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Effect of pulsed electric fields on antioxidant and polyphenolic properties of stinging nettle and its effect on bread quality

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ABSTRACT

In this study, a completely randomized design was employed to enhance the extraction of phenolic compounds from nettle plants using pulsed electric field pretreatment. The experiment involved different electric field intensities at three levels (1, 4, and 7 kv/cm) and pulse numbers at three levels (20, 40, and 60). After extraction, the total phenolic content and antioxidant activity were assessed through DPPH and FRAP. The findings indicated that increasing the electric field intensity from 1 to 7 kv/cm resulted in a reduction in total phenolic content and antioxidant activity, while increasing the number of pulses from 20 to 60 led to an increase in these values. The results of the optimized process clearly revealed that using a pulsed electric field of 1 kV/cm with 40 pulses provided the highest total phenolic content and antioxidant activity in the extract. To assess the quality attributes of bread, untreated stinging nettle extract was used as a control while plant extract treated with 1 kv/cm intensity and 40 pulses, in varying quantities (5, 15, 25, and 35 ml per 100 grams of flour), was incorporated. The findings revealed a notable difference in the total phenolic content among various bread samples. The bread enriched with 35 ml of treated extract exhibited the highest total phenolic content. Furthermore, moisture content of the bread decreased while its hardness increased during storage, and both parameters escalated with higher extract ratios up to 35 ml. The treated extract exhibited a notable enhancement in the color of both the brain and shell of the bread compared to the control. Ultimately, bread samples containing 5 and 15 ml of the treated extract were selected as optimal samples due to their superior quality attributes.

1- INTRODUCTION

Polyphenols are natural compounds found in plants that have attracted attention in the pharmaceutical, nutritional, and food industries for their antioxidant and anti-inflammatory properties. These compounds can help reduce the risk of heart disease, diabetes, and some types of cancer, and help improve brain health and strengthen the immune system. In nutrition, consuming foods rich in polyphenols, such as fruits, vegetables, and beverages such as green tea, is recommended as part of a healthy diet. In general, research emphasizes the effective role of polyphenols in preventing lipid and protein oxidation, preventing diseases, and improving health. [1].

Nettle plant with scientific name *Urtica dioica* L. And from the dark *Urticaceae* [2, 3]. Stinging nettle is a perennial plant with a wide global distribution. It grows in the Mediterranean region and the northern hemisphere [4]. Chemical analysis of this plant indicates the presence of polyphenol compounds, phenolic acids, carotenoids, minerals and other elements, which are significant due to their biological activities. Its extensive therapeutic properties include expectorant, laxative, diuretic, hemostatic and anthelmintic. In addition, it is effective in the treatment of diseases such as eczema, rheumatism, hemorrhoids, hyperthyroidism, bronchitis, diabetes and cancer [5].

As consumer demand for high-quality healthy foods rich in natural bioactive compounds such as vitamins, phenolic compounds (e.g. flavonoids), pigments, dietary fibers, etc. has increased significantly, therefore, improving the content of bioactive compounds or maintaining their stability in foods requires the application of appropriate techniques. In this context, the emergence of non-thermal technologies such as pulsed electric fields has attracted attention [7, 8, 9]. This technique causes the permeability of cytoplasmic membranes and facilitates the release of intracellular compounds from cells [2]. Pulsed electric fields can be used as a pretreatment for thermal processes in the extraction of bioactive compounds. The application of pulsed electric fields in the

extraction of bioactive compounds (e.g. polyphenols and vitamins) depends on various factors such as the properties of the raw materials, pulse length, number of pulses, rest time, intensity and application time of pulsed electric fields [10]. These short pulses with a duration of microseconds to milliseconds, by applying electrical power and creating high mechanical stress, cause reversible or irreversible permeability of biological membranes and extraction and preservation of nutrients [7, 11].

Antioxidant-rich foods play an important role in the prevention of cardiovascular diseases, cancers, and nerve cell regeneration [12]. Enriching bread dough, which is prone to lipid oxidation and microbial growth, with natural antioxidants represents a viable strategy to produce bread loaves with a higher degree of oxidative stability, leading to better sensory quality of bread during storage [13]. In 1995, the FDA¹ The use of PEF in the food industry was declared unproblematic [14]. Various studies have investigated the effect of applying pulsed electric fields on various plants, including the extraction of polyphenols from the bark of the borage plant by applying pulsed electric fields [1], the extraction of bioactive compounds from cactus fruit juice by applying pulsed electric fields [8], and the application of pulsed electric fields on the extraction of polyphenols from sorghum flour and apple flesh [15]. Some studies have also investigated the effect of adding natural antioxidants to bread, such as adding ginger powder to bread [16] and examining the antioxidant activity of bread by adding grape extract [17].

Considering the importance of medicinal plants in the production of functional foods and the development of new useful products in the food industry, and the fact that no research has been conducted so far on the extraction of polyphenols from nettle by applying pulsed electric fields and the effect of its extracted extract on bread quality, the aim of this study is to investigate the effect of pulsed electric fields on the antioxidant and polyphenolic properties of nettle and evaluate its effect on bread quality.

2- Materials and methods

2.1- Sample preparation

¹. Food and Drug Administration

In this study, the nettle plant was dried in the shade after preparation and then stored in air- and moisture-resistant plastic bags until the experiments were conducted.

2.2- Applying pretreatments and extracting extracts from samples

The samples were exposed to the pulsed electric field device made at the Mashhad Food Industry Research Institute. In this device, electric pulses in a logarithmic, unipolar form, with a duration of 0.5 milliseconds, with a chamber capacity of 4 liters and a capacitance of 8 microfarads were used to process the nettle plant. The pulse frequency was also adjusted using a digital oscilloscope [18]. The samples were exposed to field strengths of 7 and 4, 1 kV/cm, a frequency of 1 Hz and 3 levels of pulse numbers of 40, 20 and 60. The sample that was not exposed to the field was also considered as a control sample. To prepare the nettle extract, the method of Heidari-Majd et al. [1 and 19] was used, with some modifications. Thus, 10 grams of the powdered sample was mixed with 100 ml of 80% ethanol and stirred on a shaker for 24 hours at room temperature. The resulting extract mixture was filtered with Whatman paper No. 1 and then the residual ethanol was separated using a rotary apparatus under vacuum. Finally, the resulting extract was stored in a refrigerator at 2-5 °C to preserve phenolic compounds and various quality tests were examined.

