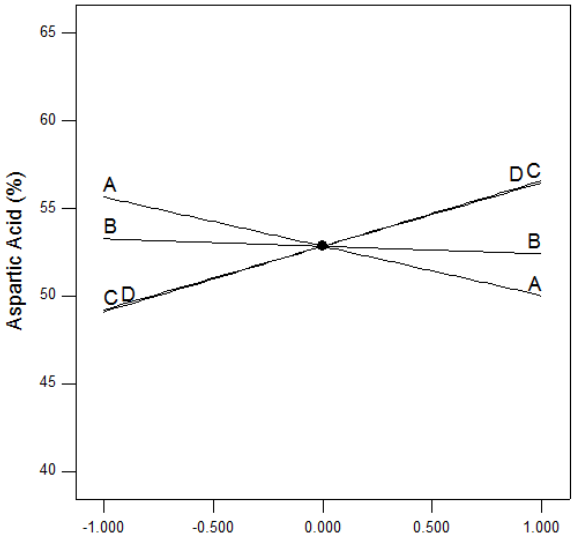

Fig. 1. Two-dimensional diagram of the effect of ultrasound-assisted extraction process variables on aspartic acid extraction (A: ethanol, B: temperature, C: pH, and D: power)

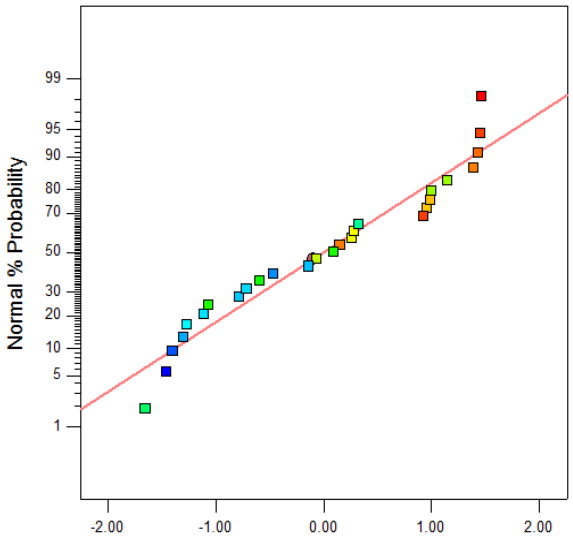

Fig. 2. Normal distribution diagram of aspartic acid extraction data in the ultrasound-assisted extraction

- **Optimization of aspartic acid extraction**

Based on the results obtained during this phase of the research and the effect of each process variable on the extraction efficiency of aspartic acid, the extraction conditions were optimized to maximize the extraction efficiency of aspartic acid. For this purpose, extraction of aspartic acid was performed by applying extraction conditions of ultrasound-assisted in three levels of ethanol as a solvent (from 0 to 50% v/v), three levels of temperature (25, 40, and 55 °C), three levels of pH (from 3 to 7), and three levels of ultrasonic range (from 30 to 70%) and the content of aspartic acid extracted from each molasses sample was evaluated using HPLC. According to the obtained results, the optimization was done based on the maximum efficiency of aspartic acid extraction, and the optimal conditions were selected based on the desirability function, and hence the limitations of the process for extracting aspartic acid from molasses samples are shown in Table 4. For this reason, the highest extraction efficiency of aspartic acid from sugar beet molasses was chosen as the optimization target, and the results of the optimization of the level of the variables are shown in Table 5 in the form of predicted data and experimental data. Therefore, it can be concluded that the optimal conditions for the highest extraction efficiency of aspartic acid from sugar beet molasses include 00.00 % ethanol, 25.09 °C temperature, 7.00 pH, and 69.99 % ultrasonic power (Table 5). Therefore, under the optimal conditions of ultrasound-assisted extraction mentioned above, the amount of aspartic acid extracted from beet molasses was 65.03 mg/100g (Table 5). The results obtained from the extraction efficiency of aspartic acid from beet molasses showed that there

is a very small difference between the experimental data and the predicted data from the regression model used in the RSM method. This indicates that the predicted model during this research to evaluate the data and optimal conditions for extracting aspartic acid from beet molasses with the highest efficiency is well fitted and the data is consistent with the predicted model. Sánchez-Zurano *et al*. (2020), studied the optimization of protein extraction from bacteria. Based on the results obtained by these researchers, it was found that there is not much difference between the experimental data and the predicted data. For this reason, these researchers stated that the proposed model for protein extraction from bacteria using the ultrasonic process fits well (Sánchez-Zurano *et al*., 2020).

- **Anti-scaling efficiency**

The results of the anti-scaling efficiency of aspartic acid extracted from molasses using the ultrasound-assisted extraction process with concentrations of 10, 25 and 50 mg/100g and different temperatures (60, 90 and 120 °C) on the sediments of sugar industry evaporators and comparing the average data based on

Table 4. Limitations of the optimization process of aspartic acid extraction from sugar beet molasses by the ultrasound-assisted extraction method

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Ethanol (%)	In range	0	50	1	1	3
Temperature (°C)	In range	25	55	1	1	3
pH	In range	3	7	1	1	3
Power (%)	In range	30	70	1	1	3
Yield (mg/100 g)	Maximize	40.02	64.02	1	1	3

Table 5. Optimal level of variables, predicted responses and experimental results for extraction of aspartic acid from sugar beet molasses by ultrasound-assisted extraction method

Variable	Optimal variable level	
Ethanol (%)	0.00	
Temperature (°C)	25.09	
pH	7.00	
Power (%)	69.99	
Response	Predicted value	Experimental value
Yield (mg/100 g)	63.44 ± 1.25^{a}	65.12 ± 1.16^{a}

* The same case showed no significant difference (p>0.05).

Table 6. Descaling efficiency of the solutions containing aspartic acid extracted from an optimum condition of the ultrasound-assisted extraction method

Treatment	Concentration (mg/L)	Temperature (°C)	Descaling efficiency (%)
1	10	60	24.24 ± 1.11^{i}
2	25	60	45.28 ± 1.21^{f}
3	50	60	70.38 ± 1.15^{b}
4	10	90	46.62 ± 1.18^{g}
5	25	90	55.49 ± 1.34^{d}
6	50	90	74.37 ± 1.42^{a}
7	10	120	29.50 ± 1.14^{h}
8	25	120	49.21 ± 1.08^{e}
9	50	120	65.34 ± 1.23^{c}

* Different letters indicate significant differences (p<0.05).

Duncan's multi-range test, it is shown in Table 6. Based on the results of data variance analysis, it was found that the anti-scaling efficiency is significantly (p<0.05) dependent on the amount of extracted aspartic acid and the process temperature. Therefore, as shown in Table 6, by increasing the concentration of aspartic acid from 10 to 50 mg/100g, the anti-scaling efficiency increases significantly (p<0.05) from 40 to 50%. It was also found that increasing the temperature of the anti-scaling process by aspartic acid extracted from molasses from 60 to 90 °C significantly (p<0.05) increased the anti-scaling efficiency. However, increasing the process temperature from 90 to 120 °C significantly (p<0.05) decreased the anti-scaling efficiency (Table 6). Aspartic acid, due to its active and reactive groups (amino group and acid group), has the ability to react with positively and negatively charged elements and ions in the structure of sediments, and in this way, it blocks the active sites of sediments for their accumulation, which leads to an increase in its anti-scaling efficiency (Chauhan *et al*., 2012). Therefore, it is obvious that by increasing the concentration of the anti-scaling agent, its ability to block the active sites of sediments to prevent the formation of crystals is blocked (Jafar Mazumder, 2020). Similarly, Hamdona *et al*. (2021) used lysine and glutamic acid as natural antifouling agents to prevent the formation of deposits. They stated that with the increase in the concentration of the antifouling agent, the efficiency of de-fouling increases, which they considered to be due to blocking the active sites of the deposits and preventing the formation of strong and compact crystals (Hamdona *et al*., 2021).

- **Morphological characteristics and EDS analysis**

The effect of using different concentrations of aspartic acid extracted from molasses using the ultrasound-assisted extraction process on the morphological characteristics of evaporator tube sediments at different temperatures was investigated. After applying 6 h of aspartic acid with concentrations of 10 to 50 mg/100g at temperatures of 60, 90, and 120 °C on the sediments of the evaporator tubes, the morphology of the sediments changed as shown in Figure 3. Based on the obtained results, it was found that the sediments of the reference sample have a uniform, regular and compact surface that has a crystalline state (Figure 3-Control). By applying aspartic acid extracted from molasses using an ultrasound-assisted process, the sediment morphology was transformed into a porous state and smaller particles (Figure 3.-1-9). By increasing the concentration of aspartic acid from 10 to 50 mg/100g, the sediments that were compact and crystalline (Figure 3-1, Figure 3-4, and Figure 3-7) turned into sediments with a porous structure and many pores, which were smaller in size (Figure 3-3, Figure 3-6, and Figure 3-9). In addition, changes in the temperature of the sedimentation process also changed the morphology of the sediments. Therefore by an increase in the temperature of the sedimentation process from 60 to 90 °C, the uniform and integrated structure of the sediments (Figure 3-1, Figure 3-2, and Figure 3-3) turned into a porous structure with smaller pieces (Figure 3-4, Figure 3-5, and Figure 3-6). However, increasing the temperature of the anti-scaling process from 90 to 120 °C reduced the porosity of the sediments and their particle size (Figure 3-7, Figure 3-8, and Figure 3-9). By reacting with the active sites of

sediments and blocking the active points of these sediments to prevent the accumulation and formation of sediments, anti-scaling agents disrupt the formation of crystals and as a result, sediments with a porous and fine structure are formed. By blocking the active points of sediments and the reaction of anti-scaling materials with the constituent elements of sediments, the growth of sediment crystals will be disrupted, which will be accompanied by a decrease in the size of sediments (Yu *et al*., 2019). Similarly, Khaled (2021), by using natural anti-scaling materials to prevent the formation of sediment, determined that by preventing the formation of sediment crystals and preventing their growth, anti-scaling materials cause the formation of small crystals that have a porous structure. This researcher considered the reason for this to be due to the reaction of sediment-forming elements with the active functional groups of the natural anti-scaling agent (Khaled, 2021).

The EDS analysis of the sediment samples treated with aspartic acid extracted with molasses was carried out to investigate the change in the composition of sediment constituents compared to the composition of control sediments (Table 7). The EDS analysis on the initial sediments of the evaporator tubes, which had not been subjected to any process, showed that oxygen, carbon, and calcium elements were the most abundant constituents of these sediments, which have values equal to 48.54%, 33.71%, and 16.08, respectively. In addition to these elements, small amounts of elements such as iron, silicon, potassium, and aluminum were observed in the structure of the control sediments, which had values equal to 0.54%, 0.52%, 0.4% and 0.21%, respectively. After using aspartic acid extracted from molasses with different concentrations and temperatures on the sediments on the surface of the evaporators, it was found that the composition of the elements forming the sediments was also dependent on the concentration and temperature of the process so the sediments treated with aspartic acid extracted with molasses contain calcium, iron, silica, potassium, and aluminum were less than the control sample. However, by applying aspartic acid to the sediments, the content of carbon and oxygen in them increased. Therefore, based on the results obtained from the EDS analysis of sediments, it was found that increasing the concentration of aspartic acid led to a decrease in the percentage of calcium and silicon elements

Table 7. Composition of elements (% w) of control sample scales in comparison with scales treated with aspartic acid extracted by the ultrasound-assisted extraction method (the codes on the table refer to the row numbers of Table 6)

Treatment	C	O	Al	Si	K	Ca	Fe
Control	33.71	48.54	0.21	0.52	0.4	16.08	0.54
1	37.12	42.52	0.18	0.17	0.18	15.54	0.84
2	35.36	45.44	0.17	0.32	0.19	13.24	1.84
3	39.41	43.52	0.18	0.17	0.29	10.94	1.62
4	35.65	44.09	0.15	0.13	0.34	14.78	1.02
5	38.43	45.34	0.21	0.19	0.24	11.41	1.28
6	41.37	41.12	0.16	0.16	0.22	9.22	1.12
7	37.15	42.66	0.12	0.14	0.20	15.33	1.28
8	38.12	44.45	0.13	0.14	0.25	12.38	1.14
9	37.42	45.37	0.12	0.15	0.30	11.45	2.07

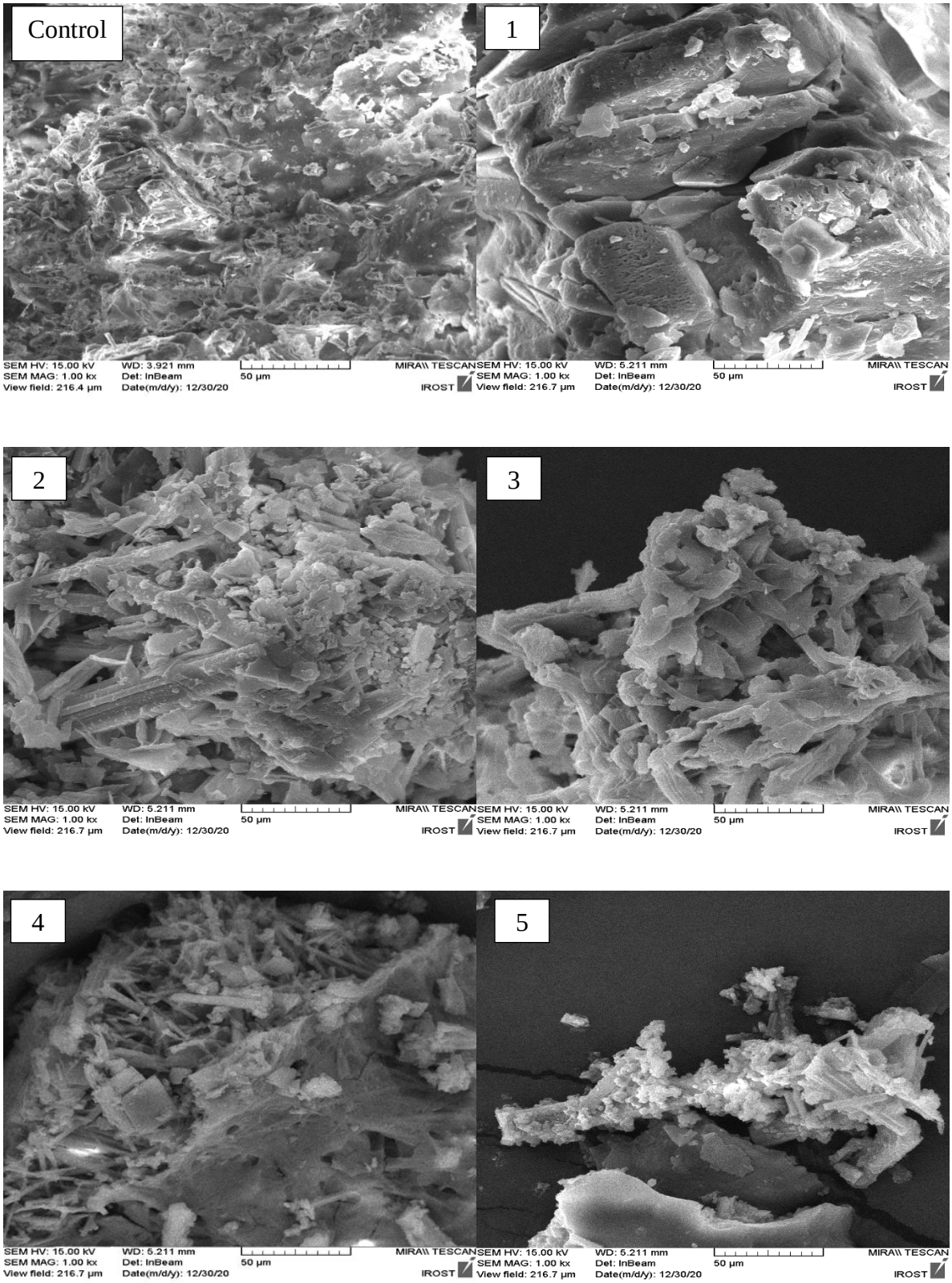

Fig. 3. The FESEM images of control sample scales compared to scales treated with aspartic acid extracted by the ultrasound-assisted extraction method (the codes on the table refer to the row numbers of Table 6) (*continued*).

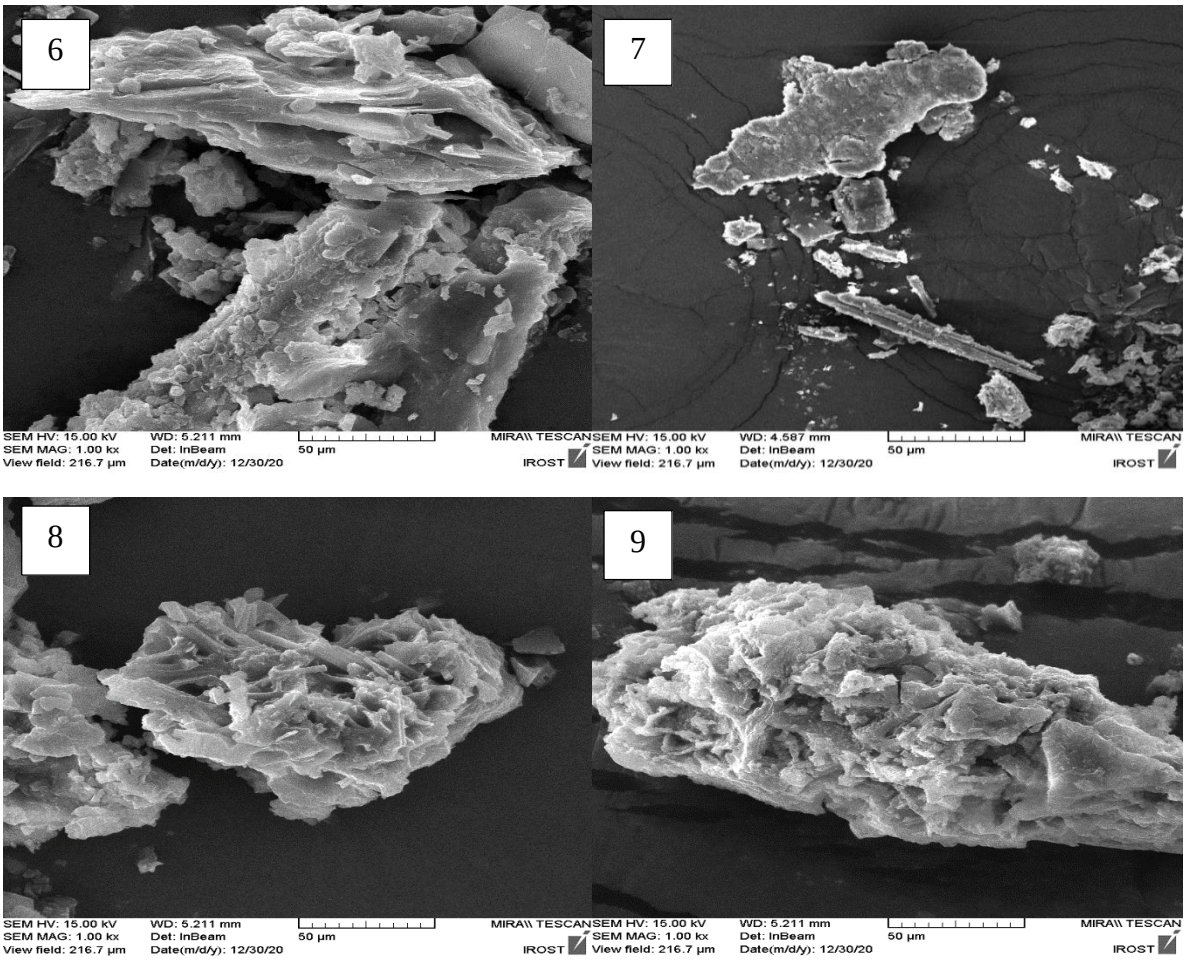

Fig. 3. The FESEM images of control sample scales compared to scales treated with aspartic acid extracted by the ultrasound-assisted extraction method (the codes on the table refer to the row numbers of (Table 6) (*continue*)

in the structure of sediments. By increasing the temperature of the anti-scaling process from 60 to 90 °C in the presence of aspartic acid as an anti-scaling agent, the content of calcium and silica decreased significantly, but with the increase of the anti-scaling temperature in the presence of aspartic acid from 90 to 120 °C, the amount of calcium and silica decreased. This is probably due to the reaction of aspartic acid with elements such as calcium and silicon. Aspartic acid can react with these elements through active functional groups and remove them from the reaction media by forming a complex between these substances. However, high temperatures (higher than 90 °C and up to 120 °C) may lead to the denaturation of aspartic acid and reduce its anti-scaling effect (Chen *et al.*, 2019).

–FTIR analysis

The FTIR spectrum of the primary sediments shows a broad absorption peak at 3392.4 cm^{-1}, which corresponds to the O-H stretching vibration (Figure 4). Also, in primary sediments, there are four specific peaks in the regions of 850.8 (out-of-plane vibrations CO_3^{-2}), 6.600 (in-plane vibrations O-C-O in calcium carbonate crystals ($CaCO_3$)), 1062.4 (Si-O symmetric stretching vibrations), and

1311.9 cm^{-1} (Si-O asymmetric bending vibrations) were observed (Figure 4). The addition of aspartic acid as an anti-scaling at different concentrations and temperatures caused a change in the location and intensity of the specific peaks of the primary sediments-by increasing the concentration and temperature of the descaling process, stable calcium carbonate crystals and silicon-containing crystals turned into unstable and weak crystals. Similarly, researchers have shown that by using Al_2O_3 as an anti-scaling agent, calcium carbonate sediments can be changed. This change is a change in the stable crystal of calcite and its transformation into unstable crystals of aragonite and vaterite (change in the position of the 700, 713, 853, and 1083 cm^{-1} peaks) (F. Wang *et al*., 2019). Also, the study showed that the anti-scaling of the landfill leachate piping system using polymer materials would change the sediment crystals in the range of 700 to 1600 cm^{-1}, which leads to an increase in descaling (Li *et al*., 2020).

Conclusion

Molasses is one of the main wastes of sugar factories, usually used to produce products with low added value. Therefore, using new extraction methods to extract its bioactive compounds can be a promising way to use these compounds. In this study, aspartic acid was first extracted from molasses as one of the bioactive components of molasses with the ability to be used as a natural anti-scaling by the ultrasound-assisted extraction method. Then, the extracted compound was used under optimal conditions as a natural anti-scaling agent on sugar industry evaporator

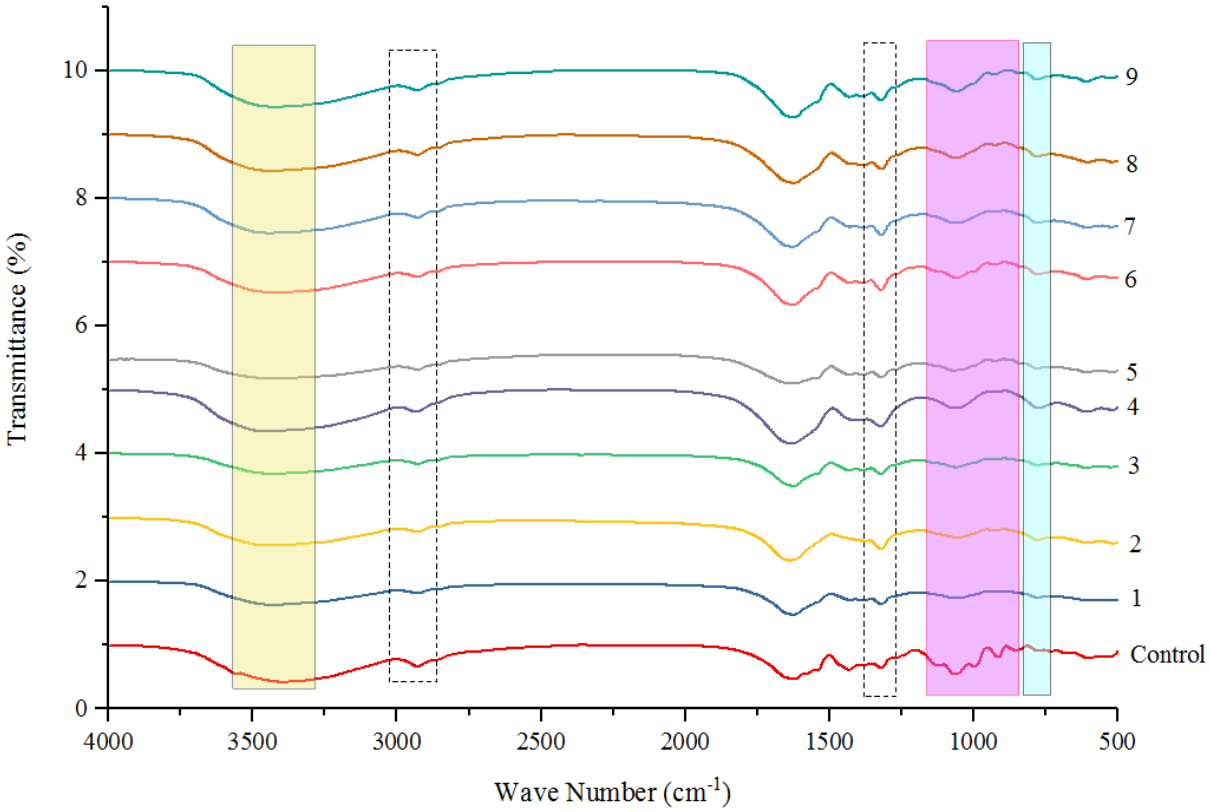

Fig. 4. FTIR spectra of control sample scales in comparison with scales treated with aspartic acid extracted by the ultrasound-assisted extraction method (the codes on the table refer to the row numbers of Table 6).

tubes. The results of the ultrasound-assisted extraction showed that the linear model is the best model to describe the behavior of aspartic acid extraction from sugar beet molasses. The optimal conditions for extracting aspartic acid using the ultrasound-assisted method include an extraction temperature of 25.09 °C, pH equal to 7.00, ultrasound power of 69.99%, and no ethanol. Aspartic acid extracted under optimal conditions with three different concentrations (10, 25, and 50 mg/100g) at three different temperatures (60, 90, and 120 °C) was applied to the sediments of sugar industry evaporator tubes. In general, the results showed that using a concentration of 50 mg/100g of aspartic acid extracted from molasses under optimal conditions at a temperature of 90 °C for 6 h effectively removes sediment from the evaporator tube. The change in the structure of the sediments formed on the tubes of the evaporators was well shown by the results of FESEM images and FTIR spectroscopy. The results of the EDS analysis also showed the change in the composition of sediments on the evaporator tubes as a result of applying aspartic acid as a natural anti-scaling agent. The use of aspartic acid at the concentration of 50 mg/100 g at the temperature of 90 °C as a natural anti-scaling agent for descaling the pipes of evaporators and heat exchangers are recommended.

