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Optimization of Probiotic Biscuit Formulation Containing Corn Flour, Buckwheat Flour, and Inulin Using Mixture Design and Evaluation of Functional and Quality Properties

Aynaz Gharebashi, Rahil Rezaei[1*], Hamid Bakhshabadi[2]

1-Department of Food Science and Technology, GKM.C., Gonbad Kavoos & minoodasht Branch, Islamic Azad University, Gonbad Kavoos, Iran

2-Department of Agriculture, Minab Higher Education Center, University of Hormozgan, Bandar Abbas, Iran

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*Corresponding Author E-

rezaei.rahil@yahoo.com

ABSTRACT

Functional foods, beyond fulfilling nutritional requirements, contribute to health promotion and disease risk reduction. This study aimed to optimize the formulation of a gluten-free probiotic biscuit using a mixture design to evaluate the effects of buckwheat flour (0–100%), corn flour (0–100%), and inulin (0–3%) on water-holding capacity, storage and loss moduli, hardness, volume, L* index, probiotic viability, and overall acceptance of the flour, dough, and final product. All responses were significantly affected by the mixture components ($p < 0.05$). The optimal formulation for maximizing probiotic count, L* index, volume, and overall acceptance while minimizing hardness consisted of 23.83% buckwheat flour, 74.16% corn flour, and 2.02% inulin, with an overall desirability of 0.769. Under these conditions, water-holding capacity reached 9.0 g/g, G' 1.39 × 10^6 Pa, G" 414,090 Pa, hardness 13.39 N, volume 4.16 cm^3, probiotic count 9.77 log CFU/g, L* index 39.85, and overall acceptance 3.91. These findings highlight the potential of the optimized formulation for developing a gluten-free functional biscuit with desirable technological quality and enhanced probiotic stability.

1- Introduction

Natural antioxidants have multiple health benefits and help prevent various chronic diseases by reducing oxidative stress. Moreover, the use of antioxidants is an effective and efficient strategy for delaying lipid oxidation and extending the shelf life of food products [1]. Natural antioxidants derived from plant sources have garnered significant attention in recent years. These antioxidants not only reduce the rate of lipid oxidation in food products but also possess various biological activities and health benefits, often demonstrating antioxidant, anti-inflammatory, anticancer, and antimicrobial properties [2]. Therefore, incorporating extracts from plant sources into food products can produce functional foods while maintaining quality [3]. Althaea officinalis L., known as marshmallow, is a valuable plant from the Malvaceae family, with flowers available in various colors, including black, white, and pink. Black marshmallow is a good source of carotenoids, anthocyanins, phenolic acids, flavonols, and polyphenols, exhibiting anti-aging activities, enzyme inhibition, and antimicrobial antioxidant functions [4]. Taraxacum officinale, or dandelion, is a perennial plant belonging to the Asteraceae family. It is found in Europe, Asia, and North America and often grows as a weed in many gardens, pastures, and wastelands [5]. Dandelion possesses numerous medicinal properties due to the phytochemical compounds in its flowers, leaves, stems, and roots. The main phytochemical compounds in this plant include carotenoids, flavonoids, polysaccharides, sesquiterpene lactones, sterols, and triterpenes [6]. Dandelion has been used for various health conditions, including intestinal diseases, colitis, stomach strengthening, menstruation regulation, cholesterol reduction, anemia treatment, rheumatism, and gout. It also exhibits antiviral, liver-protective, antifungal, antibacterial, antidiabetic, anti-obesity, antioxidant, anticancer, and anti-arthritis effects [7]. Olea europaea L., or the olive tree, is a valuable tree widely cultivated in Mediterranean regions. Due to its nutritional value and health benefits, the consumption of olives and olive oil is increasing globally [8]. During the cultivation and processing of olive fruit, a large volume of unusable by-products is generated, with leaves being the most significant by-product. Their quantity varies depending on the olive tree variety, tree age, environmental conditions, and agricultural practices [9]. Disposing of these by-products often poses environmental challenges and economic losses. However, olive leaves are valuable products containing significant amounts of beneficial phytochemical compounds, particularly oleuropein, rutin, verbascoside, hydroxytyrosol, and tyrosol. Olive leaves exhibit various biological effects, including antimicrobial and antioxidant activities, and have potential applications as a natural preservative in pharmaceutical, health, and food industries [10]. For extracting extracts from various plant sources, in addition to conventional methods such as maceration, Soxhlet extraction, and percolation, several modern techniques have been developed in recent years, including microwave-assisted extraction, ultrasound, supercritical fluid extraction, pressurized liquids, and ohmic heating. These methods generally demonstrate higher efficiency in extracting phenolic and bioactive compounds while using less solvent, offering shorter operation times, and being suitable for heat-sensitive compounds, thereby presenting advantages over conventional methods. For example, modern methods require less solvent, have shorter operation times, and can be applied to heat-sensitive bioactive compounds [11]. Noteworthy research in this field includes a study by Mahdiania-Lechayi et al. (2018), which successfully used modern processes, including ultrasound and supercritical fluid extraction, to extract plant materials with high efficiency [12]. Another study reported the superior efficiency of the

