JRIFST
Original Paper

www.journals.rifst.ac.ir
Journal of Research and Innovation
in Food Science and Technology
10 (2022) 4, 413-426
DOI: 10.22101/JRIFST.2022.299777.1271

Research Institute of Food Science and Technology

Evaluation of Antioxidant and Antimicrobial Properties of Root and Stem Bark Extracts of Three Species of Barberry in Bread

Mohammad Gorizpa[1], Fereshte Bahmanyar[2], Leila Mirmoghtadaie[3*], Faezeh Shafaei[4*]

1- MSc Graduate, Department of Agricultural Engineering, Faculty of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2- PhD. Student, Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3- Associate Professor, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran
* Corresponding authors (mirmoghtadaie@sbmu.ac.ir)
4- Assistant Professor, Department of Chemistry, East Tehran Branch, Islamic Azad University, Tehran, Iran
* Corresponding authors (faezehshafaei@yahoo.com)

Abstract

This study aimed to determine antioxidant activity, total phenol, phenolic acids and minimum inhibitory concentrations of the stem bark and root of three Barberries species (*Berberis integrima Bunge*, *Berberis vulgaris asperma* and *Berberis vulgaris orientalis*). The root extract of *Berberis vulgaris asperma* showed the highest amount of total phenol and antioxidant activity. After measuring minimum inhibition concentration, the stem extract of *Berberis vulgaris orientalis* and the root extract of *Berberis vulgaris asperma* were selected as the best extract with a high level of antimicrobial activity. Also, the root extract of *Berberis vulgaris asperma* could effectively prevent fungal and bacterial growth in a concentration of 600 and 750 ppm. Moreover, bread containing 600 ppm of *Berberis vulgaris asperma* root extract showed the highest level of overall acceptability and oxidative stability. According to the results, *Berberis vulgaris asperma* root extract was the best extract among the studied samples and the concentration of 600 ppm was the best level for enriching bread and producing functional bread with extended shelf life.

Received: 2021.08.14
Revised: 2021.10.12
Accepted: 2021.10.26
Online publishing: 2022.01.09

Keywords
Antimicrobial
Barberry
Bread
Shelf life

Introduction

From many years ago, bread has always been the main food meal of worldwide people (De Marco, Steffolani, Martínez, & León, 2014; Verardo *et al.*, 2018). The bread shelf life is restricted by different factors such as microbial growth especially the molds, moisture loss and also staling. Therefore, it seems that addition of some anti-mold agents and another chemical preservative is inevitable. However, World Health Organization (WHO) and also Food and Drug Administration (FDA) have declared the adverse effects of some of these agents on human health (Lou, Hu, Zhang, Sun, & Lu, 2012; Özgen, Serçe, & Kaya, 2009; Rostami & Gharibzahedi, 2016; Van de Velde, Grace, Esposito, Pirovani, & Lila, 2016). Considering this problem, food scientists have made efforts

to substitute natural ingredients such as plant and fruit extracts as bio-preservatives in food products (Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014; Özgen *et al*., 2009; Van de Velde *et al*., 2016; Verardo *et al*., 2018).

Berberis vulgaris is a fruity-plant, which contains nutritional and anti-nutritional compounds such as phenolic compounds, vitamin C, malic acid, acid citric and Tannins. One of the most important components in barberry fruit is a phenolic component such as anthocyanin. Phenolic components have antioxidant properties and are beneficial compounds in the diet that prevent cancer and atherosclerosis (Mortazavi, Sharifi, Maskooki, Niakousari, & Elhamirad, 2014).

Barberry fruit also contains alkaloid compounds that have antimicrobial properties (Rahimi, Maskooki, Mortazavi, Elhamirad, & Rajabzade, 2014). In addition to the fruit, the stem and root has been considered due to their phenolic compounds (Hanachi & Golkho, 2009; Özgen, Saraçoğlu, & Geçer, 2012). Medicinal studies have shown existence of some alkaloid compounds with anti-inflammatory properties in the root and stem of barberries (Imanshahidi & Hosseinzadeh, 2008; Lou *et al*., 2012; Özgen *et al*., 2009; Van de Velde *et al*., 2016). Moreover, these substances could increase the oxidation stability of oil containing cereal products due to participation of polyphenols and flavonoids in pathway of reduction of free radicals. Many studies have been conducted to investigate the radical scavenging activity of polyphenols (Chen *et al*., 2017; Da Rosa *et al*., 2014; Genskowsky *et al*., 2016). In addition, the incorporation of plant extract in food product can also limit microbial growth on it (Verardo *et al*., 2018).

The purpose of this study was increasing of bread shelf life by the inclusion of three variety (*Berberis vulgaris orientalis*, *Berberis integrima Bunge*, and *Berberis vulgaris asperma*) of barberry stem and root extracts in bread formulation. At first, total phenolic contents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were determined for all extracts and high performance liquid chromatography (HPLC) was employed to determine the kind of maximum phenolic compounds. Also, the extracts were evaluated *in-vitro* for their inhibition effect on bacteria and molds growth. Then, the extracts were incorporated into bread and subsequent the sensorial properties, microbial analysis and oxidative stability, of treated breads were investigated. Up to now, no study has been found about the effect of using different variety of barberry root and stem extract on bread shelf life.

