Original Article: Evaluation of Antioxidant and Antimicrobial Effects of Hydroalcoholic and Aqueous Extracts of *Zataria multiflora* on Increasing the Shelf Life of Lavash Bread

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Abstract

This study aimed to investigate the antioxidant and antimicrobial effect of hydroalcoholic and aqueous extracts of *Zataria multiflora* on increasing the shelf life of Lavash bread. In this research, hydroethanolic and aqueous extracts of *Z. multiflora* were added to bread flour at three levels of 1, 1.5, and 2.5 g/kg. Bread samples were baked. Tests for antioxidant effects (total phenol, total flavonoids, and free radical scavenger DPPH), microbial properties (coliform and mold count), and Organoleptic properties. Statistical analysis of the data was performed with SPSS software at the level of $P < 0.05$. The results showed the total phenol content of hydroethanolic and aqueous extracts was 34.53±3.63 and 26.12±2.11 (mg GAE/g sample) respectively. The results of the total flavonoid test in ethanolic and aqueous extracts were 263.23±10.35 and 221.24±12.13 **(**mg QUE/g sample), respectively. Comparison with BHT synthetic antioxidants was 88.34±1.81, 82.97±2.01 and 92.35±2.3(%), respectively. The results count of coliforms in Lavash bread obtained from different treatments of ethanolic and aqueous extracts during the shelf life of 10 days in 2% treatment of ethanolic extract with an average of 3.12 Log CFU/g had a lower value, and. The count of mold and yeast in the treatment of 2% ethanolic extract with an average of 5.96 CFU/g was lower. The results showed that ethanolic extract had more effects than aqueous extract.
Z. multiflora extracts have significant antioxidant and antimicrobial effects and can be suggested as a suitable alternative to synthetic preservatives.

Keywords: *Zataria multiflora*, Extract, Lavash bread, Preservation

Introduction

Phenolic compounds are classified (Phenolic formulas are categorized) into simple phenols, phenolic acids (unsophisticated phenols, phenolic acids) hydroxycinnamic derivatives, and flavonoids. Researchers have reported the function of many phenolic compounds as potent antioxidant compounds (Mazarie *et al*., 2018). Due to the health properties of antioxidants and their role in disease prevention, researchers are increasingly interested in examining the presence of antioxidant compounds in agricultural products and medicinal plants. The addition of antioxidant compounds in some products to prevent lipid peroxidation increases their shelf life and prevents the oxidation process (Shi *et al*., 2005). Flavonoids and other phenolic compounds are widely distributed in plants and their diverse biological activity including antioxidant, antimicrobial, anti-inflammatory has been reported in many studies (Jamshidi *et al*., 2010; Harbourne *et al*., 2009) and, On the other hand, these compounds have the role of reducing, chelating metals and giving hydrogen;

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Therefore, they have antioxidant effects due to their reducing effect (Safi *et al*., 2016).

Free radicals are molecules without a complete electron shell (covering) that are naturally produced in the body of living organisms, but when the production of these radicals is excessive, the substrates, including cell membranes, proteins, and nucleic acids, are oxidized. In this case, including DNA and protein degradation occurs Mazaraie *et al*. (2018) and therefore (thus) antioxidant compounds are needed to neutralize the effect (perform) of oxygen radicals (Fazeli-Nasab *et al.*, 2017).

antioxidants can prevent or delay oxidative damage to molecules such as fatty acids, proteins, nucleic acids, and pigments, by neutralizing free radicals (Mazaraie *et al*., 2020; Jafari *et al*., 2007) and on the other hand reduce the risk of cardiovascular disease and stroke and also prevent the progression of cancer (Safi *et al*., 2016).

Bread is the staple food for many people around the world, providing one of the cheapest daily sources of a significant portion of the energy, protein, minerals, and B vitamins people need (Hojjati *et al*., 2014). Of the various types of bread in the world, flatbreads are the oldest and simplest. Lavash bread is a thin sheet of bread 50-60 cm long, 25-30 cm wide, and 3-22 mm thick. The raw materials are fermented for 60-75 minutes after mixing and after bargaining and resting, they are laminated and baked at 332°C for 60-75 seconds. This bread is one of the common bread that has been used in the country since ancient times and has special fans (Salehifar *et al*., 2010).In Iran, Lavash bread has been prepared and cooked in the traditional way for thousands of years, but due to the low quality of domestic wheat and the use of incorrect dough preparation methods, and the possible use of ingredients such as baking soda, about 30% of the bread produced in the country to In case of waste, preparation of the dough is one of the technological stages of bread production that has a good effect on the quality of the final product. If the dough preparation process is defective or not prepared according to the correct principles, it can be less modified in other different stages of production (Movahhed *et al*., 2017). Bread is out of the consumption cycle due to various reasons such as doughy sides, staleness, drying, mold, and other cases. Bread can be considered an unstable substance and stale bread can be considered a process that involves physical, chemical, and organoleptic changes (Nasehi *et al.*, 2009).

