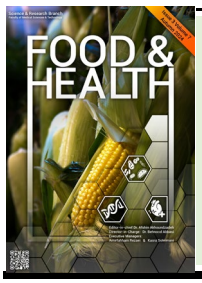

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Effect of Adding Pomeogrant Peel Essential Oil on the Microbiological, Antioxidant and Sensory Properties of Toast Bread

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ABSTRACT

Adding plant essential oils to food products has become popular among producers, industrialists, and the general public due to their antioxidant compounds and the enhancement of health benefits of foods.

In present study, the effect of adding pomegranate peel essential oil in amounts of 0.8, 0.6, 0.3, and 1% to toast bread on its physicochemical, antioxidant, sensory, and microbial properties was investigated over 10 days (first, fifth, and tenth days of production). The evaluation of physicochemical and antioxidant properties showed that the treatments containing pomegranate peel essential oil had higher moisture content, total phenol content, and antioxidant properties than the control treatment, and their pH and total microbial count were lower than the control. Regarding sensory characteristics, the treatment containing 0.6% pomegranate peel essential oil received the highest and the control sample received the lowest scores for taste, odor, texture, and overall acceptance from the tasters. Also, increasing the storage time reduced the moisture content, total phenol content, and antioxidant properties of the samples and total microbial count and the number of mold and yeast increased. The treatment containing 0.6% essential oil was the superior treatment because it received the highest score from the evaluators, and the treatment containing 1% essential oil was considered the treatment with the highest antioxidant properties and the lowest total microbial count. Adding pomegranate peel essential oil improves the physicochemical and antioxidant properties and reduces the microbial load in toast bread, and this substance can be used as a health-promoting ingredient in foods.

1. Introduction

Pomegranate, with the scientific name Granatum punica, is a member of the Punicaceae family and is one of the most important commercial crops, widely cultivated in parts of Asia, Northeast Africa, the Mediterranean, and the Middle East. Iran is the world's largest pomegranate producer, followed by India (1).

Pomegranate peel is a rich source of antioxidant compounds and is discarded as waste. Among the different parts of the pomegranate fruit, pomegranate peel has the highest amount of phenolic compounds and antioxidant activity. The main phenolic compounds found in pomegranate peel include gallic acid, punicalagin, chlorogenic acid, caffeic acid, protocatechuic acid, phloridzin, quercetin, catechin and coumaric acid. In addition to its medicinal effects, pomegranate peel has antimicrobial properties, so its effects are well known (2).

Berizi et al. (3) evaluated the antioxidant properties of methanolic extract of pomegranate peel of the Rabab variety (Punica granatum var. Rabbab). The results showed that methanolic extract of pomegranate peel is rich in phenolic compounds and has high antioxidant properties. Therefore, this plant source containing antioxidant compounds can be used as a preservative in the food industry.

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Also, in the study of the antioxidant activity of pericarp extract from 4 different pomegranate fruit cultivars, it was reported that the pericarp of pomegranate fruit contains polyphenolic compounds including alpha and beta ponicalagin and ellagic acid, which show significant antioxidant activity. The results showed that there was a significant difference in the total phenolic content and phenolic components in the four cultivars. The total phenolic content in the methanol extract was significantly higher than the aqueous extracts. The Shahvar cultivar had the highest total phenol content and showed the highest antioxidant properties among the cultivars. Overall, the results showed that the Shahvar cultivar is an excellent source of natural antioxidants and can potentially replace current synthetic antioxidants in the food and pharmaceutical industries (4).

Over the past few years, the consumption of bread, including semi-loaf and loaf breads, has increased among Iranians. On the other hand, in recent years, the use of natural ingredients such as essential oils and plant extracts in foods has become common, which, due to their antioxidant and antimicrobial properties, create health-promoting properties in foods.

Bread is the staple food of many countries around the world and provides a large portion of the daily carbohydrate, relatively low protein, negligible fat, B vitamins, vitamin E, and important minerals such as iron, calcium, zinc, phosphorus, manganese, and magnesium. Under normal conditions, bread can be stored for a maximum of one week, after which it becomes moldy and unusable. Increasing the shelf life of bread without the use of unauthorized additives has been one of the main goals in the bread industry (5).

Toast belongs to the group of bulky breads and is considered one of the most widely consumed bulky breads in Iran, especially in recent years. This bread has a pleasant aroma and a desirable taste, has a soft and uniform texture, and is suitable for preparing a variety of snacks and sandwiches.

MalekPur and Ansari (6) evaluated the effect of pomegranate peel powder on the physicochemical and sensory properties of sponge cake. Chemical analysis of pomegranate peel powder showed that this powder has high fiber, salts, especially potassium and calcium, and high phenols. In addition, replacing less than 4.5% of pomegranate peel powder can be useful in producing a cake with desirable quality properties and higher nutritional value.

Shahi et al. (1) evaluated the effect of chitosan nanocoating containing pomegranate peel extract on the physicochemical and microbial properties of pomegranate seeds during storage. The results of this study showed that the preservation of antioxidant activity, phenolic compounds and total anthocyanin in coated pomegranate seeds was higher than the control sample. The increase in total counts and mold and yeast in coated treatments was lower than the control sample. 1% nanochitosan coating containing 1% pomegranate peel extract was able to increase the shelf life of pomegranate seeds at refrigerator temperature.

Shafie Jam et al. (7) investigated the combined effect of savory essential oil and inulin in increasing the shelf life and quality of Tafton bread. The results of bread tests during 12 days of refrigerated storage showed that increasing the concentration of savory essential oil increases the microbial shelf life of bread and that using complementary natural additives together in the right ratio can produce bread with quality and health properties suitable for consumers.