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Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 17-24, 2024

Identification of Volatile Compounds Originating from Secondary Contamination and Packaging Materials in UF and White Brine Cheeses

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Received: 10 March 2024 Accepted: 30 April 2024

ABSTRACT: Identification of volatile contaminants migrating from packaging materials that might affect the quality of packaged food and might cause problems to consumer health is great importance. Soft cheeses can undergo such migration and contamination during storage. This study aimed to exclusively identify abnormal volatile compounds in Iranian cheeses that likely originated from contamination and packaging rather than endogenous components using HS-SPME GC-MS. White brine and ultrafiltrated (UF) cheeses in packages were stored for 90 days at 4 degrees Celsius (°C). Headspace-solid phase microextraction (HS-SPME) using polysulfone and mesoporous carbon nitride (MCN/Polysulfone) fiber coupled to gas chromatography-mass spectrometry (GC-MS) was employed to extract and analyze volatile compounds. Migration-based contaminants exclusively present in stored versus fresh cheeses were identified through National Institute of Standards and Technology (NIST) library matching. In total 23 unwanted volatile contaminants originating from contamination/packaging were identified, including 19 compounds in white brine` cheese (phthalates, benzenecarboxylic acids, etc.) and 13 compounds in UF cheese (phthalates, benzenecarboxylic acids, triazenes, oximes, etc.). More migrants were observed in white brine cheese. Compounds also differed based on SPME extraction method. Prolonged storage induced migration of volatile contaminants from probable packaging sources into soft cheeses. Future research should focus on refining volatile organic compound (VOC)-based detection methods to enhance early identification of spoilage and pathogenic microorganisms in cheese production.

Keywords: Cheese Ripening, Food Safety, Microbial Activity, Secondary Contamination, Volatile Organic Compounds (VOCs).

Introduction

Cheese is one of the most popular dairy products worldwide and valued for its nutritional properties and diverse sensorial characteristics. The characteristic flavor and aroma profile is the primary sensory factor determining consumer preference and market value of cheese (Delgado *et al.*, 2011). The typical volatile compounds present in cheese arise mainly from lipolysis, proteolysis and metabolic activities of starter and non-starter lactic

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acid bacteria during ripening (McSweeney, 2004). However, some volatile compounds may also originate from secondary contamination sources and interaction with packaging materials rather than from the endogenous cheese components (ÖzerKınık *et al.*, 2017; Aminifar *et al.*, 2014). Identification of these extraneous volatile compounds is important to ensure cheese quality and safety. Several studies have analyzed the volatile profiles of different cheese varieties using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). ÖzerKınık *et al*. (2017) examined volatile compounds in sheep milk cheese at 1 and 90 days of ripening, identifying extraneous compounds like benzaldehyde, 1-octen-3-ol and benzeneacetaldehyde unrelated to endogenous cheese production. Aminifar *et al.*, 2014) also detected some alcohols, esters, ketones and aromatics not associated with Liqvan cheese ripening using HS-SPME GC-MS possibly from environmental sources. These demonstrate the efficacy of HS-SPME GC-MS in detecting abnormal volatile cheese contaminants during storage.

Polyvinyl chloride (PVC) films are commonly employed for cheese packaging, which can release plasticizers like phthalates into foods (Dole *et al.*, 2010). Phthalates such as di(2-ethylhexyl) phthalate (DEHP) have been shown to migrate from packaging into yogurt and cheese based on factors like temperature, contact surface area and fat content (Nerín *et al.*, 2003; Castle *et al.*, 1989; Lyche et al., 2009). As phthalates are not covalently bound to PVC, they can readily migrate into fatty dairy products (Bradley *et al.*, 1995). Other potential packaging-related contaminants include components, adhesives, stabilizers and lubricants (Vera *et al.*, 2012). Environmental contaminants from surrounding air/contact surfaces and issues like poor employee hygiene can also secondarily introduce volatile impurities into packaged cheese post-processing (Hodgson *et al.*, 2000; Libinaki *et al.*, 2006). Therefore, identifying abnormal volatiles using analytical techniques helps determine contamination sources.

Although prior studies have profiled ripening-related cheese volatiles, limited research has focused on characterizing volatile contaminants specifically originating from secondary sources and packaging. Furthermore, misidentification of contaminants as ripening by-products can lead to incorrect inferences regarding cheese biochemistry. Dedicated characterization of contamination-derived volatiles therefore warrants investigation.

This study aimed to exclusively identify abnormal volatile compounds in Iranian cheeses that likely originated from contamination and packaging rather than endogenous components using HS-SPME GC-MS. Two fiber coatings, polysulfone and mesoporous carbon nitride (MCN/polysulfone), enabled comprehensive profiling of volatile contaminants.

Materials and Methods

- Cheese sampling

UF cheese samples were prepared using ultrafiltration process from pasteurized milk and stored in sterilized packages post treatment. White brine cheese samples on other hand were conventionally manufactured without thermal processing and brine salted before packaging. All packaged cheeses were obtained from Golpaiegan (PegahGolpaiegan, Isfahan, Iran), Iran. The cheeses were transported under refrigerated conditions on the production day to the laboratory where analysis were carried out in triplicates

order.

- **Storage study**

The cheese samples were transferred aseptically into sterilized glass containers following the purchase. The containers were then stored at refrigeration temperature (4±1°C) for a duration of 90 days. The containers were periodically opened only during headspace volatile sampling on storage days 1, 30, 60 and 90 and 120 for brine cheese and 1, 30, 60 and 90 days for analysis.

- HS-- SPME extraction

For HS extraction, 3 g of cheese samples were taken into 20 ml headspace vials sealed with polytetrafluoroethylene (PTFE)silicon septa. The vials were equilibrated at 60°C for 45 minutes in a cooling heating block to promote volatilization of compounds. The extraction was then carried out using a preconditioned (260°C for 2 hours) 85 µmMCN/Polysulfone inserted into the headspace for 45 minutes at 60°C. In should be noted that we extracted the compounds from the headspace using a Hamilton syringe and transferred them to the GC (Sabouri *et al.*, 2024).

- Direct immersion SPME extraction

Alternatively, direct immersion SPME was conducted by weighing 5 g of cheese, and transferring it into a 40 ml screw capped vial. The sample was pre-incubated at 60°C for 10 minutes before exposure to fibers - 65 µmpolysulfone and mesoporous carbon nitride (MCN/Polysulfone) (Supelco) for extracting non-polar and weakly polar compounds respectively. The fibers were directly inserted into the sample headspace for 60 minutes at the same temperature (Sabouri *et al.*, 2024).

- GC-MS analysis

The extracted volatile compounds were analyzed using an Agilent 7890B GC system (Agilent, USA) coupled with a 5977B mass selective detector (quadrupole) and equipped with either an HP-5MS or HP-624 column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The injection port was set to splitless mode at 250°C. The interface and ion source temperatures were 280°C and 230°C, respectively. Mass spectra were collected at 70 eV ionization voltage over an m/z range of 30-350. The oven temperature program had an initial isothermal hold at 40°C for 5 min, followed by a temperature ramp from 40 to 160°C at 5°C/min, then an increase to 240°C at 10°C/min with a 5 min final hold. Spectral signals were analyzed using MassHunter Workstation software and the NIST14 MS library for tentative identification based on match scores ≥80%. Relative abundances were calculated from total ion chromatogram (TIC) peak areas (Sabouri *et al.*, 2024). Compounds identified exclusively in the stored cheeses but absent in fresh samples were deduced as migrants from secondary contamination and/or packaging.

- Data analysis

The study data were summarized using descriptive statistics. Categorical variables were expressed as percentages. Quantitative variables were reported as mean ± standard deviation (SD). Experiments were conducted in triplicates to ensure reproducibility of results.

The SPSS software package was utilized to analyze the data. Statistical tests were two-sided and based on a significance level of 0.05. SPSS enabled

performance of all statistical analyses on the study data.

Results and Discussion

A total of 65 volatile compounds were identified from the UF during 90 days and brine cheeses during 120 days of storage, including alkanes, ketones, aldehydes, acids, esters, and aromatics. Out of these, 23 compounds originated from secondary contamination and packaging, as described below in different cheese types.

- **Volatile compounds originating from secondary contamination and packaging in white brine cheese**

White brine cheese showed 19 volatile compounds originating from secondary contamination and packaging materials when extracted using MCN and PSF fibers during storage. These included phthalates, benzenecarboxylic acids, adipates, siloxanes, etc. as shown in Table 1.

Phthalates such as diethyl phthalate were identified as the major contaminants from packaging on day 1 and day 90 along with benzenecarboxylic acids such as dimethyl benzene dicarboxylic acid. Adipates such as di-iso-octyladipate were also detected. Other compounds like siloxanes and benzaldehyde were present due to secondary contamination.

Phthalates such as diethyl phthalate and dimethyl phthalate were identified as some of the major contaminants in both the cheeses from possible package migration. Previous studies have also reported phthalates as common migrant contaminants from packaging materials into dairy products, especially upon long term storage. For instance, Bradley *et al.*, (1995) detected diethyl phthalate in packaged cheese shreds stored for 21 days at 8°C possibly due to migration from printing inks. Earlier, Conchillo *et al.* (Giuliani *et al.,* 2020) identified up to 16 phthalates including diethyl phthalate in plastic packed Carmenere fruit juices stored for 6 months. The authors indicated plasticizers as the primary source behind phthalate contamination. Since phthalates are used as common plasticizers, their migration from packaging materials into fatty dairy and liquid food matrices is not unusual over prolonged storage. Legislations have also been imposed by agencies such as European Food Safety Authority to monitor phthalate levels considering their toxic effects (Giuliani *et al.,* 2020). Therefore, focusing on reducing phthalate migration by using phthalate-free packaging would help minimize this risk and improve cheese quality.

- **Volatile compounds originating from secondary contamination and packaging in UF cheese**

A total of 13 compounds possibly from contamination and packaging were found in UF cheese using MCN, PSF and HS-SPME extraction during 90 days. These are presented in Table 2..

Table 1. Volatile compounds originating from secondary contamination and packaging materials in brine cheese

Compound	Day 0	Day 30	Day 90
Diethyl phthalate	Diethyl phthalate	-	Diethyl phthalate
Dimethyl phthalate	-	-	Dimethyl phthalate
Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid
Di-iso-octyladipate	-	Di-iso-octyladipate	-
Dodecamethylcyclohexasiloxane	Dodecamethylcyclohexasiloxane	-	-

Table 2. Volatile compounds originating from secondary contamination and packaging materials in UF cheese

Compound	Day 1	Day 30	Day 90
Diethyl phthalate	Diethyl phthalate	Diethyl phthalate	-
Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	-
Diethyl phthalate	Diethyl phthalate	Diethyl phthalate	-

Major contaminants identified were phthalates on day 1 and benzenecarboxylic acids during entire storage period. Other contaminants included triazenes, siloxanes, and oximes. Acetyl acetate and tetra(4-hydroxyboranylphenyl)methane were identified possibly from packaging materials using HS extraction.

- **Comparison of volatile profiles between cheese varieties**

The profiles of volatile contaminants differed between white brine and UF cheeses. While phthalates, benzenecarboxylic acids and siloxanes were common, compounds like triazenes, oximes, acetyl acetate, etc. were unique to UF cheese. UF cheese showed less number of contaminants as compared to white brine over storage.

Indeed, around 23 volatile compounds originating from contamination sources and packaging materials were identified in cheeses, which contributed to the overall volatile profiles. Better quality packaging and prevention of contamination could reduce these unwanted volatiles in the cheeses.

Benzenecarboxylic acids like dimethyl benzene dicarboxylic acid were identified throughout the 90 days of storage in both cheese varieties from possible package migration. Benzenedicarboxylic acids have been documented to migrate from polyethylene terephthalate (pvc) bottles to mineral water upon storage by Bartolome *et al.* (Bach *et al.*, 2012). PET plastic packages were indicated as a source of these compounds. Benzoic and benzenedicarboxylic acids have also been reported as migrants from adhesives (Vera *et al.*, 2012). Therefore, the benzenecarboxylic acids detected in the present cheeses might have originated from such indirect packaging components over longer storage, contaminating the product. Again, preventing this would require mitigating future migration risks while packaging.

While phthalates and benzenecarboxylic acids contaminated both cheeses, the UF cheese showed additional unique contaminants like triazenes, oximes and tetra(4-hydroxyboranylphenyl)methane compounds possibly from packaging. In comparison, more contaminants were detected in white brine cheese (19 compounds) versus UF cheese (13 compounds) during storage. The differences could be related to variations in the packaging materials and storage conditions between the cheese varieties that would impact migration behaviors. As milk processing might alter the properties of UF cheese, it could interact differently with packages compared to unprocessed white brine cheese. Besides, factors like greater surface area and higher fat content of white brine cheese could enable more sorption of migrating contaminants over time as well (Sørensen, 2006). Nevertheless, the findings highlight the impact of storage duration, packaging variations and cheese compositions on contamination risks.

The range of contaminants identified also varied based on the SPME extraction method for individual cheeses. For instance, in white brine cheese, certain

migrating compounds like adipates and siloxanes were specifically extracted only using the MCN fiber, while compounds such as furanones selectively sorbed onto the PSF fiber. The findings showcase the selectivity and variability of different extraction phases in isolating migrated volatiles based on the cheese matrix effects. Thus, utilizing multiple extraction techniques can provide a more comprehensive contaminant profile from complex dairy matrices following migration (Gong *et al.*, 2023). However, developing optimized standardized methods would be vital for effective routine monitoring.

Conclusion

This study demonstrated the ability of headspace SPME coupled with GC-MS to comprehensively identify volatile compounds originating from secondary contamination and packaging materials in two types of Iranian cheeses - white brine and UF cheese. A total of 23 extraneous volatile contaminants were detected, with white brine cheese showing higher levels of contamination (19 compounds) compared to UF cheese (13 compounds) over 90-120 days of refrigerated storage.

Major contaminants included phthalates like diethyl phthalate, benzenecarboxylic acids, adipates, siloxanes and other compounds potentially migrating from plastic packaging films, printing inks, adhesives and environmental sources. The findings highlight how prolonged storage can facilitate migration of these unwanted volatiles into fatty cheese matrices, adversely impacting product quality and safety.

Differences in the contaminant profiles between the two cheeses were evident, likely due to variations in compositional properties like fat content as well as the specific packaging materials and headspace conditions influencing migration behaviors. Utilization of multiple SPME fiber coatings enabled more comprehensive extraction of diverse volatile migrants based on their polarity differences.

Finally, the study reinforces the need for improved packaging systems that prevent migration of harmful substances into food products during storage. Monitoring of abnormal volatiles using hyphenated techniques like HS-SPME GC-MS can aid in identifying contamination sources and implementing suitable preventive controls in cheese manufacturing. Future research should focus on further refining these analytical methods for early and sensitive detection of volatile contaminants as well as correlating them with sensory defects and potential health risks. Preventing volatile migrants from secondary sources is crucial for ensuring high quality, unadulterated and safe cheese products.

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Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 25-44, 2024

Impact of Substituting Sugar with Date Juice on the Sensory and Physicochemical Characteristics of Uncoated Chewing Gum

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Received: 4 March 2024 Accepted: 24 May 2024

ABSTRACT: In contemporary confectionery industry, manufacturing of dietary and low-calorie products, notably in the realm of chewing gum production, stands as a significant challenge. Sweeteners are integral components in confectionery products, with applications extending to chewing gum as well. This research utilizes the established advantageous characteristics of dates to investigate the potential use of date juice as a replacement for sugar in the manufacturing of dietary gum. The substitution ratios tested in the experiment are, 10, 20, and 30%. The findings of the experiment indicated that the gum supplemented with date juice at a substitution rate of 20% exhibited superior quality and shelf life, as evidenced by its enhanced moisture retention, texture, and sensory attributes, particularly taste. Conversely, a higher substitution rate of 30% led to a decline in both quality and sensory characteristics, with values falling below those of the control treatment. The study encompassed physicochemical and sensory examinations, encompassing the analysis of gum content, determination of reducing sugars following hydrolysis, measurement of ash content, assessment of texture, evaluation of sensory attributes, and investigation of colorimetric properties. The findings indicated that the quantity of gum and sugar obtained following the hydrolysis of the product was consistent across all experimental treatments, displaying no statistically significant variance as compared to the control treatment. Additionally, there was a notable increase in the ash percentage index with higher levels of date juice replacement. The adhesion indices demonstrated a notable increase, while both chewability and springiness exhibited a decrease.

Keywords: Diet Chewing Gum, Date Juice, Sensory and Physicochemical Properties, Sugar Substitute.

Introduction

The use of sucrose as a natural sweetener offers numerous advantages. The link between sugar consumption and health issues, including hypertension, cardiovascular disease, dental caries, obesity, and elevated levels of blood glucose and insulin, as well as the economic and technological challenges associated with sugar production, has prompted extensive research into the potential use of alternative natural sweeteners. This line of inquiry has been explored by Alsenaien *et al*. (2015). Carocho *et al*. (2017) noted that consumers generally exhibit a preference for confectionery products that are sweetened with sugar substitutes. This preference is largely attributed to the desire to reduce calorie intake and control

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body weight. Furthermore, sugar substitutes are recognized for their potential role in managing diseases such as diabetes and lowering blood sugar levels the study by Kazemi *et al*. (2001) provide valuable insights into the topic. Iran has historically been regarded as a prominent producer of dates. As of 2013, Iran holds a prominent position in global date production with an annual output of 1,490,000 tons, as reported by statistical data. Approximately 30% of the dates cultivated in the country do not meet the quality standards required for consumption and therefore are unsuitable for the consumer market. Consequently, these dates are redirected to processing industries where they are converted into valuable products such as date juice. Date juice is considered to be a highly significant byproduct of dates, characterized by its high content of natural sugars such as fructose and glucose, while containing low levels of sucrose. In terms of physiological processes, it is notable that absorption of fructose sugar in the body does not require the presence of insulin. Hence, sugar is considered to be appropriate for individuals with diabetes and is a source of significant energy. Furthermore, date juice is rich in potassium, calcium, phosphorus, and iron, making it a valuable dietary option for supporting the nutritional needs of children, breastfeeding women, and the elderly. It is important to exercise caution when using these sweeteners and adhere to the recommended permissible limits set by authoritative organizations. The incorporation of date juice into food formulations not only serves as a viable alternative to sugar and artificial sweeteners, but also contributes to enhancing the nutritional profile of the end product (Gohari *et al*., 2005; Razavi *et al*., 2006; Mardani *et al*., 2014).

Materials and Methods

- Product Formulation

The initial step in the production of strip chewing gum involved transferring the gum base to a laboratory mixer, where it was kneaded for a duration of 30 minutes until a viscid and malleable dough was achieved. Subsequently, date juice was incorporated into each sample according to the specified weight ratios outlined in the accompanying table. The softener was incrementally introduced to the solution. The gum paste was prepared by mixing, kneading, and spreading it, and subsequently divided into small square pieces through shaping. Following the preparation of three distinct varieties of chewing gum, they were subsequently packaged in nylon bags and subjected to sensory and quality evaluations subsequent to random coding. Tables 1 and 2 present chewing gum formulation and treatments given respectively.

Table 1. Chewing gum formulation

Raw materials	%
Date syrup	In three propositions 10,20,30
Glucose	15
Gum	20-62
Glycerin	0.5
Essential oil	2
Sorbitol	1.5

Table 2- Treatment's codding(%)

Raw materials	T	T_1	T_2	T_3
Sugar	55	45	35	25
Gum	26	26	26	26
Glucose Bx=82	15	15	15	15
Date syrup	0	10	20	30
Essential oil	2	2	2	2
Glycerin	0.5	0.5	0.5	0.5
Sorbitol	1.5	1.5	1.5	1.5

- Strip Chewing Gum tests

The present study aimed to investigate several key parameters including Brix measurement of date juice, reduction sugar measurement, total gum content, sucrose

percentage, ash percentage, and moisture percentage.

- Brix measurement of date syrup

The Brix content of the date juice was quantified using a refractometer prior to conducting the experiment.

- Sugar measurement (Lin Aynon Method)

The assessment of sucrose content is a customary procedure in product analysis, and was conducted as outlined below to ascertain the presence of sucrose in the end product.

A precise measurement of 9. 5 grams of sucrose of laboratory purity was meticulously weighed and subsequently dissolved in a suitable volume of water. Subsequently, 5 milliliters of concentrated hydrochloric acid was introduced into the solution, followed by the addition of distilled water until the volume reached approximately 100 milliliters. The solution was subjected to ambient room temperature conditions for an extended duration. The experiment involved subjecting the specimens to a temperature of 12-15 degrees Celsius for a duration of 7 days, followed by a temperature of 20-25 degrees Celsius for a period of 3 days. Upon the completion of the cooling process, the resultant solution achieved a final volume of 100 milliliters. The solution referred to is the standard 10% sugar acid solution, which can maintain its efficacy for a period of up to 2 months when stored at normal ambient temperature, approximately 20 degrees Celsius.

- Measurement of reducing sugar after hydrolysis

A 10-milliliter aliquot of a 4% gum solution was transferred into a 100-milliliter flask, to which distilled water and 2. 5 milliliters of concentrated hydrochloric acid were added. The resulting mixture was subsequently subjected to a bain-marie at 70 degrees Celsius for a total duration of 10 minutes, during which time it was agitated for 3 minutes and then maintained at a constant temperature for 7 minutes. Following the cooling process, the solution was neutralized using a phenolphthalein indicator, then concentrated sodium hydroxide and one-tenth normal sodium hydroxide were added to bring the volume to 100 milliliters. The resultant solution was subsequently transferred to a burette and a titration procedure was executed. The quantity of sugar regenerated following hydrolysis in a 100-gram specimen was determined using equation 1 and expressed in units of dextrose (glucose) (Anon, 1995).

$$E = [(T \times 100 \times 100)/(\mathrm{V} \times \mathrm{W} \times 1000) \times 10)] \times 100 \quad (1)$$

where,

T= Corrected Fehling's titer in terms of dextrose in milligrams

V= The used volume of the sample solution for Fehling neutralization

W= Weight of gum in grams

- Measurement of sucrose

The quantitative analysis of the sucrose content in the sample was determined using the provided equation (Anon, 1995).

$$\text{Percentage of sucrose} = 0.95 \times (\text{Sugar before hydrolysis} - \text{Sugar after hydrolysis}) \quad (2)$$

- Total gum measurement

Approximately 2 grams of the gum sample in crushed form was precisely measured and then transferred into a beaker, using a small glass rod for

agitation. The beaker had previously been weighed and had reached a stable weight. Boiling water was incorporated into the mixture and agitated until its complete dissolution. Upon the precipitation of the insoluble material at the base of the flask, the subsequent extraction of the blue layer ensued, followed by multiple iterations of rinsing the residual contents within the flask. Subsequently, the remaining substance is to be transferred into a beaker and subjected to an oven temperature of 105 degrees Celsius, until complete desiccation is achieved. The ensuing dried substance is then allowed to cool and subsequently weighed. The percentage of total gum, defined as the sum of the gum and its filler, was determined using Equation 3 as outlined in the Iranian Standard (Anon, 1995).

$$\text{Gum} = (\,(\text{B} - \text{A})/\text{m}) \times 100 \qquad (3)$$

where,
B= The weight of the beaker with the glass rod and its dried residue
A= Weight of empty beaker with glass rod
C= Sample weight

- Moisture measurement

Initially, the plate was placed in an oven for a duration of 30 minutes at a temperature range of 100-105 degrees Celsius. Subsequently, the plate was withdrawn from the oven and transferred to a cold desiccator until it equilibrated to ambient temperature, following which its mass was determined. A specified quantity (minimum of 2 grams) of the pulverized specimen of the gum under investigation was subsequently added to the plate, and the plate was re-weighed with the sample content. The plate containing the sample content was then subjected to a temperature range of 100-105 degrees Celsius until a consistent weight was achieved. The sample was placed in a desiccator for cooling and subsequently weighed. The procedure is iterated, involving the sequential application of heat, cooling, and weighing, with the aim of achieving a consistent and stable weight. According to the Iranian Standard (Anon, 1995), it is expected that the difference in the final weight between two consecutive tests should not exceed 2 mg. Equation 4 was employed to determine the moisture content.

$$Moisture\ \% = (\text{W1} - \text{W2}/\text{W1} - \text{W}) \times 100 \qquad (4)$$

where,
W_1 = Initial weighing
W_2 = Secondary weighing
W= The total weight of the sample

- Ash measurement

The amount of ash was analysed by a furnace set at a temperature of 550-500 °C. The quantitative analysis of the ash content in the samples was determined by using a furnace set at a temperature of 500-550 °C according to equation 5 (Anon, 1995).

$$\text{Ash}\ \% = (\text{W2} - \text{W}/\text{W1} - \text{W}) \times 100 \qquad (5)$$

where,
W= Crucible weight
W_1 = The weight of the Crucible with the sample before incineration
W_2 = Crucible weight with ash

- Texture analysis

The analysis of texture was carried out utilizing the TA-XT2i Texture Analyzer Stable Micro System, which was equipped with a 5 mm cylindrical probe. The texture profile analysis parameters were measured using a compression force of 25%, and the parameters of hardness, gumminess, springiness and chewability were analyzed

with Expression PC V. 21 software, as described in a study conducted by Razavi *et al*. (2006).

- Hardness

The process of compression and deformation consists of two stages. Initially, the moving jaw of the machine compresses the sample to a degree of 70% and then returns to its original state. Subsequently, after a brief interval, the sample undergoes a second compression of 70%. Ultimately, two distinct peaks are derived, and the size of these peaks varies according to the type and properties of the products. Furthermore, the maximum force experienced during the initial stage of product compression is denoted as texture hardness (Razavi *et al*., 2006).

- Cohesiveness

The assessment of the product's second deformation in relation to its behavior during the initial deformation provides insight into the adhesion state of the sample. This analysis was conducted by dividing level 2 into level 1, as indicated in the diagram (Razavi *et al*., 2006).

- Springiness

The capacity for a product to revert to its original state following compression can be determined by calculating the ratio between the height of the peak hardness at stage 2 and the height of the peak hardness at stage 1 (Razavi *et al*., 2006).

- Chewiness

The chewing properties are derived from the combined effects of hardness, adhesion, and springness (Razavi *et al*., 2006).

Result and Discussion

- Brix measurement results of date juice

The findings of the Brix assessment of date juice indicated a numerical value of approximately 74.

- Sensory evaluation results

The analysis of the sensory evaluation results are presented in Figures 1 to 4 and indicate notable variances in the sensory attributes of the treatments subsequent to the production period and throughout a six-week storage period. The utilization of date juice in composition of chewing gum has been demonstrated to have a notable impact on enhancing taste and texture, with the results showing improvement up to 20% in the parameters studied, as illustrated in Figure 1. Elevating the substitution rate of date juice beyond 20% is anticipated to adversely affect the sensory attributes, particularly the texture of the gum, ultimately influencing overall acceptability. There were notable variations in both the aroma and flavor of the gums utilized in the post-production, as indicated by a statistically significant difference between them ($p<0.05$). The researchers conducted an evaluation in which treatments containing a 30% replacement of date juice were found to receive the lowest scores in terms of aroma and smell. Conversely, the control treatment and those with 10% and 20% replacement were deemed to have higher scores as compared to the control treatment. The influence of different periods of storage on the sensory qualities of the products showed a significant level of importance. Extending the storage time also had significant effects on the sensory characteristics of the treatments. The control group showed no significant differences as compared to the other groups until the fourth week of preservation, as evidenced by a p-value below 0.05. The extension of preservation time did not yield statistically significant differences in the treatments employing

10% and 20% preservation, particularly in terms of texture characteristics, as well as in the treatment involving a 20% replacement of date juice. The product has effectively maintained its textural attributes, flavor profiles, and olfactory properties, and has garnered the highest ratings from assessors in terms of overall acceptance. The definitive order of the treatments is as follows:

$$T_2>T_1>T>T_3$$

The findings depicted in Figure 1 demonstrate that the evaluation of the taste of the gum treatments indicates a positive correlation between the acceptability of the gum treatments and the rising percentage of date juice. As the Brix level increases, there is a corresponding increase in the acceptability of taste. The score related to taste also increases up to a 20% replacement level. This suggests that in terms of texture, particularly in juice sugar, there is potential to emulate properties similar to sugar solutions in products. The item possesses nourishment and is capable of serving as a suitable substitute in this regard (Mardani *et al*., 2014; Milani *et al*., 2011). The assessment of various chemical and sensory attributes of juice, concentrate, and juice date sugar in comparison to sugar solution revealed that the sensory evaluation indicated the juice sugar solution to possess characteristics more closely aligned with those typically sought, rendering it a more suitable product. In substitution of sucrose, an alternative consistent with the findings of the current study is sugar as suggested by (Homayouni *et al*., 2014).