2-3- Enriching bread with extract

Bulky bread, using the optimal extract of treated nettle plant and untreated extract (control) were enriched to improve the quality and performance of bread base tablets. After extraction, treated and untreated nettle samples were concentrated by screening and then diluted with water to a concentration of 1000 ppm in terms of total phenolic compounds. Then, the treated extract (in amounts of 5, 15, 25 and 35 ml per 100 g of wheat flour) was added to the initial dough along with other ingredients to evaluate the quality characteristics of bread. Accordingly, the extracts were mixed with 100 g of wheat flour along with 1.5 g of salt, 1 g of sugar, 2 g of yeast, 0.5 g of improver and 55 ml of water. After mixing all the ingredients and preparing the dough, fermentation was carried out in three stages. The initial fermentation was carried out in a fermentation chamber at 30°C and 75% relative humidity for 30 minutes. Then the doughs were divided into 100-gram portions and a second fermentation was carried out for 15 minutes. Then they were placed in baking molds and the

final fermentation was carried out for 50 minutes. Finally, the doughs were baked in an oven at 220°C for 25 minutes. After baking, the breads were cooled at room temperature for 2 hours and stored in zip-lock bags at room temperature (25°C) until further analysis. Also, a control bread sample was prepared according to the above conditions without adding any extract [12].

2.4- Tests

2-4-1- Extract tests

2-4-1-1- Determination of total phenolic compounds

The amount of total phenolic compounds present was determined by the method of Capanci et al. [20], with slight modifications, using the Folin-Ciocalteu colorimetric method. For this purpose, a standard curve of gallic acid was drawn in the concentration range of 50-1000 ppm. Then, to determine the phenol content, 2.5 ml of Folin-Ciocalteu reagent diluted 1:10 with distilled water was added to 1 ml of the extract solution with a concentration of 1000 ppm. After 2 minutes, 5 ml of sodium carbonate (75 g/l) was added to it and the resulting solution was kept in the dark at room temperature for 40 minutes. Then, the absorbance of the sample was read at a wavelength of 765 nm. By placing the results in the standard curve, the amount of total phenol in the free extract was calculated based on milligrams of gallic acid per gram of extract.

2-4-1-2- Determination of antioxidant activity by DPPH method

Antioxidant activity was evaluated using the method of Pataro et al. [21] with some modifications. The ability of phenolic compounds to lose hydrogen atoms was measured by the degree of decolorization of DPPH purple solution in ethanol. The DPPH reagent solution (25 ppm) was dissolved in ethanol. Then, 3.9 ml of 0.15 mM DPPH ethanolic solution was added to 0.1 ml of 800 ppm extract solution and stirred. After 30 minutes in the dark, the optical absorbance of the samples was read at 517 nm. Finally, the percentage of free radical inhibition was obtained by the following equation:

$$\text{Percentage of free radical inhibition} = (A_c - A_s) / A_c \times 100 \quad (\text{Relationship 1})$$

In this regard, A_c and A_s are the control absorption and the sample absorption, respectively.

2-4-1-3- Determination of antioxidant properties by FRAP method

To measure antioxidant activity by FRAP method, the method of Carvalho et al. [22] was used with some modifications. In this method, first, FRAP solution (mixture of 200 ml acetate buffer, 20 ml TPTZ solution, 20 ml ferric chloride solution and 24 ml distilled water) was prepared. After preparing the working solution, 1 ml of the above solution was placed in a cuvette². The temperature was brought to 37°C and its absorbance was measured at 593 nm to calculate the blank. Then, 100 µl of the extract with a concentration of 800 ppm was added to 3 ml of FRAP solution to initiate the reaction. The absorbance changes were measured at 593 nm and 37°C for 4 minutes. After subtracting the blank absorbance, the FRAP value was determined using the standard (0.278g FeSO₄.7H₂O/1L distilled water), converted to millimolar per liter.

2-4-2- Bread tests

2-4-2-1- Determination of total phenolic content

The content of total phenolic compounds extracted from bread using the method of Pasrija et al. [23] It was investigated with some modifications. Thus, 2 g of bread was ground and powdered for 1 minute. Then, the samples were subjected to magnetic stirring with 10 ml of water to extract polyphenols. The resulting mixture was centrifuged for 10 minutes at 15115 × g and the polyphenol content of the supernatant was measured by the Folin-Ciocaltio method.

2-4-2-2- Determination of moisture content

The AACC (2000) test was used to measure the moisture content of bread. For this purpose, the samples were placed in an oven at 130°C for 90 minutes, 2 hours after baking (day zero), on the first and third days, and then the moisture content was calculated based on the weight loss of the sample [24].

2-4-2-3- Determining the degree of tissue stiffness

Bread texture was evaluated at 2, 24, and 72 hours after baking using a texturometer, according to the method of Sahraian et al. [25]. The maximum force required to penetrate a cylindrical probe (2 cm diameter x 2 cm height) at a speed of 30 mm/min from the center of the bread was calculated as the firmness index.

2-4-2-4- Evaluation of the color characteristics of bread crust and crumb

In order to investigate the color characteristics of the crust and crumb of bread, the L* a* b* color space was used by Photoshop software. The L* component indicates the brightness of the sample and its range varies from zero (pure black) to 100 (pure white). The values of the a* component are between negative 120 and positive 120, and positive values are equivalent to red and negative values are equivalent to green. And the b* values are the same as the a* component, and negative values are equivalent to blue and positive values are equivalent to yellow [23].

2.5- Statistical analysis

A completely randomized design was used to compare the total phenolic content and antioxidant properties of nettle extract and to investigate the effect of the ratio of extract added to bread on the physicochemical properties of bread. Statistical analysis of data was performed using Spss software version 22 and analysis of variance (ANOVA) at a confidence level of 95% (0.05). *P* <) was performed. Duncan's multiple range test was used to compare the mean data and the graph was drawn using Excel 2007 software.