ultrasound method compared to conventional methods in extracting phenolic compounds from Leptospermum petersonii [13].

Although numerous studies have investigated various extraction methods and the antioxidant activity of plant species, emphasizing the importance of using natural bioactive compounds in the food industry [1, 11], most of this research has focused on a single plant species (such as black hibiscus, dandelion, or olive leaves) or solely one extraction method (such as maceration, Soxhlet extraction, ultrasound, or supercritical fluid extraction). For example, recent studies on black hibiscus [4], dandelion [5–7], and olive leaves [8–10] have typically assessed only one plant species and primarily one extraction method. However, there has been less attention on the simultaneous comparison of multiple medicinal plants using both conventional and modern extraction methods within a unified framework [11–13]. This situation has resulted in a lack of comprehensive evaluation of the efficiency of different extraction methods on multiple medicinal plants under similar experimental conditions. Furthermore, it has hindered the thorough investigation of relationships between the contents of phenolic and flavonoid compounds, phytochemical profiles, and various antioxidant activity indices. The aim of this study was to investigate the effect of three extraction methods (traditional solvent extraction, ultrasound, and supercritical fluid extraction) on the content of bioactive compounds (total phenols and total flavonoids), antioxidant activity (assessed using DPPH, FRAP, and ABTS methods), and the phytochemical profile of extracts from dandelion flowers, olive leaves, and black hibiscus flowers. Additionally, this study assessed the relationships between total phenolic and flavonoid content and various antioxidant activity indices, in order to better elucidate the role of phenolic compounds in the antioxidant response of the extracts.

2- Materials and Methods

2-1 Materials

Dandelion flowers, black hibiscus flowers, and olive leaves were purchased from Zarband Pharmaceutical Company (Iran), and their scientific names were verified by the Research Institute of Medicinal Plants. All chemicals used in this study were obtained from Merck (Germany).

2-2- Extraction of extracts by different methods, percolation, ultrasound and supercritical fluid

The selected plants were washed with distilled water and dried in the shade at room temperature (approximately 25 ± 2^0 C) away from direct sunlight for about 48 hours, until their weight stabilized. The final moisture content of the dried samples was determined based on the weight difference before and after drying in an oven at 105^0 C for 24 hours, with the moisture content being less than 10 percent. The dried plants were then powdered using an electric grinder and sifted through a 250-micron mesh sieve. The powder of each plant was stored in dark glass containers in a cool, dry place until extraction. For the extraction via percolation, 10 grams of the dried plant powder (250-micron mesh) was initially moistened with an appropriate amount of 70% ethanol and then soaked in a sealed container at room temperature for 4 hours. The wet sample was then transferred to a glass cylindrical percolator, ensuring it was completely covered by the solvent. After approximately 12 hours of initial soaking in the percolator, the outlet valve was opened, and 70% ethanol was fed from the top of the percolator at a constant flow rate of about 1-2 milliliters per minute. The resulting extract was collected from the bottom. This percolation process continued until there was minimal change in the color of the output extract, and approximately 3-4 times the weight of the dried plant was obtained as liquid extract.

The collected extracts were mixed together and concentrated in a rotary evaporator at a temperature of 45-50^0 C, and subsequently dried until a constant weight was achieved [14]. This method is reported as the "traditional solvent method" and considered representative of classic solvent extraction techniques.

