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Bioactive Components and Characterization of Extracted *Paeonia officinalis* using Ultrasonic and Microwave Assisted Maceration: Potential Evaluation as a Preservative in Panna Cotta

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Abstract

Preservatives are substances that can prevent or halt fermentation, acidification, and other processes that cause food to decompose. This study aims to extract the root of *Paeonia officinalis* with assistance of ultrasonic (40 kHz, 40 °C for 45 min) and microwave (400 watts, 40 °C, 5 min) maceration techniques, and evaluate the extraction yield, chemical compounds, antioxidant, and antimicrobial properties of the extracts. In the next phase, the best extract is incorporated at 2%, 4%, and 6% into the formulation of Panna cotta dessert to assess its effects on the physical, chemical, sensory, and microbial aspects of the product during storage. The findings reveal that the ultrasonic-assisted method improved the extraction efficiency of the extract. The extract had the highest levels of phenolic compounds (52.64±1.18 mg of gallic acid/g), antioxidant properties (76.33±0.47%), and antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The addition of the extract to Panna cotta reduces the rate of acid production and results in lower total populations of bacteria compared to the control sample at the end of storage period. The dessert containing 2% extract exhibited sensory characteristics (taste, color, odor, texture, and overall acceptance) similar to the control, while maintaining microbiological quality for a longer period. The ethanolic extract of *Paeonia officinalis* root obtained through the ultrasonic-assisted method can be introduced as an effective preservative for dairy desserts.

Keywords: Microwave, *Paeonia officinalis*, Preservatives, Rheological behavior, Ultrasonication

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Introduction

Preservatives play a crucial role in inhibiting or halting fermentation, acidification, and other processes that lead to food decomposition. The primary objective of using preservatives is to prevent the growth of microorganisms responsible for food spoilage and contamination, thereby extending the shelf life of products while maintaining their nutritional value, appearance, and organoleptic qualities (Silva & Lidon, 2016; Mirza *et al.*, 2017). However, concerns have emerged regarding the potential adverse effects of synthetic preservatives on human health, including allergies, cancer, attention-deficit/hyperactivity disorder (ADHD), brain damage, nausea, and heart disease (Gupta & Yadav, 2021). Consequently, both consumers and food producers have shown an increased interest in utilizing natural and organic additives as a safer alternative.

Paeonia officinalis is a flowering plant belonging to the Paeoniaceae family. The root of this plant contains proteins, alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, and steroids. These compounds possess antioxidant and antimicrobial properties (Dulgheru & Burzo, 2010; Park *et al.*, 2021). The extract derived from the root of *Paeonia officinalis* inhibits the acetylcholinesterase enzyme and shows effectiveness in treating Alzheimer, migraine, and neuralgia (Adhami *et al.*, 2011). Based on studies of its sensory qualities, nutrients, and antioxidant activity, it could be used as a food additive (Dienaitė *et al.*, 2019).

The quality and quantity of herbal extracts are significantly influenced by the extraction process (Qamar *et al.*, 2021; Saboora *et al.*, 2014). Ultrasonic-assisted extraction, known for its simplicity and effectiveness, is particularly advantageous. By breaking down the cell wall, this method enhances the release of extracts. However, several factors such as moisture content, particle size, and type of solvent play an important role (Ghafoor *et al.*, 2009; Alupului *et al.*, 2009). In terms of antioxidant activity and enzyme inhibition of edible mushrooms, ultrasonic treatment has been shown to yield extracts rich in ergosterol when the appropriate extraction method is employed (Milovanovic *et al.*, 2021). Microwaves, as a heating method, have also gained widespread acceptance in the food industry, including for extraction processes, due to their cost-effectiveness and efficiency. Microwave treatment facilitates the extraction process by providing rapid, efficient heating that enhances solvent penetration, improves the yield of bioactive compounds, and reduces energy consumption. This makes it a highly advantageous method for industrial extraction processes (Lu *et al.*, 2017).

Due to their nutrient content, dairy products create favorable conditions for microorganisms to grow. To maintain their quality and enhance consumer satisfaction, researchers are exploring natural preservatives as an alternative to synthetic additives. Herbal preservatives such as pomegranate peel, *Melissa officinalis*, and *Valeriana officinalis* extracts have been tested as a natural preservatives in dairy products (Mahajan *et al.*, 2015; Sanjay *et al.*, 2020).

Panna Cotta, a dairy-based dessert, is particularly susceptible to spoilage due to its high moisture content, nutrient density, and neutral pH. These characteristics create a favorable environment for the growth of spoilage microorganisms. As a result, Panna Cotta is prone to rapid deterioration, leading to changes in texture, flavor, and safety. Additionally, the dessert can undergo acidification during storage, which may negatively impact its sensory qualities and shorten its shelf life. To mitigate these issues, traditional preservatives are often employed to inhibit microbial growth and prevent spoilage. However, there is increasing public concern over the use of synthetic preservatives, which have been associated with various health risks, including allergies, cancer, and ADHD. This has spurred interest in natural and organic alternatives that can effectively preserve food products while being perceived as safer and healthier options. The incorporation of natural

preservatives with antimicrobial and antioxidant properties into dairy desserts like Panna Cotta has gained significant attention. Plant-based extracts, such as those derived from *Paeonia officinalis*, offer a promising solution. These extracts contain bioactive compounds, including phenolics and flavonoids, that can inhibit the growth of spoilage microorganisms and delay oxidation processes, thereby extending the shelf life of the product without compromising its sensory attributes.

This study examined the compounds, including their antioxidant and antimicrobial properties, in *Paeonia officinalis* extract using ultrasonic- and microwave-assisted maceration techniques. In the second phase, based on the phenolic compound, antioxidant and antimicrobial activity results, the best extract was used as a preservative at various levels in the Panna cotta formulation, and the physical, chemical, sensory, and microbiological properties of the product were evaluated during storage.