Materials and methods

Preparation of Barberry root and stem bark extract

Barberry species involving *Berberis vulgaris orientalis*, *Berberis integrima Bunge*, and *Berberis vulgaris asperma* were collected from Siah Bisheh (Mazandaran-Iran, TEH-6913), Savadkuh (Mazandaran-Iran, THE-6914) and Birjand (South Khorasan-Iran, PMP-376), respectively. They were validated by pharmacognosy branch of pharmacy faculty of Tehran University of Medical Sciences. The dried stem bark and root were powdered by laboratory-scale mill (Moulinex, Type, Dap1, CMMF 8000W, France). Then warm ethanol was mixed with 50 g of obtained root and stem bark powder. After complete homogenization, the solutions were filtered with No. 1 whatman filter paper. Then, the solvent was evaporated by a vacuum rotary evaporator (Heidolph Laboratory 4010, Germany) under the vacuum state at 40 °C and then were freeze-dried (Mashhadian & Rakhshandeh, 2005).

Microbial and chemical analysis of Barberry root and stem bark extract

DPPH radical scavenging activity (Antioxidant activity)

Antioxidant activity of the extracts were evaluated by scavenging of free DPPH radicals (Petlevski, Flajs, Kalođera, & Končić, 2013). 2 mL of different concentrations of extract involving 20, 40, 100, and 200 µg/mL was added to 2 mL of DPPH solution (0.16 mmol in methanol). Crude methanol was considered as blank. The obtained mixtures were shaken for 1 min and the following absorption was read at 517 nm after 30 min keeping the samples in a dark place. Butylated hydroxyanisole (BHA) as a synthetic antioxidant was used as a positive blank sample. The scavenging activity was calculated by the following equation:

$$DPPH\ scavenging = 100 - \left((A_{control} - A_{sample})\right) /A\_control \times 100 \quad (1)$$

$A_{control}$ and A_{sample} were defined as blank and extract absorptions.

Total phenol content (TPC) determination

TPC was determined for the extracts according to the method described by Končić, Kremer, Karlović, & Kosalec (2010). Initially, 0.5 mL of Barberry extract (with the concentration of 100 mg/mL ethanol (50%)) mixed with 0.5 mL Folin–Ciocalteu reagent (diluted with distilled water by the proportion of 10:1), and 0.5 mL sodium carbonate with the concentration of 100 mg/mL were added and were kept at ambient temperature for 1 h in order to the reaction became complete. Consequently, determination of absorptions was conducted at 760 nm. TPC was obtained by the standard curve of gallic acid and expressed as mg of gallic acid equivalent per 100 g extract (Končić *et al*., 2010).

Determination of phenolic acids by HPLC

High-pressure liquid chromatography (HPLC) (KNAUER-Germany) equipped with UV detector was employed to determine the phenolic components in the root and stem extracts of Barberries (above-mentioned varieties). Combination of acetonitrile, water, and acetic acid (2%) was selected as mobile phase with the flow rate of 0.5 mL/min. Reverse phase C_{18} column was the main system to separate the phenolic compounds. The length of the column and the packed particle sizes were 25 cm and 5 µm, respectively. Every injection was accomplished with the volume of 20 µL. To prepare the samples for analysis, 5 mL of the methanol-HPLC grade was mixed with 5 mL water. Then, 8 mL of the prepared mixture was mixed with 2 mL of hydrogen chloride (HCl) (1.2 M) and 0.5 g of dried extract was incorporated. After heating, 10 mL of methanol was added to the obtained mixture and centrifuged for 5 min by the speed of 4000 rpm. The filtered transparent fluid was applied for HPLC phenolic determination. For quantification, initially 0.1 mg of each standard was solved in 1 mL of ethanol-HPLC grade and the concentrations of 0.005, 0.02, 0.04, 0.06, and 0.1 mg/L were prepared. The obtained solutions were injected to HPLC apparatus in order to detect and determine the phenolic compositions (Mattila & Kumpulainen, 2002).

Determination of minimum inhibition concentration (MIC)

In order to determine the MIC of extracts against the microorganisms involving *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Penicillium roqueforti* (IBRC 30159), *Aspergillus niger* (ATCC 16404), micro dilution method was used. Tryptic Soy Agar (TSA) were used to cultivate and activate the bacteria, and Sabouraud Dextrose Agar (SDA) were used for molds (Mattila & Kumpulainen, 2002).