3- Results and discussion

3.1- Extract test results

3.1-1- Amount of total phenolic compounds

Figure 1 shows the effect of each PEF intensity and treatment variable on the total phenolic content in one gram of nettle extract. Compared to the control sample, at a field intensity of 1 kV/cm, the total phenolic content increased (*P* < 0.05). *P* <), which showed the highest levels of phenolic compounds in treatments 1 and 40 (0.02) ± 1.83 mg gallic acid equivalent). However, the amount of total phenolic compounds significantly decreased at field strengths of 4 and 7 kV/cm, which was consistent with the results of the number of PEF pulses (0.05). *P* <). The highest phenol loss was obtained with an intensity of 7 kV/cm and a number of treatments of 60.

². Cuvette

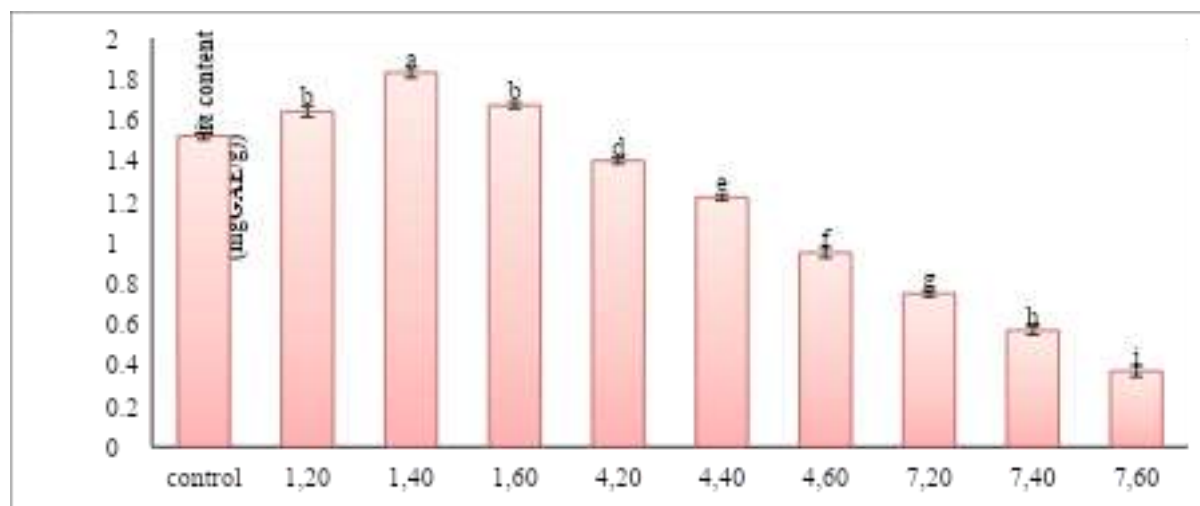


Figure 1: The effect of pulsed electric fields (1, 4 and 7 kv/cm and pulse number = 20, 40 and 60) on the total phenolic content of stinging nettle extract

a-i Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

Phenolic compounds are a large group of secondary plant metabolites whose antioxidant capacity is due to the presence of hydroxyl groups in their structure. The use of natural phenols in the food industry is increasing because these compounds delay the oxidative degradation of lipids and thus improve the quality and nutritional value of foods [26]. The changes in polyphenols of nettle extract can be influenced by the effect of pulsed electric field treatment by "electroporation"³, on biological cells⁴ explained. The membranes of biological cells may be perforated by electrical treatment and affected by the intensity and number of very short pulses [27]. With the effect of this treatment on the membranes of biological cells, the amount of bioactive polyphenolic compounds is released at low intensity and number of treatments and the content of phenolic compounds increases. However, with increasing intensity and number of treatments, these compounds have the opposite effect and with the loss of polyphenolic compounds, the content of phenolic compounds also decreases.

Carvalho et al. [22] examined the phenolic content of extracts of different species of nettle and found that each gram of nettle extract contained an average of 7.9 g/100 g of extract. Aguilar et al. [28] reported that the phenolic content of apple juice treated with a pulsed electric field at a treatment intensity of 35 kV/cm was reduced by 14.49%. The results of the above study were consistent with the results of Bi et al. [29] regarding the reduction of phenolic content with increasing pulse intensity. Luengo et al. [30]

regarding the treatment of orange peel and Aldara et al. [31] regarding grapes concluded that treatment with pulsed electric field technology resulted in the release of bioactive compounds in orange and grape peel. In these studies, extraction of polyphenolic compounds from orange peel with a pulse intensity of 1-7 kV/cm increased polyphenolic compounds by 159%, and polyphenolic compounds from grapes with a pulse intensity of 5 kV/cm increased polyphenolic compounds by 23%. This increase in polyphenol content was related to the increased extraction of some antioxidants by pulsed electric field treatment. Aldara et al. [31] suggested that grape varieties required higher electrical energy to increase skin permeability and damage the underlying layer, resulting in the release of polyphenols. On the other hand, Vorobyov and Leboca [32] stated that electric field intensity in the range of 0.5-0.8 kV/cm is usually sufficient to induce biological cells in grape juice and increase bioactive molecules. Ramboud et al. [33] also achieved similar results to this study regarding the treatment of grape seeds with pulsed electric fields.

3-1-2- Antioxidant activity level by DPPH method

In this study, the antioxidant activity of nettle extract was tested in the DPPH method at a concentration of 800 ppm. The results in Figure 2 show the effect of each of the intensity variables and PEF treatment on the amount of antioxidant activity. The DPPH method is based on the reduction of the DPPH free radical by antioxidants in the absence of other free radicals

³. Electroporation

⁴. Biological cells

in the environment, which results in the creation of a color in the environment, the intensity of

which can be measured with a spectrophotometer [34].