A primary objective in the production of packaged food is to minimize alterations during the period of storage. The findings of the investigation demonstrate that there were no discernible alterations in the sensory attributes of treatments T1 (0% date juice) and T2 (10% date juice) over the course of a six-week storage period. The findings from the assessment of the physicochemical, rheological, microbial, and sensory properties of date juice indicated that there were no notable alterations in dates throughout the storage duration in juice form, which aligns with the outcomes of the current investigation by Homayouni in accordance with the findings of (Homayouni *et al*., 2014). The assessment of texture revealed that substituting 30% of date juice resulted in a loss of the gummy consistency in gum formulations. The findings of the texture evaluation and total gum measurement indicate a significant reduction in texture scores for all treatment groups ($p<0.05$). Furthermore, the reduction in the sensory attribute ratings of the treatments demonstrates that the substantial replacement of date juice has resulted in a decline in the overall acceptance of the treatments. The judges' overall assessment of the sample under study is influenced by its various characteristics such as texture, flavor, and taste. This overall acceptance is a reflection of the judges' feelings towards the sample, indicating the importance of these specific attributes in determining the overall evaluation. The study utilized a comparison of mean scores of sensory characteristics to ascertain that the sample containing a 30% date juice substitute exhibited the lowest overall acceptance score. The findings of this study are congruent with those of previous researchers according to the study conducted by (Gohari et al., 2005). The research conducted by the authors titled "Investigation of the Impact of Replacing Sugar with Date Juice on the Physical and Sensory Characteristics of Soft Ice Cream" revealed that the substitution of 100% date

juice for sugar resulted in the lowest total acceptance score. This decrease in score was found to be highly significant, amounting to approximately 42%. One of the primary functions of sucrose, beyond its role as a sweetening agent, is to contribute to the crispness and texture of a product. However, increasing the percentage of sucrose replacement has been shown to result in a substantial decrease in these sensory characteristics, thereby significantly impacting overall consumer acceptance (Mardani, 2014). The works conducted by researchers (Mardani *et al*., 2014; Labbe *et al*., 2007) are of particular academic relevance. The function of sugar in chewing gum extends beyond imparting sweetness, as sucrose crystals also play a pivotal role in establishing a desirable texture for products like chewing gum, where textural attributes hold significance. The findings from the assessment of the flavor of the gum treatments demonstrated that the acceptability of the gum treatments increased in conjunction with higher concentrations of date juice. The rise in Brix content is positively correlated with the enhancement of taste acceptability. The score associated with taste increases significantly up to a level of 20% replacement. Specifically, in terms of texture, particularly in juice sugar there is an increased ability to replicate properties typically found in sugar solutions in products. The item possesses nutritional value and can serve as a viable alternative in the realm of sustenance. The experimental findings pertaining to gum treatments, as well as laboratory-scale gum production, indicate that the utilization of a 20% date juice substitute yields superior outcomes. This observation aligns with prior studies conducted on various products.

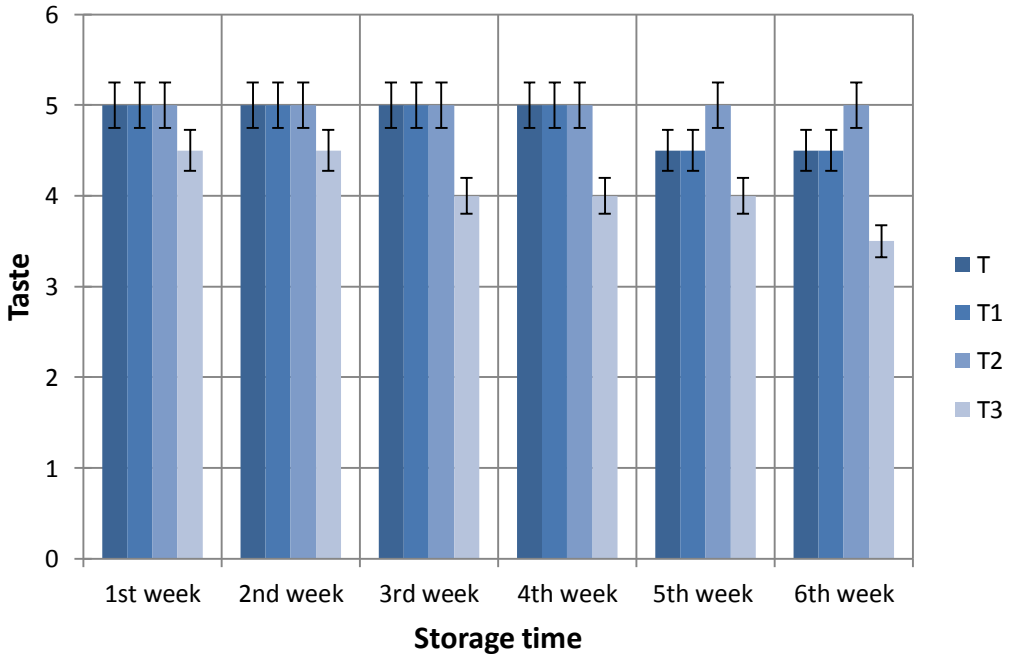

Fig. 1. The results of comparison of the average sensory evaluation index (taste) during six weeks of storage ($p<0.05$).

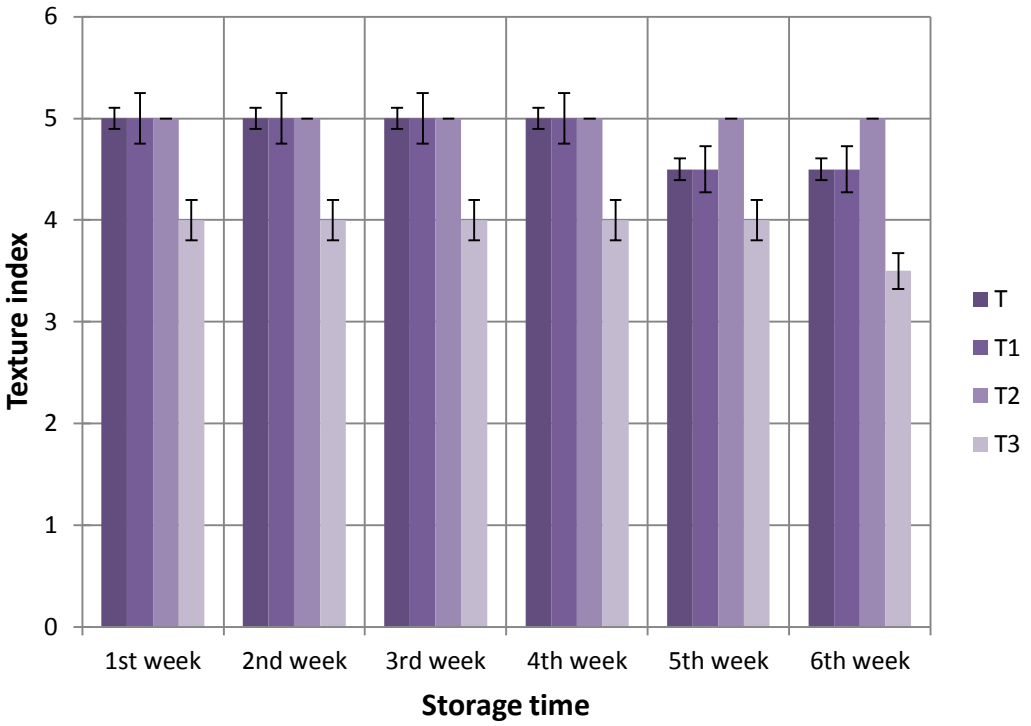

Fig. 2. The results of comparison of average sensory evaluation index (texture) during six weeks of storage ($p<0.05$).

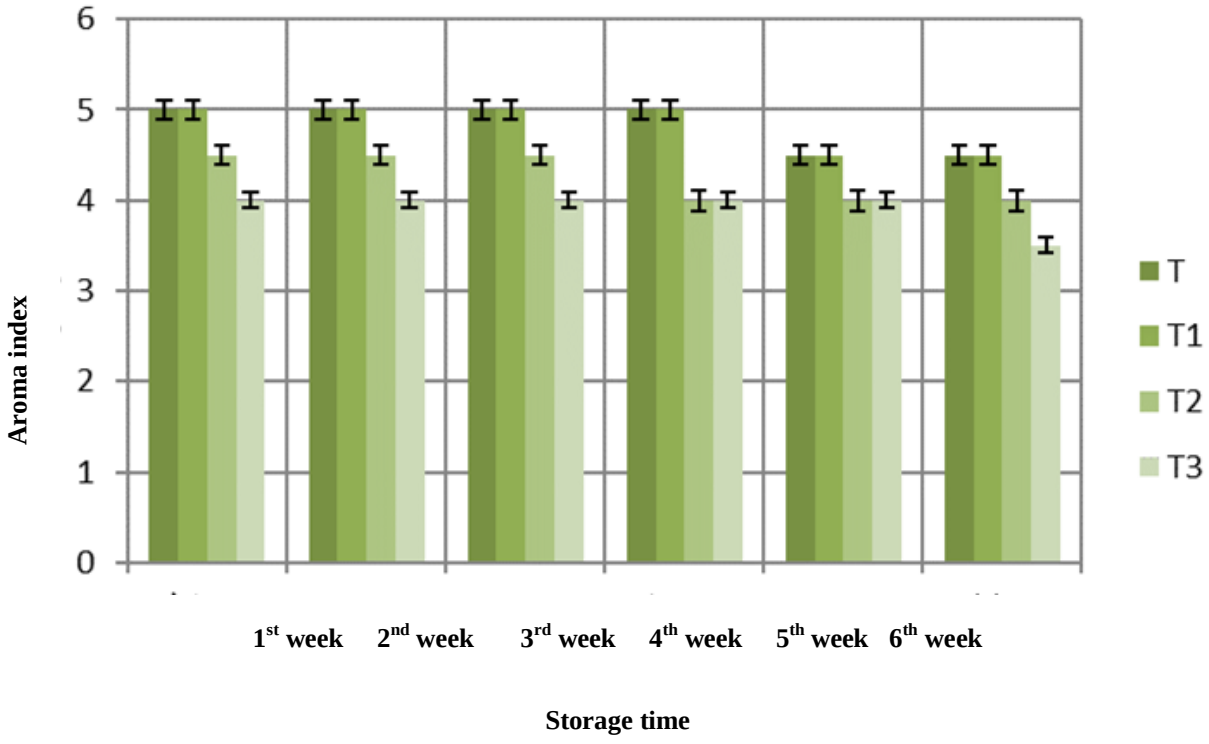

Fig. 3. The comparison results of the average sensory evaluation index (smell and aroma) during six weeks of storage ($p<0.05$).

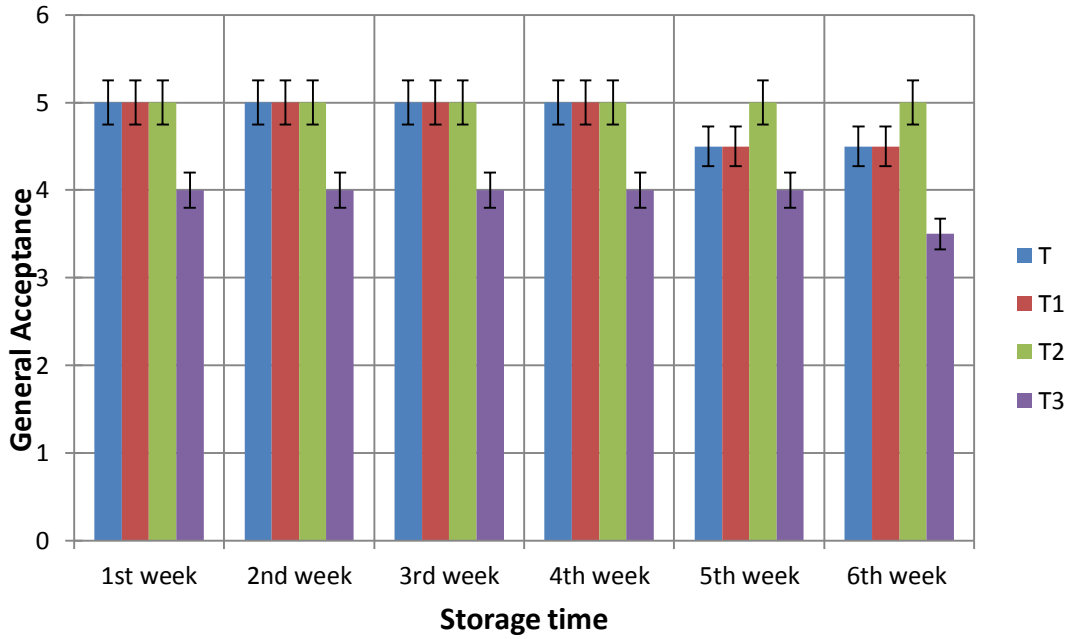

Fig. 4. The results of comparing the average sensory evaluation index (overall acceptance) during six weeks of storage ($p<0.05$).

- Color Parameters Results

The subsequent Figures (5-7) illustrate the variations in color indices within different treatments and across varying time intervals subsequent to storage. The findings indicate that the inclusion of date juice in the treatment formulations leads to a considerable reduction in the brightness index across all treatments. Consequently, there are notable distinctions between each treatment and the control group in this aspect. The a* index is observed to exhibit a color ranging from green to red, with no statistically significant changes evident post-production and throughout the duration of storage ($p<0.05$). Significant variations in redness index were observed among the different treatments, particularly in relation to the type of treatments. Furthermore, a noteworthy increase in redness index was observed with higher proportions of date juice replacing sugar, displaying a statistically significant correlation ($p<0.05$). The b* index demonstrated notable alterations in color, shifting from blue to yellow as the proportion of date juice used to replace sucrose in the gum formulation increased. This increase in date juice substitution resulted in a statistically significant rise in the yellow color index (b*) ($p<0.05$). However, the progression of time did not demonstrate any significant impact on the b* index.

The findings indicated a correlation between the augmented proportion of date juice substitution and a deepening of the coloration of the gum, as well as a rise in the yellowness index and a reduction in the brightness index. The observed phenomenon can be attributed to the Maillard reaction occurring within the simple sugars present in dates, as well as the intensity of color present in date juice.

The study "Investigation of the effect of replacing date juice sugar with invert sugar in layer cakes" determined that the substitution resulted in darker coloration of the samples as compared to the control group is in line with the present research

findings (Ishurd *et al*., 2005; Ahmadi *et al*., 2011).

The study focused on the substitution of liquid sugar with date juice in varying proportions (0, 20, 40, 60, 80, and 100%) within a biscuit formula, and its impact on the physicochemical and sensory attributes of the resulting product, determined that as the percentage of date juice replacement increased, there was a significant decrease in the L* index, which aligns with previous research findings. Mansouri (2013) and Edwards *et al*. (2018) have both contributed valuable insights to the field.

The study investigated the effects of substituting sugar with date powder and date syrup in the production of cookies. It was observed that the red index a* exhibited a significant increase, while the yellowness index showed a significant decrease as a result of the direct use of date powder and syrup. Concurrently, the current investigation exposes a contradiction in the formulation put forth by Milani *et al.* (2011).

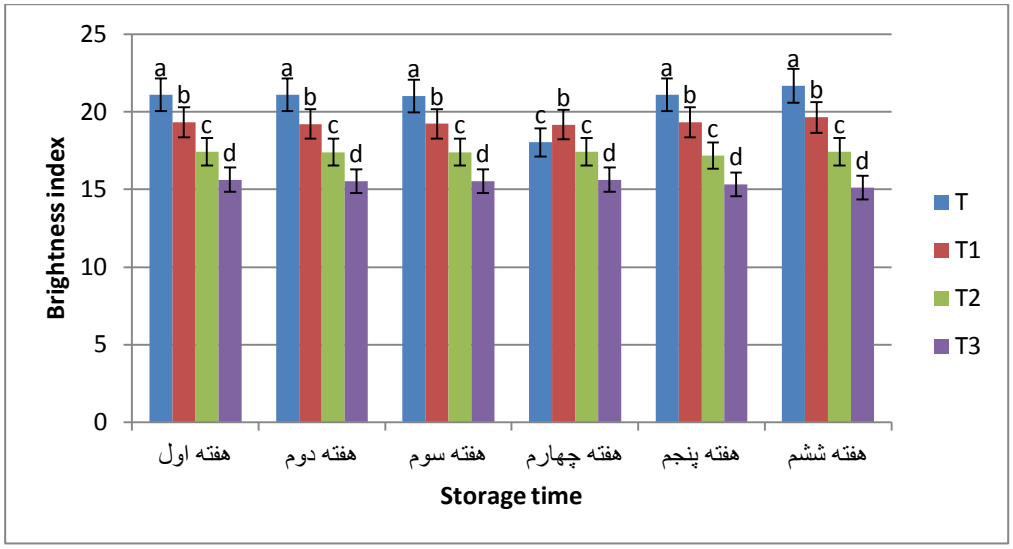

Fig. 5. The results of evaluating the brightness index of chewing gum treatments during six weeks of storage ($p<0.05$).

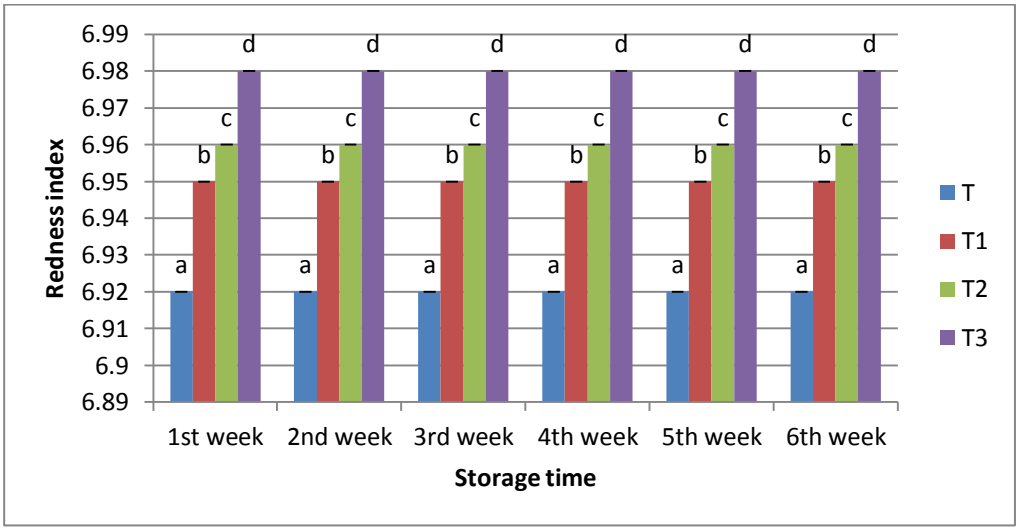

Fig. 6. Evaluation results of redness index of chewing gum treatments during six weeks of storage ($p<0.05$).

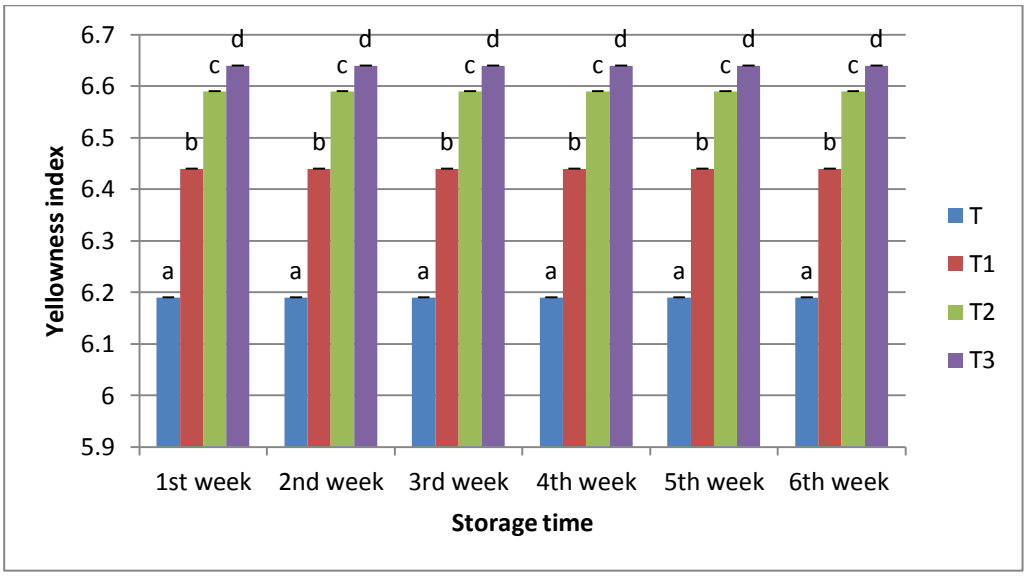

Fig. 7. Evaluation results of yellowness index of chewing gum treatments during six weeks of storage ($p<0.05$).

- Sugar measurement

Figure 8 presents various treatments in comparison to each other and the control. However, extending the duration of storage has not obtained significant impacts on any of the interventions. By augmenting the proportion of date juice replacement in the formulation of the experiment, there was a notable decrease in the total sugar percentage of the treatments ($p<0.05$). The total sugar percentage refers to the proportion of sucrose within a given sample. Date juice is found to contain a low level of sucrose, while also containing fructose and glucose. As the percentage of date juice in the formulation of chewing gums increases, the overall sugar content decreases notably. Furthermore, this reduction in sugar content becomes more pronounced with a higher percentage of substitution with date juice. The primary objective of achieving a lower sucrose content in chewing gum production is achieved more expeditiously with a formulation containing 20% date juice, as determined through experiments across various treatments. The study investigated the impact of substituting honey and date juice on the physicochemical properties, texture, and viscosity of low-fat orange yogurt ice cream dessert (Jafarpour *et al*., 2017). The findings revealed that the utilization of date juice as a substitute for sugar led to a more pronounced reduction in sugar content, attributable to the presence of reducing sugars in the date juice which enhances the activity of the starter cultures. These outcomes align with the overall decline in sugar content observed in the present study.

Researchers carried out a study to examine how replacing sucrose with date palm pulp affects the taste, physical and chemical characteristics, and ability to be stored of bread. The results of the study are consistent with earlier research carried out by Hashim *et al*., 2013; Aleid *et al*., 2013; Ghnimi *et al*., 2017).

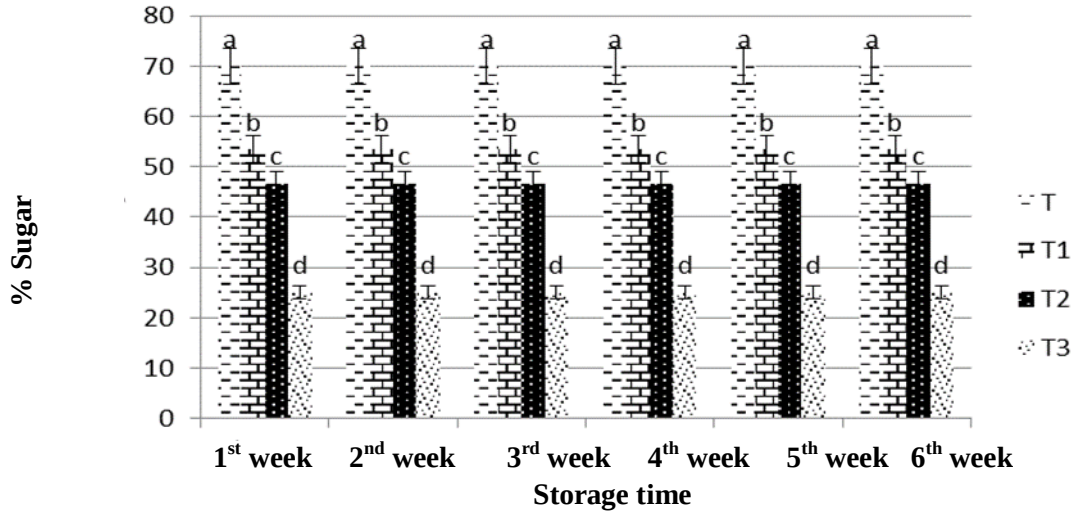

Fig. 8. Comparison of the average results of measuring sugar percentage during six weeks of storage ($p<0.05$).

- Measurement of glucose after hydrolysis

Based on the data presented in Figure 9, it can be observed that there are statistically significant variations in the levels of reducing sugars following hydrolysis in gum treatments post-production when compared to the control sample ($p<0.05$). An elevation in the sucrose substitution percentage within the treatment formulation led to a significant increase in the percentage of reducing sugar concerning the treatment T_3 demonstrate the highest percentage. Meanwhile, treatments T_2 and T_1 followed suit with lower percentages. However, there were no notable changes in the percentage of reducing sugar following hydrolysis across all treatments over a six-week maintenance period ($p<0.05$). The extent of sugar reduction following hydrolysis is contingent upon the specific sugar type and its constituent components. Date juice contains a notable proportion of rejuvenating sugars, namely glucose and fructose. The manipulation of the percentage of date juice in the formulations resulted in a significant increase in the percentage of reducing sugar after hydrolysis with an increase of 30%. This effect was found to be statistically significant at $p<0.05$ level.

The control treatment exhibited a significant decrease in reduced sugar percentage after hydrolysis as compared to other treatments, likely is attributed to the high sucrose content in the formulation. This difference was found to be statistically significant at a confidence level of $p<0.05$ and aligns with findings from previous research studies.

- Total gum measurement

Figure 10 indicates that the proportion of overall gum content in the gum treatments remained constant on the production and throughout a six-week storage period. The substitution of date juice does not result in statistically significant alterations in the quantity of total gum ($p<0.05$). Furthermore, it was observed that the duration of time had no impact on the total of gum present and the quantity of gum remained constant. The analysis of the outcomes related to alterations in the quantity of gum in chewing gum revealed that there was no significant variance in the proportion of

gum between the control group and the other experimental groups ($p<0.05$). The utilization of date juice as a sucrose substitute in the formulation has resulted in an equal percentage of base gum being used in all gum compounds. Additionally, the duration of storage for six weeks did not result in any alterations in the overall composition of the gum treatments. Thus, it can be concluded that there is no statistically significant variance between the various gum treatments. The study investigated the impact of various liquid syrups of DE grade on achieving a consistent percentage of total gum across all treatments. This finding is in accordance with the outcomes of the current study as documented by Yuda *et al*. (2018) and Mehta *et al*. (2012).