Selection of optimal extracts and preparation of bread

Among the 6 evaluated extracts, two extracts including *Berberis vulgaris*

asperma root extract and *Berberis vulgaris orientalis* stem bark extract were selected and were used in bread formulations. To assess anti-microbial properties of root and stem bark extracts, different proportions were incorporated in bread dough formulation (450, 600, and 750 ppm for *Berberis vulgaris asperma* root extract and 600, 800, and 1000 for *Berberis vulgaris orientalis* stem bark extract) based on flour weight. In order to reduce adverse effects of extract on bread yeasts activity, barberry extract was added at the final stage of fermentation. Cooking of the dough was implemented at the temperature gradient of 220 to 230 °C. After baking bread was cooled for 2 h at room temperature then was packed in polyethylene bags and stored at 25 °C. Breads were carefully stored for later analyses, and the microbial analysis and oxidation stability of bread was determined during 12 days storage at four-day intervals. All formulations were prepared in triplicate.

Sensorial evaluation of bread

Sensory analysis of the bread samples containing barberry extract was performed based on 5 points hedonic test (1= not acceptable, 5= excellent). Baked breads with certain codes were served to the evaluators under normal conditions (Daylight). The panel contained 30 untrained evaluators who assessed the breads for taste, odor and flavor, mouth-feel, color and overall acceptability (Bahmanyar, Hosseini, Mirmoghtadaie, & Shojaee-Aliabadi, 2021).

Microbiological analysis of bread

Molds determination

10 grams of grounded bread was mixed with 90 mL of peptone water. 0.1 mL of obtained uniform suspension (initial suspension; 10^{-1}) was taken and transferred to medium containing Dichloran Glycerin Selective Agar (DG_{18}). The provided cultivated plates incubated aerobically at 25 °C for 5 d. Then, the colonies were counted during 12 days storage (Murat Karaoglu, Gürbüz Kotancilar, & Gurses, 2005).

Total microbial count

The microbial total count of breads was determined during 12 days storage at 4-day intervals. 10 gram of grounded bread was combined with 90 mL of peptone water diluting solution. 0.1 mL of uniform suspension was taken and added to plates containing Plate Count Agar medium. Then, the plates were incubated at 37 °C for 24 h (Murat Karaoglu *et al*., 2005).

Peroxide value of bread

Peroxide value (PV) was ascertained as an oxidation index. To determine the antioxidant activity of incorporated extracts, the bread fat was extracted by Soxhlet method. Thereafter, the peroxide value was determined at the different times (1, 4, 8, and 12 d). The determination of the PV was performed according to the AOCS (1998). 2 g of extracted oil were mixed with chloroform (10 mL). Glacial acetic acid (15 mL) and saturated aqueous solution of potassium iodine (1 mL) were added and then shaken and stored in the dark for 5 min. Distilled water (75 mL) were then added and mixed and the free iodine was measured with a 0.01N solution of sodium thiosulphate, using a starch solution (10 g/L) as indicator (Frutos & Hernandez-Herrero, 2005).

Statistical analysis

The applied statistical design of this study was a completely randomized design with three replications. In order to study the statistically significant difference between the treatments ANOVA analysis was used. Also Duncan test was employed for comparison of data means at the significant level of 5% (P<0.05).

Results and discussion

Antioxidant activity of the extracts

The relation of absorption of different root and stem bark extracts and increasing the concentration up to 200 µg/mL is shown in Fig. (1).

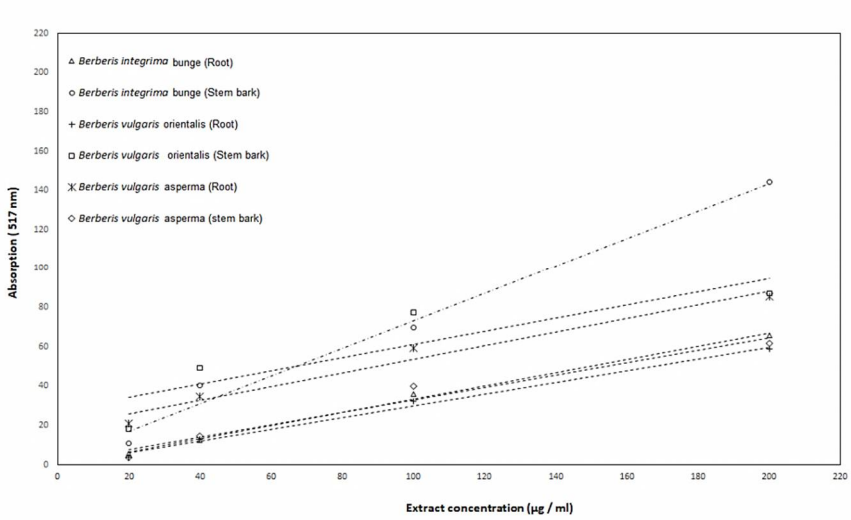

Fig. 1. Antioxidant activity of different Barberry stem and root extracts

According to Fig. (1), the absorption of extract increased with increasing its concentration. Less absorption value was observed for *Berberis vulgaris asperma* root extract that showed higher antioxidant activity. In contrast, *Berberis vulgaris orientalis* root extract showed the highest absorption value with less antioxidant activity.

