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# The effect of adding walnut green husk extract on antioxidant and antimicrobial properties of ketchup

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ABSTRACT

In this study, walnut green husk extract, which is a valuable waste of horticultural products, was used to enhance antioxidant properties and reduce microbial load of ketchup sauce. Extraction efficiency of walnut green husk extract 22.01%, total phenolic compounds 65.40 (mg gallic acid per 100 g dry husk), flavonoids 10.06 (catechin mg per 100 g dry husk), and EC50 0.17 (mg/mL) were expressed. The MBC value of this extract for *Saccharomyces cerevisiae* (0.045 mg/mL) is lower than the value of this factor in *Lactobacillus fructivorans* (0.123 mg/mL). Next, ketchup sauce samples containing control sample, containing 0.086 (mg/mL) walnut green husk extract and 0.123 (mg/mL) walnut green husk extract were prepared. The results showed that application of higher concentrations of extract (0.123 mg/mL) in the first month increased the total phenolic compounds (73.72 mg gallic acid per 100 g dry matter) and flavonoids (14.11 mg catechin per 100 g dry matter) and reduced $EC_{50}$ (0.34 mg/mL) in ketchup sauce. The results of the total counts, mold, yeast and *Lactobacillus* bacteria of ketchup sauce samples containing walnut green husk extract were significantly different from allowing control sample.

Keywords: Ketchup, Walnut green husk, Antimicrobial, Antioxidant, Functional

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## 1. Introduction

The processing of fruits and vegetables results in high amounts of waste, including husk, seed, stone and oilseed meal. Disposal of these substances usually indicates a problem that is more exacerbated by legal restrictions. However, valuable nutrients in the waste have been lost, so new aspects of using these substances as by-products for greater utilization in the production of food additives or supplements with high nutritional value have received much attention, since their recycling produces higher value products and it is also economically advantageous (He et al., 2019).

Walnut waste includes walnut leaf, green husk and hard husk. Walnut green husk includes for about 64% of the moist weight of walnut fruit. An estimated 440,000 tons of walnut green husk are annually produced and as a result, it is one of the most important walnut waste after brain extraction and currently is more used within the country for fuel and incineration. But so far, thirteen phenolic compounds that have antioxidant property include hydroxycinnamic acid (chlorogenic acid, caffeic acid, ferulic acid and synaptic acid), hydroxybenzoic acids (gallic acid, ellagic acid, syringic acid and vanillic acid), flavonoids (catechin, epicatechin and myricetin) and juglone (5-hydroxy and 1, 4-naphthoquinone) have been identified in walnut green husk (Fernandez et al., 2013). Also walnut green husk rich in ascorbic acid and having a special essence, tannin gum, alkaloids and bitter juglone has gaussian taste and is used for fungicidal and germicidal properties (Habibie et al., 2019).

Azizi shafa et al. (2016) investigated the antimicrobial effect of ethanolic and methanolic extracts of walnut leaf and green husk against *Saccharomyces cerevisiae*, *Bacillus licheniformis* and *Aspergillus niger* in dates. MIC (MBC) of ethanolic leaf extract, ethanolic and methanolic of the husk against *Aspergillus niger* were 250 (500) μg/mL. Fernandez et al. (2013) investigated the effect of solvent on the antioxidant and antimicrobial properties of walnut green husk extract. The highest extraction rate was obtained with water (44.11%) and antioxidant properties showed that the extracted sample with water and methanol had the most amounts of these properties. The extract also had the ability to inhibit the growth of gram-positive bacteria, thus, there is the potential of walnut green husk as an economic source of antioxidant and antimicrobial.

Today, tomato sauce is one of the most important food condiments that the quality of this product is related to its

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constituents (Anonymous, 2016). Hassanpour ghavifekr and Attazadeh (2017) investigated the effect of ethanolic extract of on the microbial and sensory properties of ketchup sauce. The results of this study showed that sample containing 5% and 7% of *Ferulago angulata* had a significant effect on growth reduction and overall count of microorganisms. Azari joghan et al. (2018) investigated the antimicrobial effect of *Zataria multiflora* and rosemary essences on *Bacillus coagulans* in probiotic ketchup sauce. The results showed that the minimum inhibitory concentration of *Zataria multiflora* and rosemary essences were 0.03 and 0.3%, respectively.