Materials and Methods

Materials

The root of *Paeonia officinalis* was purchased from Isfahan medicinal plant market in Iran. Chemical compounds, such as ethanol, Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, methanol, phenolphthalein, and sodium hydroxide were purchased from Merck in Germany. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Candida albicans* (J 905), and *Aspergillus niger* were obtained from Zistyakhte Company in Iran. The raw materials required to prepare Panna cotta, including milk, sugar, vanilla, gelatin, and cream, were purchased from local markets.

Extraction of Paeonia officinalis

The extraction process was carried out using the maceration method with 70% ethanol. For this purpose, 150 grams of dried *Paeonia officinalis* root were added to 600 mL of 70% ethanol and stirred at 25 °C for 2 h. The mixture was then filtered, and the extract was separated from the solvent using a rotary evaporator (40 °C, 80 rpm, 2 h) to Brix 50 (Ferioli *et al.*, 2020). The extracts were then stored in the dark at 4 °C. To evaluate the influence of ultrasonic and microwave treatments on the quantity and quality of the extracts, the solvent-sample mixture was subjected to 400 watts of microwave irradiation (LG, MH 8265), at 40 °C for 5 min with 30 s rest interval before separating the solvent. Ultrasonic waves were applied to the solvent-sample mixture in an ultrasound bath (WISD, 40kHz, South Korea) at 40 °C for 45 min with a power of 200 watts (Hoehn *et al.*, 2003). The extraction efficiency at Brix 50 was evaluated using the weight method (Hoehn *et al.*, 2003).

Characteristics of Extracts

pH of the extracts was evaluated using the pH meter (Ltd, Shanghai, Sanxin, China) and their density was determined using a digital densitometer.

Separation and identification of the extract's phytochemical compounds were performed using gas chromatography (Agilent 6890) connected to a mass spectrometer (HP 5973). The column of the device (Supelco SLB-5MS) had a length of 30 metres and an internal diameter of 0.25 mm. The sample was dissolved in methanol, and 0.6 microliters of the sample was injected into the column. The injection temperature was set to 280ºC in split mode. The column temperature started at 60 °C and then increased by 7 °C min^{-1}, maintaining it at 270 °C for 45 min. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. (Mothana *et al.*, 2013)

To determine the antioxidant activity of the extract, 0.1 mL of the sample was mixed with 3.9 mL of DPPH and incubated in the dark for 30 min. The absorbance of the samples and the control (without extract) was measured using a spectrophotometer (Shimadzu, UV-1800/2600, Japan) at a wavelength of 517 nm. The percentage of DPPH inhibition was calculated using Eq. 1:

$$Percentage\ of\ inhibition\ (\%): \left(Contrc - \left(\frac{Sample\ OD}{Control\ OD} \right) \right) \times 100 \quad (1)$$

Where 'Sample OD' is the absorbance of the sample, and 'Control OD' is the absorbance of the control.

Total phenolic compounds in the extract were analyzed using the colorimetric method with Folin-Ciocalteu reagent. To perform this analysis, 0.1 mL of the extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted with water at a ratio of 10:1) and allowed to stand in the dark for 10 min. Next, 2 mL of sodium carbonate (7.5%) was added to the mixture, and after 45 min in the dark, the absorbance of the sample was measured using a spectrophotometer at a wavelength of 765 nm. The total phenolic content of the sample was then calculated in mg kg^{-1} using a standard solution of Gallic acid (Mothana *et al.*, 2013).

Antibacterial and Antifungal Activity of Extracts

The antibacterial and antifungal effects of the extracts were determined using the well plate method. For *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), Mueller Hinton agar was utilized as the appropriate culture medium, while Potato Dextrose Agar (PDA) was used as the culture medium for *Aspergillus niger* (ATCC 9029) and *Candida albicans* (J 905). To prepare the microbial suspension, the strains were activated in nutrient broth, cultured on Tryptic soy agar, and incubated at 37 °C for 24 h. *Aspergillus niger* and *Candida albicans* were added to Potato dextrose broth, cultured linearly on PDA, and incubated at 30 °C for 24 h. A suspension of pure colonies of bacteria and fungi with a turbidity equivalent to 0.5 McFarland standard was prepared, and its absorbance was measured at a wavelength of 600 nm using a UV-Vis spectrophotometer. At an absorbance of 0.08-0.1, the concentration of bacteria and fungi was 1.5 x 10^8. To assess the antimicrobial activity of the extract, a standard microbial suspension was cultured on Mueller Hinton Agar for bacteria and PDA for fungi. The extract, sterilized using a 0.45 µm filter, and inoculated into designated holes in the cultures at concentrations of 10, 30, 50, 70, and 100%. Negative controls consisted of Mueller Hinton Broth (MHB) without the extract, while Tetracycline served as the positive control. The bacterial culture was incubated at 37 °C for 24 h and the fungus culture was kept at 30 °C for 3 to 5 days. The antibacterial activity was evaluated by measuring the diameter of the no-growth zone (mm) for microorganisms (Yeasmin *et al.*, 2016). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the extracts were determined using the tube dilution method. Pure extract (1 mL) was added to the first tube containing 1 mL of MHB and mixed by tube vortex (Behdad, Iran). Dilutions were continued in subsequent tubes to create different concentrations of the extract. Then, 10 microliters of the microbial suspension were added to each tube. The positive control contained culture media with bacteria or fungi, but no extract, while the negative control consisted of culture media without bacteria, fungi, or extract. The culture tubes were incubated for 24 h at 37 °C to allow bacterial growth. To ensure accurate assessment of turbidity in the tubes, which may be affected by the colored solution, all inoculated tubes were also cultured on Mueller Hinton agar. Specifically, 10 µL of the suspension from each tube was transferred to Mueller Hinton agar plates and incubated (Sato *et al.*, 2018). To determine the MIC and MFC of fungi, PDB and PDA were used. The cultured tubes and plates were incubated for 48 h at 30 °C and then examined (Sato *et al.*, 2018).

Preparation of Panna Cotta

To prepare the dessert, a mixture of 500 grams of pasteurized milk, 55 grams of whipped cream, 25 grams of sugar, 1 gram of

vanilla, and 14 grams of gelatin was prepared. The mixture was then heated to 37 °C for 5 min. The mixure was cooled to the room temperature, and various concentrations of *Paeonia officinalis* extract (2%, 4%, and 6% w/w) were added to evaluate their impact on the sensory, microbiological, and chemical properties of the product. The samples were stored at 4 °C in polyethylene containers for 21 days.