A factor of IC_{50} was used to evaluate the antioxidant activity of the extracts that was shown in Table (1). IC_{50} value is defined as a concentration of the extract required to inhibit 50% of DPPH free radicals which the lower value is associated with the higher anti-radical activity of the extract. According to results, less IC_{50} value was specified for *Berberis vulgaris asperma* root extract. This reduction was demonstrated higher antioxidant activity. In other words, its extract could scavenge DPPH radicals more potentially than the other extracts. In contrast, *Berberis vulgaris orientalis* root extract showed the highest IC_{50} value, so the less antioxidant activity. In addition, *Berberis vulgaris orientalis* stem extract revealed higher antioxidant activity compared to other stem extracts. IC_{50} value for this sample was obtained 139.49 µg/mL. In contrast, the stem extract of *Berberis vulgaris asperma* showed IC_{50} equal to 153.46 µg/mL that was the highest amount among the other stem extracts. In this context Končić *et al*. (2010) investigated the antioxidant activity of ethanol-based extract of root and leave of *Berberis vulgaris* L. and *Berberis Croatia Horvat*. They noted that the least amount of antioxidant activity was related to root, while the leave showed the highest scavenging activity (Končić *et al*., 2010). Also Dimitrijević, Mitić, Ranković, & Miladinović (2020) determined the antioxidant properties of Barberry by five methods and reported that antioxidant activity of *B. vulgaris* extracts was high in all of the antioxidant assays. Therefore, it was concluded that *Berberis vulgaris* could be delightful fruity-plant in terms of containing natural antioxidant constituents (Dimitrijević *et al*., 2020). As a result, the root extracts of *Berberis vulgaris asperma* and the stem bark of *Berberis vulgaris orientalis* indicated higher antioxidant activity compared to other extracts.

In agreement with our study, other researchers emphasized that the root extracts had stronger antioxidant activity in comparison with stem extracts (Končić *et al*., 2010; Swati, Sah, Harsahay, Pandey, & Aarti, 2012).

Table 1. Antioxidant activity (IC_{50} values), Total phenol and Phenolic contents of the Barberry extracts

Extract	Barberry	IC_{50} (µg/mL)	Total phenol content (µg/mL)	Chlorogenic acid (mg/g dry plant)	Ferulic acid (mg/g dry plant)
Berberis integrima bunge	Root	150.12± 1.29^{c}	81.49± 2.02^{d}	8.27 ± 0.02^{b}	101.75 ± 0.02^{a}
	Stem	140.14± 2.11^{d}	86.61± 1.82^{c}	4.29 ± 0.02^{e}	56.65 ± 0.03^{e}
Berberis vulgaris asperma	Root	89.70± 0.92^{e}	112.78± 5.11^{a}	5.93 ± 0.01^{d}	60.29 ± 0.07^{d}
	Stem	153.46± 0.51^{b}	78.38± 1.00^{e}	3.43 ± 0.01^{f}	50.67 ± 0.00^{f}
Berberis vulgaris orientalis	Root	167.24± 1.65^{a}	66.99± 0.95^{f}	8.65 ± 0.04^{a}	62.96 ± 0.06^{c}
	Stem	139.49± 1.31^{d}	94.27± 0.32^{b}	7.73 ± 0.03^{c}	77.04 ± 0.02^{b}

*Different short letters indicate statistically difference among the columns (P<0.05).

Total phenol content of the extracts

The results of total phenol content of stem bark and root extracts of Barberry were shown in Table (1). It was observed a significant differences between the TPC of different species root and stem extracts. The TPC was varied from 66.99 µg/mL in *Berberis vulgaris orientalis* root extract to 112.78 µg/mL in *Berberis vulgaris asperma* root extract. The amount of phenolic compounds measured in different extracts were as follows: root of *Berberis vulgaris asperma,* stem of *Berberis vulgaris orientalis*, stem of *Berberis integrima Bunge*, root of *Berberis integrima Bunge*, stem of *Berberis vulgaris asperma*, and root of *Berberis vulgaris orientalis*. In this context, Končić *et al*. (2010) observed that the total phenol content of root extracts of Barberry varied from 7.29 mg/g in *B. vulgaris*-Skrad to 10.34 mg/g in *B. vulgaris*-Crni Lug. Also, they reported that the phenol content in the *Berberis vulgaris* leaves was higher than the other organs extracts. Dimitrijević *et al*. (2020) studied the antioxidant properties and total phenolic of Barberry, and reported that 494 ± 2 µg GAE/mg dry extract weight of total phenolic was found in Barberry fruit. Özgen *et al*. (2012) reported that the total phenolic content ranged from 2512 to 3629 (mg/L) in barberry. The changes of barberry total phenol in mentioned studies can be due to the different types of barberry species and different region where barberry was grown. Moreover, Öztürk, Aydoğmuş-Öztürk, Duru, & Topçu (2007) reported that the total phenol content of the stem and root extracts of Rhubarb (Rheum ribes) as an edible medicinal plant were 35.71 and 25.91 µg/mg, respectively; so a comparison of the total phenol content between Rhubarb and Barberry showed that the total phenol content of barberry root and stem was higher than rhubarb as a medicinal plant (Öztürk *et al*., 2007)