In a study conducted to investigate the effect of propolis extract as a natural antimicrobial and antioxidant agent on the physicochemical, microbial and sensory properties of toast bread, as well as total phenol content and antioxidant activity, 0.5% extract showed the highest phenolic content and antioxidant activity. Propolis extract showed an inhibitory effect on mold growth in the samples. Bread with 0.5% extract had the lowest number of molds after 5 days of storage, which was not significant compared to the first day. There was no significant difference in sensory evaluation between the overall acceptance of bread samples (8).

In assaying the effect of adding pomegranate (Punica granatum) extract and juice on the physicochemical and sensory properties of ice cream, it was observed that adding pomegranate extract and juice significantly improved the concentration of zinc (Zn), magnesium (Mg), manganese (Mn), and non-heme iron (Fe) in ice cream. The result of sensory evaluation showed that ice cream with 10% pomegranate juice had the highest evaluation score by the participants (9).

In a study, the production of functional sausages using pomegranate peel and green pistachio peel extracts as two natural preservatives was evaluated. In this study, the antioxidant and antimicrobial properties of treatments containing the extracts were similar to the control or sometimes better. The sensory scores of the treatments were also not significantly different compared to the control (10).

In the present study, the effect of adding pomegranate peel essential oil on antioxidant properties, microbial load, and sensory characteristics of toast was investigated.

2. Materials and Methods

2.1. Materials and chemicals

Ripe pomegranates were purchased from the local market. Ethanol 96%, Hexane, Tween 80, chloramphenicol and anhydrous sodium sulfate were from reputable Iranian brands and culture media Nutrient Agar, DRBC Agar and RPMI Broth Sigma brand were purchased from reputable companies. All chemicals were purchased with laboratory grade and solutions were prepared freshly. The required fungal strains were obtained from Razi Serum Company.

2.2. Extraction of essential oil from pomegranate peel

Purchased pomegranate peels were dried in the shade for separation and extraction of essential oil. Essential oil was prepared by water distillation (100 g of dried pomegranate peels with 250 ml of distilled water) for 4 hours using a Clevenger apparatus. After separation of the essential oil from the water surface, dehydration was performed using sodium

sulfate and transferred to plastic microtubes with aluminum coating and a volume of 2 ml and stored at 4 °C until use (11).

2.3. Determination of Minimum Inhibitory and Minimum Fungicidal Concentration

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined using the broth microdilution method. First, a suspension of 2.5×103 spores / ml was prepared from a fresh culture of the test fungus (Aspergillus niger).

In addition, a set of 10 sterile test tubes was used, 8 tubes were used to test different dilutions of essential oil (2, 4, 8, 16, 32, 64, 128 and 256 mg/ml) and one tube was considered as a negative control (without fungus) and one tube was considered as a positive control (containing fungus). The culture medium used in the tubes was RPMI. After inoculation of a concentration equivalent to 0.5 McFarland standard of the fungal strain, the tubes were incubated at 27°C for 5 days. After incubation, the tubes were examined for turbidity resulting from the growth of the inoculated fungi and the lowest concentration at which no turbidity was observed and was completely clear was considered as the MIC. This method was repeated three times to evaluate the essential oil against the tested fungus. Using the tube dilution method, the minimum lethal concentration of pomegranate peel essential oil was determined. To determine the MFC of essential oil, a set of 10 sterile test tubes was used, 8 test tubes containing dilutions of 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 mg/ml of essential oil. One tube was considered as a negative control (without fungus) and one tube was considered as a positive control (containing fungus). All test tubes were incubated for 5 days at 27°C. After the incubation period, the tubes were examined for turbidity due to the growth of the inoculated fungus (12).

2.4. Preparation of toast samples containing essential oil

Samples of toast were prepared based on the evaluation of the results of MIC and MFC tests, with the amounts of essential oil presented in Table 1. The raw materials used to prepare toast included fancy bread flour, sugar, salt, yeast dough, egg, oil and milk. After preparing the dough and allowing it to rest, it was kneaded (about 650 grams) and molded, and the bread was baked at a temperature of 210 ° C. The produced toasts were packaged in polyethylene bags after cooling. They were evaluated at time intervals (1, 5 and 10 days after production) (8).

2.5. Determination of total phenol and antioxidant properties of samples

A sample of dried bread (3g) was mixed with 30 ml of ethanol (80% by volume) by magnetic stirrer for 30 min. The mixture was centrifuged for 15 min. The supernatant was used to determine phenolic content and antioxidant activity (three replicates for each sample). To evaluate antioxidant activity, the free radical scavenging rate was evaluated using the DPPH method in triplicate. One ml of the desired sample and 5 ml of 0.1 mM DPPH solution were mixed. After incubating the mixture in the dark for 50 min, the absorbance at a wavelength of 517 nm was evaluated by an ultraviolet-visible spectrophotometer (SU- SU-6100-Philler Scientific, USA) and graph of the percentage of free radical scavenging was drawn for the toast treatments (13).

$$\text{Radical scavenging (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$$

To determine the total phenol content, 5 ml of the supernatant was mixed with 20 ml of hexane and after 20 min of centrifugation, the fat was separated by a syringe. The total phenolic content was measured using Folin-Ciocalteau reagent. 1 ml of the reagent was mixed with 1 ml of fat-free supernatant and 10 ml of distilled water by a magnetic stirrer for 5 min. After adding 2 ml of 7.5% (w/v) sodium carbonate and incubating the mixture in the dark for 60 min, the absorbance of the sample was recorded at a wavelength of 750 nm using a UV-visible spectrophotometer (SU-6100-Philler Scientific, USA). The results were expressed as mg gallic acid equivalent/g dry weight of the sample (mg GAE/g.DM) (14).

2.6. Determination of pH and moisture of samples

The pH of bread samples was determined according to Iranian National Standard Method No. 37 and the moisture of samples was determined according to Iranian National Standard Method No. 2705 (15, 16).