Molds, yeasts, and bacteria are the most important microorganisms that have been discussed in various food and related industries such as spoilage and contamination. The most common types of molds and fungi that grow on grain-based foods and can therefore be present in the flour from which they are made are Aspergillus, Penicillium, Rhizopus, Monilia, and Yeasts (Salehifar *et al.* 2010). During their growth on food, fungi, in addition to reducing the amount of food due to the fungal contaminated portion and reducing the nutritional value, release secondary metabolites called mycotoxins (such as Aflatoxins) that have devastating and severe effects such as carcinogenicity, malignancy and They have growth retardation, inhibition of the immune system, and mutagenicity in living organisms (Movahhed *et al*., 2017; Salehifar *et al*., 2010).

Thyme medicinal plant with scientific name *Z. multiflora* Boiss. Isone of the plants of the mint family, which is one of the largest plant families in the world. Thyme has a woody stem with a height of 40-80 cm, which grows wild in the form of thick bushes on dry slopes between the boulders of different regions of Iran, Pakistan, and Afghanistan (Misaghi *et al*., 2007). Much research has been done on the inhibitory properties of thyme essential oil on pathogenic bacteria or food spoilage (MahdaviAdeli *et al*. 2020; Basti *et al.*, 2007).

So far, many studies have been done on the antibacterial and antifungal properties of this plant. In traditional Iranian medicine, a decoction of this plant has been mentioned as an analgesic, disinfectant Mahboubi *et al.* (2018), anti-flatulence, anti-parasite, and anti-diarrhea (Nazaryanpour *et al*., 2020). Pharmacological studies have shown that *Z. multiflora* extract can treat coughs caused by bronchitis and is prescribed as an antibacterial in oral health by traditional healers (Ariaee *et al*., 2018). This plant has a pleasant aroma and is a flavoring and seasoning for yogurt and many fast foods and is widely used in the preparation of many local Iranian

dishes (Khatibi *et al*., 2018). Studies on the chemical composition of *Z. multiflora* essential oil showed that its essential oil contains significant amounts of monoterpene compounds such as thymol, carvacrol, linalool, gamma-terpinene, and paracetamol (Ziaee *et al.* 2018).

Recent reports on this plant prove that its essential oil has anti-parasitic and antifungal properties and is a valuable medicine for treating cysts. Recently, Buskabadi *et al*. (2018) reported the anti-inflammatory and antioxidant effects of this plant essential oil and proved the effectiveness of this plant essential oil in improving the function of the immune system (Nazaryanpour *et al*., 2020). The essential oil of this plant has antibacterial and antifungal properties and stimulates the immune system. This plant is used to treat muscle cramps, rheumatic pains, insect bites, wound disinfection, bloating, and skin diseases (Sefidkon *et al.*, 2003).

Phenolic compounds are mainly responsible for the antibacterial and antifungal properties of extracts and essential oils (Zhang *et al*. 2009; Mashak *et al*. 2012). Therefore, the higher the levels of phenolic compounds, the higher their antimicrobial properties. These substances in *Z. multiflora* essential oil include carvacrol, thymol, andeugenol (Zhang *et al.*, 2009). Carvacrol exerts its antimicrobial effect by perforating the cell membrane of gram-positive bacteria and destroying the outer membrane of gram-negative bacteria (Mashak *et al.*, 2012). *Staphylococcus aureus* causes extraintestinal infections such as pimples, ulcers, pneumonia, toxic shock syndrome, meningitis, and staphylococcal food poisoning in the Human body.

Material and methods

The medicinal plant *Z. multiflora* was purchased in 2018 from Jahrom city located in Fars province, Iran. Chemicals used include DPPH, fullerene-calcium reagent, sodium carbonate, gallic acid, aluminum chloride, potassium acetate, ascorbic acid, and violet Redbile agar and Saburo dextrose agar media from Merck (Germany) and quercetin reagent Sigma-Aldrich (USA) and methanol from Applichem (Germany).