Due to the nutritional and functional properties of walnut green husk and the fact that annually large amounts of walnut green husk are wasted as a waste and by-product of the factories as well as due to the permeability of tomato sauces and the use of preservatives in them (despite being unauthorized), therefore, in this study was investigating the antioxidant and antimicrobial properties of processed ketchup sauce containing walnut green husk extract.

## 2. Material and Methods

### 2.1. Extracting of walnut green husk extract

Fresh green walnut was purchased from a local market in Damavand. The walnut green husk was first separated and thoroughly rinsed with water. After drying, it was cut into small pieces (smaller than 1 × 1 $cm^2$) and was dried in an oven at 60 °C for 48 h to reach constant weight. Then, flour granules were formed in the mill and 5 g of powdered sample and 25 mL of 50/50 ethanol/water solutions were extracted for 45 min at ambient temperature. The solution was filtered with whitman No. 4 filter paper. The ethanol solvent was evaporated under vacuum with a rotary evaporator and the extract was dissolved in water again to reach a final concentration of 50 mg/mL. It was then kept in the dark at 4 °C until subsequent experiment (Fernandez et al., 2013).

### 2.2. Antimicrobial activity of walnut green husk

This test was performed by using the agar well diffusion method. In this method, in a 96-wells plate, 100 µL of culture medium (Müller Hinton broth) was first added to all wells, then 100 µL of the corresponding sample extract was added to the first well, and after mix the culture medium and sample in the first 100 well, 100 µL was added to the second well and continued to dilute until the last well and the extract concentration in the wells is reduced by half. Then, from the uniform suspensions of *Lactobacillus fructivorans* and *Saccharomyces cerevisiae* yeast, McFarland 1.5 × $10^8$ (cfu/mL) was added to all wells and place the 96-well plate in a 37 °C incubator for 24 h. The first well was considered where turbidity is observed, and the previous well as MIC, which indicates the growth of microorganisms. In order to determine MBC from the first concentration that no turbidity was observed, the number of viable microorganisms was then counted by sequential dilution method (Cheraghali et al., 2018). The choice of two microorganisms

*Lactobacillus fructivorans* and *Saccharomyces cerevisiae* yeast was suggested by previous researchers (Bjorkroth & Korkeala, 1997; Dabagh et al., 2012).

### 2.3. Preparing ketchup sauce

Raw materials for the production of ketchup samples were weighted according to the formulation listed in Table 1. At first, the tomato paste with brix 30 was mixed with water and heated to boiling temperature then salt was added. The resulting mixture was heated gently for 20 min. Vinegar, sugar and walnut green husk extract were then added to samples with specified ratios of microbial test (MIC, MBC) and heated to 90 °C until reaching brix 28. Samples were then numbered, packed in glass containers and kept at 4 °C for testing (Belovic et al., 2018).

Table 1. Amounts of raw materials used to produce ketchup sauce.

| Raw materials | Percentage of consumption |
|---|---|
| Tomato paste (30%) | 45 |
| Sugar | 8 |
| Salt | 0.5 |
| Vinegar | 9.2 |
| Water | Weight reach 100 |

### 2.4. Determination of extraction efficiency of walnut green husk extract

In order to determine the extraction efficiency of the dry extract, the dry extract weight was obtained and percentage was calculated from the initial amount of walnut husk powder used during the extraction process (Sirpartravan et al., 2010).

### 2.5. Determination of antioxidant activity of walnut green husk and ketchup sauce

#### 2.5.1. Total phenolic content

Briefly, 1 mL of an aqueous solution of the extract (1 mL of extract and 75 mL of distilled water) was mixed with 1 mL of folin-ciocalteu reagent. After 3 min, the saturated sodium carbonate solution (22%) was added to the mixture and reached 10 mL with distilled water. The sample was kept in the dark for 90 min and then absorbed at 725 nm. Total phenols content was obtained as a gallic acid equation from calibration curve of standard solutions (0.01-1 mM) and expressed as mg of gallic acid per gram of extract (Fernandez et al., 2013).

#### 2.5.2. Flavonoids content

The amount of flavonoid was determined by aluminum chloride colorimetric method. To 0.5 mL of each sample (10 mg/mL), 1.5 mL of methanol, 0.1 mL of aluminum chloride solution 10% in ethanol, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added then the samples were kept for 30 min in the dark at ambient temperature. The adsorption of the mixture was read at 415 nm and expressed as the equivalent of mg of catechin per gram of extract (Sirpartravan et al., 2010).