2.7. Microbial analysis of samples

25 g of sample was homogenized aseptically in 225 ml of sterile physiological saline for 60 seconds in a pulsifier. 10-fold serial dilutions of the homogenized samples were used for total microbial count. Surface culture of the prepared dilutions was performed on nutrient agar medium and the plates were incubated at 32°C for 3 days. For total fungal count, surface culture of the dilutions was performed on DRBC agar medium and incubated at 25°C for 5 days. Nutrient agar plates containing 30-300 colonies and DRBC agar plates containing 10-150 colonies were selected for calculations. Results were reported as log10 cfu/g (17).

2.8. Sensory Evaluation of Samples

Sensory evaluation of different bread treatments was performed using a five-point hedonic test according to the parameters of texture, taste, color, odor and overall acceptance of the samples. The samples were provided to the evaluators (30 untrained evaluators between 20 and 35 years of age, both genders equally) with a blind three-digit code (using a random number table) in a complete block design, and a special evaluation questionnaire was presented to the evaluators. The sensory characteristics evaluated were scored within the range of 1 to 5 and were performed by considering the numerical

characteristics based on 1 (very undesirable), 2 (almost undesirable), 3 (almost desirable), 4 (desirable) and 5 (very desirable). Sensory evaluation was performed for each treatment on the first, fifth and tenth day after baking and at 20 ºC (18).

2.9. Statistical Analysis

In order to examine the quantitative characteristics of the data, considering the existence of 5 treatments and 3 replications, one-way analysis of variance was used, and also to compare the mean of the data, Duncan's test was used at a significance level of 5% to examine the significance of the results. Statistical analyses were performed using SPSS 22.0 software and graphs were drawn using Excel 2013 software.

3. Results and Discussion

3.1. MIC and MFC results

The results presented in Table 2 show that the MIC of Aspergillus niger for pomegranate peel essential oil was 1.551 mg/ml) and the MFC was 2.725 mg/ml ($p \leq 0.05$). Therefore, in preparing toast treatments, the results related to the MFC of Aspergillus niger were taken into account, and accordingly, pomegranate peel essential oil was added to the toast formulation in different amounts (0.3, 0.6, 0.8 and 1% by weight) and the desired tests were performed. In the control sample, the essential oil was not used.

MFC is defined as the lowest concentration of a chemical that has a lethal effect on fungi, and MIC is the lowest concentration of an antimicrobial agent that prevents the growth of a specific microorganism. This means that the microorganism is present in the environment but is unable to reproduce. The reduction in the number of microorganisms in these conditions is not due to the lethal effect of the extract, but rather due to the microorganism reaching the death phase and no longer reproducing and its number decreasing (19).

Essential oils are hydrophobic in nature and penetrate the cell membrane and mitochondria, making them permeable, as a result, the transport of ions is disrupted and the outflow and leakage of cellular contents occur, which ultimately leads to cell death. Since the recognition of terpenes in plant essential oils as primary antimicrobial factors, the mechanism of antimicrobial action of these essential oils has been attributed to them. The structure of natural phenols present in most highly active terpenes such as thymol, carvacrol and eugenol makes the theory of the relationship between the mode of activity and their phenolic structure acceptable. The mode of activity of phenolic compounds includes interference in the function of the cytoplasmic membrane and the driving force of protein and the transport of ions. In fact, there is a correlation between the chemical structure and the amount of active ingredients in essential oils and their antimicrobial properties. Usually, essential oils rich in phenolic compounds have significant antimicrobial properties. These compounds both penetrate the cell membrane and can play a role in the coagulation of cell contents. In general, the inhibitory effect of essential oils can be attributed to the presence of an aromatic ring attached to a polar group. This claim is evidenced by the widespread use of phenols, chlorophenols, and related compounds as disinfectants. The presence of a hydroxyphenol group in the structure of some compounds in essential oils facilitates the establishment of hydrogen bonds with the active sites of enzymes and increases the inhibitory activity of the essential oil (20).

Similar studies have been conducted on the antifungal and antibacterial properties of essential oils. Khaledi (21) studied the antifungal properties of the essential oil of Pomegranate peel against Fusarium culmorum and its chemical compositions. The main identified compounds of the essential oil were hexadecanoic acid (4.9%), hexadecane (9.24%), caryophyllene (5.7%), octadecane (8.6%), farnesene (3.2%), nonadecane (1.2%), tetradecanol (3.1%) and linoleic acid (5.1%). The compounds caryophyllene, hexadecane, hexadecanoic acid and linoleic acid had antifungal effects against Fusarium culmorum. The essential oil and the hexadecanoic acid compound completely inhibited sporulation and germination of Fusarium culmorum spores. The MIC of the hexadecanoic acid compound (650 ppm) was significantly lower than the values of the essential oil (1723 ppm) and the fungicides propiconazole (1300 ppm) and Cyproconazole + carbendazim (800 ppm) were effective against Fusarium culmorum.

Rahnemoon et al. (22) investigated the effect of extraction conditions on the amount of phenolic compounds and antimicrobial properties of pomegranate peel extract. In this study, pomegranate peel extract was extracted with different ratios of ethanol/water solvent (40 to 60, 60 to 40 and 80 to 20) at 25, 40 and 55 °C and times of 20, 24 and 28 hr. The extraction efficiency, amount of phenolic compounds, flavonoids and anthocyanins were measured. The MIC was measured and the antimicrobial effect of the extracts against Salmonella Enteritidis, Escherichia col(E.Coli), Listeria monocytogenes, Staphylococcus aureus, Aspergillus niger and Saccharomyces cerevisiae was determined by disc diffusion method. The results showed that the highest extraction efficiency (50.1%), total phenolic compounds (349.518 mg/g gallic acid/g dry extract), flavonoids (250.124 mg rutin/g dry extract), anthocyanins (252.047 mg cyanidin-3-glucoside/100 g extract) and the strongest antimicrobial properties were obtained at an ethanol to water ratio of 60:40, temperature of 25°C and time of 24 hr. All extracts had antimicrobial properties against the microorganisms studied, and Staphylococcus aureus was the most sensitive to pomegranate peel extrac.