Characteristics of the Product during the Storage Period

Acidity and pH of Samples

pH of the samples was measured at different time points (1, 7, 14, and 21 days) during the storage of the product at 4 °C using a digital pH meter (Elmentron pH-meter CP-501, Netherlands).

To determine the acid content in the sample, 9 grams of the dessert were titrated in distilled water with 0.1 N Sodium hydroxide. The acid content was reported as mg of citric acid per 100 g of the sample (AOAC 942.15).

Micribiological Properties

To assess the microbiological properties of the samples, a bacterial culture test was conducted using PCA. After 24 h of incubation at 35 °C, colony-forming units (CFUs) were counted. The proliferation and enumeration of yeasts and molds were carried out using Dichloran Rose-Bengal Chloramphenicol agar. Colony counting was performed after 72 h of incubation at 35 °C. Surface culture and incubation at 37 °C for 24 h was performed for *Staphylococcus aureus* on Mannitol Salt Agar, *Escherichia coli* on Eosin Methylene Blue Lactose Sucrose Agar, and Salmonella species on Salmonella Shigella Agar. (Chauhan *et al.*, 2020; Nottagh *et al.*, 2020).

Texture Analysis

Texture of the samples was evaluated using a back extrusion test. A texture analyzer (Santam/stm 20, Iran) equipped with a cylindrical probe of 29-mm diameter at a speed of 60 mm/s was utilized. The samples were placed in containers with a diameter of 36 mm, and their texture was assessed through a reciprocating pressure cycle at 4 °C (VanWees *et al.*, 2020).

The rheological evaluation of the samples was conducted using a Rheometer (Anton Paar Physica MCR 301, Austria). The apparent viscosity was measured across a range of 0.01-100 shear rates at 4 °C (Rezvani *et al.*, 2020). To assess the flow behavior of the samples, the values of shear stress versus shear rate were fitted using the power law equation (Eq. 2) in the Cure Expert 1.4 environment. The coefficient of determination (R^2) was determined to evaluate the fitness of models (Adeli & Samavati, 2015).

$$\delta = K(\gamma)^{n_p} \quad (2)$$

Where δ is the shear stress (Pa), γ is the shear rate (S-1), K is the consistency coefficient (Pa.sn) and n represents the flow behavior index (dimensionless).

The viscoelastic properties of the product were assessed using Cone & Plate geometry in an oscillation test. The strain sweep test was conducted at a constant frequency to evaluate the linear viscoelasticity. Furthermore, in the frequency sweep, the rheological parameters of the product were examined across a frequency range of 0.1 to 50 Hz (Rezvani *et al.*, 2020).

Sensory Analysis

A sensory evaluation was conducted 24 h after production, involving 20 panelists who participated in a 5-point hedonic scale testing. The samples were assessed and compared based on taste, color, texture, odor, and overall acceptance. The highest and lowest scores were assigned to the excellent and poor samples, respectively, for each attribute (Clark, 2009).

Statistical Analysis

Experiments were performen in triplicate and the results for the comparison of extracts from different methods were analyzed using a completely randomized design. In the second part of the research, the

qualitative characteristics of the samples were evaluated and compared using a completely randomized design-factorial test. Mean comparisons were performed using the minimum significant difference (LSD) at the 5% significance level.

Results and Discussion

The Extraction Efficiency and Compounds of the Extract

The extraction efficiency was assessed based on the weight of the extracts at Brix 50. The maceration method yielded an extraction efficiency of 14.7%, while maceration with assistance of microwave and ultrasonic achieved extraction efficiencies of 15.7% and 19%, respectively. A significantly higher extraction efficiency ($p<0.05$) was observed with ultrasonic-assisted extraction compared to the other methods. This can be attributed to the ability of ultrasonic waves with frequencies higher than 20 kHz to induce oscillations within the plant material and mechanically disrupt the cell walls, facilitating solvent access and the release of active components (Chemat *et al.*, 2017). Microwave-assisted extraction utilizes electric and magnetic fields generated by electromagnetic microwaves, which generate heat through bipolar rotation and ion conduction (Shirsath, 2012; Bagade & Patil, 2021). The heated solvent in the presence of microwave energy disrupts the matrix, leading to enhanced mass transfer. This explains the higher extraction efficiency observed in microwave-assisted extraction compared to the traditional maceration method (Shirsath, 2012; Bagade & Patil, 2021).

Table 1 presents the compounds identified in *Paeonia officinalis* extracts, including monoterpenes (trans-Linalool oxide, alpha-Terpinene), sesquiterpenes (Caryophyllene oxide, Humulene, alpha-Guaiene), and benzopyrans (Heneicosane, Encecalan, Benzaldehyde). The sesquiterpenes and benzopyrans were the predominant compounds in the *Paeonia officinalis* extract. Maceration yielded six identified compounds, with benzoic acid (19.04%), Heneicosane (15.81%), Humulene (6.93%), Benzaldehyde (3.99%), alpha-Terpinene (3.29%), and 1,3-dimethyl-benzene (1.29%) being the major components. In the microwave-assisted extract, five compounds were identified, with benzoic acid (14.44%), Benzaldehyde (5.74%), Humulene (4.49%), Heneicosane (3.30%), and Encecalan (2.64%) being the quantitatively significant ones. The ultrasonic-assisted extract contained seven identified compounds, with Caryophyllene oxide (35%), Benzoic acid (21.14%), Humulene (8.42%), Benzaldehyde (5.80%), alpha-Guaiene (4.22%), Encecalan (2.53%), and trans-Linalool oxide (1.95%) being the prominent. Notably, the ultrasonic-assisted extract showed higher concentrations of Benzoic acid, Benzaldehyde, and Humulene compared to other methods. Furthermore, the ultrasonic-assisted extract contained special compounds such as caryophyllene oxide, alpha-Guanine, and trans-linalool oxide, known for their antioxidant properties. The application of ultrasound in the solid-liquid phase creates a cycle of contraction and expansion, leading to the formation and collapse of bubbles (cavitation). This phenomenon induces oscillation of solid and liquid particles, enhancing the diffusion of soluble material from the solid phase to the solvent (Blanco-Llamero *et al.*, 2022). The increased extraction efficiency of the active compounds can be attributed to emulsification and tissue damage caused by ultrasound. Therefore, the ultrasonic-assisted extraction method exhibited significantly higher extraction efficiency for specific components of *Paeonia officinalis*, particularly Benzaldehyde, Benzoic acid, alpha-Guanine, Caryophyllene oxide, trans-linalool oxide, and Humulene, known for their antioxidant and antimicrobial activity.