#### 2.5.3. DPPH inhibition activity

The radical scavenging ability of the extracts was monitored using the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) by the method of Noor shah et al. (2018). Aqueous solutions of sample extracts (0.01–2 mg/mL) were prepared. Extract solutions (0.3 mL) were mixed with 2.7 mL of DPPH solution ($6 \times 10^{-5}$ M methanol) and shaken vigorously and kept in the dark at room temperature for 60 min (until absorbance values stabilized). The reduction of the DPPH radical was measured by absorbance reduction at 517 nm and the DPPH inhibitory effect was calculated as percentage of discoloration using Equ. 1:

$$\text{Free radical trapping}(\%) = \frac{A_C - A_S}{A_C} \times 100 \qquad (1)$$

In this respect, $A_C$ and $A_S$ are control adsorption and sample adsorption, respectively. The 50% concentration limit ($EC_{50}$) of the extract can be calculated based on the graph of percent inhibitory effect of the extract concentration in solution.

### 2.6. Microbial sauce test

*Lactobacillus fructivorans*, *Penicillium glaucum* and *Saccharomyces cerevisiae*, after activation in their suitable culture media, were added to ketchup sauce samples at concentrations of $6.4 \times 10^4$ cfu/g, $1.8 \times 10^4$ cfu/g and $10^4$ cfu/g, respectively (Dabagh et al., 2012). Microbial tests including total counts, mold and yeast counts and *Lactobacillus* were performed according to Iranian national standards Nos. 10899-1, 5272-1, 8923-1, and 8897.

### 2.7. Sensory evaluation

After the basic training, 10 people (25-30 years old) were selected as evaluators and by using the hedonic method (5 points), the ketchup sauce samples were evaluated for appearance, color, texture, taste, mouthfeel and consistency. Finally, the mean of these factors was expressed as overall sensory evaluation. Thus, a maximum score 5 was considered to be a good sample and a minimum score 1 was considered to be a bad sample (Rahman et al., 2018).

### 2.8. Statistical analysis

At first, normality test was performed for all data, and if it was normal, variance analysis was performed for data. A factorial test in a completely randomized design was used and minitab software was applied to investigate the effect of independent variables such as increase in extract concentration and shelf life on the dependent variable (tests examined).

## 3. Results and Discussion

### 3.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Amount of MBC for *Saccharomyces cerevisiae* (0.045 mg/mL) is lower than this factor in *Lactobacillus fructivorans* bacterium (0.123 mg/mL) due to the presence of peptidoglycan wall and more bacterial resistant structure than yeast (Table 2). Since the aim of the present study was to increase the shelf life of ketchup sauce and to identify two target microorganisms, and then the use of MIC and MBC numbers of *Lactobacillus fructivorans* as the amount of extract used in later stages.

The number and position of the hydroxyl groups are key factors in the antimicrobial activity of phenolic compounds, flavonoids, quercetin and its derivatives. The antimicrobial activity of flavonoids is the result of the ability to form a complex with the cell wall, thereby preventing the growth of microorganisms. Phenolic compounds inhibit enzymatic activity by reacting with sulfhydryl groups and nonspecific interactions with proteins, and thus exhibit their antimicrobial activity, also polyphenols are able to form heavy soluble complexes with proteins, thereby attaching to the bacteria and destroying the acceptors present on the bacterial cell surface (Almajan et al., 2011). Quercetin and its derivatives also inhibit bacterial growth through inhibition of the DNA gyrase enzyme (Cheraghali et al., 2018).

Ghahen et al. (2012) confirmed the antimicrobial effect of walnut husk on *Escherichia coli*. Blessington et al. (2014) investigated the antimicrobial effect of walnut and its husks on the *Enterobacteriaceae* bacteria family and demonstrated its antimicrobial effects. Cheraghali et al. (2018) observed the effect of the walnut husk extract on *Bacillus cereus* and *Staphylococcus aureus* with maximum inhibition area of 14.45 and 15.33 mm, respectively, however, this extract was ineffective on *Escherichia coli* and *Salmonella murium* bacteria that indicated the extract has no effect on gram-negative bacteria. In the present study, the aim is acid resistant gram positive spore bacteria that have selected *Lactobacillus fructivorans* bacterium as the indicator and according to Table 2, the antimicrobial effect of the walnut husk extract on that has been observed. In the case of gram-positive bacteria, antimicrobial substances easily damage the cell wall and cytoplasmic membrane, leading to the leakage and coagulation of the cytoplasm (Duffy & Power, 2001).