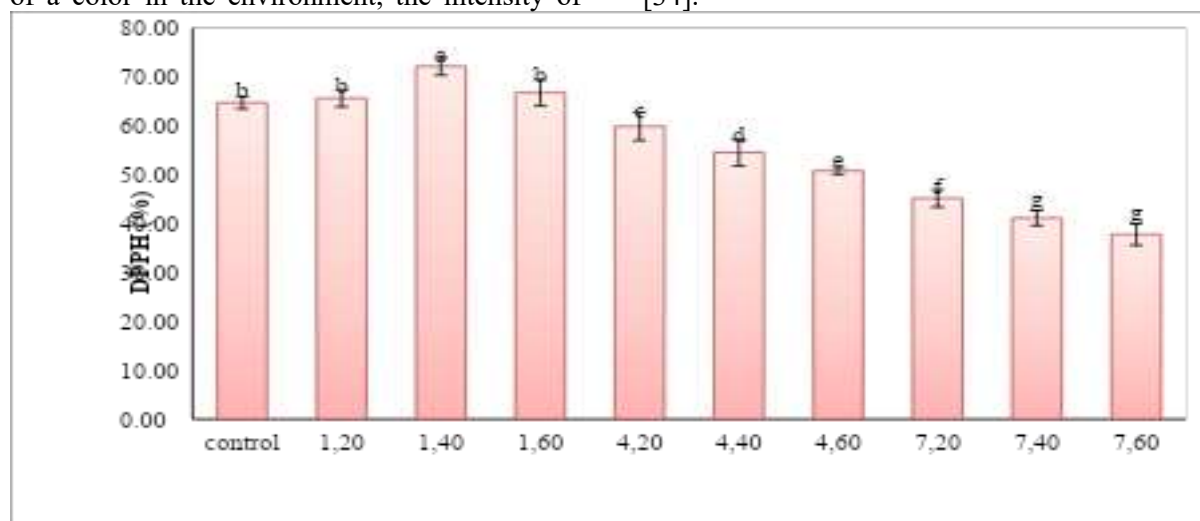


Figure 2: The effect of pulsed electric fields (1, 4 and 7 kv/cm and pulse number = 20, 40 and 60) on the free radical scavenging activity of stinging nettle extract

a-g Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

As shown in Figure 2, the percentage of DPPH reduction at a pulse intensity of 1 kV/cm increased compared to the control sample, which was significant for treatments 1 and 40. Also, the study of changes in the percentage of reduction of nettle extract showed that the intensity of the electric field had the greatest effect on the amount of antioxidant activity and its effect was negative. This means that with increasing the intensity of the electric field, the percentage of reduction decreased significantly ($P < 0.05$). $P < 0.05$ had.

3-1-3- Antioxidant activity level by FRAP method

Figure 3 shows the results of antioxidant activity by FRAP assay, which was in line with the results of DPPH reduction percentage. Increasing the number of pulses also had a similar effect on this property ($P < 0.05$). $P < 0.05$. These results were highly correlated with the percentage of DPPH reduction. This correlation of antioxidants most likely indicates that the compounds in nettle extract have a high ability to reduce heavy metals, which can prevent some of the oxidative pathways catalyzed by metals [22].

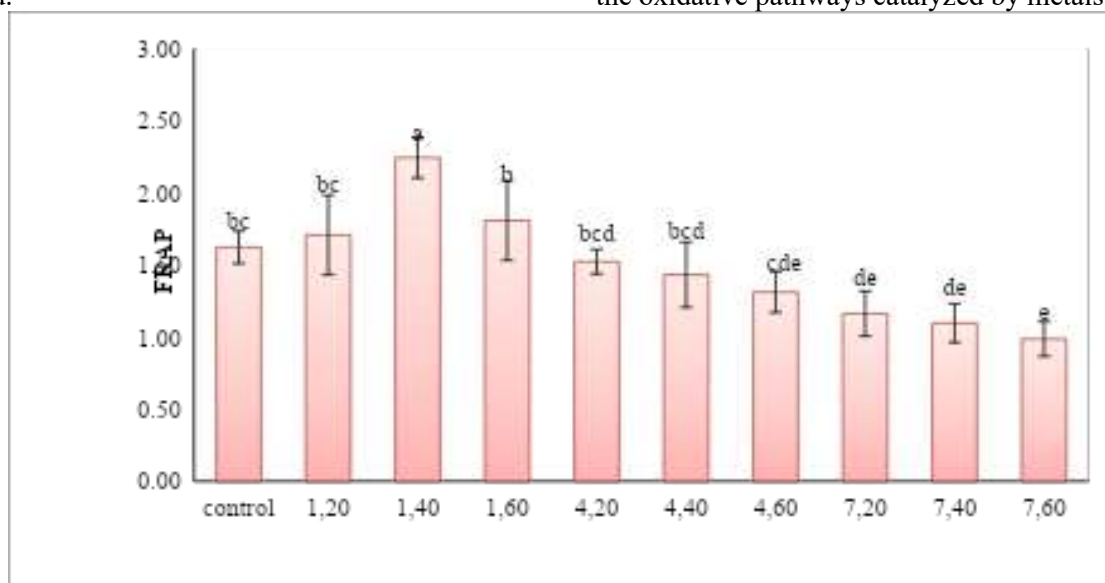


Figure 3: The effect of pulsed electric fields (1, 4 and 7 kv/cm and pulse number = 20, 40 and 60) on the antioxidant activity (FRAP) of stinging nettle extract

a-e Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

Comparison of the results showed that with increasing electric field intensity and pulse number, the amount of total phenolic compounds initially increased due to the release of these compounds, but with higher intensity of these two variables, the amount of these compounds decreased due to the destructive effect of high electric field intensities. Due to the direct relationship between phenolic compounds and the level of antioxidant activity, the level of antioxidant activity also decreased in line with the reduction of polyphenolic compounds. The FRAP method uses a redox reaction that is accompanied by a color change. When the reducing agent of the reaction (antioxidant) donates its electron, a colored substance is produced that indicates the progress of the reaction and can be easily evaluated [35].