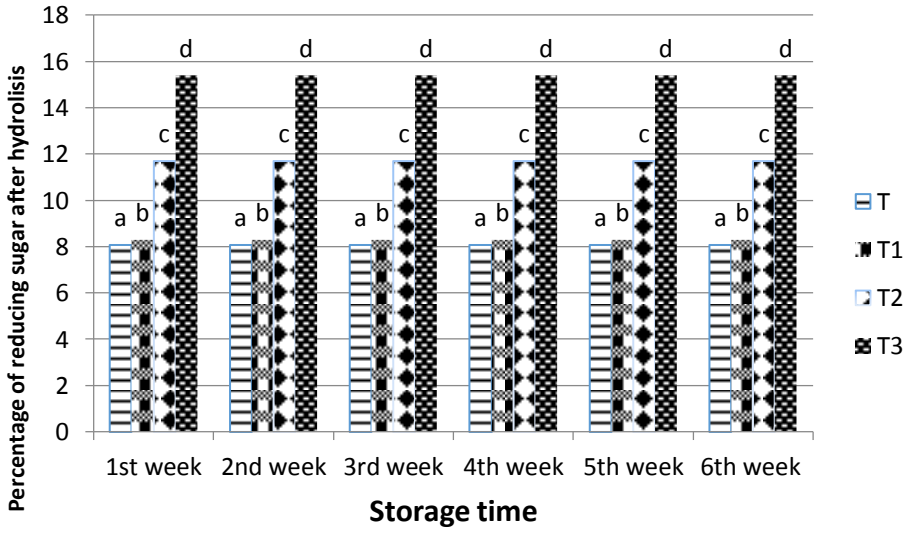

Fig. 9. Comparing the average results of measuring the percentage of reduced sugar after hydrolysis during six weeks of storage ($p<0.05$).

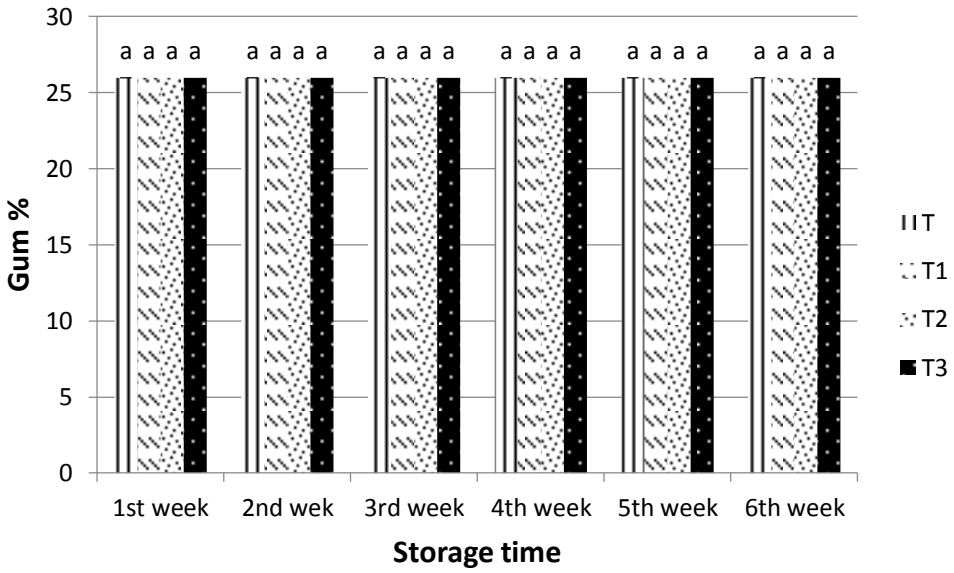

Fig. 10. Comparison of the average results of measuring the percentage of total gum during six weeks of storage ($p<0.05$)

- Moisture measurement

The analysis of the findings depicted in Figure 11 indicates that there were statistically significant variations in the moisture contents among the treatments starting from the initial day of production ($p<0.05$). During the initial three weeks of storage, there were no notable variations in the moisture content among the treatments. However, starting from the third week of storage, a significant decrease in moisture content was observed in the treatments. Notably, treatments with a higher concentration of date juice exhibited a slower rate of moisture loss. The statistical analysis of the mean values revealed a statistically significant difference in moisture content between the different gum treatments, as indicated by a p-value of less than 0.05. The findings from the Duncan's test indicated that there were significant variations in the moisture content of the samples in comparison to the control sample. Moreover, it was observed that the moisture content increased proportionally with the rise in the substitution of date juice sugar in the chewing gum. The elevation in moisture can likely be attributed to the competitive nature of water absorbent compounds present in the formulation. It appears that altering the variety of sugar has proven to be successful in modifying the moisture content of the product. The majority of the sugar present in date juice consists of regenerating monosaccharide sugars (glucose and fructose) and negligible quantities of sucrose. In general, the majority of sugars produce solutions with high viscosity due to their strong hydrophilic nature and ability to dissolve. The presence of the hydroxyl group facilitates the potential for hydrogen bond formation with molecules of water. An examination of the molecular compositions of sucrose, fructose, and glucose suggests that the elevated concentration of functional groups in the sugars found in date juice, as compared to sucrose, contributes to heightened hydrogen bond formation. The consequent reduction in the mobility of unbound water leads to increased absorption and retention of moisture, ultimately resulting in higher moisture content in the treated materials. The substitution of sucrose with date juice in gum formulations has been the focus of research in studies carried out by Damodaran *et al*. (2007), Mäkinen *et al*. (2014), and Amerine *et al*. (2013).

The results of the study are consistent with those reported by the researchers in their examination of the effects of replacing liquid sugar with invert sugar in layer cakes. Ishurd *et al*. (2005) and Haahr *et al*. (2003) have noted that a rise in the proportion of invert sugar used as a substitute is linked with a concomitant increase in the level of moisture in cakes.

The incorporation of date powder in the production of chocolate toffee resulted in similar findings. The conclusions drawn from the study suggest that the substitution of more than 25% of date juice in the chocolate toffee recipe results in significant changes in the moisture characteristics of the chocolates. Furthermore, the findings of the experiments carried out in this study align with the outcomes reported by Ahmadnia and Sahari in their previous research (Hull *et al.* 2010), Al Hagbani *et al.* 2018, and Ahmadnia *et al*. conducted an examination of multiple topics within their individual areas of expertise.

- Ash measurement

Figure 12 presents the ash content during examined period. The correlation between the percentage of ash in the treatments and the extent of date juice replacement in the treatment formulations

was found to be directly proportional. As depicted in Figure 12, the augmentation of the replacement percentage of date juice in the gum formulation resulted in a notable escalation in the ash content of the treatments post-production. The duration of storage of the treatments did not have a statistically significant impact on the percentage of ash. Additionally, as the storage time increased, none of the treatments exhibited any significant deviations from the ash percentage observed on the day after production ($p<0.05$). The ash content of the product is significantly influenced by the fiber and pectin compounds, with variations based on the purification and extraction methods employed. The findings indicate a notable increase in the percentage of ash with the incorporation of a higher proportion of date juice in the treatment formulations. The control treatment yielded lower ash content attributed to the presence of sucrose. Conversely, the incorporation of date juice and fiber at increased proportions led to a substantial rise in the percentage of these compounds. The elevation of the ash percentage of the product demonstrates a concurrent increase. Previous studies have reported a noteworthy rise in the ash percentage of the treatments, correlating with higher levels of date juice replacement (Sahari *et al*., 2008; Yuda *et al*., 2018).

- Texture analysis

The assessment of gum texture interventions is presented in Table 3. The findings indicate that there were notable distinctions in chewing capability between the various gum treatments, as well as in comparison to the control treatment, as evidenced by the statistical significance ($p<0.05$). The findings indicate that the substitution of date juice in place of sucrose at concentrations of 10% and 20% resulted in a significant increase in the chewability of gum treatments ($p<0.05$). A notable increase in chewability is observed with the implementation of a 30% sucrose substitution. The treatment with T_3 resulted in a decrease. The duration of storage for six weeks did not produce statistically significant effects on the quantity of chewing gum ability ($p<0.05$).

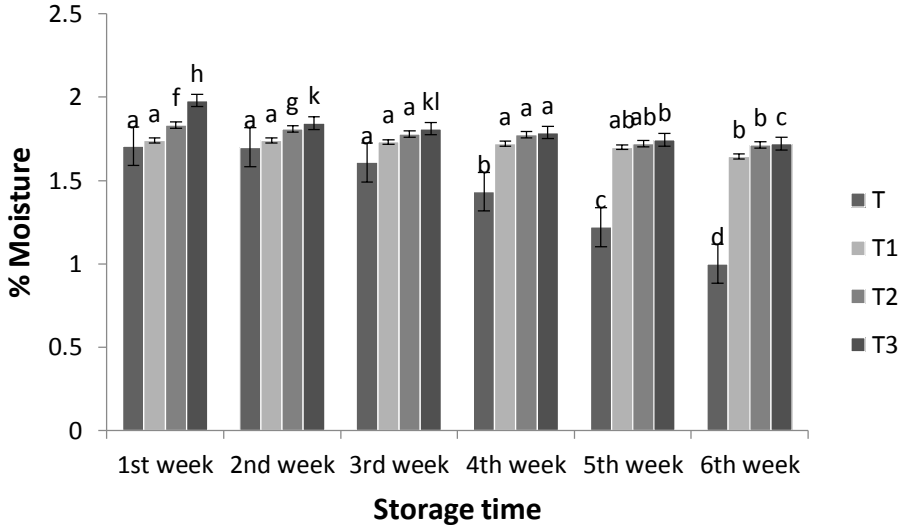

Fig. 11. The results of comparing the average moisture percentage of gum treatments during six weeks of storage ($p<0.05$)

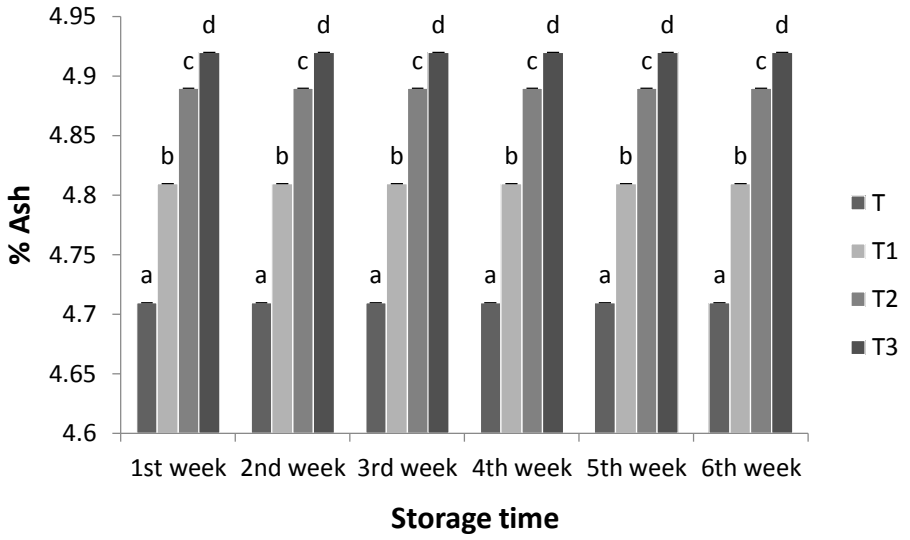

Fig. 12. The results of comparing the average ash percentage of chewing gum treatments during six weeks of storage ($p<0.05$)

The analysis of the hardness of chewing gum treatments indicates that an increase in the percentage of sucrose substitution in the gum formulation leads to a reduction in the hardness of the gum treatments post-production ($p<0.05$) Furthermore, over a six-week storage period, the hardness of the gums remains constant at the same ratio. The minimum level of hardness is found to be correlated with T_3 treatment, while the maximum level of hardness is observed in the control treatment.

Substituting sucrose with date juice resulted in notable alterations in the adhesion index across the various treatments. As demonstrated in Table 3, the incorporation of date juice in the composition of the treatments resulted in a significant increase in adhesion. Specifically, the treatment utilizing a 30% replacement of date juice exhibited the highest adhesion rate, whereas the control treatment yielded the lowest adhesion rate. The treatments employing 10% and 20% substitution of date juice exhibited minimal distinctions from the control treatment. Specifically, substituting sucrose with date juice at a 20% level did not result in substantial variances in treatment adhesion ($p<0.05$).

The capacity for springiness is influenced by additional texture properties, including hardness and adhesion. There is a significant decrease in the springiness of treatments by increasing adhesion and reducing hardness. The decline in springiness becomes apparent at the replacement level of 20% and above. The treatment with the highest level of adhesion (T_3 treatment) exhibits the least degree of springiness. The springiness of T_1 and T_2 treatments shows a significant increase when the hardness of these treatments is reduced through the substitution of 10% and 20% ($p<0.05$). Furthermore, over a six-week period of storage, no statistically significant differences were observed between the various treatments on the first day following production ($p<0.05$).

The findings of the texture assessment demonstrate alterations in the gum's texture as a consequence of substituting sugar with date juice. The textual alterations can be attributed to the inherent properties of date juice. Date juice, as a

sweetener, can contribute to the viscosity, shape retention, and elasticity of gum due to its high solids content. This phenomenon has been demonstrated in various studies (Al Hagbani *et al*., 2018; Sultan *et al*., 2016; Apar *et al.*, 2004). The findings presented in Table 3 demonstrate that the retention of elevated levels of moisture results in a reduction in the hardness of gum treatments. This issue relates to improving the chewiness of date juice gum treatments by substituting 10 and 20% of the original ingredients used. The augmentation of adhesion in elevated concentrations of date juice corresponds to a 30% reduction in the elasticity and resilience of the texture, resulting in a subsequent diminution in masticatory function. Furthermore, the resilience of the gum experiences a notable reduction as the percentage of adhesion increases, due to its correlation with the rise in moisture content in the T_3 treatment. The preservation of chewing gum treatments through packaging and limited moisture exchange with the surrounding environment prevents notable alterations in the texture attributes of chewing gum within the initial six weeks of storage following production ($p<0.05$). Several studies have shown consistent findings regarding the impact of liquid syrup consumption on the gum production process and the assessment of gum quality characteristics (Al Hagbani *et al*., 2018; Haahr *et al*., 2003).

Table 3. Comparing the average results of evaluation of histometric indicators of treatments during six weeks of storage ($p<0.05$)

Storage time	Treatments	Chewing ability ($N.mm^{-1}$)	Hardness (N)	Cohesiveness	Springiness (mm)
1st week	T	6±0.01 a	7.8±0.02 a	1±0.02 a	0.9±0.02 a
	T_1	6.3±0.02 b	7.4±0.01 b	1±0.49 a	0.92±0.01 b
	T_2	6.6± 0.04 c	7.2±0.04 c	1±0.98 a	0.94±0.02 c
	T_3	5.8± 0.2 d	7±0.02 d	1.5±0.02 b	0.7±0.02 d
2nd week	T	6±0.01 a	7.8±0.02 a	1±0.02 a	0.9±0.02 a
	T_1	6.03±0.1 b	7.4±0.01 b	1±0.09 a	0.92±0.03 b
	T_2	6.6±0.02 c	7.2±0.04 c	1±0.53 a	0.94±0.01 c
	T_3	5.8±0.02 d	7±0.02 c	1.5±0.88 b	0.7 ±0.02 d
3rd week	T	6±0.01 a	7.8±0.02 a	1±0.02 a	0.9±0.02 a
	T_1	6.3±0.03 b	7.4±0.01 b	1±0.04 a	0.92±0.03 b
	T_2	6.6±0.03 c	7.2±0.04 c	1±0.03 a	0.94±0.03 c
	T_3	5.8±0.02 d	7±0.02 d	1.5±0.94 b	0.7±0.02 d
4th week	T	7.8±0.02 a	7.8±0.02 a	1±0.02 a	0.9±0.02 a
	T_1	7.4±0.01 b	7.4±0.01 b	1±0.04 a	0.92±0.02 b
	T_2	7.2±0.05 c	7.2±0.04 a	1±0.02 a	0.94±0.03 a
	T_3	7±0.02 d	7±0.02 d	1.5±0.9 4 b	07±0.02 d
5th week	T	6±0.01 a	7.8±0.02 a	1±0.02 a	0.9±0.02 a
	T_1	6.3±0.02 b	7.4±0.01 b	1±0.29 a	0.92±0.02 b
	T_2	6.6±0.06 c	7.2±0.04 c	1±0.76 a	0.94±0.04 c
	T_3	5.8±0.02 d	7±0.02 d	1.5±0.96 b	0.7±0.02 d
6th week	T	6±0.01 a	7.8±0.02 a	1±0.02 a	0.9±0.01 a
	T_1	6.3±0.03 b	7.4±0.01 b	1±0.02 a	0.92±0.03 b
	T_2	6.6±0.04 c	7.2±0.04 c	0.99	094±0.05 c
	T_3	5.8±0.02 d	7±0.02 d	1.5 b	0.7±0.02 d

Conclusion

Sucrose is celebrated for its natural sweetness and robust functional properties, making it a widely utilized ingredient across various food products. Nevertheless, the prevalent use of sucrose in food items has been found to diminish the intake of essential nutrients such as vitamins, minerals, amino acids, and fatty acids. Conversely, the correlation between sugar consumption and various health ailments such as elevated glucose and blood sugar levels, cardiac conditions, dental caries, and obesity, along with concomitant economic and technological factors, has prompted an upsurge in research efforts to identify a viable sugar substitute to be used as alternative. Concurrently, there is a concerted effort to develop value-added products in this aspect.

Cereal-based products, including bread, cakes, biscuits, and confectionery items like chewing gum, are extensively utilized worldwide and have become integral components of the contemporary diet. Many of these products, particularly chewing gum, contain excessive quantities of sugar. Various agricultural products, including corn, date fruit, figs, and grapes, have been identified as potential natural and viable alternatives to sucrose. Furthermore, incorporating date juice in food formulation not only decreases the sucrose content, but also offers various advantages such as enhancing the nutritional profile of food items, generating high-value products, utilizing cost-effective and easily accessible raw materials, and consequently aiding local processing sectors. The sensory (taste) and tactile (firmness and mouthfeel) attributes of the product are of considerable importance. Several issues pertaining to optimization arise when substituting sucrose in food products, including negative impacts on taste, physical attributes of the product, consumer acceptance, and legal restrictions within this domain. This research examined the sensory and physicochemical attributes, including texture, chewability, elasticity, and flavor, of chewing gum treated with different levels of date juice replacements. The treatments that replaced 20% and 10% of date juice were found to be the most favorable based on the mentioned characteristics.

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Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 45-58, 2024

The Effect of Plant Essential Oils on Some Physiochemical Traits and Enzymatic Activity of Cherry (Prunus Avium L. CV Takdaneh mashhad) in Postharvest Conditions

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Received: 10 February 2024 Accepted: 22 April 2024

ABSTRACT: Maintenance and quality of fresh cherry fruit during storage is important therefor the aim of present study is to estimate the effects of post-harvest thyme, basil and mint (TEO, BEO and MEO) essential oils (EOs) on the biochemical traits and shelf life of cherry fruit during storage at 1±0.5 ℃ and 90 to 95% humidity. Fruit were dipped in deionized water (control), (EOs) at 250 and 500 µl l^{-1} concentrations for 5 min and evaluation of traits were performed on 0, 5, 10 and 15 days after harvest. All treatments had a significant effect on the measured variables. Firmness, anthocyanins and superoxide dismutase activity were improved with 500 µl l^{-1} MEO treatment. Fruit treated with 500 µl l^{-1} TEO exhibited the highest cell membrane stability index, phenol, vitamin C, catalase, polyphenol oxidase activity during the storage period. The maximum TSS and pH was observed at 500 µl l^{-1} BEO. At the end of the storage period, the lowest fresh weight loss and relative fresh weight were observed in the 500 µl/l TEO and Total acidity was in 250 µl l^{-1} MEO treatment, respectively. Over the cold storage, 500 µl l^{-1} TEO was found to be the best treatment to maintain fruit quality in terms of postharvest life with 21 days. This experiment revealed that post-harvest treatment with 500 µl l^{-1} thyme, basil and mint essential oil prolonged the storage-life and preserved the valuable marketing characteristics of cherry fruit.

Keywords: Basil, Cherry, Essential Oil, Mint, Shelf Life, Thyme.

Introduction

Cherry belongs to Rosaceae family and the genus *Prunus*, a non-climacteric fleshy drupe cultured in the temperate zones within the world. Sweet variant (*P. avium*) is one of the three major cultivars of cherry fruits (Iezzoni, 2008). Various species are demanded in overseas market namely Takdaneh Mashhad which is the most popular for export. The fruit is rich in health-promoting components such as vitamins B1, B2, A, D, potassium, magnesium, calcium and organic acid, anthocyanin, total phenolic content and antioxidants. The sweets varity contain higher rates of sugar and lower amount of TA (13-25% SSC, 0.4-1.5% TA) that affect the sweetness and flavor. Five kinds of sugars are generally present in sweet cherries; glucose, sucrose, fructose, maltose, and sorbitol (Usenik *et al.*, 2008). Although, they are extremely decayable

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and very problematic to employ after harvest. Cherries are sensitive to water loss, softening, rottenness and stem browning. They are also susceptible to different physiological and microbial disorders. Various postharvest methods have been expanded to increment the shelf life and market cost of cherries such as controlled and modified atmosphere storage (MAP), irradiation, edible coatings and some chemical materials. Other methods and technologies for processed cherry productions such as dehydration, freezing and canning have also been assumed for preservation of cherries (Shah *et al*., 2018).

Essential oils include complex, volatile and natural materials that are produced by different parts of plants as a secondary metabolite and have various assignment. In most situation, it has antimicrobial, allelopathic, antioxidant and bioregulatory attributes. Numerous studies displayed that factors associate with postharvest ripening, like color and firmness were remarkably postponed in treated cherries with combination of essential oils, plus respiration level and moisture loss (Zapata *et al.*, 2017). In this aspect, the conducted works have reported the positive effect of plant essential oils on improving the quality of fruits on postharvest conditions for example the positive effect of using *Thymus vulgaris*, *Foeniculum vulgare* and *Satureja hortensis* oils on *Vitis vinifera* (L.) cv. Tabarzeh and *Thymus vulgaris* and *Lavandula angustifolia* oils on apple Jonagold cultivar reported by *ABDOLAHI et al.* (2010) and *RABIEI et al*. (2011), and also Mohamed and El-Badawy (2013) and Salimi *et al.* (2013) reported the effect of *Thymus vulgaris* and *Syzygium aromaticum* oils and *Ocimum basilicum*, *Mentha longifolia* and *Carum copticum* oils on the quality of washington navel orange and *Vitis vinifera* fruits, respectively. Therefore, due to the risk of unsuitable applications of chemicals and the health of consumers application of natural products namely essential oils to improve the quality and extend of shelf life is pre to need.

Materials and Methods

- Plant material and treatment

Uniform commercial cherry was purchased according to commercial harvest and fruit color change without mechanical and pest or diseases damage. Pure essential oils of mint (*Mentha piperita*) (MEO), basil (*Ocimum basilicum*) (BEO) and thyme (*Thymus vulgaris*) (TEO) were provided from the Essential Oil Company (Portland, OR, USA). Post-harvest EOs treatments were applied with three replications. Fruit were dipped for 5 min in 250 and 500 µl l^{-1} mint (MEO), basil (BEO), thyme (TEO) and distilled water (control). All fruits were kept in the storage with 1±0.5°C and 90-95% humidity. Samples traits were measured in 0, 5, 10 and 15 days after storage.

- Assessed traits

Fresh weight of nectarine fruits were recorded in the first, 5, 10 and 15 days using a digital scale with an accuracy of 0.01%. Weight loss was estimated in each replication and was noted initially and 5, 10 and 15-days during storage (Danaee and Abdossi, 2015).

In orders to determined cell membrane stability index, the samples were placed at Benmarry Jar in 30 °C for 60 min, then the EC_1 level was recorded by EC meter. The Falcons were then transferred in an autoclave at 120 ° C for 20 min at 1.2 atm. After cooling, the EC_2 was recorded. Finally, cell membrane stability index was expressed as a percentage (Dareini *et al*.,

2014). Fruit firmness was monitored using penetrometer (TA-XTPlus, Stable microsystem Co. Ltd., UK) with an 8 mm diameter flat probe. Total Acidity (TA) values of solutions were measured with titration method using 0.1 M NaOH to the endpoint pH: 8.3 and the results were expressed as percentage of citric acid. Total Soluble Solids (TSS) were demonstrated using a digital refractometer (Atago Co., Tokyo, Japan) and pH of juice squeezed from fruit was determined in 50 ml samples of pulp with a digital pH meter; CP - 505 Clmeriron (Imani and Danaee, 2023). In order to the anthocyanin content of nectarine fruits, a certain amount of fruit was ground with methanol extraction solution and hydrochloric acid. The samples were poured into a test tube and exposed to 4ºC for 24 h and centrifuged for 5 min at 5000 rpm. The adsorption rate of the extract was read using spectrophotometer at 530 and 657 nm (Meng and Wang, 2004). Vitamin C content was measured by two-steps oxidation-reduction titration. The content of Vitamin C was measured by titrimetric method and calculated in mg (ascorbic acid) $100g^{-1}$ FW (Hosseinzadeh Rostam Kalaei *et al*., 2022). In order to measure the amount of phenol, 0.5-1 g of sample was used, which was ground in 80% ethanol and centrifuged at 10000 rmp for 20 minutes. The absorbance of the samples at 650 nm was determined by the control reagent (Malik *et al*., 1980).

The superoxide dismutase activity was measured by NBT method, as explained by Soroori *et al*. (2021). Activity of catalase was expressed in the method of *BAILLY et al.* (2004) and polyphenol oxidase was assayed following the procedure outlined by *POLLE et al*. (1994).

In order to measure the shelf life, the fruits were kept at 0.5±1°C and 85-90 % relative humidity. Symptoms such as placed, spoilage and mold contamination were noted in relation to the shelf life parameters.

- Statistical Analysis

The factorial experiment was performed in a completely randomized design with 3 replications. The data were analyzed using SPSS software. Data values were compared using LSD test at 1 and 5% levels.

Results and Discussion

The result of analysis of variance shows that the effect of treatment and days was significant on relative fresh weight, fruit weight loss, cell membrane stability index, tissue firmness, total soluble solids, titratable acidity, anthocyanin content, vitamin C, antioxidant activities at 1% level and phenols at 5% level. The effect of treatment on postharvest life at different concentrations of treatment was significant at the level of 1% (Table 1).

- Morphological traits

The results of the experiment indicated relative fresh weight, fruit weight loss, cell membrane stability index and tissue firmness decreased in all treatments during storage but declining trend in control treatment was more than others. Relative fresh weight was decreased after harvesting and during storage period but it was highest to 63.73% with 500 µl l^{-1} TEO (Figure 1). Fruit weight loss increased during fruit storage; but minimum level was achieved to 11.41 % in treated fruit with 500 µl l^{-1} TEO (Figure 2).

Weight loss of fruits is due to the incline of water vapor pressure among the fruit and the circumambient air, which is normally decreased by both epidermal cell layer and cuticle. Therefore, edible coating proceed as a surplus tissue which also cover the stomata conduce a reduction in

transpiration and in turn, to a decrease in weight loss. This leads to the principal useful impact of edible coatings, which has been illustrated in an immense area of fruits such as *Prunus armeniaca*, *Capsicum annuum*, Prunus persica, sweet cherry and *Persea americana*, among others (Maftoonazad and Ramaswamy, 2005; Maftoonazad *et al*., 2008). These obstacle characters also decrease the selective penetrance to O_2 and CO_2 of the fruit surface being a gain in CO_2 levels in the fruit layers and a decline in O_2 concentration, which might be accountable for the minimize respiration level in the alginate coated fruits. 500 µl l^{-1} MEO were more effective than other sources and decreased softening rate, the difference was observed by the 15_{th} day with 13.27 N m^{-2} (Figure 3). Essential oils increased the amount of cell membrane stability index in all applied treatments and the highest level (63.73%) was observed in 500 µl l^{-1} TEO (Figure 4).