### 3.2. Extraction efficiency

The extraction methods and the antioxidant activity of the extracts correlate with each other, due to the different polarity of the compounds, which extraction efficiency is also decreased with decreasing polarity of the solution. In this study, the extraction efficiency of walnut green husk extract was 22.01%. Fernandes et al. (2013) assigned the highest extraction efficiency and phenolic compounds among a variety of extraction solutions including water, ethanol, methanol, water-ethanol, to flood water extraction method with water-methanol. Sanagol et al. (2018) compared three methods traditional extraction, microwave and ultrasound and in the traditional method, they used an equal mixture of water and ethanol.

### 3.3. Total phenolic compounds of walnut green husk

The solvent used is an important factor in the overall content of phenols and flavonoids, and two-component solvent systems are more desirable in extracting phenolic compounds from plant samples than single-component solvent systems (Chew et al., 2011). Table 3 shows the results of the total amount of phenolic and flavonoid compounds of the target extract, respectively (65.40 mg gallic acid per 100 g of dry husk and 10.06 mg of catechin per 100 g of dry husk). Rahimi panah et al. (2010) obtained the total phenolic content of the walnut husk extract in the range of 700.33 to 2178.63 mg/100 g. Noshiravani et al. (2015) reported phenolic compounds of walnut green husk by soxhlet and soaking methods

89/07 and 38.7 mg/g gallic acid respectively. Also, the flavonoid content of walnut green husk by soxhlet and soaking methods was expressed as 17.81 and 1.59 mg/g catechin, respectively. In a study by Fernandez et al. (2013), the extracts were extracted with the highest total phenolic content, 50% ethanol and methanol and the lowest water content. Sandel et al. (2018), by flooding method, determined the amount of phenolic compounds in the geographical area of walnut ranging from 170.49 to 220.87 mg/g dry weight in gallic acid.

### 3.4. *Antioxidant activity*

The results of the concentration required for 50% adsorption were expressed as $EC_{50}$ values (mg/mL) in Table 3. Low $EC_{50}$ values indicates the increased antioxidant activity. Researchers have identified the amount of phenolic and flavonoid compounds as important factors in determining the antioxidant capacity of plant extracts.

In the present study, phenolic compounds and flavonoids softened plant cell wall tissues during soaking in aqueous-alcoholic solvent, which is usually associated with an increase in the solubility of bonded phenolic compounds. Since these compounds have high solubility in polar solvents, they have entered the solvent phase under the influence of concentration gradients. The walnut husk extract contains phenolic and flavonoid compounds. These compounds have the ability to neutralize free radicals because of their hydroxyl groups. The antioxidant activity of phenolic compounds depends on the number of hydroxyl groups in their aromatic ring and the negative correlation between total phenol content and $EC_{50}$ values in DPPH free radical scavenging assay indicates the direct effect of phenols on this activity. The number of hydroxyl groups involved in the antioxidant structure is usually not a determining factor, but rather the position of the hydroxyl groups and the presence of other functional groups such as double bonds, the combination of hydroxyl groups and the presence of ketone groups play important roles in antioxidant activity (Cheraghali et al., 2018). Dolatabadi et al. (2017) investigated the antioxidant activity of walnut green husk extract using DPPH free radical scavenging ability and found the highest amount of phenolic compounds with 49.66 mg/g (in gallic acid) corresponding to the walnut green husk of Hezar jarib region with water-ethanol solvent was 1:1 volume ratio at 24 h.