Preparation of thyme extracts: Hydroalcoholic and aqueous extracts of thyme were obtained by percolation method using a percolator. To extract, the plant was first ground, 50 grams of the ground plant was carefully weighed and poured into a decanter with cotton at the end. Then, for the hydroalcoholic extract, 80% ethanol solvent was added to it regularly and it was tried that the solvent penetrated evenly in the whole plant mass. Deionized water was used as the solvent for the aqueous extract. Then percolation was performed for 24 to 48 hours while the solvent completely covered the plant mass. Erlenmeyer-containing solvents were then transferred to a rotary apparatus to concentrate the liquid extract. To maintain the quality of the extracts, they were stored in the refrigerator until use (Alsalvar *et al*., 2005).

Measurement of Total Phenol Content

In this method, the amount of total phenolic compounds was measured by the Folinciocalteu method (Ordonez *et al*., 2006). In this method, 0.5 ml of each extract with a concentration of 50 µg/ml was removed. Then 2.5 ml of Folinciocalteu solution was added to it. After 5 minutes, 2 ml of a Sodium carbonate solution was added and rested. 2 hours later, the absorbance of the samples was measured by ultraviolet spectrophotometer at 760 nm against Blanc. To draw the standard gallic acid curve, standard gallic acid solutions with concentrations of 400, 200, 100, 50, and 25 µg/ml were prepared. The results were then expressed as equivalent values to the gallic acid standard. In this way, the average absorption in the line equation obtained from drawing the standard curve of gallic acid was placed and the result was reported as the total phenolic content of the extract based on the equivalent of "mg of gallic acid per gram of extract".

Measurement of Total Flavonoid Content

In this method, the number of total flavonoid compounds of all extracts was done based on the Chang method and using an Aluminum chloride reagent (Chang *et al.*, 2002). In this experiment, first 0.5 ml of each extract with a concentration of 100 µg/ml was taken, then 1.5 ml of methanol and 100 ml of Aluminum chloride, 100 ml of potassium acetate

M 1, and 3 ml of water were added to it. After 30 minutes, the absorbance of the samples was measured at 415 nm relative to the blank by a visible-ultraviolet dual spectrophotometer. Quercetin was also considered as a standard with concentrations of 15.62, 31.25, 62.5, 125, and 250 µg/ml to draw the calibration curve.

DPPH Test

The electron or hydrogen atom donation ability of extracts was measured based on the rate of free radical scavenging activity of 2-diphenyl-1-picryl hydrazyl (DPPH) (Kartal *et al*., 2007). In this study, different concentrations were prepared. Thus, the extract solution with a concentration of 800 µg/ml was used to dilute and stocks with concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 were made (dilution method). This was done for all extracts. In the next step, 1 ml was transferred to a new tube after each stock, and 1 ml of PDPH was added to each. The control solution contained 1 ml of methanol and 1 ml of DPPH. One control was observed for each extract. It was then kept in the dark for 15 minutes and then the absorption of the samples at 517 nm at different concentrations of different extracts was read
The percentage of free radical inhibition was calculated using the following formula:

$$R = \left(\frac{A_B - A_S}{A_B} \right) \times 100$$

AB=Blank absorption (DPPH absorption diluted with methanol in a ratio of 1: 1)
AS=sample or standard absorption

Baking Lavash Bread

The Lavash bread flour used was selected by Iranian National Standard No. 103. To prepare different treatments, 1, 1.5, and 2 grams of hydroalcoholic (ethanolic) and thyme aqueous extracts were added and prepared per kilogram of flour. Calcium propionate at a rate of 1 g/kg flour was used as a control sample. After completely mixing the ingredients in the blender, baking bread samples in the oven at a temperature of 550-600°C was done in about 1 minute (ISIRI, No. 103-2018).

Determining the Characteristics of Lavash Bread

In this study on Lavash bread samples containing different concentrations of thyme extracts, tests including determination of antioxidant properties (total phenol, total flavonoids, and antioxidants), microbial properties (coliform count and mold count), and review of Organoleptic characteristics (flavor, aroma, texture, color, and total acceptance) were performed for 10 days at the time of production (zero days) as well as at 2-day intervals. Bread samples were stored at 25°C (at room temperature) and relative humidity.