In a study of the effect of hydroalcoholic extract of pomegranate peel on biofilm formation by Pseudomonas aeruginosa, the findings showed that in all treatments, the bacteria was unable to produce biofilm in the first six hours. The amount of biofilm formed in the 12-hour treatment was moderate for concentrations of 0.01 and 0.05 g/mL. In the 18 and 24 hr treatments, a concentration of 0.001 g/mL of pomegranate peel extract showed moderate and strong inhibition of biofilm formation, respectively The duration of exposure of bacteria to biofilm formation and the percentage

of reduction in biofilm formation had a direct relationship, which can be due to different growth phases. The growth kinetics study also revealed that in most treatments, growth increased until about 15 hours and then decreased due to the effects of different treatments. The treatments had the greatest effect on biofilm formation after 18 hr. In this regard, the results of the percentage reduction in biofilm formation also showed that the greatest reduction occurred in 18 hours. The results of the microscopic slides also fully confirmed the above (23).

In the study of the antibacterial interaction effects of rosemary essential oil and lavender essential oil on two gram-positive bacteria and three gram-negative bacteria in a laboratory environment, it was observed that at dilutions of 1, 0.5, and 0.25, the inhibitory effect of lavender on five different bacteria was greater and more significant than that of rosemary, and dilution 1 of the essential oils had the greatest inhibitory effect on Proteus mirabilis. Also, comparing the different effects of lavender and rosemary essential oils on five different bacteria with each other showed that dilutions of 1, 0.5, and 0.25 of lavender essential oil had the greatest inhibitory effect on Proteus mirabilis, and its inhibitory effect was greater and more significant than that of rosemary. In examining the MIC and MBC levels of lavender and rosemary essential oils, it was observed that the bacteriostatic effect of the essential oils on bacteria except Enterococcus faecalis was similar, however, the bactericidal effect of the essential oils on all bacteria except Staphylococcus epidermidis was similar (24). Research has shown that the aerial parts of the lavender plant have a stronger antimicrobial effect than other parts of the plant (25). Of course, it has been found that the leaves of this plant, in addition to diterpenes, contain large amounts of cyclic alcohols, flavonoids, and organic acids such as carnosic acid and saponins, among which saponins have effective antibacterial properties. The lavender plant contains 26 different substances, of which linalyl acetate and linalool are among the most abundant constituents of its essential oil (26). In a study of the antibacterial effect of ethanolic extract of lavender, it was stated that the ethanolic extract of this plant was effective on two bacteria: Staphylococcus aureus (gram-positive cocci) and E.Coli (gram-negative bacillus), so that the MIC of Staphylococcus aureus and E.Coli was 12.5 and 25 mg/ml, respectively, and the minimum bactericidal concentration of Staphylococcus aureus and E.Coli was 25 and 50 mg/ml, respectively. The results showed that ethanolic extract of lavender had more inhibitory and lethal effects on gram-positive bacteria (27). Also, in the study of the antimicrobial properties of lavender essential oil, it was observed that this essential oil was effective against E.Coli, Shigella sonnei and Enterobacter aerogenes. Candida albicans was sensitive to this essential oil and inhibition zones were clearly visible. Most studies conducted on the effect of plant essential oils on spoilage microorganisms and food pathogens show that the antimicrobial effect of such essential oils on gram-positive bacteria is greater than on gram-negative bacteria (28). Also, in a study conducted to investigate the chemical composition of lavender essential oil and waste residues, and their antibacterial and antifungal effects, the main compounds identified included monoterpenes, sesquiterpenes, and some aliphatic compounds. Lavender essential oil showed good antibacterial activity against Bacillus subtilis, Pseudomonas fluorescens, Xanthomonas campestris, Erwinia carutovara at a concentration of 300 µg/ml, and Erwinia amylora, Candida utilis at a concentration of 150 µg/ml, respectively. Also, the residual water and ethanol extracts of solid waste residues had high antimicrobial activity against Aspergillus niger, Alternaria alternata, Penicillium chrysogenum, and Pseudomonas aeruginosa at concentrations of 0.75-6 µg/ml, 0.125-0.08 µg/ml, and 0.05-4 µg/ml, respectively (29).

3.2. Results of moisture measurement of toast samples

Based on statistical analyses, the moisture content of the treatments was significantly different compared to the control treatment. After 10 days of storage, the moisture content of each treatment decreased significantly, and the interaction between variables on the moisture content of the samples was statistically significant ($p<0.05$). The results of moisture measurement showed that the sample containing 1% pomegranate peel essential oil had the highest moisture content and the control treatment had the lowest moisture content. Also, with increasing storage time, the moisture content decreased, such that the highest moisture content of the samples was observed on the first day and the lowest moisture content was observed on the last day (the tenth day) (Fig. 1).