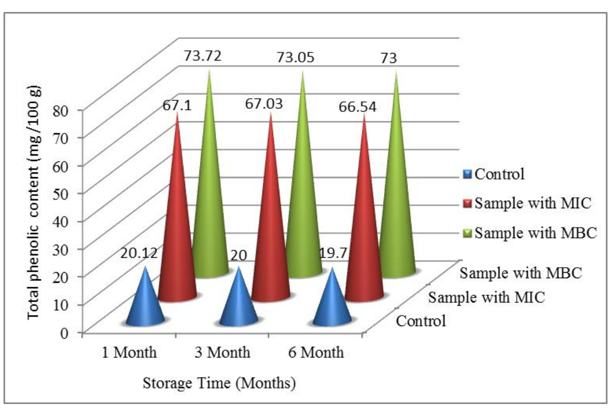


Fig. 1. Results of comparing mean interactions of different amounts of extract and time on total phenolic content (mg GAMEs/100 g dry matter) of ketchup samples.

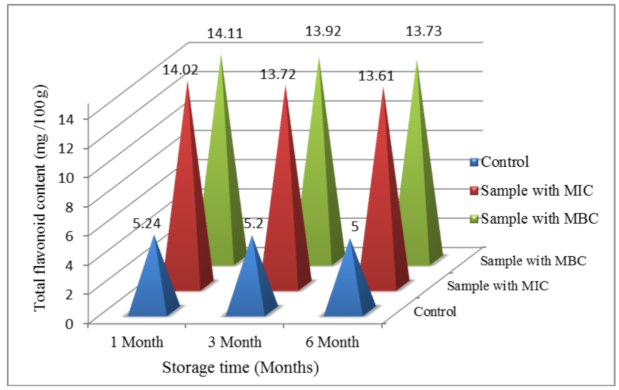


Fig. 2. Results of comparing mean interactions of different amounts of extract and time on total flavonoid content (mg catechin/100 g Dry matter) of ketchup samples.

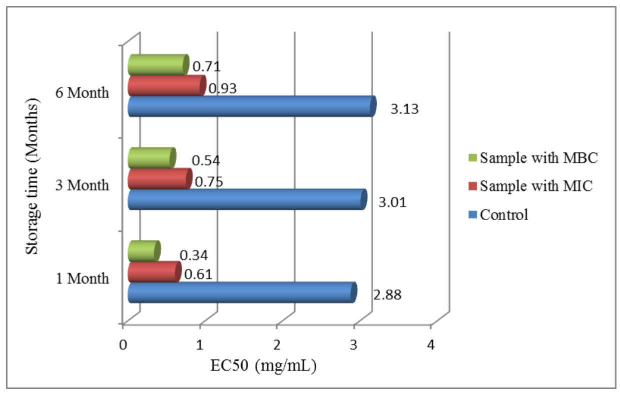


Fig. 3. Results of comparing mean interactions of different amounts of extract and time on $EC_{50}$ (mg/mL) of ketchup samples.

### 3.5. *The amount of total phenolic compounds and flavonoids*

The Fig. 1 and 2 show the mean values of the total phenolic and flavonoid concentrations of the ketchup samples with the shelf life effect, respectively. The results of variance analysis showed that the effect of walnut bark extract concentration on extraction of phenolic and flavonoid compounds was significant at 0.05 level during shelf life. As shown in Fig. 1 and 2, the total phenolic compounds and flavonoids increased with increasing concentrations of walnut green husk extract. As it was shown, the sample containing MBC had the significantly highest amount of total phenolic and flavonoid compounds in all three shelf life and after that, the sample contained MBC and at all times the control sample had the lowest total phenolic compounds ($p < 0.05$). All treatments showed a significant decrease in the amount of total phenolic and flavonoid compounds over time, which is consistent with the results of previous investigators because time has an effect on reducing these compounds. It is likely that the polymerization of phenolic compounds with low molecular weight and the formation of insoluble compounds have a reason for the reduction of these compounds over a shelf life of six months. In addition, the formation of insoluble complexes of polyphenols with proteins or carbohydrates converts them into unmeasured forms (Saxena et al., 2003).

### 3.6. Capability of DPPH free radical scavenging ketchup sauce containing walnut green husk extract

The results of variance analysis showed that extract concentration and time had a significant effect on ($P < 0.05$) inhibition of free radicals. The ability of the extracts to inhibit free radicals also increased as the concentration increased. In comparison, the sample containing the highest extract (0.123 mg/mL) had the lowest $EC_{50}$ and thus had the highest ability to trap DPPH radicals compared to the other samples.