Table 1- Components of aqueous-alcoholic *Paeonia officinalis* extracts

Compounds	Molecular Formula	*Retention Time* (min)	*Kovats Index*	Extraction method		
				Maceration	Microwave-assisted	Ultrasonic-assisted
2-hexenal	$C_6H_{10}O$	3.84	850	6.95%	-	2.50%
1,3-dimethyl-benzene	C_8H_{10}	4.576	897	1.29%	-	-
Benzaldehyde	C_6H_5CHO	5.741	960	3.99%	5.74%	5.80%
α-terpinene	$C_{10}H_{16}$	7.602	1021	3.29%	-	-
trans-Linalool oxide	$C_{10}H_{18}O_2$	7.181	1071	-	-	1.95%
Benzoicacid	$C_7H_6O_2$	9.023	1164	19.04%	14.44%	21.14%
α-guaiene	$C_{15}H_{24}$	10.984	1260	-	-	4.22%
2-butyl-2-octenal	$C_{12}H_{22}O$	12.043	1287	3.85%	-	-
Humulene	$C_{15}H_{24}$	13.802	1350	6.93%	4.49%	8.42%
caryophyllene oxide	$C_{15}H_{24}O$	14.55	1475	-	-	35%
Heptadecane	$C_{17}H_{36}$	15.99	1566	28.1%	33.16%	5.28%
Phytone	$C_{24}H_{48}O_7$	17.36	1609	-	4.05%	1.47%
Heneicosane	$C_{21}H_{44}$	19.56	1741	15.81%	3.30%	-
Encecalan	$C_{14}H_{16}O_3$	23.56	2056	-	2.64%	2.53%

Characteristics of the Aqueous-alcoholic Extract of *Paeonia officinalis*

Table 2 presents the quality characteristics of the aqueous-alcohol extracts of *Paeonia officinalis*. The extraction method had a significant impact on the pH of the extracts. The ultrasonic-assisted method produced extracts with significantly higher pH values compared to other methods ($p<0.05$). Additionally, the maceration method yielded extracts with significantly higher pH values than the microwave-assisted method ($p<0.05$). This variation in pH can be attributed to the differential solubility of *Paeonia officinalis* components, including phenols, flavonoids, anthocyanins, tannins, organic acids, carbohydrates, sugars, and other compounds, under different extraction conditions (Batinić *et al.*, 2022). The composition of compounds such as carbohydrates and proteins also contribute to changes in the pH of the extract (Ebringerová & Hromádková, 2010). The density of the extracts obtained through the ultrasonic-assisted method was significantly higher ($p<0.05$) compared to those produced through maceration. However, no significant difference in density was observed between the extracts obtained through ultrasonic and microwave-assisted extraction ($p<0.05$). As a result, the maceration extracts exhibited the lowest density, whereas the ultrasonic-assisted extracts had the highest density, as shown in Table 2. The variation in extract density can be attributed to the impact of extraction conditions on the extraction of different quantities and qualities of *Paeonia officinalis* compounds, such as phenols, flavonoids, anthocyanins, tannins, carbohydrates, sugars, and others (Batinić *et al.*, 2022).

The phenolic content and antioxidant activity in the extract obtained through ultrasound-assisted extraction was significantly higher ($p<0.05$) compared to the extract obtained through maceration. However, there was no significant difference between the ultrasound and microwave-assisted methods in this regard ($p<0.05$). Notably, the extract obtained through maceration exhibited the lowest total phenolic content as shown in Table2.

The superior extraction efficiency of phenolic components and antioxidant activity can be attributed to the shear forces and high energy produced by ultrasonic waves, which effectively disintegrate cell walls, enhance

mass transfer, and facilitate the release of their contents. Additionally, ultrasonic extraction reduces particle size, promoting better contact and diffusion of the solvent into the tissue (Kumar *et al.*, 2021). On the other hand, in microwave-assisted extraction, the energy from electromagnetic waves is converted to heat, leading to the evaporation of water and an increase in pressure within the cells. This process causes cell disintegration and facilitates the release of active compounds into the solvent, thereby enhancing the extraction yield of active compounds such as polyphenols (Kumar *et al.*, 2011). In a study carried out on comparing microwave-assisted extraction with traditional method for extraction of phenolic compounds from walnut leaves, it was found that microwave-assisted extraction demonstrated higher efficiency and required less time compared to the traditional method (Salimi & Majd, 2012).

Table 2- Physicochemical properties of *Paeonia officinalis* extract

Extraction method	Extraction efficiency (%)	pH	Density ($g.cm^{-3}$)	Phenolic compounds (mg of Gallic acid/g)	Antioxidant activity (%)
Maceration	14.73±0.33 ^{c}	4.28±0.01 ^{b}	1.07±0.07 ^{b}	46.60±0.87 ^{b}	42.06±1.34 ^{b}
Ultrasonic-assisted extraction	18.97±0.34 a	4.31±0.01 ^{a}	1.22±0.03 ^{a}	52.64±1.18 ^{a}	76.33±0.47 ^{a}
Microwave-assisted extraction	15.69±0.34 ^{b}	4.22±0.01 ^{c}	1.11±0.01 ^{ab}	49.73±1.03 ^{ab}	46.66±3.14 ^{b}
LSD	0.67	0.02	0.14	3.29	6.33

*In each column, means with different letters are significantly different at the 5 percent level of the LSD test.