The preservation of firmness in covered fruits might be demonstrated by delayed decay of cell wall membrane, mostly water in soluble and NaOH insoluble pectin, because of the impact of the internal fruit atmosphere with supreme CO_2 and minimum O_2 on subtractive the activity of the cell wall hydrolases responsible for fruit softening (Valero and Serrano, 2010). Hence, the overall consequence displays a delay in the postharvest ripening/maturation process in alginate-coated cherries, leading to retention of organoleptic and nutritive quality factors, purposefully with the addition of essentials oils.

Loss of firmness might be expected with increased activity of cell wall degrading enzymes such as polygalacturonate and pectin methyl esterase. On the other hand, reducing the amount of fruit juice during storage increases the pressure of cellular turgor and reduces the firmness of fruit tissue. The use of compounds in the essential oils of medicinal plants such as carvacrol, siamic acid and anethole by increasing the antioxidant activity and strengthening the host's defense system reduces the rate of aging, softening and increasing tissue resistance to disease. As a result, relative fresh weight, cell membrane stability index and fruit tissue firmness, preservation and fruit weight loss percentage are also lower than the control treatment (Banani *et al*., 2018).

It is widely accepted that the most important quality parameters determining sweet cherry acceptability by consumers are red color, firmness and flavor which is mainly related to the ratio between TSS and TA, and they show important differences among cultivars and maturity stages (Díaz-Mula *et al*., 2009).

- Biochemical traits

Our finding indicated that total soluble solids, anthocyanin content, titratable acidity, antioxidant capacity, vitamin C and phenol declined in all treatments during storage time but decreasing style in control treatment is more than others except titratable acidity. Total soluble solids in 500 µl l^{-1} BEO treated fruit with 13.93 ºBrix was highest at the 15_{th} day. Total acidity with 0.95% was highest in 250 µl l^{-1} BEO treatment after 15_{th}. Anthocyanin content was significantly greater in 500 µl l^{-1} MEO treatment than the other treatments with 9.35 mg g^{-1} FW after 15_{th} day. Treatment of 500 µl l^{-1} TEO with 3.21 mg 100 g^{-1} FW, demonstrated maximum vitamin C content after 15_{th} day. Phenol content were influenced by 500 µl l^{-1} TEO treatment since its value obtained 31.72 mg 100 g^{-1} DW during fruit storage (Table 2). *Thymus capitates* L oil has been applied as barrier for plant diseases of

some fruits (Tzortzakis and Economakis, 2007; Abd-AllA *et al.,* 2011; Hyun *et al*., 2015) and considerably maintained the vitamin C content and quality of the orange fruit (Fatemi *et al.,* 2011). *Mentha piperita* L. oil employed to decrease decay levels and greatly implemented vitamin C, raised acidity and preserved quality of the orange fruit (Fatemi *et al.,* 2011) and illustrated positive impacts on TA, TSS, weight loss percentage, increased shelf life of plum fruits (Aminifard and Mohammad, 2013). The enhancement level of total soluble solids in this study was up to fifth day. Based on the results of other investigations the impact of essential oils on total soluble solids can be derived from features of essential oil have do not a positive efficacy on total soluble solids during storage (Maqbool *et al*., 2010). According to our data, the optimum concentration of essential oils is capable to protect the total acidity rate to the end of the storage period. This result is similar to those of (Yousuf and Srivastava, 2017). Various concentrations of essential oils during the keeping period show significant impact on pH changes. According to the previous results, it is not possible to establish a straight correlation between pH and total acidity due to the changes in the buffering capacity of organic acids, the application of organic acids in the reaction leads to lower enzymatic levels of respiration (Bico *et al*., 2009). Phenolic compounds are one of the important plant metabolites that are synthesized by the schemic acid pathway and play an important role in neutralizing the excess of free radicals. The essential oils stimulate induction resistances and as a result phenolic compounds are reduced at a slower rate than the control. According to similar research results, it can be noted that most of the antimicrobial activity of the essential oils is directly associated with the prevention of the production of phenolic compounds, which makes it possible to use plant essential oils to reduce phenolic compounds and reduced the effect of free oxygen (Tzortzakis, 2007).

As a result, reducing the amount of phenol will hamper ruinous effects and lower quality. Regarding the results of the experiment concerned with the total anthocyanin content, it should be mentioned, that by applying the essential oil treatment, total anthocyanin has increased. Essential oils with high antioxidant properties probably preserves and stabilizes the color markers (anthocyanin content) which is the main pigment in cherries, and prevents its decomposition. Similar results were observed by Oz and ulukanli, (2012).

Catalase activity of cherry fruits at 15th day after harvest with 8.85 unit enzyme g^{-1} FW was highest at 500 µl l^{-1} TEO treatment. 500 µl l^{-1} TEO treatment with 9.13 unit enzyme g^{-1} FW increased POD at 15th day after harvest and the highest SOD was at 500 µl l^{-1} MEO treatment after 15 day harvested (Table 3). Antioxidants delay the oxidation of biomolecules such as lipids, proteins, carbohydrates, and deoxyribonucleic acid by inhibiting the release of electrons into free radicals. The antioxidant capacity of fruits is related to enzymatic compounds (superoxide dismutase, catalase, peroxidase, glutathione reductase, etc.) and non-enzymatic (carotenoids, ascorbic acids, phenols, flavonoids, etc.) depending on plant growth conditions, including environmental conditions, harvest time which may affect the antioxidant capacity. Plant essential oils improve the activity of antioxidant enzymes (Dar *et al.,* 2015). It has been reported that bioactive compounds and antioxidant activity show changes during cold storage of sweet

cherry cultivars (Serrano *et al.*, 2009).

During postharvest storage, 500 µl l^{-1} TEO was found to be the best treatment to maintain fruit quality in terms postharvest life with 21 days (Figure 6). The results of the study is consisting with the findings of Hosseini *et al.* (2015) on the effect of essential oil of marjoram on (*Prunus avium* L. cv Takdaneh Mashhad). Kamyab and Mahidashti *et al.* (2018) effect of peppermint extract on banana, Saedi and Asgharzadeh (2017) effect of thyme and Clov on Prunus Avium L. CV Takdaneh mashhad. Also the result of Şerban *et al.* (2011) showed that *Lavandula hybrida* oil, *Anethum graveolens* L. oil and *Coriandrum sativum* L. oil indicated antibacterial and high antifungal activity against different bacteria and fungi species. Our experience is in line with recent studies on treatment with essential oil compound that have proven to induce the inductive-defensive system and increase the shelf life of fruits and vegetables (Bill *et al.*, 2017; Vithana *et al.*, 2019).

Table 1. Analysis variance of application of MEO, BEO, TEO on some physicochemical attributes of *Prunus avium* L. cv Takdaneh Mashhad

		Mean Square												
	DF	**Relative fresh weight**	**Weight lose**	**Cell membrane stability index**	**Firmness**	**TSS**	**TA**	**pH**	**Anthocyanin**	**Vitamin C**	**Phenol**	**Catalase**	**Superoxide desmutase**	**peroxidase**	**Shelf life**
Essential oils	6	147.215**	15.716**	117.318**	21.116*	18.217**	3.261*	9.045**	16.385**	6.526*	56.762**	14.196*	8.417**	18.056*	25.216**
Days	3	98.216**	6.312**	87.043**	9.710**	7.439**	1.012**	4.119*	7.520*	1.974*	37.912**	5.412**	3.762*	9.184**	-
Essential oils *Days	18	115.315**	11418**	104.216**	17.212**	12.093**	1.917**	6.171**	11.095**	4.219**	44.285*	8.087**	6.073**	14.458**	-
Error	42	4.51	3.12	5.18	2.03	4.51	3.85	4.12	6.81	3.65	4.12	5.04	6.01	4.65	3.28
CV (%)	-	9.54	`12.25	10.43	11.52	12.25	9.54	9.27	11.26	8.75	11.26	10.22	11.53	12.25	9.76

ns, * and ** indicates non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

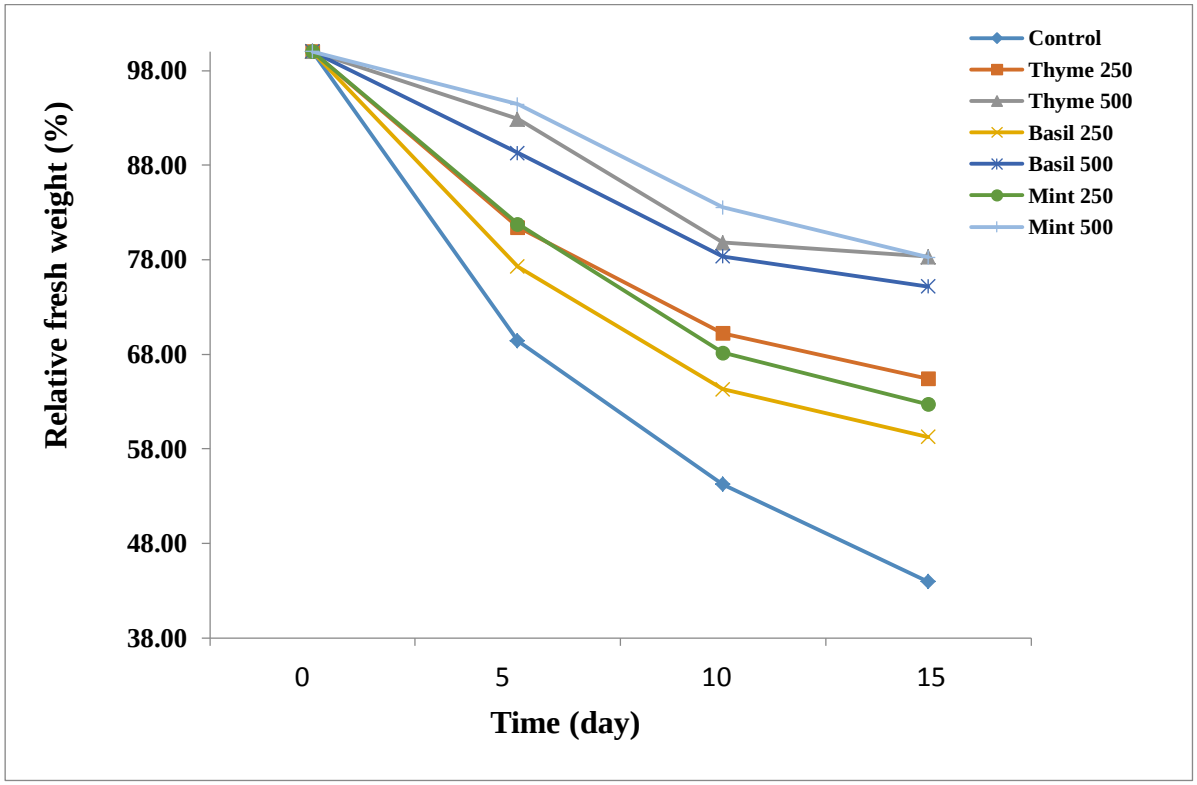

Fig. 1. Effect of application of MEO, BEO, TEO on relative fresh weight of *Prunus avium* L. cv Takdaneh Mashhad.

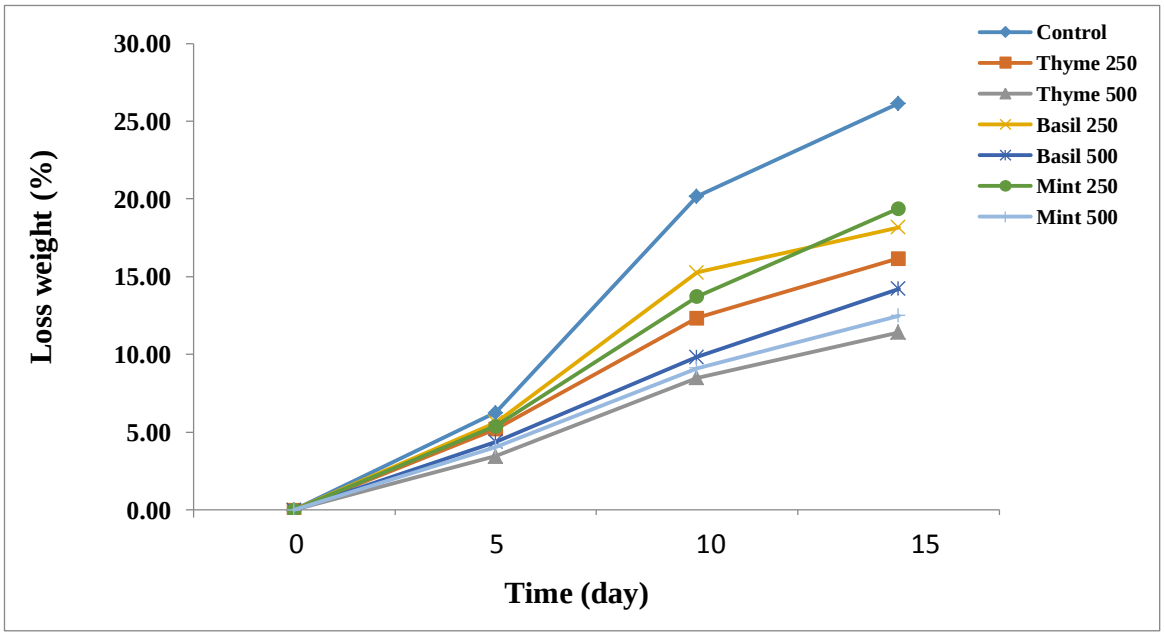

Fig. 2. Effect of application of MEO, BEO, TEO on loss weight of *Prunus avium* L. cv Takdaneh Mashhad.

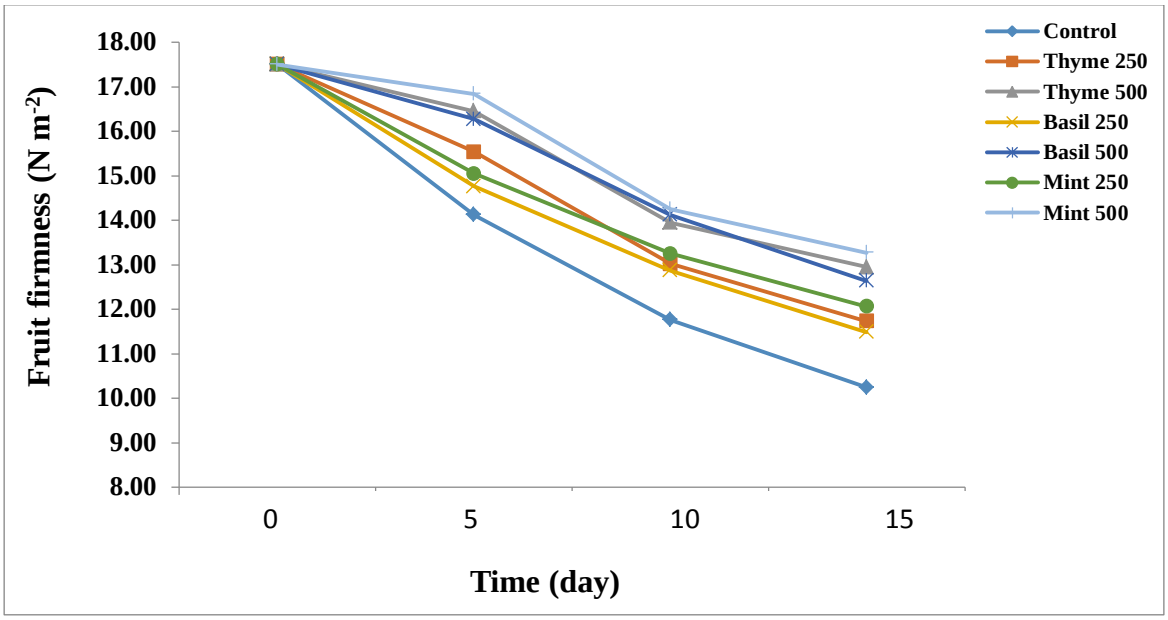

Fig. 3. Effect of application of MEO, BEO, TEO on furit firmness of *Prunus avium* L. cv Takdaneh Mashhad.

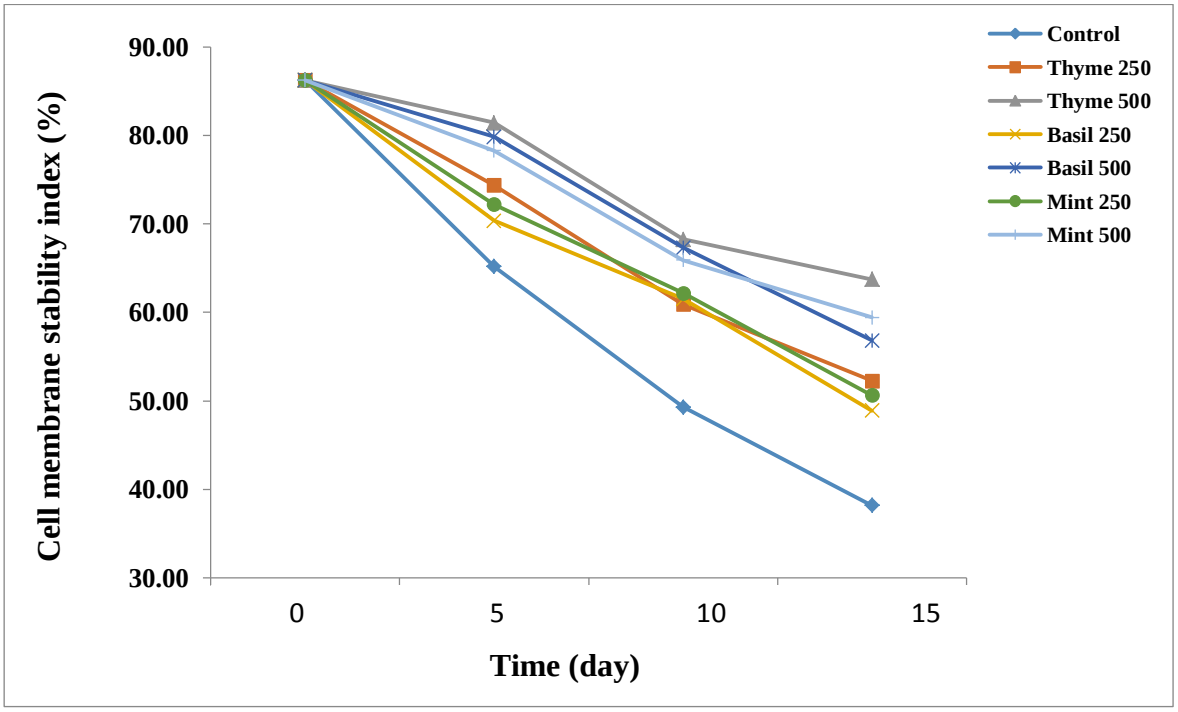

Fig. 4. Effect of application of MEO, BEO, TEO on cell membrane stability index of *Prunus avium* L. cv Takdaneh Mashhad.

Table 2. Effect of application of MEO, BEO, TEO on some physicochemical attributes of *Prunus avium* L. cv Takdaneh Mashhad

Storage time (day)	Treatment (µl l^{-1})	TA (mg 100 ml^{-1})	TSS (°Brix)	pH	Vitamin C (mg $100g^{-1}$ FW)	Anthocyanin (mg g^{-1} FW)	Phenol (mg g^{-1} DW)
At harvest	Control (distilled water)	1.45±0.33	15.66±0.18	4.76±0.14	4.27±5.89	12.45±3.43	42.17±0.43
	Thyme 250	1.45±0.28	15.66±0.14	4.76±0.09	4.27±2.18	12.45±4.34	42.17±0.30
	Thyme 500	1.45±0.10	15.66±0.20	4.76±0.09	4.27±3.34	12.45±12.01	42.17±0.24
	Basil 250	1.45±0.10	15.66±0.24	4.76±0.03	4.27±9.20	12.45±2.23	42.17±0.24
	Basil 500	1.45±0.08	15.66±0.14	4.76±0.06	4.27±19.05	12.45±5.09	42.17±0.17
	Mint 250	1.45±0.09	15.66±0.14	4.76±0.06	4.27±19.05	12.45±5.08	42.17±0.17
	Mint 500	1.45±0.08	15.66±0.14	4.76±0.06	4.27±19.05	12.45±5.80	42.17±0.17
5 days	Control	1.36±0.23	13.45±0.28	3.04±0.14	2.67±5.89	9.66±3.54	30.27±0.43
	Thyme 250	1.02±0.28	14.42±0.14	3.89±0.09	3.46±12.18	10.87±3.32	35.76±0.30
	Thyme 500	0.87±0.22	15.12±0.20	4.32±0.09	4.05±3.34	11.98±3.43	39.83±0.24
	Basil 250	1.27±0.14	14.56±0.24	3.51±0.03	3.11±9.20	10.42±0.03	32.85±0.24
	Basil 500	0.95±0.07	15.23±0.14	4.53±0.06	3.75±19.05	11.35±0.09	38.35±0.17
	Mint 250	1.21±0.07	14.07±0.14	3.75±0.06	3.23±19.05	10.51±0.07	34.19±0.17
	Mint 500	0.75±0.07	14.89±0.14	4.27±0.06	3.87±19.05	11.96±0.06	41.56±0.17
10 days	Control	1.12±0.13	12.62±0.28	2.58±0.14	2.04±5.89	8.14±0.82	24.19±0.43
	Thyme 250	0.78±0.03	13.39±0.14	3.48±0.09	2.98±12.18	9.57±0.14	30.17±0.30
	Thyme 500	0.69±0.12	14.01±0.20	3.87±0.09	3.56±3.34	10.67±0.30	35.39±0.24
	Basil 250	1.05±0.09	13.02±0.24	2.94±0.03	2.76±9.20	9.19±0.13	27.63±0.24
	Basil 500	0.71±0.08	14.35±0.14	4.02±0.06	3.39±19.05	10.42±0.13	29.34±0.17
	Mint 250	0.94±0.08	13.18±0.14	2.93±0.06	2.82±19.05	9.63±0.13	34.19±0.17
	Mint 500	0.52±0.08	13.76±0.14	3.61±0.06	3.35±19.05	11.15±0.30	35.12±0.17
15 days	Control	0.91±0.33	11.23±0.28	1.85±0.14	1.25±5.89	7.41±0.27	20.37±0.43
	Thyme 250	0.65±0.22	12.78±0.14	2.67±0.09	2.59±12.18	8.62±0.24	27.26±0.30
	Thyme 500	0.50±0.21	13.76±0.20	3.75±0.09	3.21±3.34	9.01±0.18	31.72±0.24
	Basil 250	0.69±0.22	12.38±0.24	2.69±0.03	2.14±9.20	8.24±0.18	26.45±0.24
	Basil 500	0.95±0.08	13.39±0.14	3.78±0.06	2.87±19.05	8.84±0.17	30.39±0.17
	Mint 250	0.85±0.09	12.64±0.14	2.83±0.06	2.65±19.05	8.39±0.21	27.61±0.17
	Mint 500	0.38±0.09	13.25±0.14	3.52±0.06	3.09±19.05	9.35±0.22	31.05±0.17

Data are the mean ± standard error (n=4).

Conclusion

The results of this study indicated that the application of thyme, basil and mint essential oils especially in 500 µl l^{-1} in postharvest could improve the quality and shelf life of the fruits. In this study essential oils are considered as a good postharvest tool to increase the shelf life of sweet cherry cultivars with beneficial effects in terms of increasing the antioxidant potential.

Table 3. Effect of application of MEO, BEO, TEO on enzymic activity of *Prunus avium* L. cv Takdaneh Mashhad

Storage time (day)	Treatment (µl l^{-1})	Catalase (Unit enzyme g^{-1} FW)	Superoxide desmutase (Unit enzyme g^{-1} FW)	POD (Unit enzyme g^{-1} FW)
At harvest	Control (distilled water)	11.38±0.28	4.95±0.09	13.25±2.88
	Thyme 250	11.38±0.22	4.95±0.08	13.25±2.18
	Thyme 500	11.38±0.23	4.95±0.08	13.25±3.23
	Basil 250	11.38±0.22	4.95±0.08	13.25±9.21
	Basil 500	11.38±0.16	4.95±0.08	13.25±19.12
	Mint 250	11.38±0.16	4.95±0.09	13.25±19.12
	Mint 500	11.38±0.16	4.95±0.08	13.25±19.11
5 days	Control	8.61±0.20	3.24±0.11	10.27±5.11
	Thyme 250	9.97±0.12	3.96±0.01	10.92±12.77
	Thyme 500	10.96±0.22	4.61±0.01	12.18±3.65
	Basil 250	9.53±0.21	3.64±0.20	10.71±9.23
	Basil 500	10.48±0.24	4.27±0.11	11.94±1.16
	Mint 250	9.61±0.13	3.83±0.10	11.17±1.34
	Mint 500	10.76±0.13	4.25±0.10	11.63±1.17
10 days	Control	6.94±0.08	2.48±0.11	7.49±4.33
	Thyme 250	8.13±0.18	3.12±0.08	8.82±2.77
	Thyme 500	9.58±0.18	3.59±0.18	10.32±2.19
	Basil 250	7.93±0.22	3.01±0.12	8.65±3.44
	Basil 500	9.12±0.25	3.38±0.16	9.91±2.19
	Mint 250	8.32±0.25	2.95±0.16	8.29±4.44
	Mint 500	9.24±0.25	3.76±0.16	9.95±3.99
15 days	Control	5.62±0.23	2.11±0.11	6.23±1.98
	Thyme 250	7.52±0.17	2.76±0.19	7.75±1.99
	Thyme 500	8.85±0.19	3.04±0.19	9.13±2.44
	Basil 250	7.26±0.19	2.59±0.13	6.97±3.55
	Basil 500	8.31±0.18	2.89±0.01	8.95±3.22
	Mint 250	7.04±0.18	2.51±0.03	6.83±2.23
	Mint 500	8.15±0.18	3.12±0.03	8.76±2.44

Data are the mean ± standard error (n=4).