As we know, phenolic compounds have been verified to have potent antioxidant activity (Dziki *et al*., 2014; Gundogdu, 2013; Özgen *et al*., 2012); therefore, extracts with higher total phenol content should have more antioxidant activity. As the results of this study, the *Berberis vulgaris asperma* root followed by *Berberis vulgaris orientalis* stem extract showed the highest amount of total phenolic.

Determination of phenolic acids by HPLC

The chlorogenic acid and ferulic acid were the common phenolic acids of Barberry extract that were detected by HPLC (Gird *et al*., 2017; Gundogdu, 2013). The results of HPLC analysis are presented in Table (1). A significant differences were observed among the detected phenolic compounds (P<0.05) in different variety and different organs extracts. The chlorogenic acid content was varied from 3.43 to 8.65 mg/g of dry plant and the highest level of chlorogenic acid was observed in root extract of *Berberis vulgaris orientalis* while the lowest was detected in stem extract of *Berberis vulgaris asperma*. As represented in Table

(1), ferulic acid was another abundant phenolic acid in plant extracts. It was ranged from 50.67 in stem extract of *Berberis vulgaris asperma* to 101.75 mg/g of dry plant in root extract of *Berberis integrima bunge*. Consistent with these results, Belwal *et al.* studied the optimization of phenolic extraction of *Berberis asiatica* fruits and observed 3.112 (g/kg dry weight) chlorogenic acid in extraction of *Berberis* (Belwal, Dhyani, Bhatt, Rawal, & Pande, 2016). Therefore, based on HPLC findings, it can be claimed that the amount of chlorogenic acid in root extracts was more than stem extracts. Also, the amount of ferulic acid was more than chlorogenic acid in all types of studied barberry extracts. Contrary to this result, Gundogdu (2013) reported 0.752 and 0.02 g/kg chlorogenic acid and ferulic acid respectively in *Berberis vulgaris* fruit, and chlorogenic acid was the Major phenolic compound in *Berberis*. This contrast may be related to the barberry growing area.

Minimum inhibition concentration of extracts

Fig. (2), shows minimum inhibition concentration of different barberry extracts. The maximum anti-microbial effect against *Staphylococcus aureus* was related to root extract of *Berberis integrima Bunge*. Although, it's anti-fungal effect was the lowest in case of the *Aspergillus niger* growth. Riazi, Zeynali, Hoseini, & Behmadi (2015) compared the minimum inhibitory concentration of the barberry extract and the dried residue of red grape and noted that the barberry extract was more effective than the dried residue of red grape on *E. coli* and S. aureus. They also found that barberry extract was more effective on *Staphylococcus aureus* than *Escherichia coli* (Riazi *et al*., 2015). The lowest inhibitory effect achieved for *Berberis vulgaris orientalis* root extract, which was evaluated *on E. coli* and *Staphylococcus aureus*. Nevertheless, *Berberis vulgaris orientalis* stem extract was the most effective on *Penicillium roqueforti*, *Aspergillus niger*, and *Bacillus cereus*.

The MIC results of the *Berberis vulgaris asperma* extract showed that the *Berberis vulgaris asperma* root extract was more effective than the stem extract and the molds were more sensitive than the bacteria. This result could be related to the higher antioxidant activity of *Berberis vulgaris asperma* root extract and more total phenol content of it with potential antimicrobial activities. The maximum inhibitory effect of its root extract was on *Penicillium roqueforti* and the least inhibitory effect was observed on *Staphylococcus aureus*. The *Berberis vulgaris asperma* stem extract had the maximum inhibitory effect on *Aspergillus niger*. However, no significant effect was observed on *Penicillium roqueforti*. Among the bacteria, *Bacillus cereus* showed more sensitivity than other bacteria to both stem and root extract of *Berberis vulgaris asperma*. Same results were observed for *E.coli*, *Bacillus cereus* and *Penicillium roqueforti* after using *Berberis integrima Bunge* root and stem extract. MIC of the *Berberis integrima Bunge* root extract was equal to 590 ppm for all considered microorganisms. Stem extract of *Berberis integrima Bunge* had the maximum inhibitory effect on *Aspergillus niger*, although did not have the same effect on *Staphylococcus aureus*. In this context, Aliakbarlu, Mohammadi, & Khalili (2014) studied the antioxidant and antibacterial activity of spice extracts that are widely used in the Iranian diet and showed that barberry extract was second in the minimum inhibitory concentration among 10 extracts studied (Aliakbarlu *et al*., 2014). Therefore, barberry extract can be considered as a desirable antimicrobial compound and according to Fig. (2), it can be stated that the minimum inhibitory concentration of root extract is lower than the stem. In other words, barberry root extracts show antimicrobial properties at lower concentration.