Antimicrobial Properties of *Paeonia officinalis* Extract

Table 3 presents the impact of *Paeonia officinalis* extracts derived through different methods and their concentrations on microorganisms activity. The diameter of the zone of inhibition of *Escherichia coli* and *Staphylococcus aureus* by ultrasonic-assisted extract was significantly larger than the other extracts ($p<0.05$). The microwave-assisted extract exhibited greater antimicrobial properties compared to the maceration extract. However, in some concentrations, there was no significant difference in the antimicrobial activity between the maceration and microwave-assisted extracts ($p<0.05$). In the case of microwave-assisted extraction, the rapid rise in temperature and internal pressure accelerated the breakdown of cell walls and facilitated the release of antimicrobial compounds from tissue into the solvent (Swaathy *et al.*, 2014). At most concentrations, there were no significant variations among the *Paeonia officinalis* extracts produced using different methods in terms of their efficacy at inhibiting the growth of *Candida albicans* ($p<0.05$).

Table 4 displays the impact of different extraction methods on the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of *Paeonia officinalis* extract against microorganisms. The antibacterial activity of the maceration and ultrasonic-assisted extracts was greater than that of the microwave-assisted extract against *Escherichia coli*. The ultrasonic-assisted extract exhibited the lowest MIC and MLC values against *Candida albicans* and *Aspergillus niger* ($p<0.05$), while all extracts showed similar inhibitory and lethal effects on *Staphylococcus aureus* ($p<0.05$).

Table 3- Non-growth halo diameter of microorganisms near the different concentrations of *Paeonia officinalis* extracts

	Method	Concentration				
		10%	30%	50%	70%	100%
Escherichia coli	Maceration	13.50±2.12 a	19.00±1.41 a	15.50±0.71 b	19.00±2.83 a	24.00±1.41 b
	Ultrasonic-assisted	16.00±1.41 a	20.00±1.40 a	25.00±2.83 a	26.00±1.41 a	29.00±1.41 a
	Microwave-assisted	10.00±0.14 b	12.00±1.41 b	14.50±0.70 b	17.50±3.53 a	22.00±1.40 b
Staphylococcus aureus	Maceration	23.50±2.12 a	33.50±2.12 a	36.00±1.41 a	38.50±0.71 a	42.00±1.41 a
	Ultrasonic-assisted	25.00±1.41 a	30.00±2.83 a	37.50±3.54 a	32.50±3.54 a	42.00±2.83 a
	Microwave-assisted	20.00±1.41 a	16.50±2.11 b	20.00±1.41 b	20.00±1.41 b	24.00±1.41 b
Candida albicans	Maceration	10.00±0.71 a	14.00±1.41 a	21.50±2.12 a	21.50±0.71 a	28.50±2.12 a
	Ultrasonic-assisted	10.50±0.71 a	14.00±1.41 a	19.50±0.71 a	20.00±0.41 a	28.50±2.12 a
	Microwave-assisted	11.50±2.41 a	15.50±2.12 a	18.00±2.83 a	16.00±1.40 b	28.00±2.83 a

*In each column, means with different letters are significantly different at the five percent level of the LSD test.

Table 4- Effect of the *Paeonia officinalis* extract on the MIC and the MLC of microorganisms (µL/mL)

Extraction Method	*Escherichia coli*		*Staphylococcus aureus*		*Candida albicans*		*Aspergillus niger*	
	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC
Maceration	12.50 b	10.00 b	25.00 a	12.50 a	25.00 a	12.50 a	50.00 a	30.00 a
Ultrasonic-assisted	12.50 b	10.00 b	25.00 a	12.50 a	12.50 b	6.25 b	25.00 b	20.00 b
Microwave-assisted	25.00 a	12.50 a	25.00 a	12.50 a	25.00 a	12.50 a	50.00 a	25.00 ab
LSD	2.91	1.84	2.95	1.60	3.95	1.67	5.95	6.36

*In each column, means with different letters are significantly different at the five percent level of the LSD test. Mean ± standard deviation

Consequently, we observed a higher level of antimicrobial activity in the ultrasonic-assisted extract compared to others. In this regard, it has been reported that the utilization of ultrasonic technology, through its ability to disrupt cell membranes and improve mass transfer, led to an increased extraction rate of compounds such as Benzoic acid and Benzaldehyde with antimicrobial and antioxidant properties from the roots of *Paeonia officinalis* (Lu *et al.*, 2017).

Characteristics of Panna Cotta during the Storage Period

pH is a critical factor that significantly impacts the quality of dairy desserts. As per the Iranian standard (IDS 1143), the acceptable pH range for dairy desserts is ranged between 3.6 and 6.8. In this study, the pH values of the control and other treatments fell within this standard range. The presence of different concentrations of aqueous-alcoholic extract from *Paeonia officinalis* root exerted a noticeable influence on the pH changes of Panna cotta during a 21-day storage period. The samples with extract exhibited lower pH values compared to the control. This increased acidity in the extract-containing samples during the initial storage period can be attributed to the presence of carboxyl groups in triterpenoids and organic acids, as well as the acidic nature of the compounds found in the root extract of *Paeonia officinalis*, including phenolic compounds (Stagos, 2019).

Over the course of 21-day storage period, the pH of the samples experienced a significant decrease ($p<0.05$) (Fig. 1-a). It can be attributed to microbial activity, which leads to the

production of acids (Barbieri *et al.*, 2019), as well as the breakdown of ester groups and their conversion into acids. The proliferation of acid-resistant non-pathogenic bacteria also contributes to pH fluctuations in the product (Stagos, 2019). However, the incorporation of the aqueous-alcoholic extract of *Paeonia officinalis* effectively mitigated the rate of pH reduction during storage, likely due to the extract's ability to inhibit microbial activity (Barbieri *et al.*, 2019). Consequently, the addition of the aqueous-alcoholic extract of *Paeonia officinalis* prevented excessive pH decline in Panna cotta during storage, resulting in higher pH values compared to the control after 7 days of storage. In a similar vein, the molded yogurt demonstrated an increase in acidity during storage, although the sample containing the highest concentration of spinach extract exhibited the lowest acid content (Ahmad *et al.*, 2022). In the other study, the acidity of ketchup was unaffected by different concentrations (0.2%, 0.5%, and 0.8%) of aqueous and ethanolic extract of black Hollyhocks during storage (Yourdkhani & Jafarpour, 2021).