Haqjoo et al. [6] evaluated the colloidal and antioxidant properties of nanoliposomes containing nettle extract, and the antioxidant activity was 50.94%. Mottab et al. [36] compared the factors affecting the extraction process of antioxidant compounds from nettle leaf and stem extracts using microwaves with extracts extracted using traditional methods. The results showed that the amount of antioxidant compounds in the organic solvent extract was much higher than the content of the aqueous extract. Also, the antioxidant activity of nettle leaf extract was 82%. Since ethanol has a direct effect on the sample tissue, it causes the cell wall of phenolic compounds to break. Jin et al. [37] studied the treatment of blueberries with pulsed electric fields at a pulse intensity of 2 kV/cm and the results indicated that the amount of polyphenolic compounds and antioxidant activity increased by 25% with the treatment, which was consistent with the results of this study. Ferrari et al. [38] also achieved similar results in a study of pomegranate juice [38]. Plaza et al. [39] observed that the pulsed electric field process did not change the antioxidant capacity of orange juice before and after treatment, which was consistent with the results of Odriozola et al. [40]. Gu et al. [41] observed

that the pulsed electric field was the important factor that had the greatest effect on anthocyanin content when pomegranate juice was treated with pulsed electric fields at 35 and 38 kV/cm at 281°C.

2-3- Optimal extract

Among the treatments with different intensity and number of pulses on nettle plant, nettle treatment with intensity of 1 kV/cm and number of 40 pulses was used in different amounts to evaluate the quality of bread due to high polyphenolic compounds and high antioxidant activity of the extract. Since nettle is a rich source of various polyphenols that have numerous medicinal benefits, in this study the performance of bread containing optimally treated nettle extract was investigated by evaluating the total phenolic content and compared with adding 15 ml (at a concentration of 1000 ppm) of untreated extract (control) to the bread.

3-3- Bread test results

3-3-1- Content of phenolic compounds in whole bread

The graph of the effect of adding different proportions of nettle extract on the content of phenolic compounds in bread samples is shown in Figure 4. The results showed that phenolic compounds increased significantly (0.05) when the extract was added to bread. *P* The sample contained 5 ml of the treated extract with the lowest phenol content (41/mg GAE/g03). ± 0.0) and the sample containing 35 ml of the treated extract had the highest total phenol content (81.03 mg GAE/g ± 0.0). In comparing the control extract with the treated extract, the amount of phenolic compounds in bread containing 5 ml of the treated extract was higher due to the higher amount of extract, although it was not statistically significant (0.05).*P*>) but compared to bread containing 15 ml of treated extract (in the same amount), it had significantly lower phenolic compounds, which was due to the effect of PEF on the release of phenolic compounds (0.05).*P*<).

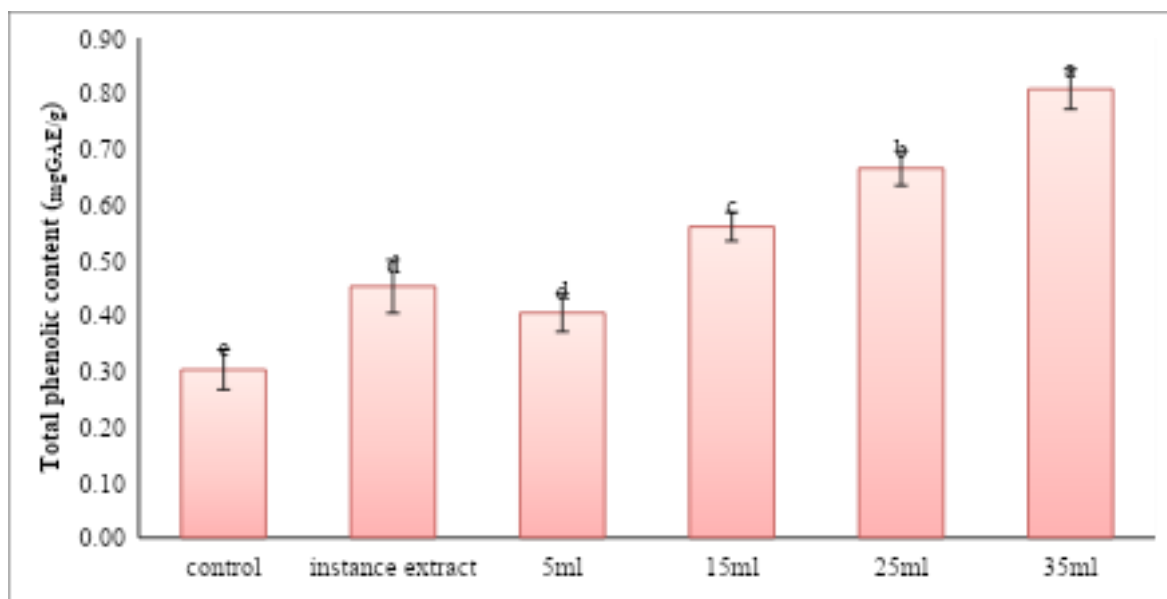


Figure 4. The effect of different concentration of stinging nettle extract on the total phenolic content of bread

a-e Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

Peng et al. [17] examined the change in the amount of phenolic compounds by adding grape seed extract to bread. The bread in question had more phenolic compounds than the control sample. Increasing the level of grape seed extract used increased the amount of phenolic compounds. Bayano et al. [42] examined the amount of phenolic compounds in bread containing chicory and celery extracts. According to the results of this study, the amount of phenolic compounds increased compared to the control bread. Bread containing chicory extract had more phenols on the dry surface of the bread crust than on the wet surface, and the dry surface of the bread core also had more phenols than on the wet surface of the bread core. This result was also true for bread containing celery extract. Pasrija et al. [23] reported an increase in the amount of total phenols in breads containing green tea extract. According to the report of Horswald et al. [43], some of the phenolic compounds in the extract may be destroyed due to heat during baking, which is why in this study the amount of total phenolic

compounds in bread after baking was lower than before baking.

3-3-2- Bread crumb moisture content

Figure 5 shows the effect of adding different proportions of nettle extract on the moisture content of bread crumb on 3 different days. According to the results of statistical analysis, there was a significant difference ($P < 0.05$) between the moisture content of breads on different days. ($P > 0.05$) was not present. However, with the addition of nettle extract, the moisture content increased compared to the control bread. So that the samples containing 25 and 35 ml of extract significantly ($p < 0.05$) ($P < 0.05$) had higher moisture content than other samples, which was also significantly different during storage. According to the results obtained, the lowest moisture content on day zero was for the sample containing 5 ml ($3.81 \pm 31.1\%$) of the treated extract. This amount was due to the presence of nettle extract from the control sample ($1.01\% \pm 31.2\%$) was higher. With increasing amount of extract, the percentage of moisture increase also increased.