Determining the Presence of Coliforms

To investigate the presence of coliforms, McConkey Agar and violet-red bill Agar medium were used by Iranian National Standard No. 9263. In this method, 1 ml of the prepared dilutions were taken by the sampler and transferred to a sterile petri dish. About 15 ml of the culture medium, the temperature of which is not more than 45°C, is transferred to the Petri dishes, and to mix the sample with the culture medium, the petri dish is rotated several times to close the medium. After closing the environment, the containers are inverted at 30°C for 24-48 hours and after this time the test result is determined by counting the colonies (ISIRI, No. 9263-2007).

Determination of Mold Growth Rate

The study of Aspergillus mold growth rate was performed using Sabouraud dextrose agar-bearing medium with chloramphenicol and by Iranian National Standard No. 2-10899. In this method, as above, the method of culturing pour plate was used. The difference was that the incubator temperature was 25°C and its duration was 72 hours (ISIRI, No. 10899-2 2008).

Evaluation of Organoleptic Properties

Evaluation of Organoleptic properties was performed using the 5-point hydronic scale method. Bread samples, including 6 samples treated with different concentrations of ethanolic and aqueous extracts of thyme, and a control sample were evaluated by fifteen trained detectives (food industry students) in terms of flavor, aroma, texture, and total acceptance.

Statistical Analysis

Statistical analysis of data was performed with SPSS software version 22. To analyze the quantitative values obtained from the tests, after checking the normality of the data using the Kolmograph-Smirnov test, a one-way analysis of variance in a completely randomized factorial statistical design was used. Duncan's test was also used to compare the means in cases where the overall effect of the treatments was found to be significant.

Results and Discussion

Antioxidant Activity

The results of studying the total phenol content of ethanolic and aqueous extracts of *Z. multiflora* in Table (1) show that they had 34.53±3.63 and 26.12±2.11 mg equivalent of gallic acid per gram of sample, respectively. The results of the total flavonoid test in ethanolic and aqueous extracts were 263.23±10.35 and 221.24±12.13 mg equivalent to quercetin per gram of sample, respectively. Ethanol and aqueous compared to BHT synthetic antioxidants were 88.34±1.81, 82.97±2.01, and 92.35±2.3 respectively.

In this regard, Sharafati Chaleshtari *et al*., (2013) reported the antioxidant properties of *Z. multiflora* extract and its antimicrobial effect on *Staphylococcus aureus*. The flavonoid content was 131.23±4.50 mg/g quercetin. The antioxidant activity of free radical scavenging DPPH was 71±4%.

In one study, the flavonoid content of thyme hydroalcoholic extract was reported to be 32 mg/g and its free radical scavenging was reported to be 71% (Fatemi *et al*., 2012). According to a study by Sharififar *et al*. (2007), the polar fraction of *Z. multiflora* extract has a high antioxidant effect, so IC50 = 16.2 µg/ml for the extract was calculated against IC50 = 18.2 µg/ml. The high antioxidant activity of polar fractions is due to the presence of large amounts of phenolic acids and flavonoids, while the non-polar phase is less active due to the absence of such compounds. On the other hand, the presence of different antioxidant compounds in the polar phase causes a synergistic effect on the activity of these compounds (Sharififar *et al*., 2007).

Table 1 Results of antioxidant parameters tests

Extract	Total phenol (mg GAE/g sample)	Total flavonoid (mg QUE/g sample)	Antioxidant activity (%)
Hydroalcoholic	34.53±3.63 a	263.23±10.35 a	88.34±1.81 b
aqueous	26.12±2.11 b	221.24±12.13 b	82.97±2.01 c
BHT	-	-	92.35±2.3 a

*Numbers (mean ± standard deviation) with common letters in each column are not statistically significantly different from each other (P<0.05).

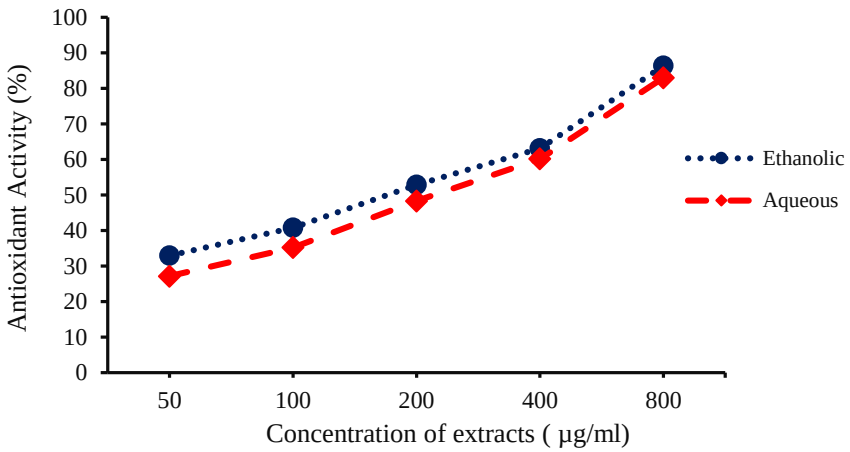

Fig. 1 Comparative study of DPPH free radical scavenging percentage in different concentrations of ethanolic and aqueous extracts