There were no significant changes in the firmness of the samples produced with different concentrations of the extract and the control up to 7 days of storage ($p<0.05$), and there were no significant differences observed in samples at 14 days. However, on the 21st day of storage, a significant increase in sample stiffness was observed ($p<0.05$). The samples containing 2% and 4% of the extract exhibited a lower increase in stiffness compared to the others, resembling the control samples more closely at the beginning of production (Fig. 1-b). This increase in sample stiffness can be attributed to the phenomenon of water transfer from the gel structure of the product during the storage period, resulting in a denser texture (Djaoud *et al.*, 2020). Additionally, the interaction between the compounds in the extract, particularly the phenolic components, with proteins or polysaccharides is another factor contributing to the increased sample stiffness (Jridi *et al.*, 2015).

The total bacterial count in the samples with different concentrations of the extract and the control remained unchanged over 14 days ($p<0.05$). However, on the 21st day of storage, there was a significant decrease in the total bacterial of the samples with different extract concentrations compared to the control ($p<0.05$). This indicates that the extract is effective in reducing the overall bacterial count and maintaining the microbial quality of Panna cotta during extended storage periods ($p<0.05$). Higher concentrations of the extract (4% and 6%) were effective in reducing the bacterial population and preserving the microbial quality of the Panna cotta (Fig. 1-c). *Staphylococcus aureus*, *Escherichia coli*, and Salmonella were not observed in the samples. The presence of antimicrobial compounds in the alcoholic extract of *Paeonia officinalis*, such as Benzoic acid and Benzaldehyde, inhibits the growth of microorganisms, slows down their population growth, and ultimately prevents product spoilage. The interaction between polyphenols and proteins alters the biological activity of microorganisms and inhibits their growth and activity. Therefore, the polyphenol content in the extract limits the growth and activity of bacteria (Walter, 2021).

Rheological Properties of Panna Cotta

Fig. 2 illustrates the flow behavior of Panna cotta at the beginning (day 1) and end (day 21) of the storage period. The samples exhibited shear-thinning behavior, as indicated by a decrease in apparent viscosity with increasing shear rate. There were no significant differences in the flow behavior and consistency coefficients of the samples containing the extract and the control based on the Power-law model. However, the apparent viscosity of samples increased at the end of the storage period (day 21) compared to the initial viscosity, and the samples with higher extract concentrations had a significantly lower flow behavior index (Table 5). The increase in viscosity during storage is attributed to the reorganization of proteins and their hydration (Rezvani *et al.*, 2020).

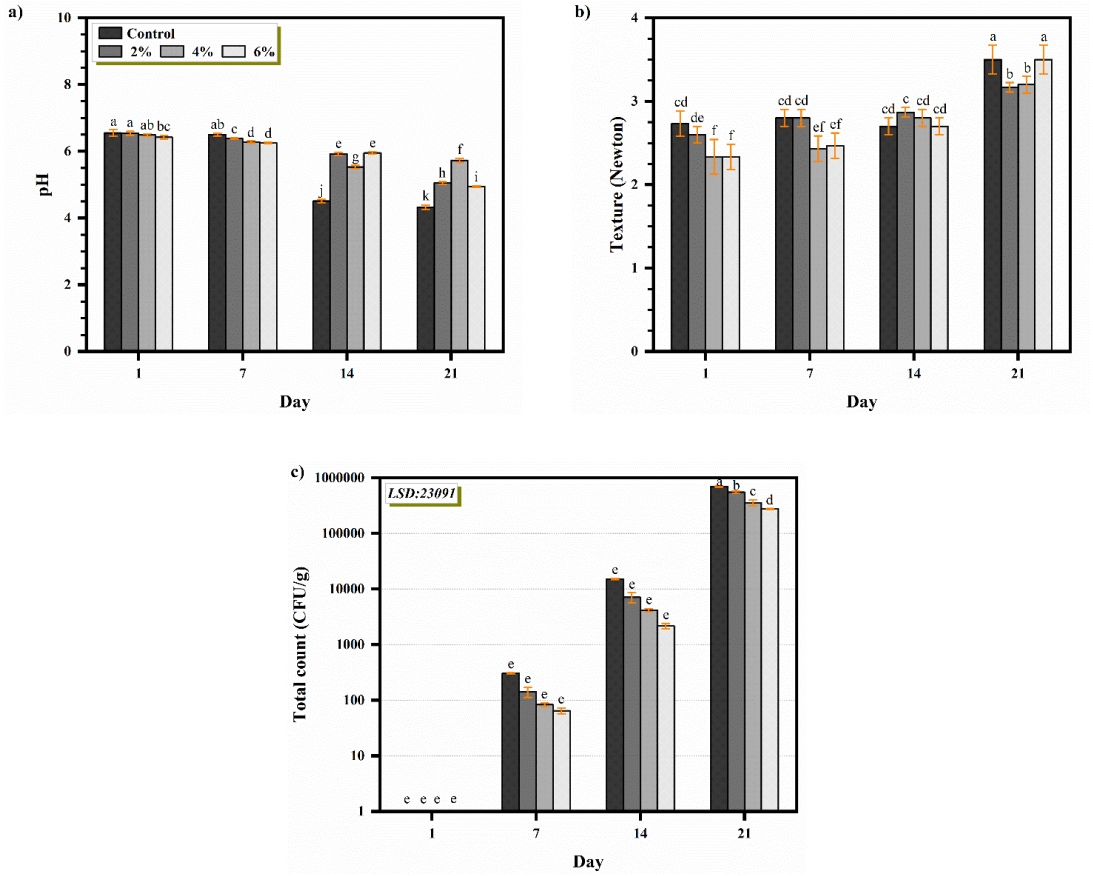

Fig. 1. Effect of different concentrations of aqueous-alcoholic extract of *Paeonia officinalis* and storage time on the quality of Panna cotta (each color is indicated in Fig. 1a).