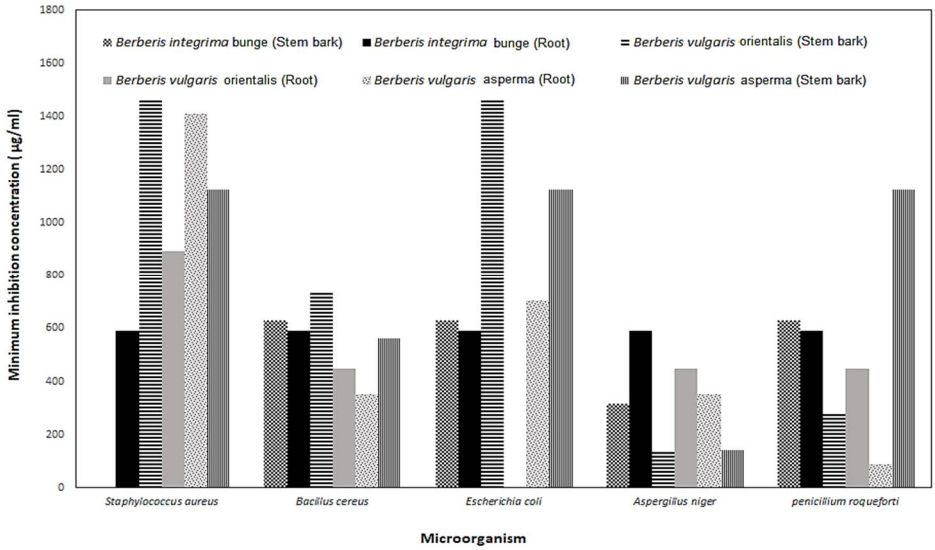

Fig. 2. Minimum Inhibition concentration of different Barberry stem and root extracts

Sensorial evaluation of bread

Two extract with highest total phenolic content and antimicrobial properties (*Berberis vulgaris asperma* root and *Berberis vulgaris orientalis* stem extracts) were choosed to evaluate the sensorial properties of bread (Table 2). However, increasing the extract concentration resulted in a reduction of taste and total acceptability of bread.

The results demonstrated that the inclusion of root extract of *Berberis vulgaris asperma* in bread formulation was more acceptable than the *Berberis vulgaris orientalis* stem extract. Based on the panelists' declaration, the samples with high concentrations of extracts had bitter taste and flavor. There is no significant difference among the total acceptability of samples with 450 and 600 ppm of *Berberis vulgaris asperma* root extract. Contrary to these results, Jaberi, Kaban, & Kaya (2020) observed that the addition of barberry (*Berberis vulgaris* L.) extracts up to 3% in chicken frankfurters did not make a significant difference in the overall acceptability and flavor of the product (Jaberi *et al.*, 2020). Among the various concentrations of *Berberis vulgaris asperma* root, the bread with 600 ppm of this root extract was acceptable to consumers as bread containing natural antioxidant and antimicrobial compounds. In this context, Bourekoua, Gawlik-Dziki, Różyło, Zidoune, & Dziki (2021) noticed that partial replacement of the rice flour with 3% Acerola fruit powder as a source of natural antioxidant in gluten free bread could satisfy the panelists.

Table 2. Sensory analysis of breads with Barberry extracts

Bread samples	Concentration (ppm)	Taste	Odor and Flavor	Mouth-feel	Color	Overall acceptability
Blank	0	4.05 ± 0.82^{a}	4.50 ± 0.99^{a}	3.79 ± 0.70^{a}	4.05 ± 0.82^{a}	4.50 ± 1.21^{a}
Roots (*Berberis vulgaris asperma*)	450 ppm	3.90 ± 0.85^{a}	3.85 ± 0.11^{b}	3.07 ± 1.10^{b}	3.90 ± 0.71^{a}	3.90 ± 0.40^{b}
	600 ppm	3.85 ± 1.14^{a}	3.88 ± 1.31^{b}	2.85 ± 0.21^{b}	4.10 ± 0.62^{a}	3.50 ± 0.20^{b}
	750 ppm	2.50 ± 0.51^{b}	3.70 ± 0.91^{b}	2.63 ± 1.10^{b}	3.80 ± 0.32^{a}	3.05 ± 0.10^{c}
Stems (*Berberis vulgaris orientalis*)	600 ppm	2.90 ± 0.61^{b}	3.60 ± 0.11^{b}	2.50 ± 1.10^{b}	4.07 ± 0.67^{a}	3.50 ± 0.29^{b}
	800 ppm	2.50 ± 0.96^{b}	3.68 ± 0.45^{b}	2.15 ± 0.91^{b}	3.90 ± 0.62^{a}	2.20 ± 1.15^{c}
	1000 ppm	1.10 ± 0.71^{c}	3.70 ± 0.81^{b}	1.63 ± 1.10^{c}	3.75 ± 0.73^{a}	1.50 ± 1.20^{d}

*Different short letters indicate statistically difference among the columns (P<0.05).