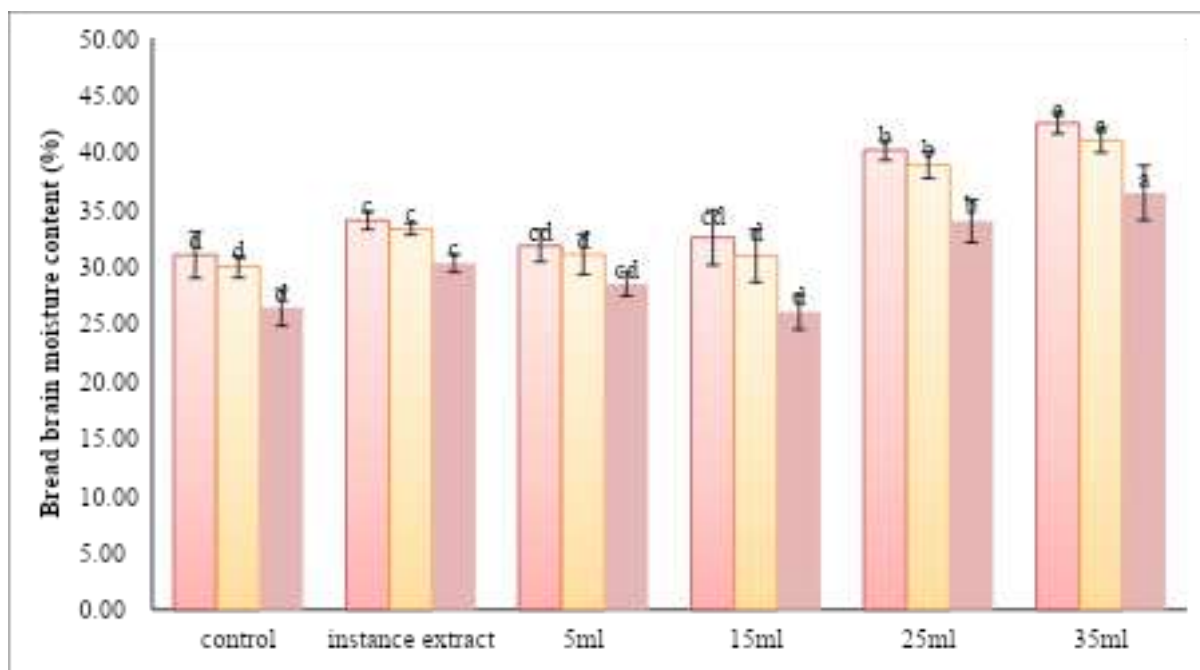


Figure 5. The effect of different concentration of stinging nettle extract on the bread brain moisture content (%)

a-d Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

The decrease in moisture content during storage indicates the onset of staleness in bread and the loss of the ability to retain moisture during storage, which leads to a decrease in the moisture content of the samples. The moisture content is of particular importance during the storage period of food products. Baking time, evaporation of water from the surface and center of the dough, causes changes in moisture content and water activity. Natural baking of bread can lead to a decrease in dough moisture content [23]. Pasrija et al. [23] reported an increase in the moisture content in bread using free extract. They attributed this difference to the presence of dietary fibers and polyphenols in green tea extract and their possible role in moisture retention. The findings of these researchers were in accordance with the results obtained from this study. Sivam et al. [44] considered the increase in bread moisture content in the presence of extract containing polyphenolic compounds to be due to interactions between water and hydroxyl, carbonyl or amine groups with fibers and polyphenolic compounds, as well as flour protein. In the study by Lim et al. [21], who evaluated the addition of different percentages of turmeric powder to bread, a significant difference (0.05) was found ($P >$) in the moisture

content of breads. In the study of Sarabandi et al. [45], the effect of adding malt extract and its role as a sugar source on dough characteristics and quality properties of bread was investigated. The results showed that adding malt extract significantly increased the moisture content of bread. Sahraian et al. [25] showed that by increasing the percentage of rice bran and soy flour in the baguette bread formulation, the moisture content increased significantly (0.05). $P <$ increased at the 5% level. They attributed this increase to the high fiber content and the hydroxyl groups of rice bran and soy flour in their structure and their ability to bind with water molecules in the formulation, which are able to increase the moisture content of the final product.

3-3-3- Bread crust hardness

Figure 6 shows the effect of adding different proportions of nettle extract on the firmness of bread crumb on 3 different days. The results showed that adding different proportions of extract affects the firmness of bread crumb texture, such that with increasing the amount of extract added to bread, the firmness increased. In this study, the firmness of bread crumb for the control sample was between 0.2 ± 0.84 to $0.4 \pm$ It was 2.47 Newtons measured over 3 days.