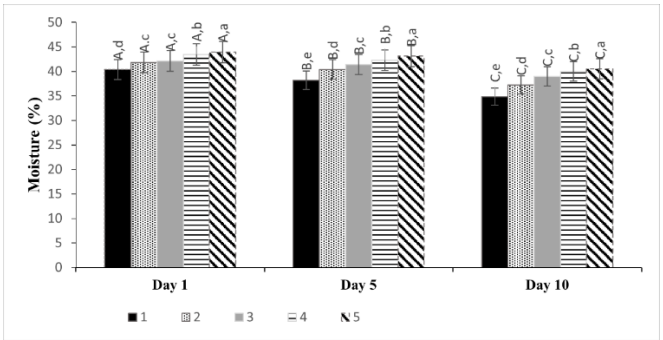


Fig. 1. Moisture content of toast samples. Different capital letters indicate significant differences between different days and Different small letters indicate significant differences between different treatments ($p<0/05$).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

The reason for the increase in moisture content in treatments containing essential oil is the presence of many hydrophilic compounds such as polyphenols, caffeine and polar amino acids, which absorb water and increase the moisture content of bread. On the other hand, due to the high polyphenol content, it has active polyhydroxyl structures that have a high tendency to form hydrogen bonds with water, thus increasing the ability

to retain moisture and maintain it in the bread texture. The moisture content of bread in all treatments was higher than the control sample during storage. The reason for this can be explained as follows: due to the hydrophilic nature of its compounds, pomegranate peel essential oil formed hydrogen bonds with water and, with its stability in the system, maintained moisture during baking and storage of bread, which can also be effective in delaying the staleness of bread (30).

In this regard, Hoseinzadeh and Shirazinejad (31), in a study they conducted on the antioxidant and antimicrobial properties of grape seed extract in sponge cake, stated that adding grape seed extract increased the moisture content in the cake, which is consistent with the results of this study.

3.3. Results of pH measurement of toast samples

Based on statistical analyses, the pH of the treatments was significantly different compared to the control treatment, and during 10 days of storage, the pH of the samples increased significantly, and the interaction of variables on the pH value of the samples was also significant ($p<0.05$). The results of pH measurement showed that the highest pH value in all days of storage belonged to the control treatment and the lowest pH belonged to the sample containing 1% pomegranate peel essential oil. During the storage period, the pH of all treatments increased significantly (Fig. 2).

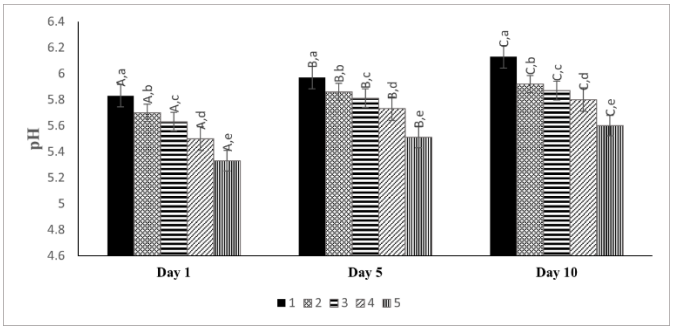


Fig. 2. pH results of toast samples**.** Different capital letters indicate significant differences between different days and Different small letters indicate significant differences between different treatments ($p<0/05$).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

Treatments containing pomegranate peel essential oil had the lowest and the control sample had the highest pH. The lower pH in samples containing pomegranate peel essential oil may be due to the presence of weak organic acids and phenolic compounds in the essential oil. In a study conducted by Karami Moghadam et al. (32), the bactericidal and antimicrobial effects of pomegranate peel extract were investigated in mechanical films containing sodium caseinate. In this study, it was reported that pomegranate peel had an antimicrobial effect and this prevented the activity of microorganisms. In another study, in line with the results of this study, Naghavi and Seyed Alangi (33) investigated the effect of adding red pomegranate peel powder and cardamom on the sensory and rheological properties of oil cake and stated that adding pomegranate peel caused a decrease in pH.

3.4. Results of total phenol and antioxidant properties of samples

Based on statistical analyses, the total phenol content of the treatments was significantly different compared to the control treatment, and during 10 days of storage, the total phenol content of each treatment decreased significantly, and the interaction of variables on the total phenol content of the samples was also statistically significant ($p<0.05$). The results of measuring total phenol content showed that the highest total phenol content belonged to the sample containing 1% pomegranate peel essential oil and the lowest value with a significant difference belonged to the control treatment. Treatments containing pomegranate peel essential oil had the highest total phenol content. Also, with increasing storage time, the total phenol content decreased, such that the highest total phenol content of the samples was observed on the first day and the lowest on the last day (Fig.3).

The percentage of DPPH inhibition of the treatments was significantly different compared to the control sample, and during 10 days of storage, the percentage of DPPH inhibition of each treatment decreased significantly, and the interaction of variables on the DPPH inhibition of the samples was also significant ($p<0.05$). The results of measuring the percentage of DPPH inhibition showed that the highest percentage was observed by the sample containing 1% pomegranate peel essential oil and the lowest was observed by the control sample. During the storage period, a significant decrease in the percentage of DPPH inhibition was observed in all treatments (Fig. 4).

Pomegranate peel is rich in phenolic compounds from the polyphenol group and is considered a natural source of antioxidants (34). Additionally, vitamins and glutathione have been found in high amounts in pomegranate peel (35). Polyphenols are able to neutralize free radicals, however, the antioxidant activity in pomegranate peel is more affected by phenolic compounds, including ellagitannins, and less influenced by the amount of anthocyanins and ascorbic acid (36).

The control sample showed very little antioxidant activity due to the lack of phenolic and anthocyanin content. In addition, the antioxidant activity of toast samples decreased during storage due to the decrease in phenolic and anthocyanin compounds, which was consistent with the results of Jaster et al. (2017) (37).

In general, the effect of antioxidants in inhibiting DPPH free radicals is due to their ability to donate hydrogen. Free radical inhibition is one of the best-known mechanisms by which antioxidants can inhibit lipid oxidation. In the DPPH free radical inhibition test, DPPH radicals react with antioxidants and their amount is reduced. There is a direct

relationship between polyphenol concentration and anti-radical activity. At high concentrations, as the number of hydroxyl groups of phenolic compounds increases, the probability of donating hydrogen to free radicals increases, and the inhibitory power of the compound increases. Hydroxyl groups, by donating hydrogen, change DPPH radicals from dark purple to light yellow (38).