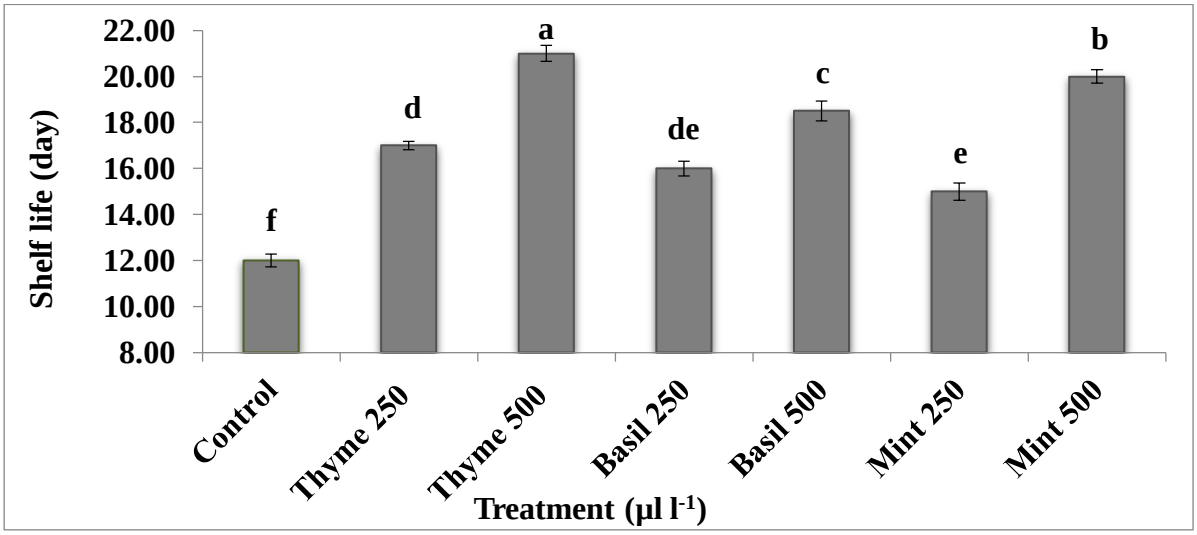

Fig. 5. Effect of application of MEO, BEO, TEO on shelf life of *Prunus avium* L. cv Takdaneh Mashhad, vertical bars indicate standard error (n=4)

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Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 59-69, 2024

Effects of Freeze-Drying Process on Decontamination and Quality Characteristics of Conventional Crystal Sugar for Possible Use in Pharmaceutical Products

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Received: 17 July 2023 Accepted: 21 April 2024

ABSTRACT: The increasing demand for pharmaceutical sugar requires higher quality (specifically microbial level) of standards compared to the sugar commonly used in the food industry. While different thermal processes are applied for microbial decontamination of crystal sugar, they usually destruct and degrade its bio-active compounds, that are undesirable. This research was conducted to evaluate the effects of freeze-drying (at – 42^{o}C, and pressure of 0.1 mbar) on crystal sugar contaminated highly with heat resistant bacteria of *G. stearothermophilus* (9×10^{5}) and also most common fungi of *A. niger* (9×10^{4} CFU/g). The results of this study showed that the non-thermal process of freeze-drying could eliminate > 99% of its original *Gs* and *An* microorganisms successfully. Physicochemical tests have indicated that the freeze-dryer process has made 10.4% decrease in crystal size and 23.5% decrease in ash content, that brings the characteristics of sugar crystals closer to international pharmaceutical standards. Furthermore, the microbial load of crystal sugar after freeze-drying process were below the permissible levels identified in European Pharmacopeia. In fact, there is a potential to use freeze-drying technique to decontaminate the conventional crystal sugar and make it appropriate for using (as ingredient) in pharmaceutical products.

Keywords: Hybrid processes, Lyophylization, Pharmaceutical Sugar.

Introduction

Microorganisms are the most important and well-known creatures, while not visible, exist in nature in various forms, and human have spent many years trying to destroy them. The science history has recorded, deaths of millions children and adults due to epidemic microorganisms' that cause diseases such as tuberculosis, plague, cholera, diarrhea, etc. in different regions of the world. Microorganisms cause diseases in different ways such as: breathing, skin crashes and digestion. In gastrointestinal disease tract, except for a few sterile foods, all foods contain one or more types of microorganisms. Some

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microorganisms cause food spoilage and foodborne diseases. Inorder to study the role of microorganisms in food and control them when necessary, is important to isolate them in pure culture and find suitable ways to destroy them (Ray & Bhunia, 2014). Over the years, scientists have proposed different methods to struggle microbial contamination, which the most well-known are thermal methods, such as pasteurization by Louis Pasteur and sterilization by Joseph Lister, which temperatures about 100 degrees and more are used (Jay, 2000). Drying is one of the oldest methods used to preserve food products. However, drying process may slightly or severely affect the properties of food, especially when it is carried out at high temperatures (Harguindeguy et al., 2019).

Since thermal methods have significant effects on food and destroy functional substances such as vitamins, over time, non-thermal methods opened their way to food industry. In recent years, with increasing complexity of food, products offered in modern food industry markets, increasing demand for food safety and quality standards, development and compliancy of effective non-thermal technologies is a priority for food industry and consumers. Freeze-drying or lyophilization is one of processes that has considered in relation to drying food while maintaining its quality. Lyophilization is a process which water is removed from food in the form of ice under low pressure by sublimation. This process has many applications to produce high quality food and pharmaceuticals (Nowak & Jakubczyk, 2020). Among microorganisms *Geobacillus stearothermophilus* (GS), is one of the most resistant bacteria, which used as an indicator to sterilize. This bacterium is rod-shaped, gram-positive and thermophilic, and widely found in soil, hot springs, and ocean sediments, and known as contaminant in food industry. Since this bacterium is an indicator to sterilize, its destruction by non-thermal methods can indicate the removal of all pathogenic bacteria (Burgess *et al*., 2017; Wang *et al*., 2020). *Aspergillus Niger* (AN) fungus is one of other well-known indicators due to its abundance and generality in food industry. *Aspergillus niger* is the cause of black mold disease in fruits and vegetables and is one of the biggest food contaminants in the world (Kalalian et al., 2020). Pharmaceutical sugar is pure sucrose that is produced based on international standards such as IP[1], EP[2], JP[3], USP[4], BP[5]. Pharmaceutical sugar is widely used in pharmaceutical products. Pharmaceutical sucrose used as sweetener, bulking agent and binder in chewable tablets. Pharmaceutical sugar used to remove unpleasant taste and create necessary viscosity in syrups. Pharmaceutical sugar is used to cover some drugs in form of dragees, therefore unpleasant taste and smell of above drug can be hidden (Patil et al., 2021; Porter, 2021). In this study, two microorganisms, *Geobacillus stearothermophilus* and *Aspergillus Niger*, have been used as targets in measuring disinfection power of lyophilization process in production of pharmaceutical sugar.

Materials and Methods

GS bacteria with ATCC number 7953 (PTCC 1713) AN fungus with ATCC number 9142 (PTCC 5010) were obtained from Iran Scientific and Industrial Research Organization. After preparing desired strains of bacteria and fungi, their

[1] Indian Pharmacopeia
[2] European Pharmacopeia
[3] Japanese Pharmacopeia
[4] The United State Pharmacopeia
[5] British Pharmacopeia

required culture media was prepared according to information in the product sheet of ATCC Company. Culture mediums NA (Nutrient agar) and PDA (Potato Dextrose agar) were obtained from Merck Chemical Company.

Analysis tests performed using the following devices:

NMR[1] (Brukers, Ultra shield, 500MHz USA) was employed to show the probable changes in the molecule structure and ICP[2] was used to measure the heavy metals in samples before and after process., (Varian-OES-730-ES). In the case of ICP all the samples must be in aqueous medium and should be filtered through a 0.2 µm filter in order to avoid the presence of micro-precipitates that could damage the equipment. A liquid sample is introduced using a peristaltic pump to ensure constant, stable flow. Commonly, a nebulizer with a high-speed flow of gas (usually argon) to shatter small droplets of liquid into an aerosol was employed. This aerosol is then introduced into a spray chamber that removes the larger droplets. The sample is then injected into a plasma chamber that is approximately 10,000 degrees. This extreme temperature breaks everything in the sample apart into the basic elements. Every element is released at a specific wave length). FTIR (Thermo Nicolet Nexus, 870 FT-IR USA) to investigate probable changes in chemical radicals, SEM (Seron Technology - AIS2300) to show the probable changes in physical appearance of crystals and microorganisms and PSA[3] to measure the crystal size changes (Sympatec Helos/KF) are employed.

First, lyophilized microorganisms transferred from their vials to Nutrient Broth culture medium, and then liquid sample containing *Geobacillus stearothermophilus* cultured on Nutrient agar plates as surface culture and incubated in a 44°C incubator for 48 hours. Liquid samples containing *Aspergillus Niger* cultured on plates containing Potato Dextrose Agar as surface culture and incubated in incubator at 25°C for one week. Then, each of grown samples passaged several times to use in aforementioned experiments (Anon, 2009). The standard equivalent of one McFarland used to prepare bacterial suspension, and the standard equivalent of half McFarland used to prepare fungal suspension. The combination of 0.048 M barium chloride solution and 0.18 M sulfuric acid used to prepare the McFarland standard. One and a half McFarland standards have optical density = 0.01 ± 0.17 and optical density = 0.08-0.13 at the wavelength of 625-620 nm respectively (Habeeb et al., 2007; Andrews, 2006). Bacterial suspension equivalent to McFarland's standard was prepared and sprayed by TLC Atomizer in 10 ml of physiological serum solution and then diluted to 10^6. Three plates cultured from each dilution and then colonies counted. After calculations, it was found that there are 10^5 x 3 CFU/puff of bacteria in each puff of bacterial suspension and 10^4 x 3 cfu/puff of fungus in each puff of fungal suspension.

Pharmaceutical sugar in the world is produced according to ICUMSA45 standards and sent to pharmaceutical factories, and special baby food factories. Pharmaceutical sugar standard based on the latest edition of ICUMSA45 is described in Table 1. Prototype of sugar (A) which considered as the basis for Freeze Dryer (FD) process has the specifications as described in Table 1. In this study, ICUMSA45 standards, 2019 edition, considered as reference for

[1] Nuclear Magnetic Resonance
[2] Inductively Coupled Plasma
[3] Particle Size Analyzer

production of pharmaceutical sugar by freeze dryer process. Also solid food substrate, namely sucrose, used. Sucrose has chosen as basic material for the investigation due to its high consumption in food industry and special uses in the pharmaceutical industry. The sucrose used obtained from the Hekmetan sugar factory. Each 50 grams of sucrose sprayed with 3 puffs of bacterial or fungal suspension by TLC Atomizer and then placed in freezer at - 20 degrees Celsius for 24 hours. Then placed in Siemens freezer for 24 hours at temperature of - 42 degrees Celsius, pressure of 0.1 mbar and condenser temperature of - 58 degrees Celsius and then after 24 hours, microbial tests related to GS and AN was performed according to the method described by Liu *et al*. (2021).

GS bacteria testing has done based on ICUMSA standard method No. GS2/3-49(1998) and AN fungus testing has done based on ICUMSA standard method No. GS2/3-47(1998) (Anon, 2009). 10 grams of sugar and 100 ml of sterile water were added to sterile erlenmeyer and diluted by magnet stirrer. Then the spread culture method was used for culturing in 55°C for 48 h for GS and 25°C for 5 days for AN.

Chemical tests performed based on the ICUMSA reference book according to the following methods:

The principles of determining polarization number GS1/2/3/9-1 (2009), is the measurements of the optical rotation of the sample solution, compared with the optical rotation of the normal solution of sucrose. For determining amount of invert number GS2/9-5 (2007), a solution of the sugar is heated in a boiling water bass with an alkaline copper reagent. The cupric ions are reduced to insoluble cuprous oxide by the reducing sugars present. After cooling the residual cupric ions are titrated with the EDTA using murexide as indicator. For determining amount of sulfur dioxide number GS2/1/7-33 (2009), the color of sulphite/rosanilin complex is measured photometerically at a wavelength of 560 nm, after reaction with formaldehyde. For ash determination test No. GS2/3-17(2002), the specific conductivity of a white sugar solution at a concentration of 28gr/100gr is determined. The equivalent Ash is calculated by the application of a conventional Factor. The Principal of the pH determination test No. GS1/2/3/4/7/8/9-23(2009), is the potentiometric measurements of pH. The electrodes are standardized with buffer solution, rinsed with distilled water and immersed in the sugar solution. The reading is taken after five minutes when the equilibrium potential across the electrodes is judged to have been reached. For color determination test No. GS2/3/9 (2005) and GS2/3/10 (2007), white sugar is dissolved in distilled water to give a 50% sugar solution. The solution is filtered and then the absorbency is measured at a wavelength of 420 nm and then solution color is calculated. Determining percentage of total phenol has done by SINGLETON method.

Results of tests and experimental design of this study analyzed with SPSS version 26 software.

Results and Discussion

Sugar infected with GS bacteria and AN fungus after passage of time and conditions mentioned for freeze dryer process, were tested according to ICUMSA international standard method (Anon, 2009) in terms of contamination after process.

The results of bacterial and fungal tests show that effect of freeze dryer on bacterial and fungal cells is significant and it has been able to reduce the bacterial load up to 10^5 cfu/gr and fungi load up to 10^4 cfu/gr.

Because the contamination created intentionally and the reduction of its load is quite significant (Figure 1) and compared to the ICUMSA45 international standard, sugar obtained from the freeze dryer process completely accepted in terms of its sterility and absence of bacterial and fungal contamination.

Table 1. Values of physicochemical and microbiological variables in control sugar, sugar obtained from freeze-dryer process and standard range

No	Analysis/test	Blank	Freeze Dried	Limit
1	Taste	Natural Sweet	Natural Sweet	Natural
2	Smell	Natural Smell	Natural Smell	Natural
3	Colour of the solution	71.69±3.55	70.12±0.32	45 ICUMSA
4	Moisture content	0.02±0.006	0.027±0.002	0.06% m/m
5	Conductivity ash	0.018±0	0.014±0	0.04% m/m
6	Polarization	99.81±0.036	99.81±0.02	99.7°Z min
7	invert sugar content	0.018±0	0.014±0	0.04% m/m
8	Coliforms	ND	ND	10 cfu/10g
9	Thermophilic Bacteria	ND	ND	
10	Mesophilic Bacteria	<10	<10	100cfu/gr
11	Yeast and Mould	<10	<10	20 cfu/10g
12	Sulphur dioxide (SO2)	<0.5	<0.5	15 mg/kg
13	Arsenic (As)	ND	ND	0.5 mg/kg
14	Lead (Pb)	0.13	9.25	0.5 mg/kg
15	Copper (Cu)	0.55	3.6	1.0 mg/kg
16	Total Phenol	38.07±2.29 (mg/kg)	15.02±2.81 (mg/kg)	
17	PSA	817.24±5.09	732.17±6.22	500-800µm
18	pH	5.08±0.01	5.3±0.01	

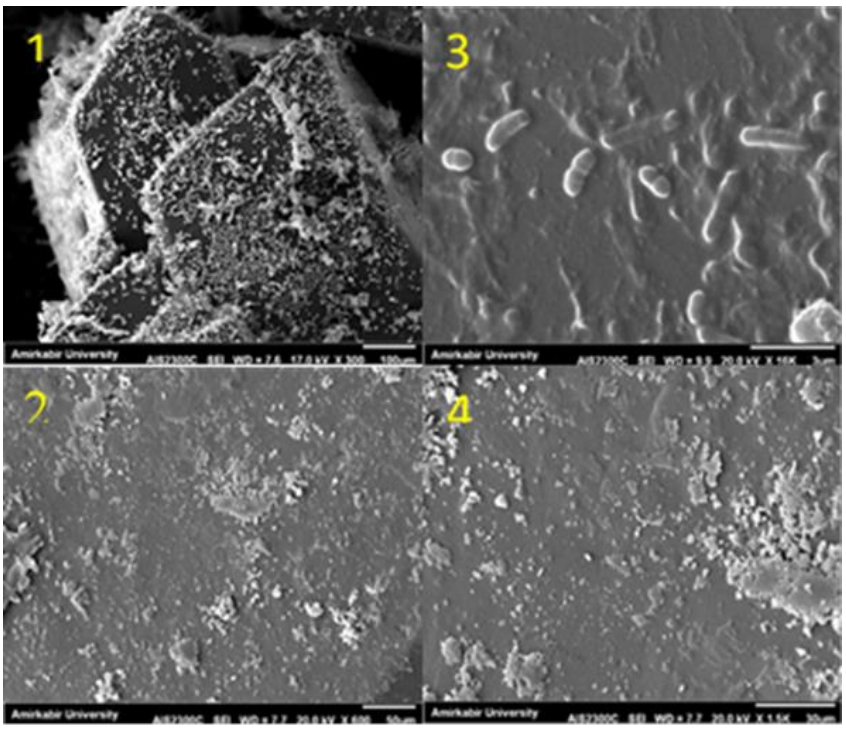

Fig. 1. Sugar: infected with AN Fungus before FD [1], AN Fungus after FD [2], infected with GS bacteria before FD [3], GS bacteria after FD[4].

In investigation of resulting changes of process (Table 2), considering that, amount of polarization(Pol) which is main variable in sugar tests, no significant difference was observed compared to the original sample in relation to changes of Pol. No significant difference observed in investigation of crystal color changes before FD and after FD. The amount of invert did not change significantly after process. Regarding the amount of sulfurous anhydride, since the amount of this substance was zero in the initial sample, there was not observed in the sample after the process. Existence of moisture in the form of crystals during sublimation causes acids in crystal, dehydrated and settle inside the crystal that seems to increase the pH after process and causes significant difference between the averages before and after process (Van Den Berg, 1966).

Regarding ash after freeze dryer process, the amount of ash has significant difference is compared to the original sample and one of the causes is dehydration of salts in crystal. Since the method of measuring ash is conductometry, in this method, soluble salts that can ionized, measured, therefore amount of ash has decreased with dehydration of salts and their structure (Thorat and Suryanarayanan, 2019).

Sucrose itself is a hygroscopic substance. After freeze-dryer process, in addition to sucrose, dehydrated salts are also included in the crystal structure of sucrose, which leads to increase in hygroscopic properties. This probably causes the moisture content of sugar to increase a little after process during transfer as compared to before, that is due to correct transfer and in accordance with principles. This increase is not statistically significant and is still within range of ICUMSA standards (Li et al., 2018). In the conditions of the freeze dryer, due to sublimation action, active site of enzymes changes during removal of moisture in solid form. Sometimes this is accompanied by movement of substrate towards the active site of enzyme that leads to the reduction of phenolic compounds in the sample. One of the enzymes that can be present in crystal is polyphenol oxidase enzyme, some of which enters the process through extract and remains in crystal. During sublimation, pigment of sugar, which is melanoidin, decomposed by enzyme under the conditions mentioned above, and this leads to reduction of phenolic compounds and color of

sugar crystal after the freeze-dryer process, and decreasing color is not statistically significant and is in standard range (Papoutsis et al., 2017; Asadi, 2007). In freeze dryer process, a polymer (rubber) container with the same diameter as the freeze dryer plates was used for freezing. Based on studies, this type of container is made of isoprene polymer or 2-methyl1-3-butadiene. In order to make these containers, in addition to isoprene, salts of elements of groups 4-8 of the Periodic Table or acyl and alkyl compounds of elements of groups 1-3 of the Periodic Table are used, depending on the factory's formulation (Anon, 2007).

If these containers exposed to extreme temperature changes such as high heat or very low cold, the diffusion coefficient of materials increases between 6-7 times. This causes migration of elements and monomers from container to food (Richardson et al., 2012). As seen in the results, samples obtained from freeze dryer have more copper and lead than the control sample. This

increase in amount of heavy metals is probably due to migration of elements from container containing sample during freezing and considered secondary

pollution, which can be eliminated by changing the type of container. During freeze dryer process, pressure resulting from sublimation in removal of solid moisture causes some sugar crystals to break and reduce their size (Levin et al., 2021). Test measuring crystal particles (Figure 2) along with images taken by the electron microscope (SEM) prove this hypothesis (Figure 3).

Table 2. Comparison of the average physicochemical properties in the design of two dependent samples with samples A control sugar and FD sugar obtained from the freeze-dryer process.

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PolA - PolFD	-.006667	.051316	.029627	-.134143	.120809	-.225	2	.843
Pair 2	ColorA - ColorFD	1.573333	3.906153	2.255219	-8.130089	11.276756	.698	2	.558
Pair 3	Ash.A - Ash.FD	.004167	.000058	.000033	.004023	.004310	125.000	2	.000
Pair 4	pH.A - pH.FD	-.213333	.025166	.014530	-.275849	-.150817	-14.683	2	.005
Pair 6	MoistureA - moistureFD	-.007000	.005568	.003215	-.020831	.006831	-2.178	2	.161
Pair 7	TotalphenolA - TotalpheolFD	22.876667	.676782	.390740	21.195448	24.557885	58.547	2	.000
Pair 8	PSA.A - PSA.FD	85.230000	9.362035	5.405173	61.973416	108.486584	15.768	2	.004

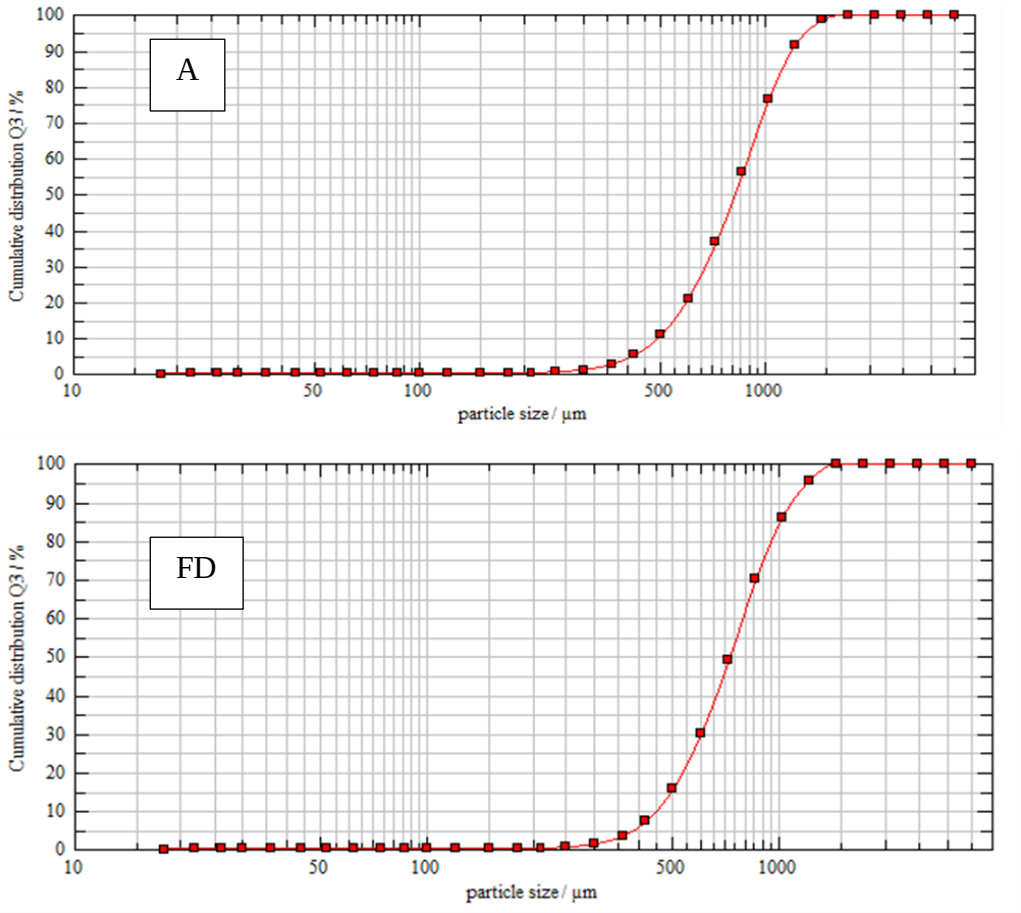

Fig. 2. Particle size distribution of sugar crystals after freeze dryer process.

In order to investigate was structural changes in sucrose molecule, FTIR (Fourier Transform Infrared Spectroscopy) was also performed. Comparing chemical groups of the control sugar (Figure 4 A), with sugar after process (Figure 4 FD), shows no changes were made in chemical groups of sucrose structure, and the main groups of CH and OH are still visible.

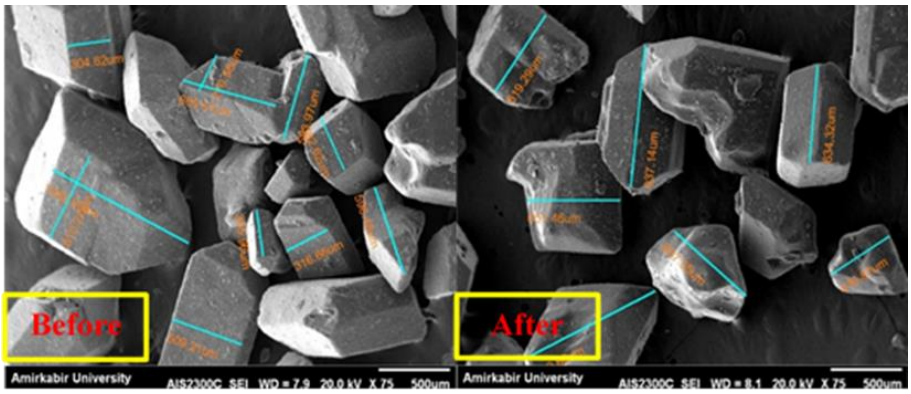

Fig. 3. Change in particle size of sugar crystal before and after FD by SEM**.**

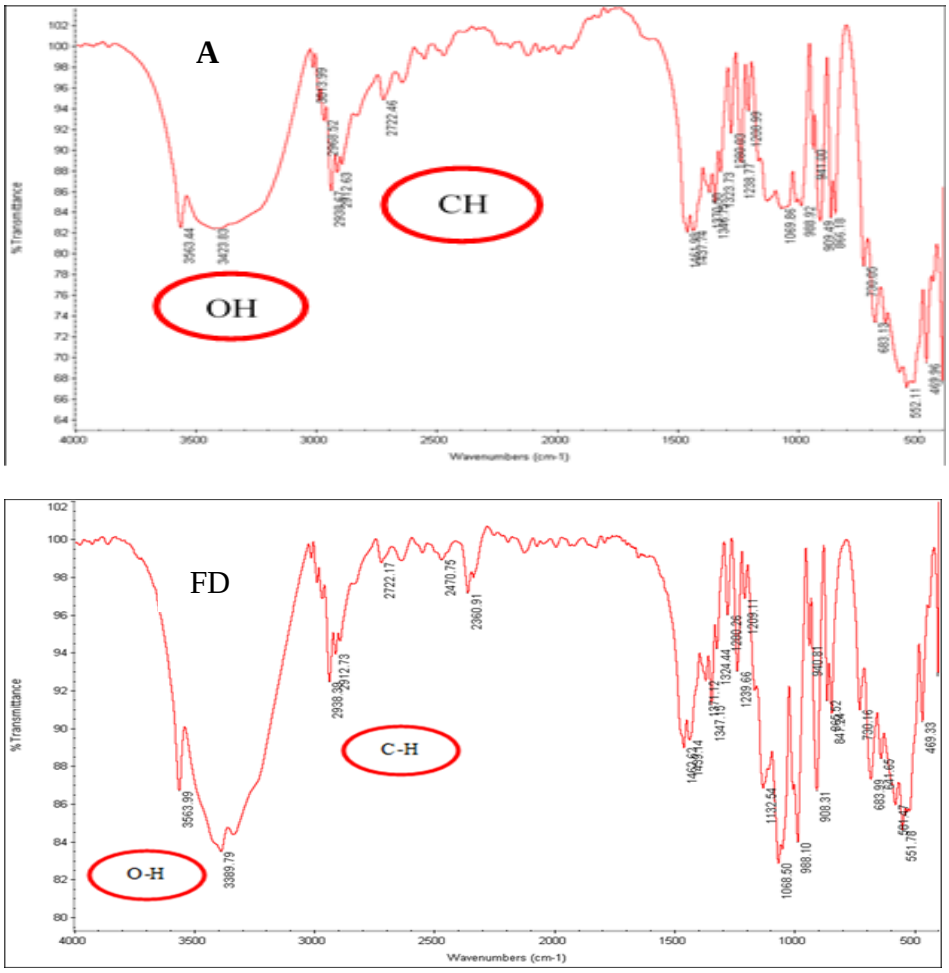

Fig. 4. Fourier transform infrared spectroscopy of sugar before freeze-drying A and after freeze-drying FD.