Also, the effect of retention on DPPH trapping ability was significant ($P < 0.05$). Previous studies have reported a high correlation between the ability to trap free radicals and the amount of phenolic compounds for grains, fruits, vegetables, and beverages (Arabshahi et al., 2007).

The results of Fig. 3 show that the sample containing MBC had the lowest $EC_{50}$ in the first month and thus had the highest ability to trap radical DPPH than the other samples because it contained the highest amount of extract and the control sample had the highest $EC_{50}$ in the sixth month and thus had the lowest ability to trap DPPH radicals compared to the other samples. Parakashi et al. (2016) enriched ketchup sauce using tomato and acerola and their results showed that sample containing 75% acerola pulp and 25% tomato pulp had the highest amount of total phenolic compounds that this sample had the highest amount of antioxidant activity (0.192 $EC_{50}$ mg/mL).

### 3.7. Microbial tests

The Table 4 shows the effect of adding walnut green husk extract on the total counts, mold, yeast and *Lactobacillus* bacterium after one, three and six months of shelf life. The results of the analysis showed that the amount of mold (mold allowing limit in the national standard (cfu/g) is 10 in the sauce) and yeast (yeast allowing limit in the national standard (cfu/g) is 10 in the sauce) in the ketchup samples were less than standard. But regarding the total plate counts and the presence of *Lactobacillus* bacterium, it can be said that the control sample without any additives in the first month had higher levels of contamination more than allowing limit (the allowing total count in national standard $10^4$ per gram of sauce and the allowing limit number of heterofermentative lactic acid bacterium in the 0.1 g negative sauce and the acid resistant bacterium was less than 10 (cfu/g)), which total counts significantly decreased over time and reached the allowing limit. The staining of the colonies indicates the presence of gram-positive, catalase-positive, rod and spore bacteria that can be transmitted through secondary pollution or contaminated air.

Concentration and structural properties of phenolic compounds such as the type, number and position of the substituent groups on the benzene ring affect the type and mechanism of antimicrobial action of the plant extracts (Cosmolescu et al., 2010). In the field of evaluation on antimicrobial activity of walnut green husk extracts, limited research has been done, including Oliviera et al. (2008), Mikulic-Petkovsek et al. (2013) and Salejda et al. (2016) that they have demonstrated the antimicrobial role of walnut green husk extract in both in vivo and in vitro conditions. However, its antimicrobial effect as part of the food product formulation has not been found in scientific sources. The results of these studies indicate that the antimicrobial effects of plant extracts are mainly related to the presence of phenolic compounds. Various mechanisms have been expressed to justify the antimicrobial behavior of phenolic compounds. Phenolic compounds by interfering with the bilayer phospholipid membrane, affect the permeability of microbial cell membranes and cause the release of intracellular compounds (Dengles et al., 2012).

In addition, they lead to changes in membrane function for electron transfer or uptake of nutrients and disruption of protein synthesis and enzyme activity (Bajpai et al., 2019). Also, it has been shown that flavonoid compounds can affect the synthesis of proteins, lipids, and especially nucleic acids. Researchers believe that hydrogen bonding between hydroxyl groups of phenolic compounds and nucleic acids in microbial cells can be effective in deactivating DNA molecules. The decrease in microbial contamination of the samples during shelf life is due to the effect of the new network formed on the ketchup sauce samples and the decreasing trend of pH over the shelf life of six months.

The results of microbial test by Dabagh et al. (2012) showed that 200 and 250 ppm nisin with 750 ppm sodium diacetate had good inhibitory effects on the growth of *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Penicillium glaucum* microorganisms in French sauce. The number of microorganisms in the total counts was in the standard range of salad sauce and according to the results, nisin and sodium diacetate could prevent the growth of spoilage microorganisms during the shelf life of French sauces.

### 3.8. Sensory evaluation of ketchup sauce

Table 5 shows the effect of using walnut green husk extract on the sensory properties of ketchup sauce based on the results of the evaluators as a general evaluation. The results showed that the walnut green husk extract has an influence on the taste and aroma of the final product due to its specific taste and bitterness. But the sample containing less extract during shelf life without significant difference with control sample has a good sensory evaluation score.