Microbial analysis of bread

In this section, bread as a cereal-based product was developed and then the effect of incorporated barberry extracts on molds and bacteria were evaluated. The changes in total count and molds count of bread containing *Berberis vulgaris asperma* root and *Berberis vulgaris orientalis* stem extract during 12 days storage are summarized in Table (3). As is shown in the Table (3), total count and mold count of bread with both extracts of *Berberis vulgaris asperma* roots and *Berberis vulgaris orientalis* stem showed a progressive increase throughout the 12 days storage, but this increase was less in samples containing Barberry extracts than control sample. In general, microbial activity decreased with increasing extract concentration. The obtained results of microbial counts in present study are consistent with the results reported by Pattison & Von Holy (2001). These authors investigated the effect of natural antimicrobials on bakery yeast activity and reported that yeast activity decreased with increasing selected natural antimicrobial concentrations.

Also, the results showed that the concentrations of 600 and 750 ppm of *Berberis vulgaris asperma* root prevented completely from mold and bacteria growth up to 8th day of storage. The results were consistent with what was mentioned above about the MIC for mold growth. As was illustrated in Fig. (2), the root extract of *Berberis vulgaris asperma* could prevent the growth of the mold in lower concentration. The concentrations involving 800 and 1000 ppm of *Berberis vulgaris orientalis* stem extract exhibited a more preventive effect in comparison with 600 ppm. Generally, the root extract of *Berberis vulgaris asperma* revealed more inhibition property against microbial growth in bread compared to the *Berberis vulgaris orientalis* stem extract. Guynot *et al*. (2003) demonstrated that phenolic ingredients in plant extract were an important factor to inhibit the growth of microorganism. Therefore, more antimicrobial properties of bread containing *Berberis vulgaris asperma* root can be related to the higher amount of total phenol and lower IC_{50} value in *Berberis vulgaris asperma* root compared to the *Berberis vulgaris orientalis* stem extract (Table 1).

Table 3. Microbial culturing results of breads containing Barberry extracts

Bread samples	Day	Microorganism	0 ppm	450 ppm	600 ppm	750 ppm
Berberis vulgaris asperma roots extract	1	Mold	-	-	-	-
		Total count	-	-	-	-
	4	Mold	++	+	-	-
		Total count	+	+	-	-
	8	Mold	++	+	+	-
		Total count	++	+	+	-
	12	Mold	++	+	+	+
		Total count	++	++	+	+
Berberis vulgaris orientalis stems extract	1	Mold	-	-	-	-
		Total count	+	+	+	-
	4	Mold	++	+	-	-
		Total count	+	+	+	+
	8	Mold	++	++	+	-
		Total count	++	++	++	+
	12	Mold	++	++	+	+
		Total count	++	++	++	++

-, +, and ++ indicate no microorganism growth, low microorganism growth (lower than 20 colonies), and high microbial growth (higher than 20 colonies), respectively.

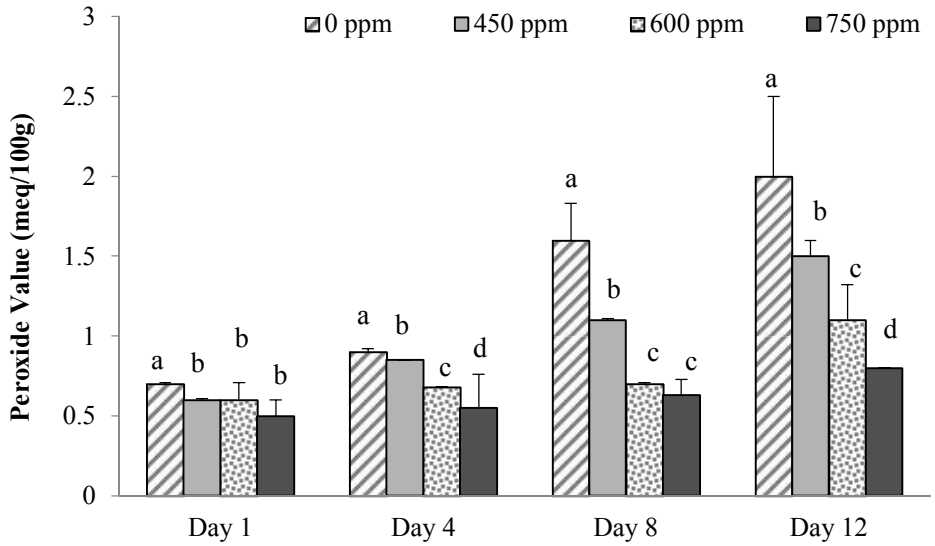

Fig. 3. Peroxide value of bread containing up to 750 ppm root extract of *Berberis vulgaris asperma* during 12 days Dissimilar letters in each day showed a significant difference between different concentrations (P<0.05).