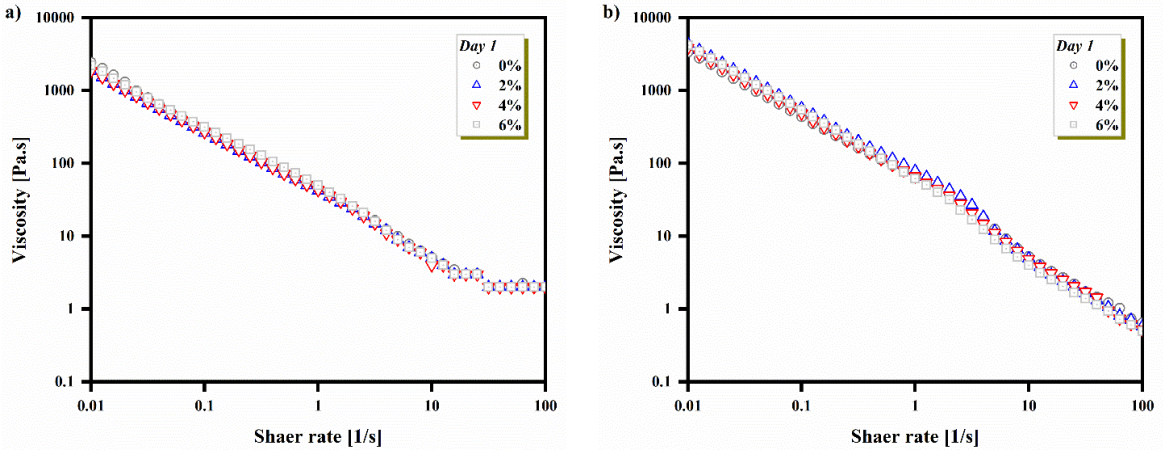

Fig. 2. Apparent viscosity of Panna cotta at (a) the beginning and (b) the end of the storage period

Table 5- Rheological parameters of the power law model in different samples

Storage period (Day)	Extract (%)	Rotation test	
		Consistency coefficient (K)	Flow behavior index (n)
1	0	40.205±3.344 ^{c}	0.265±0.021 ^{a}
	2	37.3±0.862 ^{c}	0.24±0.00 ^{ab}
	4	41.585±4.504 ^{c}	0.21±0.028 ^{bc}
	6	46.545±1.251 ^{c}	0.15±0.0141 ^{d}
21	0	64.225±6.795 ^{b}	0.165±0.021 ^{cd}
	2	81.31±4.751 ^{a}	0.13±0.00 ^{d}
	4	64.91±4.907 ^{b}	0.14±0.028 ^{d}
	6	62.745±1.661 ^{b}	0.081±0.024 ^{e}
LSD		9.27	0.047

*In each column, means with different letters are significantly different at the five percent level of the LSD test. Mean ± standard deviation

In the frequency sweep, the storage modulus (G') was higher than the viscous modulus (G"), indicating that the samples exhibited solid viscoelastic behavior. The slight changes in G' and G" with increasing frequency suggest the presence of a semi-gel-like structure in the samples. At the beginning of the storage period (day 1), the rheological properties of samples with different extract concentrations were similar. However, at the end of the storage period (day 21), the dessert containing 2% extract exhibited the highest elastic modulus (G'). On the other hand, the sample with 6% extract showed the highest damping factor (tan delta), indicating its weaker elastic behavior (Fig. 3).

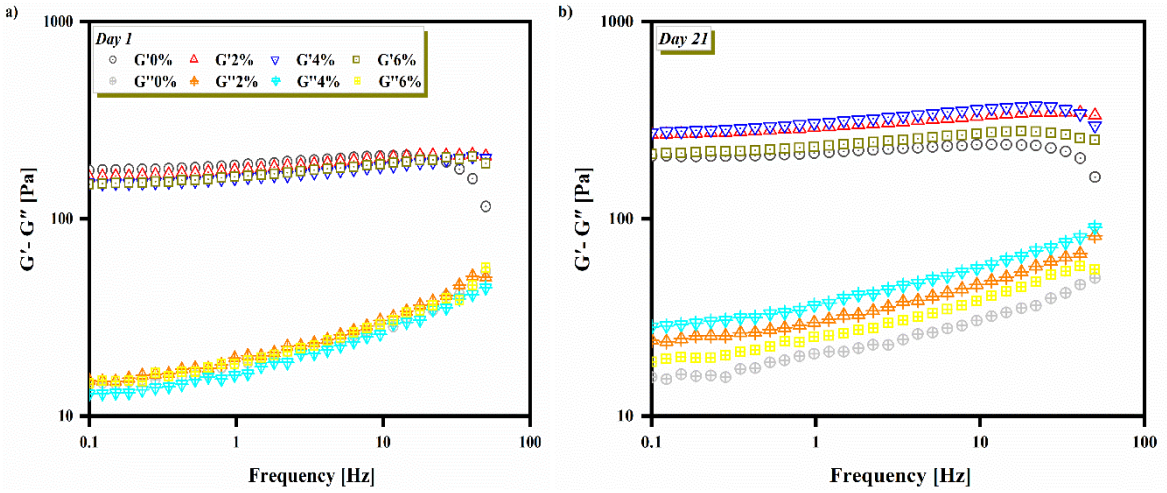

Fig. 3. Frequency sweep of different samples at (a) the beginning, and (b) the end of storage period

Sensory Analysis

The results of the sensory evaluation for Panna cotta prepared with different extract concentrations are presented in Fig. 4. The control and the sample with 2% extract received high scores and did not show significant differences in terms of taste, color, odor, texture, and overall acceptance ($p<0.05$). The sample with 4% extract had a similar color and odor to the control, and was preferred over the sample with 6% extract in terms of taste, color, odor, texture, and overall acceptance ($p<0.05$). Therefore, the sample with 2% extract closely resembled the control in sensory characteristics (taste, color, odor, texture, and overall acceptance). The presence of a bitter, astringent, or woody taste reduced the organoleptic perception of samples with high extract concentrations (Walter, 2021), leading to a decrease in overall acceptance. Similarly,

high concentrations of other extracts and essential oils used as preservatives in food have been reported to negatively affect the organoleptic quality of products. For example, the use of ginger extract in concentrations above 5% in yogurt has been found to significantly reduce overall acceptance (Ahmadi, 2020; Raikos, 2018).