Azeri Joghan et al. (2018) reported the results of sensory evaluation of the sauce samples in that the taste of *Zataria multiflora* was more successful than rosemary in terms of taste and odor. Thus, in addition to being a very good natural preservative with high antimicrobial effect on the bacteria, *Zataria multiflora* essential oil was also accepted by consumers. Hassanpour ghavifekr and Attazadeh (2018) reported that in terms of sensory evaluation, evaluators' satisfaction of samples containing 5 and 7% ethanolic extract of *Ferulago angulata* was higher than the control sample of ketchup sauce.

Table 2. Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentrat ion (MBC).

| Target microorganisms | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| *Lactobacillus fructivorans* | 0.086 | 0.123 |
| *Saccharomyces cerevisiae* | 0.024 | 0.045 |

Table 3. Results for amount of phenolic compounds, flavonoid and $EC_{50}$ (mg/ml) and extract of walnut green husk.

| Experiment | Value |
|---|---|
| Total phenolic compounds (mg/100 g) | 65.40 ± 0.19 |
| Flavonoid content (mg/100 g) | 10.06 ± 0.23 |
| $EC_{50}$ (mg/mL) | 0.17 ± 0.02 |

Table 4. Results of comparing mean interactions of different amounts of extract and time on microbial test of ketchup samples.

| Storage time | | Control | Sample with MIC | Sample with MBC |
|---|---|---|---|---|
| 1 Month | Total count | $4\times10^4$ | <10 | <10 |
| | Mold | <10 | <10 | 10 |
| | Yeast | <10 | <10 | <10 |
| | Lactobacillus | $3\times10^2$ | Neg | Neg |
| 3 Month | Total count | $10^3$ | <10 | <10 |
| | Mold | <10 | <10 | <10 |
| | Yeast | <10 | <10 | <10 |
| | Lactobacillus | 10 | Neg | Neg |
| 6 Month | Total count | 10 | <10 | <10 |
| | Mold | <10 | <10 | <10 |
| | Yeast | <10 | <10 | <10 |
| | Lactobacillus | Neg | Neg | Neg |

* The values listed in the table were the number of microorganisms per gram of ketchup sauce (cfu/g).

Table 5. Results of comparing mean interactions of different amounts of extract and time on sensory evaluation of ketchup samples.

| Samples | 1 Month | 3 Month | 6 Month |
|---|---|---|---|
| Control | $5.00 \pm 0.00^{a}$ | $4.01 \pm 0.02^{b}$ | $3.54 \pm 0.06^{d}$ |
| Sample with MIC | $5.00 \pm 0.00^{a}$ | $3.96 \pm 0.05^{bc}$ | $3.49 \pm 0.04^{de}$ |
| Sample with MB | $3.69 \pm 0.03^{c}$ | $3.55 \pm 0.07^{d}$ | $3.39 \pm 0.02^{e}$ |

## 4. Conclusion

In this study, first the walnut husk extract was extracted by hydro-alcoholic method and extraction efficiency of walnut green husk extract 22.01%, the total amount of phenolic compounds 65.40 (mg gallic acid per 100 g dry husk), target extract flavonoid 10.06 (mg catechin per 100 g dry husk) and $EC_{50}$ values in 0.17 DPPH free radical scavenging assay (mg/mL) were expressed. The MIC and MBC values of this extract for *Saccharomyces cerevisiae* were 0.024 and 0.045 mg/mL, respectively, and these factors were reported for *Lactobacillus fructivorans* 0.086 and 0.123 mg/mL, respectively. The results showed that application of higher concentration of extract (0.123 m/mL) in the first month increased the total phenolic compounds (73.72 mg gallic acid per 100 g dry matter), flavonoid (14.11 mg catechin per 100 g dry matter) and reduced $EC_{50}$ values (0.34 mg/ml) in ketchup sauce. However, there was a significant decrease in total phenolic compounds and an increase in $EC_{50}$ value over a 6 month shelf life. The effect of addition of both concentrations of walnut green husk extract on total counts, mold, yeast and *Lactobacillus* bacteria in ketchup showed less than the allowable limit after one, three and six months of shelf life. However, in the control sample in the first and third months the total counts and *Lactobacillus* bacterium were higher than allowed that results indicating the antimicrobial effect of the extract on the samples. The results of sensory evaluation of samples containing extract were reported lower than the control sample but acceptable. The overall results of the study showed that the use of walnut husk extract is an effective combination in the production of functional ketchup sauce.

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