DPPH free radical scavenging assay is one of the suitable methods to evaluate the antioxidant properties of various compounds. In this study, it was found that pomegranate peel essential oil has DPPH free radical scavenging properties and this property increased with increasing concentration of pomegranate peel essential oil. These results were consistent with the results of Vaithiyanathan et al. (39).

There is a positive correlation between the antioxidant activity of pomegranate peel and the amount of phenolic compounds, flavonoids, and anthocyanins in it (40). The DPPH free radical scavenging property of pomegranate peel essential oil is probably due to the ability to hydrogenate the hydroxyl groups of the phenolic compounds present in this essential oil. In addition, antioxidant compounds are able to stop the free radical cycle in the oxidation process (3). Pomegranate peel is rich in antioxidants, and as a result, increasing its amount is able to inactivate more DPPH free radicals. Kanatt et al. (41) pointed out the significant antioxidant activity of pomegranate peel extract . Also, Cam et al. (42) added pomegranate peel extract to the formulation to increase the functional properties of ice cream and observed an improvement in antioxidant activity in the product. It is worth noting that the antioxidant property of pomegranate peel remains even after heating. Srivastava et al. (43) also studied the effect of dried pomegranate peel powder on the properties of biscuits and observed that the antioxidant activity of biscuits increased.

Dried and extracted pomegranate peel has a very high phenolic content and antioxidant properties. According to the results of this study, pomegranate peel powder has a high concentration of phenol and DPPH free radical scavenging activity (44).

The decrease in antioxidant activity of samples during storage can be attributed to the formation of complexes between phenolic compounds and sample proteins (45,46).

In addition, the reduction in phenolic compounds is probably due to the degradation and hydrolysis of polyphenols into aromatic acids such as phenylacetic, phenylpropionic, and benzoic acids by microorganisms (45).

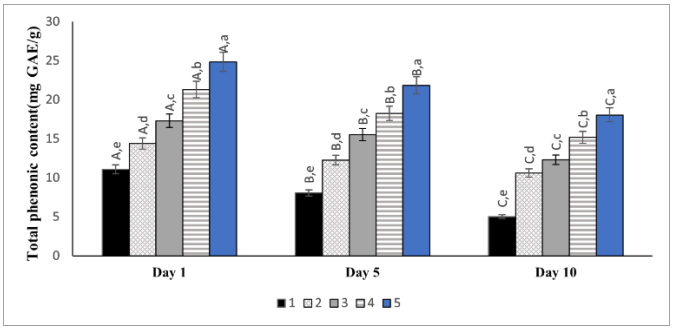


Fig.3. Total phenol content of toast samples**.** Different capital letters indicate significant differences between different days and Different small letters indicate significant differences between different treatments (p<0/05).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

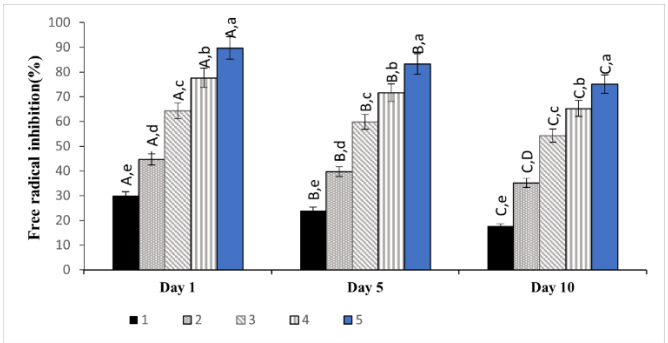


Fig. 4. DPPH free radical scavenging results of toast samples. Different capital letters indicate significant differences between different days and Different small letters indicate significant differences between different treatments (p<0/05).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

3.5. Microbiological evaluation results

Based on statistical analyses, the total microbial population and the number of mold and yeast in the treatments were significantly different compared to the control sample and increased significantly in all treatments during 10 days of storage, and the interaction of variables on the total microbial population and the number of mold and yeast in the samples was also significant (p<0.05). The results showed that the sample containing 1% pomegranate peel essential oil had the lowest total microbial, mold and yeast population. During the storage period, a significant increase in the total microbial count and the number of mold and yeast was observed in all treatments (Fig.5a and Fig.5b).

In general, the treatments containing pomegranate peel essential oil had the lowest total microbial, mold and yeast population, and the highest number was observed in the control sample. In all treatments, the lowest total microbial, mold, and yeast population was observed on the first day and the highest on the last day.

The lower microbial population in samples containing essential oil can be attributed to the antibacterial and antifungal properties of pomegranate peel essential oil, which is related to the presence of gallic acid, ellagic acid, and punicalagin, which have strong antimicrobial activity. The mechanism of

action of the essential oil is related to the reaction of phenolic compounds with membrane proteins and their precipitation and inhibition of the glycosyl transferase enzyme, which ultimately leads to the destruction and breakdown of the microbial cell membrane (47).

In addition, the antimicrobial effect of essential oils has been attributed to their ability to penetrate the membrane into the cell, inhibit cell functional properties, and their lipophilic properties (48).

Phenolic compounds also cause permeability of the lipid membrane of the cell wall and mitochondria, ultimately leading to the release of cellular contents and cell death or preventing its growth and proliferation (20).

Other compounds present in pomegranate peel essential oil also have antimicrobial properties. In the study of the antifungal properties of pomegranate peel essential oil against Fusarium culmorum, the compounds caryophyllene, hexadecane, hexadecanoic acid, and linoleic acid identified in the essential oil had antifungal effects against Fusarium culmorum. The essential oil and the hexadecanoic acid compound completely inhibited sporulation and germination of Fusarium culmorum spores (21).

Flavonoids and anthocyanins also have antimicrobial properties. Silvan et al. (49) stated that phenolic acids, catechins, proanthocyanins and flavonols have antimicrobial properties.