Antimicrobial Effects

Presence of Coliforms

The results of logarithm study of the number of coliforms in lavash bread obtained from different treatments of ethanolic and aqueous extracts during the shelf life of 10 days in Table (2) show that different treatments of ethanolic and aqueous extracts have effects They are antimicrobial against coliforms, so that at the end of the storage period, the control sample with an average 5.22 log CFU/g had the highest amount of coliform infection, while in other treatments with increasing the percentage of extract, the number of coliforms was less. So that on the tenth day, the treatment of 2% ethanolic extract with an average of 3.12 Log CFU/g was less. The results showed that ethanolic extract had more effects than aqueous extract.

In this regard, Goodarzi *et al*. (2006) by examining the effect of aqueous and alcoholic extracts of *Z. multiflora* on *Escherichia coli* found that Alcoholic extract of *Z. multiflora* had significant effects on *E. coli* strains at a concentration of 0.78 mg/ml of the inhibitory and lethal effects on the studied bacteria. Shabanpour *et al*. (2012) investigated the effect of *Z. multiflora* Boiss. On the shelf life of salted and vacuum-packed salmon rainbow trout (*Oncorhynchus mykiss*) in the refrigerator, with microbial, chemical, and organoleptic properties. *Z. multiflora*extract increased the shelf life of fish fillets by about 5-6 days.

The results of counting coliforms of Lavash bread in the zero and first days in this study indicate that the coliforms do not grow, in this regard, Heidari *et al*. In 2018 in the study of microbial contamination of flour used in bakeries and Lavash bread Semi-industrial packages in Bandar Abbas showed that dont contaminated with coliforms and *E. coli*.

Mold Growth Rate

Molds, yeasts, and bacteria are the most important microorganisms that have been discussed in various food and related industries such as spoilage and pollution. The most common types of molds and fungi that grow on grain-based foods and can therefore be found in the flour from which they are made are Aspergillus, Penicillium, Rhizopus, Monilia, and Yeast (Karami *et al*. 2013).

To evaluate the antifungal properties of ethanolic and aqueous extracts, the Aspergillus mold counting method of bread samples containing different concentrations of these compounds was used. The results are shown in Table (3). That obtained from the addition of ethanolic and aqueous extracts to bread showed that different treatments of ethanolic and aqueous extracts had antifungal effects, so that at the end of the storage period of the control sample with an average of 8.56 CFU of the highest contamination The fungus was present, while in other treatments with increasing the percentage of the extract was a less fungal, so that on the tenth day, treatment 2% ethanolic extract with an average of 5.96 CFU/g was less.

The results showed that ethanolic extract had more effects than aqueous extract. According to the results, it can be said that, among the different concentrations of thyme extracts, high concentrations will be more effective and can reduce the growth of mold up to about 3 logarithms.

Heidari *et al*. 2018 in the study of microbial contamination of flour used in bakeries and semi-industrial packaged lavash bread in Bandar Abbas showed that according to the national limit (10^2 CFU/g for mold in bread), the number of molds in 24% of bread samples were exceeded. Also, 25% of the total microorganisms and 10% of the molds of the flour samples exceeded the national permissible limits (10^5 CFU/g for total microorganisms and 5×10^3 CFU/g for molds).

Nouri *et al*. (2016) in a study comparing the antifungal effects of *Z. multiflora* and pomegranate extract with nystatin on *Candida albicans* reported that *Z. multiflora* extract had significant antifungal effects, although nystatin had a mean inhibitory zone diameterof 17 mm, *Z. multiflora* extract with an average inhibitory zone diameter of 13.25 mm had significant antifungal effects compared to pomegranate flower extract with an average of 11 mm.