H-NMR test was also performed to ensure that changes in the structure did not occur. Comparison of H-NMR results (Figure 5) with registered standard diagrams confirms no change in the structure and accuracy of FTIR.

Conclusion

The results of this research show that with FD process, there is a possibility of reducing indicator microorganisms in food contamination, specifically two species of GS bacteria and AN fungus, in sugar produced by sugar factories using the conventional method. It can achieved with regard to pharmaceutical sugar standards in terms of microbial indicators by adding the FD complementary process. According to the available facilities in this research, suitable conditions for the FD process suggests, temperature conditions of -42°C, time of 24 hours and pressure of 0.1 mm bar. One of the most important achievements of this research is that there is no undesirable change on the structure of sugar crystal. Other physical and chemical indicators under application of FD process, percentage of sucrose as the main parameters, color and moisture did not have any significant changes. Physical and chemical parameters of ash, pH, particle size and invert, with degree of changes obtained, are still within the acceptable range of pharmaceutical sugar standards. Changes in some elements, including lead and copper, that probably caused by secondary pollution, are under control with predictable measures. Therefore, it is possible to propose FD process in batch form to sugar factories at the end of the sugar production line with the aim of producing pharmaceutical sugar.

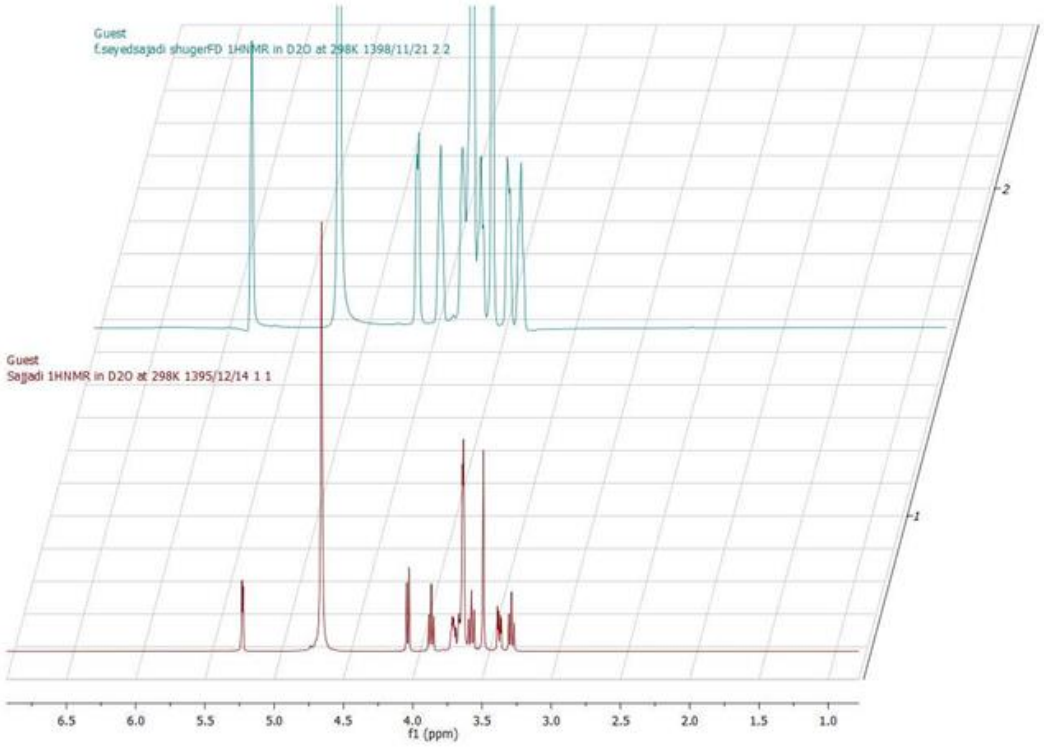

Fig. 5. Comparison structure of sugar before and after Freeze dryer process by NMR.

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Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 2, 71-86, 2024

The Effect of Olive Leaf Extract on Physicochemical, Microbial and Shelf-Life Characteristics of Chicken Nuggets

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Received: 24 February 2024 Accepted: 26 May 2024

ABSTRACT: The primary objective of this study was to examine the effects of olive leaf extracts on the quality characteristics and shelf-life of chicken nuggets .Chicken nuggets were treated with aqueous and ethanolic extract of olive leaves (at two levels: 0.25 and 0.35%) and the results were compared with chicken nuggets without additives (control). The treatments were stored in the freezer for 6 months and on days 0, 45, 90, 135 and 180, physicochemical characteristics (pH, moisture content, cooking efficiency and color indicators) and microbial qualities (total count, salmonella and total Forms) of the samples were evaluated. The addition of ethanolic extract to nugget formulation increased the pH during storage, and the pH values of all tests gradually increased. By increasing the concentration of olive leaf extract in the samples, the moisture content and cooking efficiency increased significantly. During storage, moisture content and cooking efficiency of nugget samples decreased. By the addition of olive leaf extracts to the nuggets, the color indices were improved. Microbial tests revealed that the salmonella and coliforms in all the tested samples were discent. The addition of aqueous and ethanol extracts of olive leaves to the nugget formulation reduced the microbial load, therefore the bacterial count of the samples containing the extracts was essentially lower than the control. Sensory evaluation showed that the lowest sensory score was related to the sample containing 0.35% ethanol extract, but there was no noteworthy distinction between other tests. It can be concluded that olive leaf extract can be utilized as a normal preservative in chicken nugget formulation.

Keywords: Durability, Microbial Load, Nugget, Olive Leaf Extract.

Introduction

The request of prepared meals has become popular in recent years. One of the foremost imperative foods in this aspect are foods made from dough products. Pastry products such as chicken nuggets are produced and covered with a coating and are affected by the preliminary heat process. Therefore, consumers only need a gentle final cooking (frying, microwave or traditional oven) to consume these kind of foods. (Adedeji *et al*., 2011; Chen *et al*., 2009). Today, in meat industry, many chemical additives are used to process meat products to mitigate the proliferation of pathogenic microorganisms and elongate product durability. As in recent years, there has been a growing concern regarding the safety of chemical additives and there has been a notable focus on natural bioactive compounds derived from traditional and medicinal plant sources.

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Numerous investigations have been directed towards exploring the anti-oxidant and antimicrobial activities exhibited by natural compounds present in plants, with a specific focus on their applicability in the meat and poultry industry. (Davidson *et al*., 2013; Natheer, 2010).

Olive fruit, oil, and leaves have a rich historical background regarding nutritional, medicinal, and traditional applications. The consumption of Olive products holds a significant position within Middle Eastern dietary patterns which have high nutritional and medicinal value due to the presence of polyphenols. (Soni *et al*., 2006; Taamalli *et al*., 2012). Olive leaf polyphenols have garnered significant research attention due to their potential to promote health and exhibit unique properties, including, anti-cancer, anti-arteriosclerosis, anti-inflammatory, and antimicrobial activities (Lee and Lee, 2010; Sudjana *et al*., 2009; Wang *et al*., 2008). Studies have demonstrated that olive leaf extract harbors bioactive compounds that exhibit potent antimicrobial activity against bacterial pathogens. Oleuropein is the primary phenolic compound present in olive leaf extract, responsible for bitter taste observed in both olives and olive oil. It possesses antimicrobial properties and is capable of inhibiting the growth of various foodborne pathogens, including Campylobacter jejuni, Helicobacter pylori, and Staphylococcus aureus (Sudjana *et al*., 2009). Given the confirmed antimicrobial activity of olive leaf extract in various sources, the present research aims to assess the impact of olive leaf extract on the physicochemical, microbial, and shelf life characteristics of chicken nuggets.

Materials and Methods

- **Materials**

Chicken breast, wheat flour and other ingredients needed to make chicken nuggets were purchased from the local market. The culture medium required for this research was purchased from Quelab Company. Ethanol and other chemicals used in the experiments were obtained from Merck Chemical Company, Germany.

- **Preparation of aqueous and ethanol extracts of olive leaves**

After washing the olive leaf with water, it was dried in an oven. The electric mill was employed for the purpose of grinding samples. A quantity of fifty grams of the powdered substance was mixed with one liter of distilled water with a 70% ethanol content (in a 70:30 ratio) and allowed to blend for a duration of 48 hours. The solution underwent agitation for a period of 24 hours at room temperature using a magnetic stirring apparatus. Following the filtration process, the resultant extracts were subjected to concentration in a water bath utilizing reduced pressure via a rotary evaporator, maintained at a temperature of 40°C. Then the resulting extracts were poured into glass containers covered with aluminum foil and kept at 4°C until the next use (Mirzaei *et al*., 2019).

- **Preparation of nugget samples**

Treatments including: control sample (T_0) , chicken nuggets containing 0.25% olive leaf aqueous extract (T_1), chicken nuggets containing 0.35% olive leaf aqueous extract (T_2), chicken nuggets containing 0.25% 0% olive leaf ethanol extract (T_3) and chicken nuggets containing 0.35% olive leaf ethanol extract (T_4) were prepared and analyzed. In order to produce nugget samples, first boneless chicken breast (60%) with ice (23%) was cut for 30 seconds by a cutting machine. Typical ingredients utilized in the production of commercial nuggets include

15% potato flakes, 1% salt, and 1% albumin. The combination of all constituents was carried out conjointly with the aim of achieving a homogeneous blend. Subsequently, chicken nugget samples were meticulously fashioned into predefined forms, measuring 1 x 3 x 5 cm and possessing a mass of 25 grams. These samples were carefully stored in a freezer maintained at the temperature of -18 degrees Celsius. Subsequently, the fragments were submerged in the pre-prepared batter (93.57% wheat flour, 1.17% salt, 0.24% bicarbonate, 2.34% yeast, and 1.17% xanthan gum) for a duration of fifteen seconds. (Teruel *et al*., 2015).

- **Microbial tests**

In order to perform microbial tests, firstly, based on the national standard of Iran No. 1-8923 for the total count of bacteria, the sample solution was prepared with the desired dilutions. In order to count total bacteria (Total count), as per the methodology outlined by the Iranian National Standard No. 5272-1. The presence of Salmonella bacteria in the samples was determined using the Iranian National Standard No. 1810 and the overall count of the nugget samples' forms was assessed through adherence to the methodology delineated by the Iranian National Standard No. 11166.

- **pH measurement**

In order to obtain the pH measurements, the samples were introduced into a container with a volume of 150 ml, into which 90 ml of distilled water had been previously added. Subsequently, the mixture was homogenized by stirring with a glass rod. Then, the pH of the sample was read and recorded by a pH meter at ambient temperature (Najafi *et al*., 2012).

- **Measurement of moisture content**

The samples were ground twice by a meat grinder. The empty plate was weighed and then 5-8 grams of the sample was transferred to the plate and weighed again. The sample was subjected to a temperature of 103°C in an oven and left to dry for a duration of two hours. Then the plate with all the contents was cooled in a desiccator until reaching the ambient temperature and weighed approximately 0.001 grams. The operation of heating and cooling and weighing was repeated until the result of two consecutive weightings (with a difference of one hour) is not more than 0.1% of the sample weight. Equation No. 1 was used to calculate moisture percentage by weight (Najafi *et al.*, 2012):

Equation (1)

$$\text{Moisture content} = [(M_1-M_2) / (M_1-M_0)] \times 100$$

where;

M_0: The weight of an empty plate is denominated in grams;

M_1: The pre-drying weight of the plate and the sample;

M_2 The weight of the plate and the sample:

- **Calculation of cooking efficiency**

In order to evaluate the cooking efficacy, the nugget specimens were first weighed and subsequently subjected to frying at a temperature of 180°C for a duration of 5 minutes. Over the course of this process, the internal temperature at the core of each sample attained at 80°C. Subsequent to frying, the specimens were allowed to cool at ambient temperature for a duration of one hour. The fried specimens were then subjected to weighing procedures to determine their post-cooking weight. The calculation of cooking efficiency was performed using Equation 2. (Kim *et al*., 2015).

Equation (2)

Cooking efficiency= [Raw sample weight/ Cooked sample weight] × 100

- **Determination of color indicators**

The colorimetric test was performed using Hunterlab colorimeter. The color indices a* (redness intensity), b* (yellowness intensity) and L* (brightness intensity) were determined by placing the samples inside the tank of the device. (Babuskin *et al*., 2014).

- **Sensory evaluation**

In the current study, sensory evaluation of nugget samples was conducted utilizing a seven-point hedonic test. In order to do this, the nugget samples were fried in frying oil until golden and placed hot in coded containers. The sensory evaluation form was provided to the evaluators and 20 semi-professional evaluators rated the sensory characteristics of the nugget samples. (Carpenter *et al*., 2012).

- **Statistical analysis**

The data obtained were subjected to statistical analysis using the software SPSS 16.0, specifically employing one-way analysis of variance (ANOVA). The disparities among the treatments were analyzed using Duncan's multiple test, with a significance level of 95% ($p < 0.05$) being observed.

Results and Discussion

- **pH Values of nugget samples**

The findings derived from the statistical analysis indicate that both the treatments applied and the duration of storage have a statistical impact on the pH levels of chicken nuggets. The latter evidenced by a p-value of less than 0.05. The alterations in the mean pH readings of the control group and the experimental groups, which consisted of varying concentrations of aqueous and ethanolic extracts of olive leaves, were monitored over a six-month period of storage in a freezer. The results are illustrated in Figure 1. On the day of production, the inclusion of alcoholic olive leaf extract resulted in a notable increase in pH levels ($p<0.05$). Conversely, the integration of aqueous extract led to a reduction in pH levels, although this decrease was deemed insignificant ($p > 0.05$). During the initial 45 days of production, no notable alterations were detected in the pH levels of the nugget samples. However, an evident increase in pH levels was registered between the 45th day and the final day of their frozen state, that attained a notable statistical significance ($p < 0.05$). On the final day of the experiment, the pH levels were observed to be significantly higher in the control sample (6.19 ± 0.03) and the sample containing 0.25% aqueous extract (6.17 ± 0.04), where as the sample containing 0.35% alcoholic extract exhibited the lowest pH. The measured pH value was found to be (6.0 ± 0.04) with an estimated uncertainty.

In Figure 2, the effect of the treatments used on the average pH values of chicken nuggets is presented. As it can be observed, there was no significant difference between the pH values of the control and the levels of 0.25 and 0.35 olive leaf aqueous extract ($p >0.05$) , but the difference between different concentrations of the alcoholic extract was significant ($p <0.05$) .) and had the lowest pH.

The results of the present study align with the findings of the research conducted by Banerjee and colleagues in 2012. Various concentrations of broccoli extract were utilized in the production of goat meat nuggets, where by the consequential pH levels of the nuggets were evaluated. It was observed that the incorporation of said extract resulted in a marked reduction in

the pH value of the nugget. They stated that the reason for this decrease can be related to the relatively lower pH of broccoli extract compared to the normal pH of goat meat (Banerjee *et al*., 2012).

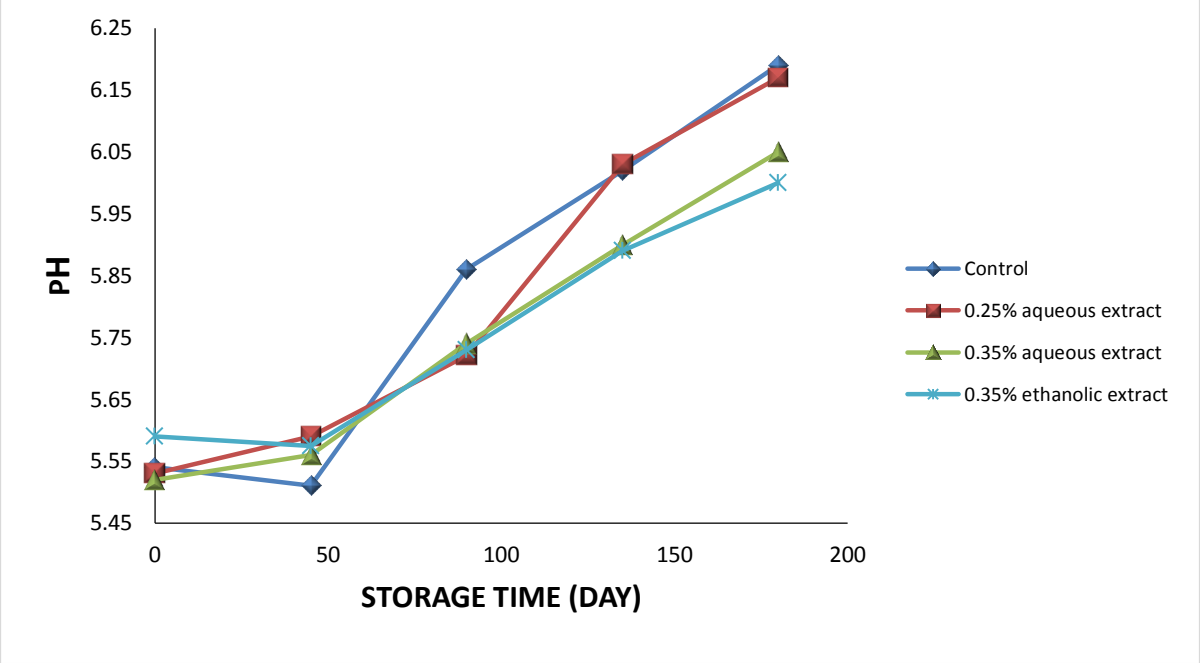

Fig. 1. Changes in the average pH values of different chicken nugget samples during s torage.

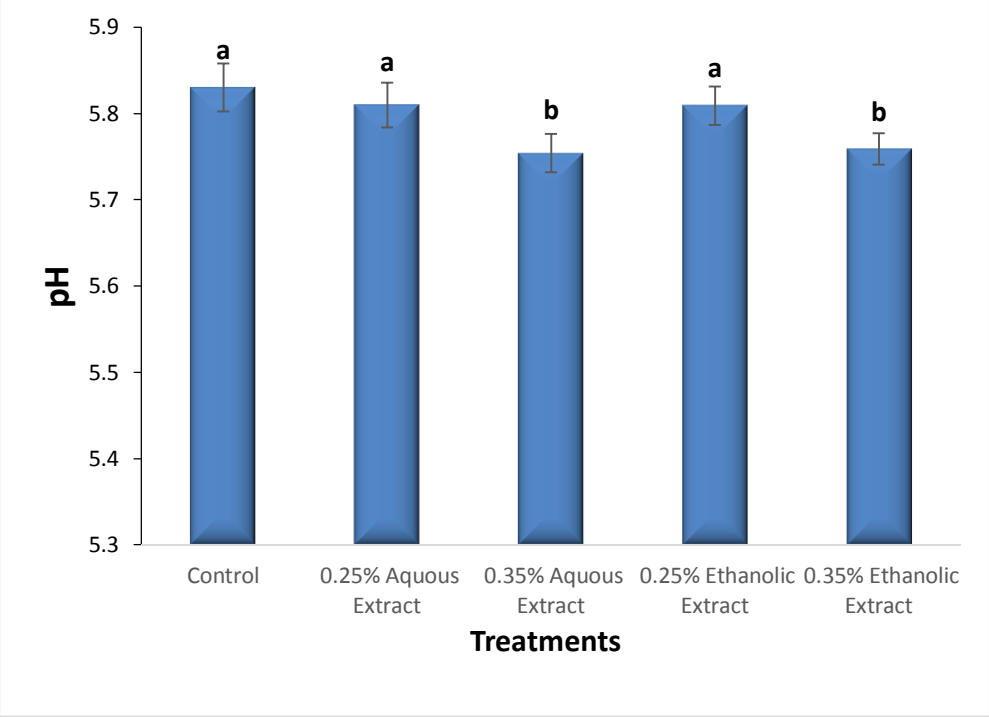

Fig. 2. The effect of the type of treatment used on the average pH values of chicken nuggets**.**
* Similar letters indicate the absence of significant differences between the values (p >0.05).

- **Moisture content of nugget samples**

The findings of the statistical examination of the data have demonstrated a noteworthy impact of the applied treatments and length of storage on the moisture level of chicken nuggets ($p < 0.05$). The alterations observed in the mean levels of moisture content between the control specimen and those incorporating diverse concentrations of aqueous and ethanol extracts of olive leaves over a period of storage are illustrated in Figure 3. During the initial day of experimentation, a marked elevation in the moisture content was observed upon augmenting the dosage of olive leaf extracts in the formulation of the nuggets. Throughout the duration of storage, the moisture content of all nugget samples exhibited a progressive decline. However, it was observed that the control sample and the sample supplemented with 0.25% olive leaf aqueous extract experienced a notably greater rate of moisture loss as compared to the remaining treatments studied ($p < 0.05$). Such findings suggest that the incorporation of olive leaf aqueous extract has the potential to induce a noteworthy influence on the reduction of moisture content in nugget samples during storage. On the final day of storage, the two aforementioned samples exhibited the least amount of moisture, while the sample that contained 0.35% aqueous extract displayed the maximum amount of moisture (46.83% ± 0.52%).

The findings in Figure 4 demonstrate the impact of the treatments administered on the mean moisture level of chicken nuggets. As it is evident from the depicted figure, the control sample exhibited the lowest moisture content. The application of aqueous extract derived from olive leaves resulted in a marginal, non-significant enhancement in moisture content ($p > 0.05$). Nonetheless, a noticeable increase in the total moisture content was observed with a rise in the concentration of the extract ($p < 0.05$). The introduction of an alcoholic extract into chicken nugget samples, alongside an associated increase in concentration, demonstrated a significant effect on the moisture content of the samples ($p < 0.05$).

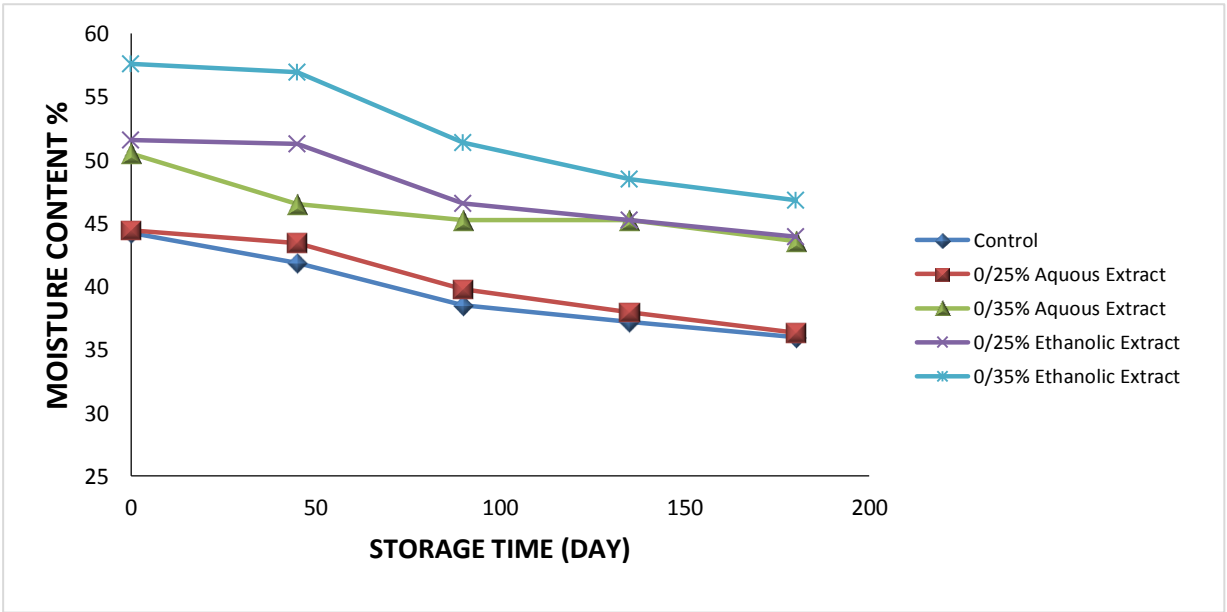

Fig. 3. Changes in the average moisture content of different chicken nugget samples during storage time.

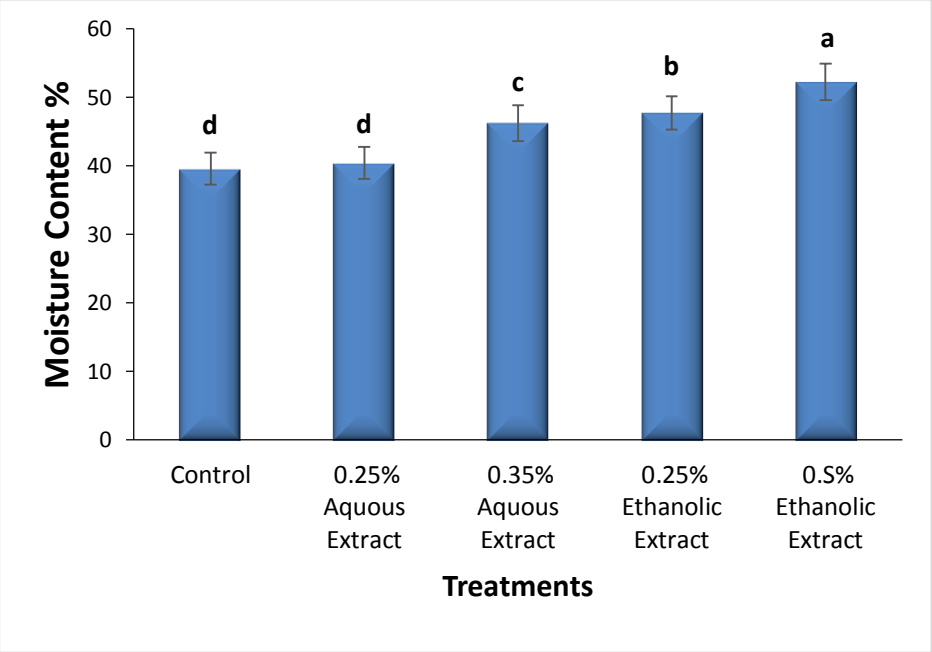

Fig. 4. The effect of the type of treatment used on the average moisture content of chicken nuggets**.**

* Different letters indicate a significant difference between the values ($p < 0.05$).

The enhancement of water retention capacity resulting from the inclusion of olive leaf extracts is hypothesized to be linked to the improvement in oxidative stability and the mitigation of pH variations in meat products throughout the storage period. (Young *et al*., 2003).

- **Cooking efficiency of nugget samples**

The findings derived from the statistical examination of the data have indicated that both the applied treatments and the duration of storage have had a statistically substantial impact on the cooking proficiency of chicken nuggets, with a p-value below 0.05. Figure 5 displays the alterations observed in the mean cooking efficiency values of the control sample and samples containing varying concentrations of aqueous and ethanol extracts derived from olive leaves over the course of storage. On the day of production, the lowest level of cooking efficiency was observed in the control sample (76.22 ± 0.56 percent), while the inclusion of the desired treatments in the formulation of the nuggets resulted in a statistically significant improvement in cooking efficiency ($p < 0.05$). Over the course of time, the cooking efficiency of all samples of nuggets exhibited a decline, with a notably steeper decline observed in the control group compared to the samples containing extracts. On the final day of storage, it was observed that the cooking efficiency of the samples exhibited significant variability. Specifically, the specimen that contained an alcoholic extract concentration of 0.35% demonstrated the highest cooking efficiency, with a mean value of 79.71% ± 0.94%. In contrast, the control sample exhibited the lowest cooking efficiency, with a mean value of 70.78% ± 1.65%.