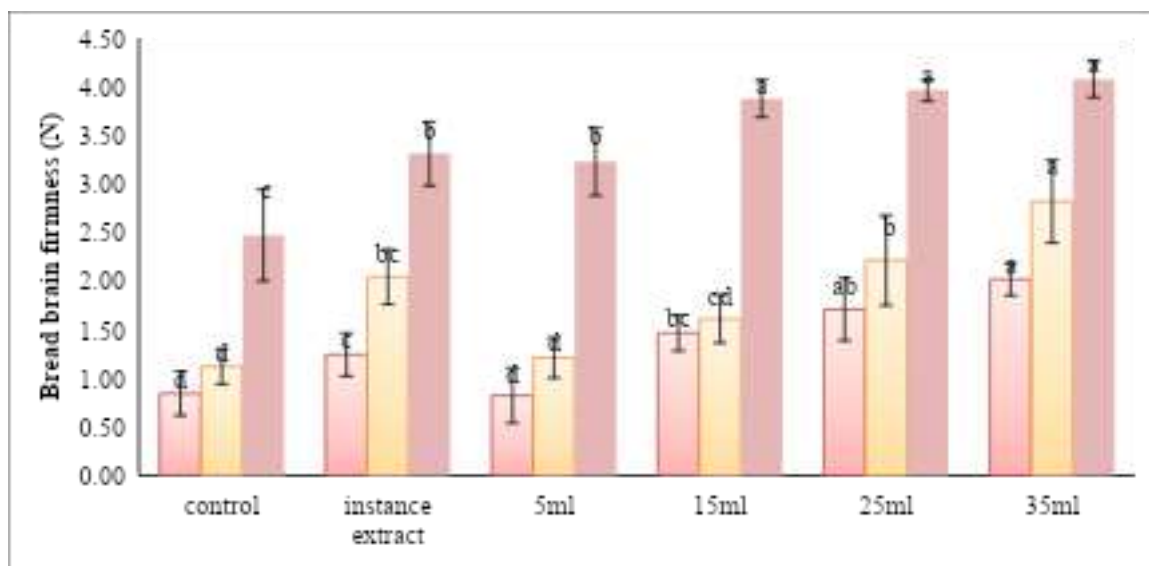


Figure 6. The effect of different concentration of stinging nettle extract on the bread brain firmness (N) a-d Mean values among samples not followed by a common letter differ significantly ($P < 0.05$).

Measuring the firmness of bread crumb is an important characteristic in assessing bread quality [16]. During starch gelatinization, the relatively swollen granules, by creating an elastic state, allow gas expansion in the network. Hence, the texture and structure of the product depend on the gelatinization of starch [23]. As can be seen, the firmness of the bread crumb gradually increased, the reason for which can be sought in the crystallization of starch components, especially amylopectin, during product storage. In addition, the moisture of the product migrated from the crumb to the surface and crust of the sample, leading to the creation of a hard and dry crumb in the bread [46]. By adding nettle extract to the bread, the amount of this increase in firmness increased. Due to the presence of phenolic compounds in the extract, the number of gas cells remained in the dough texture, which in turn can be effective in increasing the firmness of the product texture. With the further increase in the amount of extract, the firmness of the texture also increased. Balestra et al. [16] showed that adding 4.5% ginger powder to bread had the

lowest crumb firmness and adding 6% ginger powder had the highest crumb firmness, which was consistent with the results of the study by Wadhwa et al. [47], who showed that adding 5% ginger powder to bread improved the quality characteristics of bread. Pasrija et al. [23] investigated the texture firmness of breads enriched with green tea extract. The results indicated that the polyphenols in the extract, in competition for moisture with the gluten network and thinning the network, caused a relative increase in the texture firmness of the bread samples. Although this increase in firmness was significant ($P < 0.05$), $P >$ was not. According to the research of Hoogiten et al. [48], humidity has a direct effect on the firmness of bread crumb in such a way that the firmness increases with decreasing humidity. Finally, the results of bread crumb firmness were also in line with the results of specific volume. In this way, bread samples with lower specific volume had higher firmness.

4-3-3- Color of bread crumb and crust

In Table 1, the color values L^* (luminance), a^* (red vs. green) and b^* (yellow vs. blue) The kernels of the bread samples are shown.

Table 1. The effect of different concentration of stinging nettle extract on the bread brain color properties

Treatments	brain color properties		
	L^*	a^*	b^*
control	93.1 ± 0.1^a	-4.46 ± 0.2^d	21.86 ± 0.9^c
Instance extract	80.03 ± 0.5^b	1.73 ± 0.5^{cd}	24.45 ± 0.7^b
5ml	90.84 ± 0.6^a	-2.8 ± 0.6^{cd}	22.02 ± 1.1^c
15ml	80.32 ± 1.3^b	1.99 ± 1.06^c	25.39 ± 0.6^b
25ml	77.61 ± 1.2^c	2.49 ± 0.7^b	28.21 ± 1.1^a
35ml	76.52 ± 0.7^d	3.12 ± 0.2^a	29.56 ± 1.5^a

a-d Mean values in the same column not followed by a common letter differ significantly ($P < 0.05$).

Regarding the color of the bread crumb, as the results show, the value of the L index* which indicates the brightness of the bread, had a decreasing trend with the addition of colored nettle extract. With the further increase of the treated extract to the bread, due to the effect of the treatment in increasing the amount of active ingredient and color of the nettle plant extract, the brightness of the samples decreased, which was a significant difference (0.05). $P < 0.05$ had with each other. This value in terms of indices a* and b* It had an upward trend compared to the control bread.

Bayano et al. [48], in a study on the color characteristics of bread crumb enriched with various types of vegetable extracts, concluded that bread containing chicory, celery, cabbage, fennel, olive leaf and grape pomace extracts had a lower brightness index than control bread. However, by adding these extracts to the bread, the a index* and b* In all cases except the sample containing celery extract, the trend of increase compared to the control bread was shown, which was consistent with the results of this study. In

the report of Lim et al. [12], by adding different percentages of turmeric powder to bread, the L index was increased.*Reduced to indices a* and b* was added to the control bread. This ratio increased with increasing percentages added. Also, Pasrija et al. [23] considered the reason for the color change to be the nature and initial color of the extract used in bread production. In their study, they found a decrease in the L index* (brightness) and increasing the a indices* and b* reported in breads containing green tea extract.

In Table 2, the color values of the crust of bread samples such as L* (luminosity), a* (red vs. green) and b* (yellow vs. blue) is shown. According to the results obtained, the control sample has the highest brightness index (L*), and the lowest index b* and a* Because the control sample did not have any additives, the color of the breads produced was reduced by adding nettle extract. Thus, the L index* Gradually decreasing, but the value of the index a* and b* For the samples, it increased with increasing active ingredient or extract.

Table 2. The effect of different concentration of stinging nettle extract on the bread shell color properties

Treatments	Shell color properties		
	L*	a*	b*
control	71.98±0.7 ^a	12.34±0.7 ^d	52.36±0.7 ^{and}
Instance extract	69.37±0.5 ^b	13.95±0.5 ^c	55.9±0.5 ^c
5ml	71.41±0.9 ^a	12.7±0.5 ^d	54.01±0.4 ^d
15ml	69.18±0.9 ^b	14.46±0.3 ^c	56.7±0.7 ^{bc}
25ml	65.84±0.8 ^c	17.53±0.6 ^b	57.7±0.7 ^b
35ml	61.86±0.6 ^d	19.68±0.8 ^a	59.06±1.3 ^a

a-d Mean values in the same column not followed by a common letter differ significantly ($P < 0.05$).