(e.g.2012a, b) in the study of the effect of thyme powder, extract, and essential oil on *Aspergillus niger* mold and yeast *Geotrichum candida* in yogurt and its shelf life showed that thyme powder has an

inhibitory effect only at 1% level and thyme essential oil on It has the best 1% inhibition against *Aspergillus niger* mold and geothermic candidiasis yeast. Therefore, the antimicrobial effect of powder was less than essential oil and extract (e.g. 2012 a, b).

In a study on the quality of bread produced in local bakeries and packaged bread in Dakar, Bangladesh, it was found that the number of molds in unpackaged and packaged bread was 1.05 $\times 10^5$ CFU/g and 11.01$\times$ 10^5 CFU/g, respectively. Also, the number of coliforms in unpacked and packaged bread was 1.36 $\times 10^4$ CFU/g and 1.36$\times$ 10^5 CFU/g, respectively (Khanom *et al*., 2017).

Table 2 The logarithm of the total number of coliforms (Log CFU/g) of bread samples with different treatments of ethanolic and aqueous extracts and *Z. multiflora* plant during shelf life

		0	1	3	5	7	10
Control		0	0	1.397±0.024 dA	2.499±0.034 cA	4.096±0.029 bA	5.222±0.123 aA
Ethanolic	1%	0	0	1.000±0.021 dC	1.478±0.135 cB	2.932±0.105 bC	4.125±0.103 aC
	1.5%	0	0	0.903±0.065 dD	1.079±0.014 cD	2.361±0.210 bDE	3.716±0.305 aDE
	2%	0	0	0.59±0.101 dF	0.885±0.036 cE	1.876±0.101 bF	3.120±0.231 aF
Aqueous	1%	0	0	1.113±0.060 dB	1.631±0.076 cB	3.210±1.010 bB	4.650±0.036 aB
	1.5%	0	0	1.000±0.045 dC	1.276±0.104 cC	2.691±0.043 bD	4.011±0.010 aD
	2%	0	0	0.810±0.104 dE	1.041±0.112 cEF	2.380±0.111 bE	3.707±0.012 aE

*Numbers (mean ± standard deviation) with large common letters in each column are not statistically significantly different from each other (P<0.05).

**Numbers (mean ± standard deviation) with small common letters in each row are not statistically significantly different from each other (P<0.05).

Table 3 The logarithm of the total number of Aspergillus mold (Log CFU/g) of bread samples with different treatments of ethanolic and aqueous extracts and *Z. multiflora* plant during shelf life

		0	1	3	5	7	10
Control		0	0	3.681± .036 dA	5.124±.063 cA	6.929±0.072 bA	8.560±0.214 aA
Ethanolic	1%	0	0	3.039±.041 dC	3.901±0.101 cCD	6.048±0.112 bC	7.011±0.062 aDE
	1.5%	0	0	2.761±.021 dD	3.076±.098 cD	5.653±0.102 bDE	6.397±0.089 aE
	2%	0	0	2.124±.065 dE	2.679±0.073 cE	3.897±0.132 bG	5.965±0.152 aF
Aqueous	1%	0	0	3.531±.026 dB	4.826±0.054 cB	6.606± 0.041 bB	7.353±0.062 aB
	1.5%	0	0	3.491±.032 dB	4.785±.062 cB	5.954±0.032 bD	7.021±0.53 aC
	2%	0	0	2.411±.032 dD	3.715±.062 cD	4.824±0.032 bF	6.125±0.052 aE

*Numbers (mean ± standard deviation) with large common letters in each column are not statistically significantly different from each other (P<0.05).

**Numbers (mean ± standard deviation) with small common letters in each row are not statistically significantly different from each other (P<0.05).

Organoleptic Evaluation

Flavor

Flavor is the first and most important organoleptic feature and is the first characteristic by which the consumer can be drawn to the desired product and reuse it. Therefore, in presenting and evaluating the formulation of a new product, we must always pay attention to this important index and do our best to present a product with a suitable oral taste. Figure (2), which shows the changes in the taste of bread samples with different concentrations of ethanolic and aqueous extracts at the end of the tenth day, can be expressed as samples of bread treated with 1.5% and 2% Thyme ethanolic extract obtained a higher score and had a statistically significant difference at the level of 95% (α=0.05) compared to the

concentration of 1% ethanol and various aqueous extract treatments. Examination of the flavorof the extracts leads us to the conclusion that the ethanolic extract of thyme had a more suitable flavor compared to the aqueous extract of thyme

Aroma

The results of the analysis of variance of the data related to the aroma of bread samples with different concentrations of thyme extracts are shown in figure (3). Bread samples containing 2% ethanol extract of thyme and bread samples without extract (control sample) at the end of the tenth day had the highest and lowest odor points, respectively.