Figure 6, shows the effect of the treatments used on the average values of chicken nugget cooking efficiency. As stated previousely and can be seen in this figure, the lowest cooking efficiency was related to the sample without additives (control) and the use of aqueous and alcoholic extracts of olive leaves caused a

significant increase in the cooking efficiency. The study revealed a statistically significant increase ($p < 0.05$) in cooking efficiency with higher levels of extracts. The findings suggest that the utilization of alcoholic extract resulted in a greater enhancement of cooking efficiency in chicken nuggets than the utilization of aqueous extract.

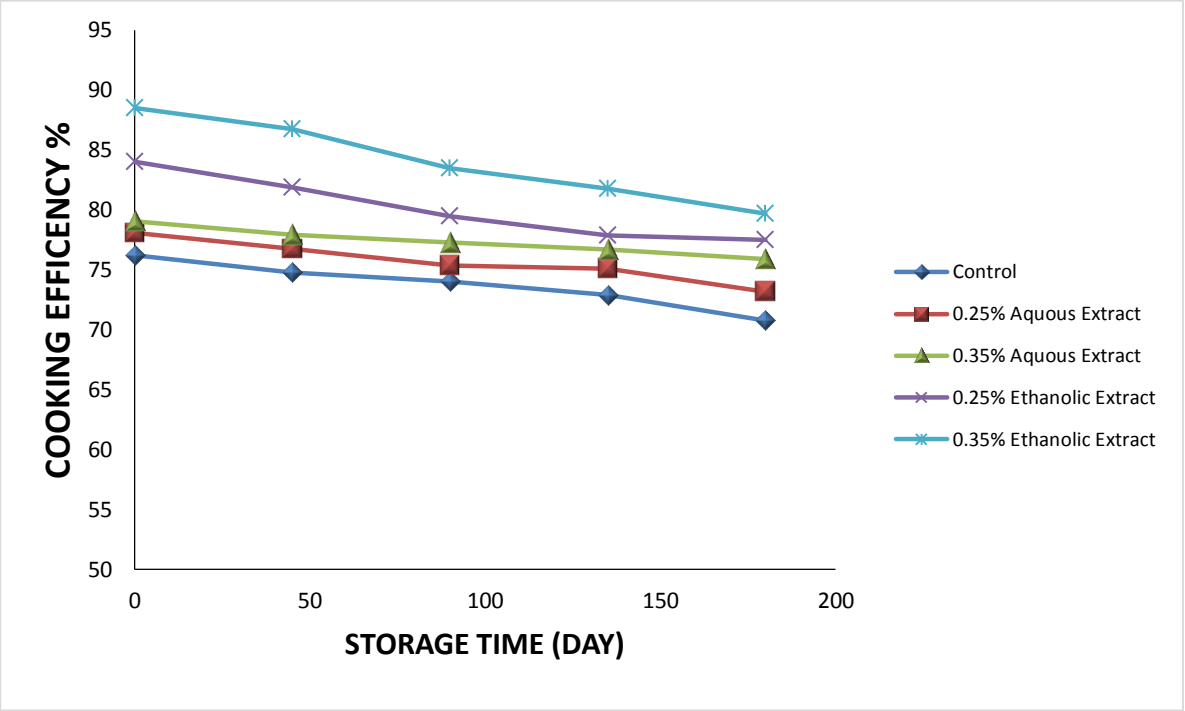

Fig. 5. changes in the average values of percentage cooking efficiency of different samples of chicken nuggets during storage time.

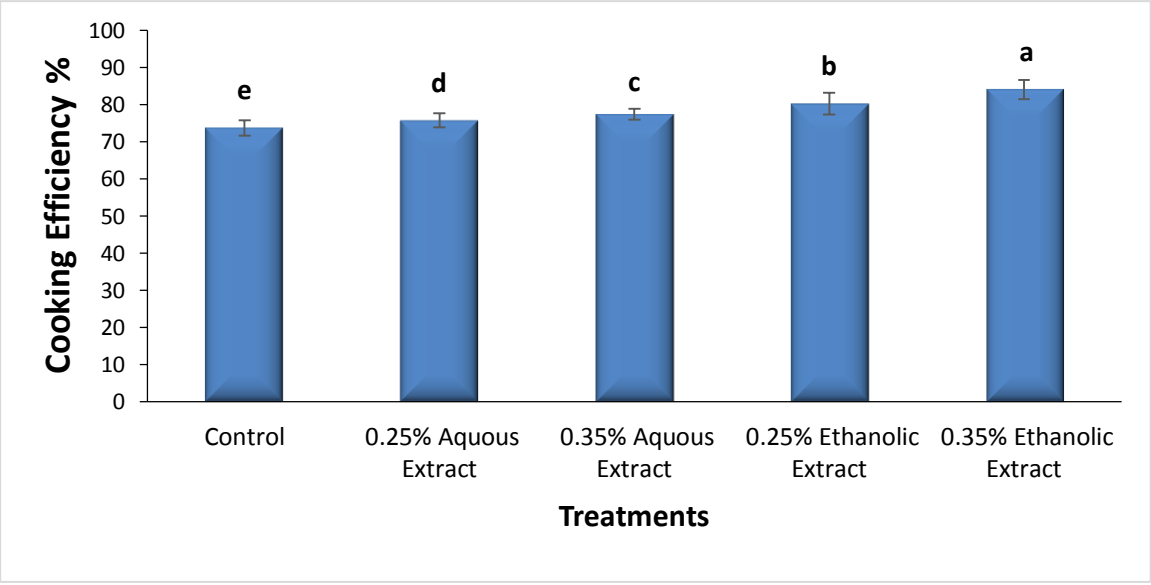

Fig. 6. The effect of the type of treatment used on the average values of the percentage of chicken nugget cooking efficiency
*Different letters indicate a significant difference between the values ($p < 0.05$).

The study of Banerjee *et al.* (2012) was related to the influence of broccoli powder extract on the qualitative characteristics of goat meat nuggets. The findings revealed that the introduction of the broccoli extract did not have a worthly impact on the cooking efficiency of the nuggets. Bani Safar (2013) stated that the incorporation of ethanol extract of olive leaf into veal hamburger resulted in a notable augmentation of cooking efficiency, whereby an increase in the quantity of the extract introduced into the preparation elicited a proportional rise in the cooking efficiency. Furthermore, the augmentation of cooking efficiency attributed to the inclusion of olive leaf extracts was found to correlate with a commensurate increase in water retention capacity, leading to greater moisture retention within the final product. (Bani Safar, 2013).

- **Determination of color indicators of nugget samples**

The statistical analysis of the collected data revealed that both the employed treatments and the duration of storage presented a significant statistical impact on the brightness of the chicken nuggets ($p < 0.05$). The present study illustrates the alterations in the mean values of L* pertaining to both the control group and the experimental treatments incorporating varying concentrations of aqueous and ethanolic extracts derived from olive leaves, over a period of six months of storage within a freezing environment. The graphical representation of these findings is depicted in Figure 7. On the day of production, it was found that the control sample had the lowest quantity of L*. The addition of aqueous and alcoholic extracts of olive leaves was found to markedly enhance the quantity of L* ($p < 0.05$). In the control group, over the course of 45 days from the initial production date, the parameter L* displayed a notable increase followed by a substantial decrease, concluding at the final day of evaluation ($p < 0.05$). Over the course of 90 days following production, the intensity of color brightness in samples consisting of aqueous and alcoholic extracts derived from olive leaves demonstrated an initial increase, followed by a subsequent decrease. On the final day of experimentation, the sample demonstrating the highest and lowest L* values were those containing 0.35% alcoholic extract (70.88 ± 0.77) and the control sample (64.19 ± 1.17), respectively.

In Figure 8, the effect of the treatments used on the average b* values of chicken nuggets is shown. As it is indicated, the lowest intensity of yellow color was related to the control sample and the addition of aqueous and alcoholic extracts of olive leaves to the nugget formulation caused a significant increase in the value of b* . The increase in the concentration of extracts, the amount of b* increased significantly ($p < 0.05$). Among the two types of olive leaf extracts, the amount of b* in the alcoholic extract was higher than the aqueous extract.

Figure 9 presents the impact of the employed treatments on the mean a* values of chicken nuggets. As evident from this study, the minimal concentration of a* was found to be associated with the two variations of alcoholic olive leaf extract, while the aqueous extracts exhibited a higher concentration of a* in comparison to the control group. The findings indicate that variations in the concentration of aqueous and alcoholic extracts were not observed to have a statistically significant influence on the level of a* ($p>0.05$).

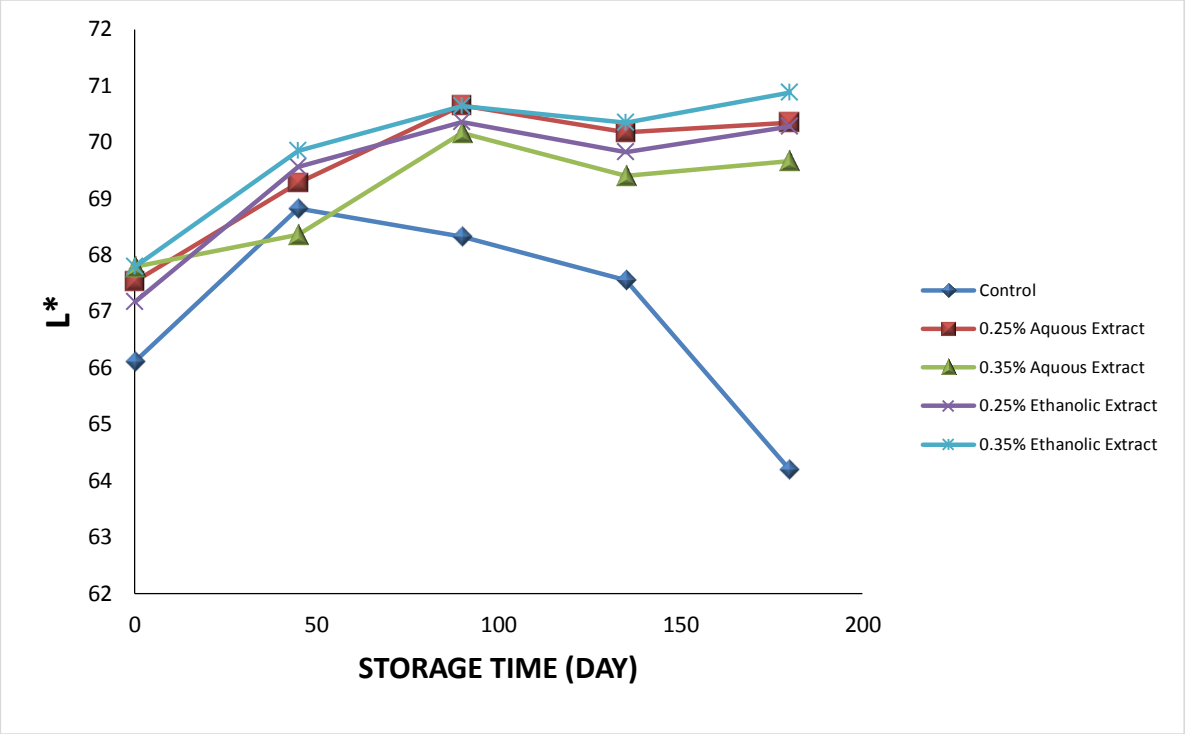

Fig. 7. Changes in the average L* values of different chicken nugget samples during storage time

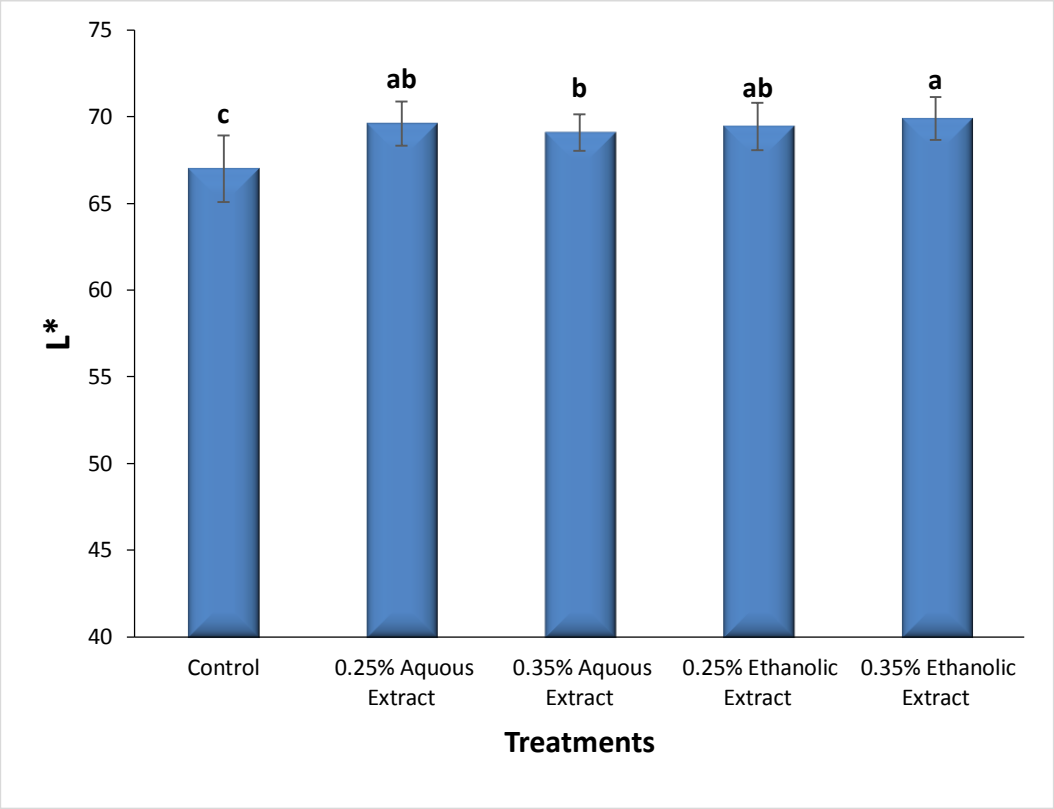

Fig. 8. The effect of the type of treatment used on the average b* values of chicken nuggets

* Different letters indicate a significant difference between the values ($p < 0.05$).

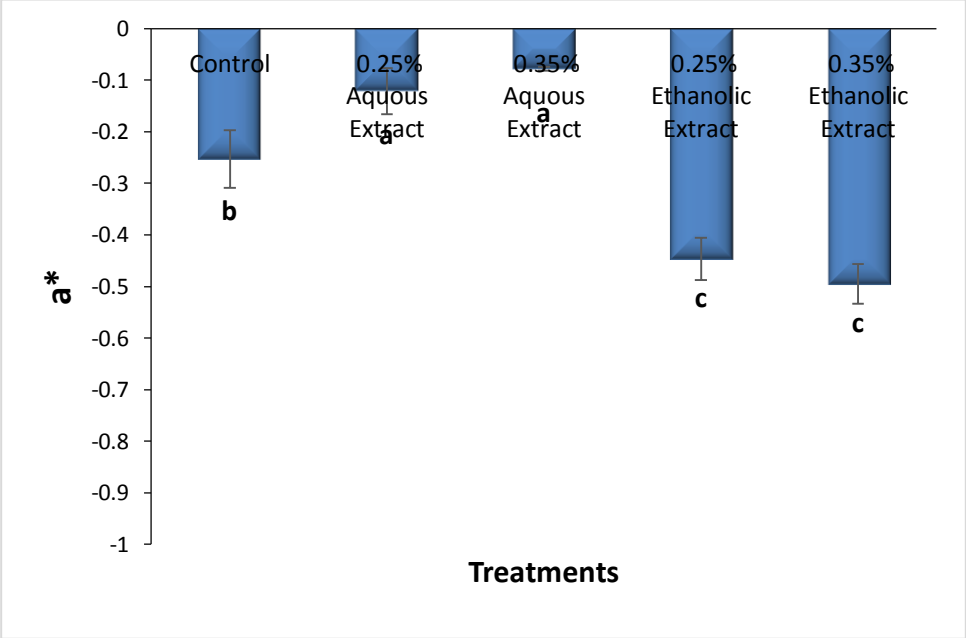

Fig. 9. The effect of the type of treatment used on the average a* values of chicken nuggets

* Similar letters indicate the absence of significant differences between the values ($p > 0.05$).

Tzurier *et al.* (2007) in their investigation discovered that phenolic extracts were efficacious in obstructing the metamorphosis of myoglobin and its conversion to ferrilmyoglobin state. This outcome serves as a compelling indication of the reaction occurring between certain phenolic compounds and heme protein reduction reactions (Tesoriere *et al.*, 2007). Zhang *et al.* (2006) treated raw chicken meat with extracts of different spices and observed that during the storage time, the brightness of the color of the samples containing the extracts increased, while the color of the control sample became darker. Singh and colleagues. The impact of incorporating clove powder, garlic, and ginger on the qualitative characteristics of raw chicken meat was investigated in a study by Singh *et al.* (2014). The outcomes revealed that the inclusion of ginger had a negative effect on the brightness of the meat, while the incorporation of garlic and cloves had a positive effect, resulting in an increase in the brightness of the meat samples. The addition of ginger and garlic also reduced the intensity of the red color of the samples.

- **Microbial evaluations**

The statistical analysis of the data indicates that both treatments employed and the duration of storage exerted a statistically significant impact on the total bacterial count in chicken nuggets ($p < 0.05$). Figure 10 displays the alterations in the mean logarithmic values of the overall bacterial count for the control group, as well as for the samples infused with diverse concentrations of aqueous and ethanol extractions of olive leaves over the course of storage. On the initial day of experimentation, the control sample indicated to have the greatest logarithmic value of total bacterial count per gram at 2.454 ± 0.064 log CFU/g. The inclusion of both aqueous and alcoholic extracts of olive leaf in the nugget formulation demonstrated a significant reduction in bacterial count ($p < 0.05$). The outcome of the present study indicates that the

administration of alcoholic extract exhibited a statistically significant decrease in the microbial burden of the nugget, as compared to the aqueous extract. Throughout the period of storage, a substantial increase in the logarithmic value of the concentration of bacteria per gram of the analyzed samples was observed. However, it was observed that the rate of increase in the control sample surpassed that of the samples containing various extracts. It was therefore noted on the final day of analysis that the microbial load of the control sample was the most elevated. (log CFU/g 4.435 ± 0.134).

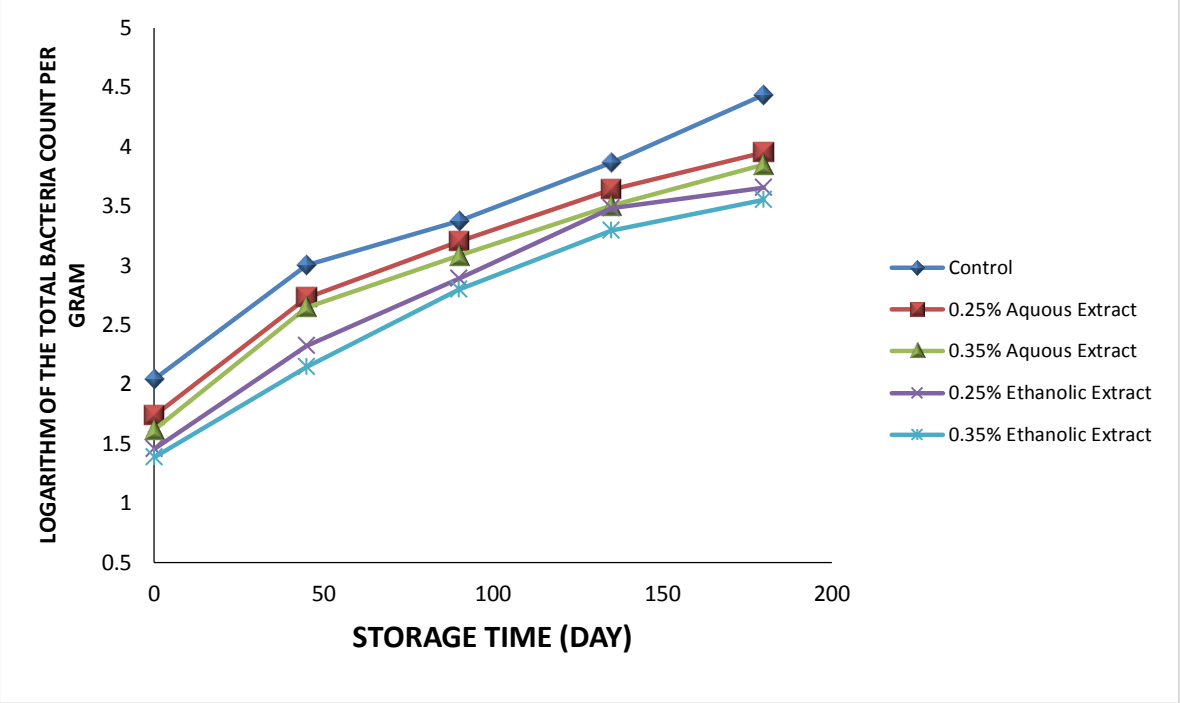

Fig. 10. Changes in the average logarithm of the total bacterial count per gram of different nugget samples during storage time.

The results of checking the presence of salmonella bacteria and also the total forms during six months storage in all samples were negative.

Baker (2014) examined the impact of olive leaf extract as an antimicrobial agent on lamb patties while undergoing refrigerated storage. The findings revealed that the incorporation of olive leaf extract conferred a significant delay in microbial proliferation throughout the refrigerated preservation of patties. The results of the observation demonstrate that the total bacterial count in the control samples exceeded that of the samples treated with the extract. Furthermore, as the storage period progressed, the number of bacteria increased. Aliabadi *et al*. (2012) reported that the use of olive leaves had a beneficial effect on the control of microbial infections.

- **Sensory evaluations**

The statistical analysis of the data indicates that the administered treatments had a significant impact on the collective acceptance score of chicken nuggets ($p < 0.05$). Figure 11 presents a comparative analysis has been presented regarding the mean acceptance scores of the control

group and the groups treated with varying concentrations of aqueous and ethanol extracts of olive leaves. The sample containing 0.35% alcoholic olive leaf extract demonstrated the lowest overall acceptance score of 5.95 ± 0.31, while the overall acceptance scores of other treatments showed no significant difference ($p > 0.05$).

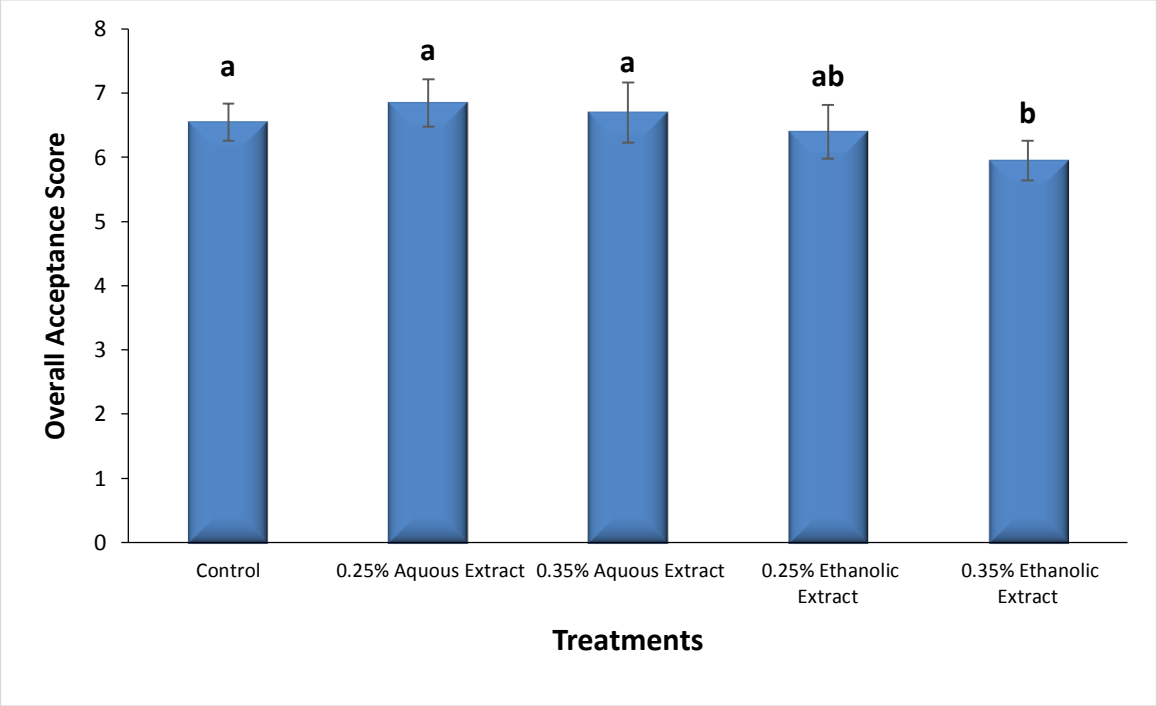

Fig. 11. Average overall acceptance scores of different samples of chicken nuggets.

* Similar letters indicate the absence of significant differences between the values ($p > 0.05$).

A study was conducted by Zhang *et al*. (2016) on the influence of various spice extracts on the sensory characteristics of chicken meat. The research revealed that the samples that incorporated the spice extracts scored superior sensory scores, in contrast to the control sample. Bani Safar (2013) reported that incorporating both aqueous and ethanolic extracts of olive leaves at a concentration of 0.15% resulted in a significant improvement in the overall acceptance of hamburgers. As these treatments had the highest texture, taste and color scores, it is expected that further investigations be carried out for this applications in the chicken nuggets production.

Conclusion

The addition of olive leaf extracts caused a significant increase in pH ($p < 0.05$) . During storage time, the pH of the nugget samples increased. The incorporation of olive leaf extracts into the nugget formulation resulted in an augmentation of the moisture content and cooking efficacy of the specimens. Over time, the moisture content and cooking efficacy of all nugget samples exhibited a gradual decline. The employment of aqueous and ethanolic olive leaf extracts in nugget samples demonstrated a noteworthy impact on color indicators, as evidenced by a substantial increase in the brightness and yellowness of the nugget ($p < 0.05$). During the course of production,

it was observed that the samples infused with extract exhibited lower levels of a* as compared to the control group. Analysis conducted during the storage period indicated that the levels of a* continued to decrease albeit at a slower rate in the samples containing extract. In contrast, the control group displayed a higher rate of decrease in a*. Furthermore, it was found that the extract contributed towards enhancing the color stability of the chicken nuggets. Over the course of storage period, there was a significant increase in the total bacterial count of the analyzed specimens. Nevertheless, the rate of escalation in the control sample was found to be significantly greater than the samples that had been supplemented with the extract. Ethanol extract of olive leaf had higher antimicrobial activity than aqueous extract. The results of the sensory evaluation indicated that the exclusive use of a 0.35% ethanol extract derived from olive leaves resulted in a significant reduction in sensory attributes. However, the degree of acceptability in relation to the score was upheld. In summary, the results indicate that the application of olive leaf extracts had a positive effect on the qualitative and microbial characteristics of chicken nuggets.

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