The color characteristics of bread crust are one of the most important indicators of overall product quality and acceptance. In general, the reactions resulting from Maillard browning during baking as a result of the reaction between reducing sugars and proteins are considered to be effective factors in creating the desired color in the bread crust. These results indicate a significant effect of the compounds in the extract and its initial color on the color indicators of the final products. The findings were consistent with the results of researchers who used different extracts or plant powder in bread [16 and 49]. Sahraian et al. [25], in investigating the effect of mixing wheat flour

with rice bran and soy flour on the color properties of baguette bread, concluded that by increasing the amount of rice bran and soy flour in the formulation, the amount of L components* and b* Increased.

4- Conclusion

Fat oxidation causes oxidative stress, which leads to a decrease in the nutritional value and sensory properties of food products and adverse effects on human health. So far, carcinogenicity and negative effects of synthetic antioxidants on human health have been identified. Therefore, today a new approach has emerged for the use of natural

antioxidants, including plant extracts, in foods. In this study, nettle extract was used as an antioxidant source. Increasing the electric field intensity and the number of pulses initially led to an increase in the total phenolic compounds and antioxidant activity of nettle extract, but with a further increase in these variables, these two parameters decreased. The extract obtained from the pulsed electric field process with an electric field intensity of 1 kV/cm and a pulse number of 40 was selected as the optimal extract. The produced samples showed a significant difference ($0.05 > P$) in the content of total phenolic compounds in different amounts during baking. Bread samples containing treated and untreated nettle extract increased in terms of moisture and firmness of bread texture with increasing extract ratio up to 35 ml. By adding extract to bread, the color of the core and crust had a significant difference compared to the control bread. Finally, bread containing 5 and 15 ml of treated extract had better characteristics than other breads. Finally, it can be concluded that the effect of pulsed electric field at low intensity and pulse number was positive on nettle plant and caused an increase in its phenolic and antioxidant compounds. By adding nettle polyphenolic extract to bread, the phenolic compounds of bread increased and its consumption is recommended as a functional product.

Data access

The data obtained and used in this study will be available upon request from the corresponding author.

Conflict of interest

The authors declare that there are no financial or non-financial conflicts of interest related to the publication of this article.

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Authors' Contributions

Maryam Najafian Mehrabad: Conducted experiments, collected data, participated in data analysis, and prepared the initial draft of the article; Zahra Mazloun: Collected and analyzed data, and scientifically reviewed the article; Mehdi Gharekhani: Designed and directed the research, supervised the implementation of the study, interpreted the results, edited and scientifically reviewed the article, and was responsible for correspondence; Kazem Alirezaloo: Participated in the design of

the research, analyzed and interpreted the results, and scientifically reviewed the article, and was responsible for correspondence.

Statement on the use of artificial intelligence

No artificial intelligence tools were used in the preparation and writing of this article. All scientific content, writing, data analysis, and interpretation of results were done by the authors.

5-Resources

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تأثیر میدان‌های الکتریکی پالسی بر خواص آنتی‌اکسیدانی و پلی‌فنولی گیاه گزنه و اثر آن بر کیفیت نان

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چکیده

اطلاعات مقاله

در این تحقیق به منظور بهینه‌سازی فرآیند استخراج ترکیبات فنولیک از گیاه گزنه در قالب طرح کاملاً تصادفی به کمک پیش تیمار میدان الکتریکی پالسی، از ۳ سطح شدت میدان الکتریکی (۱، ۴ و ۷) و ۳ سطح تعداد پالس (۲۰، ۴۰ و ۶۰)، استفاده گردید. بعد از اعمال پیش تیمارها، فرایند عصاره گیری انجام شد و میزان ترکیبات فنولیک و فعالیت آنتی‌اکسیدانی به دو روش DPPH و FRAP اندازه‌گیری گردید. نتایج نشان داد که تغییر شدت میدان الکتریکی از ۱ kv/cm تا ۷ باعث کاهش ترکیبات فنولیک کل و فعالیت آنتی‌اکسیدانی و تغییر تعداد پالسها از ۲۰ تا ۶۰ باعث افزایش این مقادیر گردید. با توجه به نتایج بهینه‌سازی فرآیند می‌توان بیان نمود که میدان الکتریکی پالسی با شدت میدان ۱ kv/cm و تعداد پالس ۴۰، منجر به حداکثر میزان ترکیبات فنولیک کل و فعالیت آنتی‌اکسیدانی عصاره گردید. به همین منظور عصاره گیاه گزنه تیمارنشده به عنوان نمونه کنترل و عصاره گیاه تیمارنشده با شدت ۱ kv/cm و تعداد ۴۰ پالس، در مقادیر مختلف (۵، ۱۵، ۲۵ و ۳۵ میلی لیتر به ازای ۱۰۰ گرم آرد) برای ارزیابی ویژگی‌های کیفی نان تولیدی افزوده شد. نتایج نشان داد که تفاوت معنی‌داری در محتوای ترکیبات فنولیک کل نمونه های مختلف نان، در طول پخت وجود داشت. به طوری که نان حاوی ۳۵ میلی لیتر عصاره تیمارنشده از بیشترین محتوای ترکیبات فنولیک کل برخوردار بود. همچنین مشخص گردید که میزان رطوبت و سفتی مغز نان با افزایش زمان نگهداری به ترتیب کاهش و افزایش یافت و این دو پارامتر با افزایش نسبت عصاره تا ۳۵ میلی لیتر افزایش پیدا کردند. عصاره تیمارنشده به طور قابل توجهی رنگ مغز و پوسته نان را نسبت به شاهد افزایش داد. در نهایت نمونه های نان حاوی ۵ و ۱۵ میلی لیتر عصاره تیمارنشده به دلیل دارا بودن ویژگی های کیفی بهتر به عنوان نمونه های بهینه انتخاب شدند.

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