Texture

The results obtained from figure (4) showed a significant difference in terms of the texture of bread samples with concentrations of 1% and 2% thyme extract at the probability level of $P < 0.05$, and the control sample obtained the lowest score, which can be related to excessive moldiness of bread without extract and disintegration of its texture.

Color

In evaluating the color score of bread prepared with different percentages of ethanolic and aqueous extracts of thyme infigure (5), only 1% of the aqueous extract and the control sample had a statistically significant difference with each other and other treatments, while different treatments There was no statistically significant difference between ethanolic and aqueous extracts of thyme. Although a significant difference in the color of all treatments was observed compared to the control sample, the reason for this is mold, which leads to green and black spots on the bread and causes the color and its natural state to disappear.

Total Acceptance

total acceptance is an indicator that includes all the organolepticcharacteristics of a product and in other words expresses the level of satisfaction and consumer acceptance of the product. According to Figure (6), some of the prepared bread samples had statistically significant differences from each other, so that the treatment of 2% ethanolic extract and the control sample had the highest and lowest total acceptance points, respectively. The results of organoleptic evaluation of Lavash bread containing ethanolic and aqueous extracts of thyme showed that in the study of falvor, aroma, color, texture and total acceptance of 2% treatments of thyme extract and the control sample had the highest and lowest scores, in this regard, patience *et al*. (2010) in examining the Organoleptic properties of samples containing hyssop and echinacea extracts in the cake stated that cake samples containing both extracts retained the sensory properties (falvor, aroma, color, texture and total acceptance) well during storage (e.g. 2010 a, b).

Nikbakht *et al*. (2013) investigated the sensory properties of oilcake made from sage extract. They concluded that adding the extract of this plant to the oily cake improves the sensory properties of the cake and this stale plant delays the cake without adversely affecting the taste, texture, and color of the product. *Salvia officinalis*extract not only had no negative effects on these properties but with increasing storage time, properties such as texture, color, taste, total appearance, and finally the overall acceptance of the product were reported to be much better than the control sample (Nikbakht *et al.,* 2013). Therefore, these results are consistent with the results of our research.

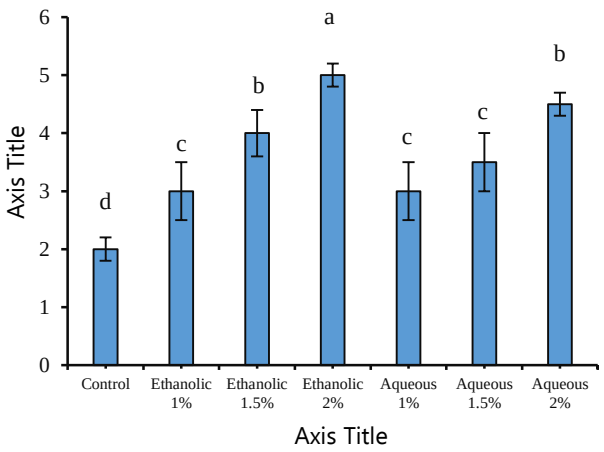

Fig. 2 Changes in taste score in bread samples with different concentrations of ethanolic and aqueous extracts of thyme on day 10

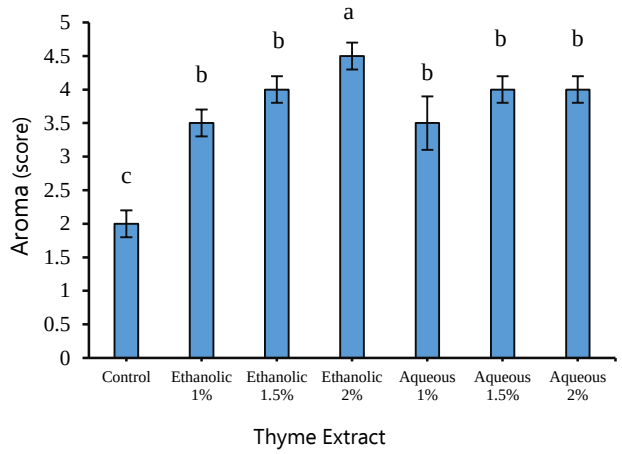

Fig. 3 Changes in odor score in bread samples with different concentrations of ethanolic and aqueous extracts of thyme on day 10