During the storage period, an increase in microbial population was observed in all treatments, which can be attributed to the decrease in the amount of phenolic compounds in the samples over time.

The diffusion of phenolic compounds of pomegranate peel extract into the cells increases due to the hydrophobicity of their active compounds. And since the amount of phenolic compounds decreases with time, the antimicrobial activity also decreases and causes an increase in the total count of microorganisms (50). In this regard, Ibrahium (51), in investigating the antimicrobial and antioxidant effects of pomegranate peel extract, reported the same results as this study.

In addition to pomegranate peel essential oil, its extracts also have antimicrobial properties, and similar results have been obtained in the research conducted in this regard. In one of these studies, Mehdizadeh et al. (52) investigated the effects of an edible starch-chitosan composite film containing a combination of pomegranate peel extract and Kakuti essential oil on the population of aerobic mesophilic bacteria, lactic acid bacteria, and Pseudomonas in red meat during 21 days of storage. At the end of 21 days, a 11.7 logarithmic cycle decrease was observed in the population of aerobic mesophilic bacteria compared to the uncoated group. In the population of lactic acid bacteria, the best effect was observed for the film containing 2% essential oil and half a percent extract, which was 29.3 logarithmic cycles different from the control group. On the other hand, in the sample coated with a film containing 2% essential oil and 1% extract, at the end of the 21st day, a 6.92 logarithmic cycle reduction in the population of Pseudomonas bacteria was observed compared to the control group without coating. This study showed that the composite film containing extract and essential oil has high antimicrobial properties and by improving some physical properties of the film, it can be used for packaging and increasing the shelf life of meat.

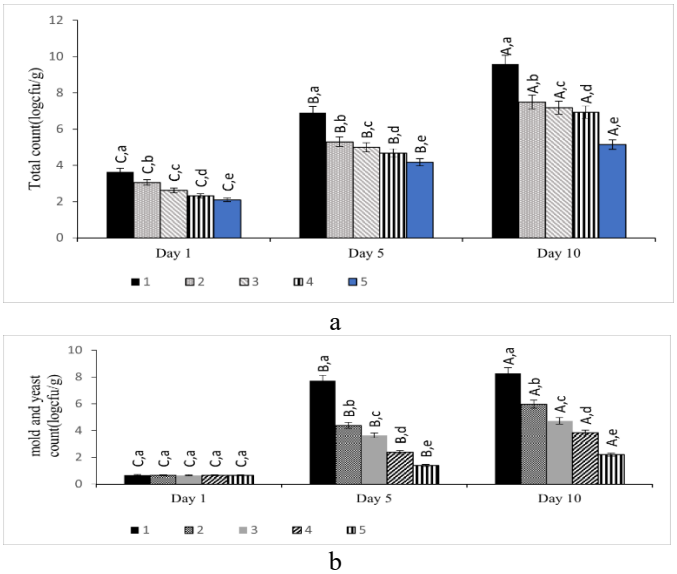


Fig .5. Microbiological evaluation results of toast samples: **a**) total colony count **b**) mold and yeast count**.**Different capital letters indicate significant differences between different days and Different small letters indicate significant differences between different treatments (p<0/05).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

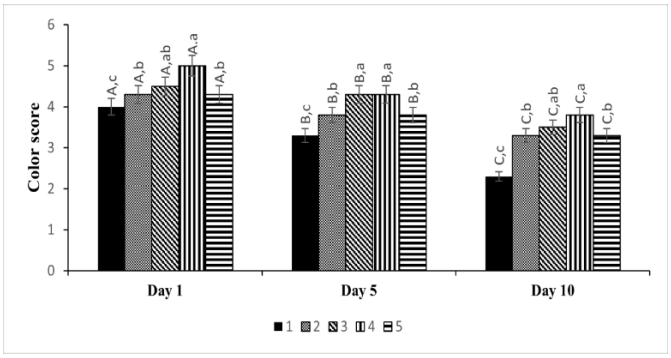
3.6. Results of sensory evaluation of toast samples

Based on statistical analyses, the sensory characteristics (color, taste, odor, texture and overall acceptance) of the treatments were significantly different compared to the control treatment, and during 10 days of storage, the sensory score of each treatment decreased significantly, and the interaction of variables on the sensory score of the samples was also significant (p<0.05). The results of sensory evaluation showed that during the storage period, the highest color score belonged to treatment 4 (containing 0.8% pomegranate peel essential oil) and the lowest score belonged to the control treatment. (Fig. 6a) During the storage period, the highest taste score belonged to treatment 3 (containing 0.6% pomegranate peel essential oil) and the lowest score belonged to the control treatment. In all treatments during storage, the taste score decreased significantly. (Fig. 6b) During the storage period, the highest odor score belonged to treatment 3 (containing 0.6% pomegranate peel essential oil) and the lowest score belonged to the control treatment. (Fig. 6c) During the storage period, the highest texture score belonged to treatment 3 (containing 0.6% pomegranate peel essential oil) and the lowest score belonged to the control treatment. (Figure 4.7d) During the storage period, the highest overall acceptance score belonged to treatment 3 (containing 0.6% pomegranate peel

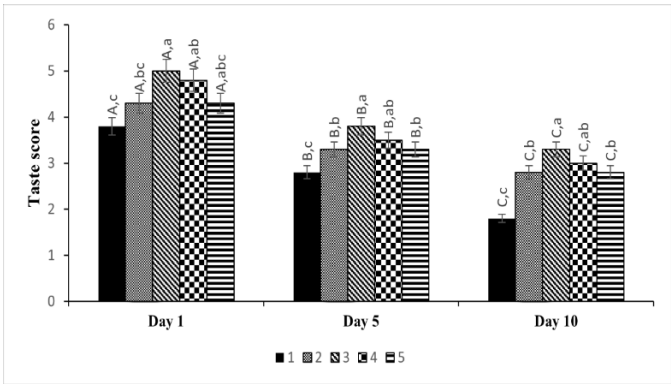
essential oil) and the lowest score belonged to the control treatment (Fig.7).