Oxidation stability of bread

Since, the bread containing *Berberis vulgaris asperma* root was demonstrated more inhibition property against microbial growth and was more acceptable by panelist; it was selected as the best extract for further analysis.

Therefore, the oxidation stability was performed only for breads containing *Berberis vulgaris asperma* root extracts during 12 days storage (Fig. 3). As shown in Fig. (3), the peroxide value of all samples increased during the storage. The blank sample had 0.7 (meq/100 g) peroxide value on the first day that raised to 2 (meq/100 g) at the day 12 and the peroxide value varied from 0.5 to 0.8 (meq/100 g) in the highest concentration of extract during storage period. Moreover, the peroxide value was decreased with increasing extract concentrations at the end of storage and the oxidation process was significantly decreased in breads containing extracts that may be due to high antioxidant activity and total phenolic of *Berberis vulgaris asperma* root extract. In this vein, Jaberi *et al*. (2020) reported that the addition of three concentrations of *Berberis vulgaris* extract in chicken frankfurters could be delayed lipid oxidation.

Conclusions

This study highlighted that *Berberis vulgaris asperma* root and *Berberis vulgaris orientalis* stem extracts can improve the shelf life of bread. The results indicated the highest amount of total phenol and the antioxidant property was related to *Berberis vulgaris asperma* root and *Berberis vulgaris orientalis* stem extracts. Also, these two types of extracts showed more inhibition effect against molds and bacteria in the MIC test. However, bread containing *Berberis vulgaris asperma* root extract had more total acceptability than bread containing *Berberis vulgaris orientalis* stem. Also, the root extract of *Berberis vulgaris asperma* effectively prevented microbial growth in a concentration of 600 and 750 ppm and could delay lipid oxidation of bread containing 600 ppm extracts. Overall, the addition of 600 ppm *Berberis vulgaris asperma* root extract is recommended in bread formulation.

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ارزیابی خواص آنتی‌اکسیدانی و ضدمیکروبی عصارهٔ پوست ریشه و ساقهٔ سه گونهٔ زرشک در نان

محمد گریزپا[1]، فرشته بهمنیار[2]، لیلا میرمقتدایی[3]*، فائزه شفاعی[4]*

۱- دانش‌آموختهٔ کارشناسی ارشد، گروه مهندسی کشاورزی، دانشکده علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۲- دانشجوی دکتری، گروه علوم و صنایع غذایی، انستیتو تحقیقات تغذیه و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران

۳- دانشیار، گروه علوم و صنایع غذایی، انستیتو تحقیقات تغذیه و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران

* نویسندهٔ مسئول (mirmoghtadaie@sbmu.ac.ir)

۴- استادیار، گروه شیمی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

* نویسندهٔ مسئول (faezehshafaei@yahoo.com)

چکیده

این مطالعه با هدف تعیین فعالیت آنتی‌اکسیدانی، میزان فنول کل، اسیدهای فنولیک و حداقل غلظت بازدارندهٔ پوست ساقه و ریشهٔ سه گونهٔ زرشک (*بربریس انتگریما بانگ*، *بربریس ولگاریس آسپرما* و *بربریس ولگاریس اریتنالیس*) انجام شد. عصارهٔ ریشهٔ *بربریس ولگاریس آسپرما* بیشترین میزان فنول کل و فعالیت آنتی‌اکسیدانی را نشان داد. پس از اندازه‌گیری حداقل غلظت بازدارنده، عصارهٔ ساقهٔ *بربریس ولگاریس اریتنالیس* و عصارهٔ ریشهٔ *بربریس ولگاریس آسپرما* به‌عنوان بهترین عصاره با داشتن بالاترین فعالیت ضدمیکروبی انتخاب شدند. همچنین عصارهٔ ریشهٔ *بربریس ولگاریس آسپرما* می‌تواند به‌طور مؤثر از رشد قارچ و باکتری در غلظت ۶۰۰ و ۷۵۰ پی‌پی‌ام جلوگیری کند. همچنین نان حاوی ۶۰۰ پی‌پی‌ام عصارهٔ ریشهٔ *بربریس ولگاریس آسپرما* بالاترین سطح پذیرش کلی و پایداری اکسیداتیو را نشان داد. بنابراین به‌طورکلی براساس نتایج، عصارهٔ ریشهٔ *بربریس ولگاریس آسپرما* بهترین نوع عصاره در بین نمونه‌های موردمطالعه بود و غلظت ۶۰۰ پی‌پی‌ام بهترین سطح برای غنی‌سازی نان و تولید نان فراسودمند با قابلیت ماندگاری بالا بود.

واژه‌های کلیدی: خاصیت ضدمیکروبی، دورهٔ ماندگاری، زرشک، نان