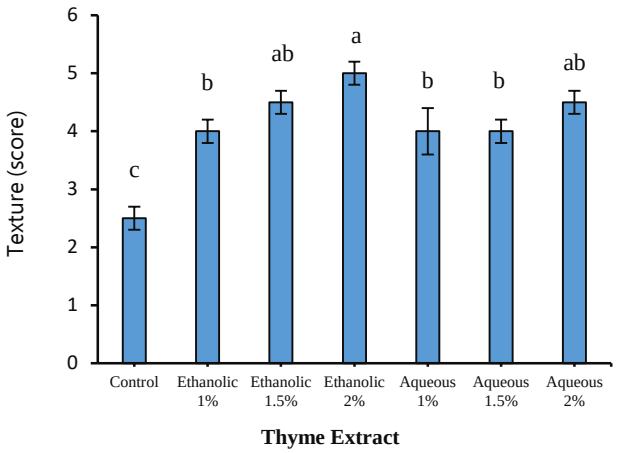

Fig. 4 Texture score changes in bread samples with different concentrations of thyme extract and powder on day 10

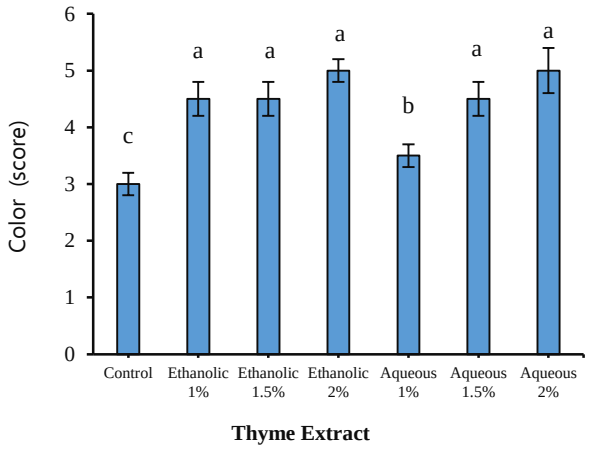

Fig. 5 Changes in color score in bread samples with different concentrations of thyme extract and powder on day 10

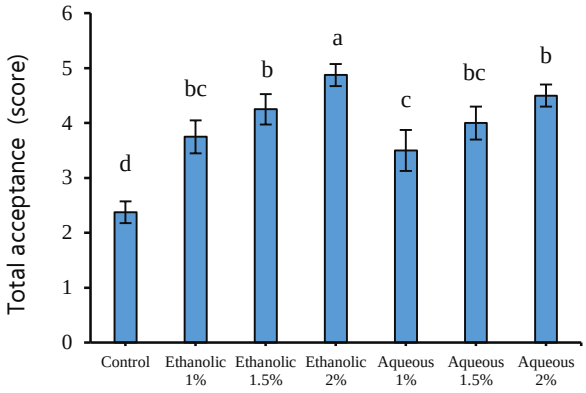

Fig. 6 Changes in the total acceptance score of bread samples with different concentrations of thyme extract and powder on day 10 of storage

Conclusion

Due to the importance of wheat flour in the diet and high volume of consumption as a raw material for baking bread, in this study, the preservation effect of ethanolic and aqueous extracts of thyme on the microbial properties of lavash bread was investigated. The present study showed that hydroethanolic and aqueous extracts of thyme had significant antimicrobial effects on coliforms and molds, which was due to the antioxidant effects on the extracts of this plant.

Food contamination of coliforms, especially *E. coli*, is very important for microbial contamination. The absence of coliform bacteria in bread samples on days zero and one may indicate that these samples may have been produced in a healthy environment. Increasing the level of microbial contamination, including mold growth in control samples, indicates that the number of molds has increased over time between the production of these samples and their consumption. The average moisture content of bread samples was 22.4%. It seems that in addition to moisture, which is one of the main causes of microbial activity in food, other factors also increase the contamination of bread samples with mold. Of course, one of the most important factors in mold

spoilage of food products is a lack of control over water activity. That is, the lower water activity, the less likely the molds to grow and multiply. In other words, water activity is usually directly related to moisture content. In general, the hydroalcoholic and aqueous extracts of *Z. multiflora* were able to reduce the microbial contamination of Lavash bread by about 3 logarithms during 10 days, so it can be said that the extracts of *Z. multiflora* have been very good antimicrobial effects. Has been, and can be a good alternative to chemical preservatives in food.

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