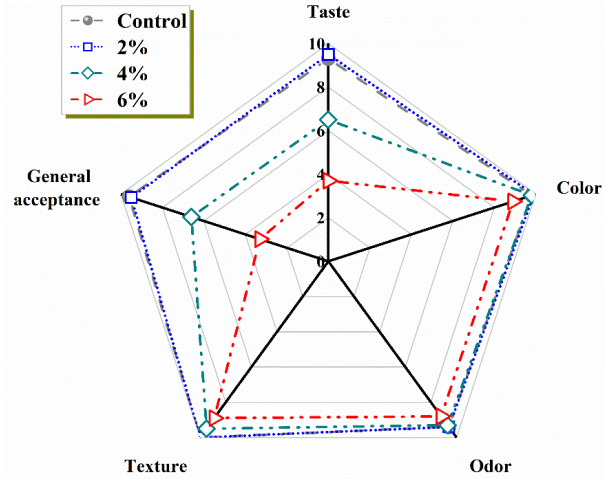

Fig. 4. Sensorial properties of Panna cotta samples

Conclusion

The findings of the current investigation demonstrated that the ultrasonic-assisted method exhibited superior extraction efficiency and yielded more potent compounds compared to the microwave-assisted method and traditional maceration. The ultrasonic-assisted extract showcased the highest content of phenolic compounds, exceptional antioxidant properties, and notable antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Furthermore, the pH, acidity, and firmness of the Panna cotta, formulated with varying concentrations of the extract, exhibited lower changes during storage compared to the control. The total bacterial population was also significantly lower in these samples compared to the control. Notably, the Panna cotta prepared using 2% *Paeonia officinalis* extract closely resembled the control in terms of texture, viscosity, and sensory characteristics, encompassing taste, odor, color, texture, and overall acceptance. It is concluded that ultrasonic-assisted extraction improves the yield and quality of bioactive compounds from *Paeonia officinalis* and can be used as a natural preservative in dairy desserts, such as Panna cotta. Future research on the long-term shelf life and effectiveness of *Paeonia officinalis* extract, along with other natural preservatives in various dairy products and storage conditions are recommended.

Declaration of Conflicting Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

This article is extracted from Mrs. **Fallahpour Sichani**'s thesis presented at Islamic Azad University, Isfahan (Khorasgan) Branch under the supervision of Dr. Abbasi. Scientific and statistical analysis corrections were made by **Hajar Abbasi**.

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مقاله پژوهشی

جلد ۲۰، شماره ۶، بهمن-اسفند، ۱۴۰۳، ص. ۱۳۶-۱۱۹

بررسی ترکیبات و خواص کیفی عصاره *Paeonia officinalis* استخراج شده به روش غوطه‌وری با اعمال امواج ماوراءصوت و مایکروویو: ارزیابی بالقوه عصاره به‌عنوان یک نگهدارنده در دسر پاناکوتا

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چکیده

نگهدارنده‌ها موادی هستند که می‌توانند از تخمیر، اسیدی شدن و سایر فرآیندهایی که موجب فساد غذا می‌شوند، جلوگیری یا آن‌ها را متوقف کنند. این پژوهش با هدف استخراج عصاره ریشه گیاه *Paeonia officinalis* با کمک اعمال روش امواج فراصوت (۴۰ کیلوهرتز، ۴۰ درجه سانتی‌گراد به‌مدت ۴۵ دقیقه) و مایکروویو (۴۰۰ وات، ۴۰ درجه سانتی‌گراد، ۵ دقیقه) در روش غوطه‌وری و ارزیابی بازده استخراج، ترکیبات شیمیایی، خواص آنتی‌اکسیدانی و ضدمیکروبی عصاره‌ها انجام گرفت. در مرحله بعد، بهترین عصاره به میزان ۲، ۴ و ۶ درصد به فرمولاسیون دسر پاناکوتا اضافه شد تا اثرات آن بر ویژگی‌های فیزیکی، شیمیایی، حسی و میکروبی محصول در طول مدت نگهداری بررسی شود. یافته‌ها نشان می‌دهند که اعمال روش امواج فراصوت راندمان استخراج عصاره را بهبود می‌بخشد. این عصاره دارای بالاترین سطوح ترکیبات فنلی (۱/۱۸±۵۲/۶۴ میلی‌گرم اسید گالیک در هر گرم)، خواص آنتی‌اکسیدانی (۰/۴۷±۷۶/۳۳ درصد) و فعالیت ضد میکروبی در برابر *Escherichia coli*، *Staphylococcus aureus* و *Candida albicans* بوده است. افزودن عصاره به پاناکوتا نرخ تولید اسید را در محصول کاهش می‌دهد و منجر به کاهش جمعیت کل باکتری در مقایسه با نمونه شاهد در پایان دوره نگهداری می‌شود. دسر حاوی ۲ درصد عصاره ویژگی‌های حسی (طعم، رنگ، بو، بافت و پذیرش کلی) مشابه نمونه شاهد داشته در حالی‌که کیفیت میکروبیولوژیکی آن برای مدت طولانی‌تری حفظ گردید. عصاره اتانولی ریشه *Paeonia officinalis* که با اعمال روش امواج فراصوت در روش غوطه‌وری استخراج شده است، می‌تواند به‌عنوان یک نگهدارنده مؤثر برای دسرهای لبنی معرفی شود.

واژه‌های کلیدی: پائونیا، رفتار رئولوژیکی، فراصوت مایکروویو، نگهدارنده‌ها

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