In general, treatment number 3, which contained 0.6% pomegranate peel essential oil, had the highest taste, odor, texture, and overall acceptance scores. Also, with increasing storage time, the color, taste, odor, texture, and overall acceptance scores of the toast treatments decreased, such that the treatments obtained the highest sensory test scores on the first day and the lowest scores on the last day. In this regard, Ahmed et al. (46) also reported that during storage, the sensory properties scores of yogurt enriched with ethanolic extract of argil leaves (a type of medicinal plant) decreased.

The decrease in aroma and flavor scores at high levels of essential oil concentration is due to the increase in the concentration of bitter compounds such as polyphenols and caffeine and aroma and flavor-producing compounds present in the essential oil and the excessive participation of amino acids and flavonoid compounds of pomegranate peel essential oil in the Maillard reaction. Of course, the color of the peel, aroma and flavor of the treatment containing 0.6% were desirable due to the favorable effect of flavonoid compounds and amino acids on the Maillard reaction and were more acceptable compared to other treatments. The lowest level of firmness was related to the treatments containing pomegranate peel essential oil and the highest firmness was related to the control treatment. The reason for the softer texture of these treatments compared to the control sample can be attributed to the effect of the essential oil on the moisture content of the bread crumb and starch retrogradation, which is an important factor in bread staleness. In a study conducted by Ghanbari et al (30) on the effect of green tea hydroalcoholic extract on the rheological properties of dough and staleness of Berberi bread, they reported similar results to the present study.



a



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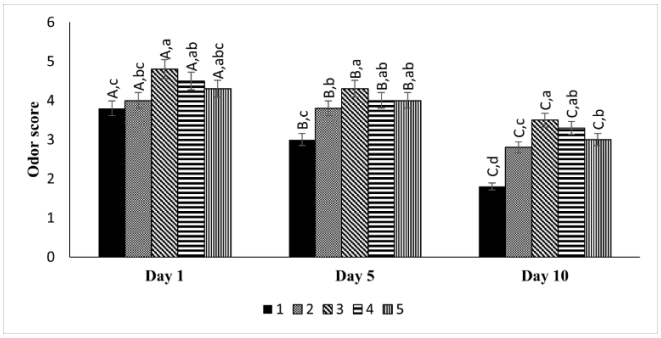

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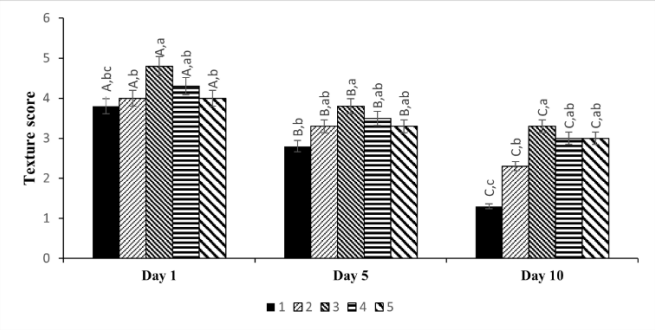

d

Fig. 6. Results of sensory evaluation of toast samples: **a**) color **b**) taste **c**) odor **d**) texture**.** Different capital letters indicate significant differences between different days (p<0/05). Different small letters indicate significant differences between different treatments (p<0/05). **1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

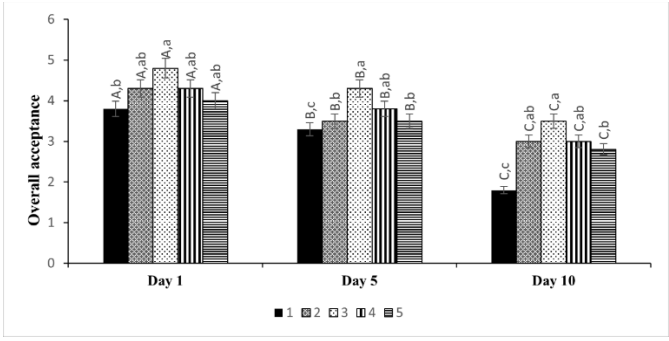

Fig. 7. Results of overall acceptance evaluation of toast samples.Different capital letters indicate significant differences between different days (p<0/05).Different small letters indicate significant differences between different treatments (p<0/05).**1**: Control, **2**: Treatment containing 0.3% pomegranate peel essential oil, **3**: Treatment containing 0.6% pomegranate peel essential oil, **4**: Treatment containing 0.8% pomegranate peel essential oil, **5**: Treatment containing 1% pomegranate peel essential oil.

4. Conclusion

In this study, the effect of adding pomegranate peel essential oil on antioxidant, sensory and shelflife properties of toast was investigated over a period of 10 days (days 1, 5 and 10). Evaluation of physicochemical and antioxidant properties showed that treatments containing pomegranate peel essential oil had higher moisture content, total phenol content, antioxidant properties and lower pH and total microbial count than the control sample. In terms of sensory properties, the treatment containing 0.6% pomegranate peel essential oil received the highest taste, odor, texture and overall acceptance scores from the evaluators, and the control treatment had the lowest score. Also, storage until day 10 reduced the moisture content, total phenol content, antioxidant properties, toast treatments. And the total microbial population and the number of mold and yeast increased.

The results of the present study showed that adding pomegranate peel essential oil improves the quality and physicochemical, antioxidant, antimicrobial and sensory properties of toast and this essential oil can be used as a health-promoting agent in foods. Also, the sample containing 0.6% essential oil with the highest sensory score and the treatment containing 1% essential oil with the highest antioxidant and antimicrobial properties were considered as the superior sample.

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