For the extraction using ultrasound, the method described by Shoghahi et al. (2025) was utilized with minor modifications [15]. In this procedure, 1 gram of plant powder was mixed in a 50-milliliter centrifuge tube with 10 milliliters of 70% ethanol. The mixture was treated in a bath-type sonicating device, model BANDELIN SONOREX digitec (DT510H) manufactured by Germany country], with a a frequency of 20 kHz at a temperature of 40^0 C for 10 minutes. After extraction, the resulting solutions were filtered through Whatman No. 1 filter paper, and their solvent was evaporated in a rotary evaporator (Heidolph, Germany) at a temperature of 45^0 C, similar to the traditional solvent method. The concentrated extracts were dried until a constant weight was achieved and stored in tightly sealed glass containers covered with aluminum foil at -18^0 C until testing was conducted [15].

For the extraction using supercritical fluid, 20 grams of plant powder was combined with 100 milliliters of ethanol as a co-solvent. The extraction was conducted using supercritical CO2 at a pressure of 100 bar and a temperature of 35^0 C for 30 minutes, with a supercritical CO2 flow rate of 1 mL/min. After the extraction process was completed, the solution was centrifuged at 3000 rpm for 10 minutes, and the upper phase was collected. This upper phase was then filtered through Whatman No. 1 filter paper, and the solvent was evaporated using a rotary evaporator at a temperature of 45^0 C [12]. The resulting extracts were poured into tightly sealed glass containers covered with aluminum foil and stored in a freezer at -18^0 C until further use.

2.3- Measurement of total phenol content

To determine the total phenolic content of the extracts according to the Folin-Ciocalteu method, 100 microliters of the extract was mixed with 500 microliters of Folin-Ciocalteu reagent. After 60 seconds, 1 milliliter of a 20% (w/v) sodium carbonate solution was added. Then, 10 milliliters of distilled water were added to the mixture, and it was allowed to stand in the dark for 30 minutes. Subsequently, the absorbance of the sample was recorded using a UV-Vis spectrophotometer at 765 nanometers. The total phenolic content of the olive leaf extract was determined using a standard curve of oleuropein, while the total phenolic content of the black hibiscus and dandelion flower extracts was determined using a standard curve of Gallic acid. The results were reported as grams per 100 grams of dry weight (DW) of extract. The total flavonoid content was expressed in terms of mg QE per gram of dry weight [16].

2-4- Investigating antioxidant activity by DPPH radical scavenging method

To investigate the antioxidant activity of the extracts by DPPH radical scavenging method, the extract (0.3 mL at a concentration of 1 mg/mL) was mixed with 0.004% DPPH methanolic solution and stirred vigorously. The mixture was kept at room temperature in the dark for one hour, and then the absorbance of the samples was recorded at 517 nm. The solution without sample was used as a blank. The percentage of DPPH radical scavenging of the extracts was obtained by the following equation [17]:

Relation (1):

$$\text{DPPH inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of DPPH solution without the presence of extract and A_{sample} is the absorbance of DPPH solution in the presence of extract.

2.5- Measurement of antioxidant activity by ABTS radical scavenging method

To evaluate the antioxidant activity of the extracts using the ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) method, ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L) solutions were mixed in a 1:1 (v/v) ratio. The resulting mixture was kept in the dark at room temperature for 16 hours to prepare the ABTS radical solution. Subsequently, the ABTS radical solution was diluted with distilled water until it reached an approximate absorbance of 0.7 (at a wavelength of 734 nanometers). Each extract was prepared at a concentration of 0.5 mg/mL and then diluted 100-fold with water. The diluted ABTS radical solution (280 microliters) was mixed with the diluted extract (20 microliters) and transferred to a microplate. Additionally, a blank solution and a control were prepared. The blank solution consisted of the diluted solvent (20 microliters) and the ABTS radical solution (280 microliters), while the control contained the diluted extract (20 microliters) and distilled water (280 microliters). The microplate was kept in darkness at room temperature for 30 minutes, and the absorbance was recorded at 734 nanometers. The results were expressed as milligrams of Trolox equivalents (mg TE/g DW) per gram of dry weight of the extract [18].

2-6- Measurement of antioxidant activity by the iron reducing power (FRAP) method

First, a buffer solution (25 milliliters) was mixed with TPTZ solution (2.5 milliliters) and FeCl3·6H2O solution (2.5 milliliters). The resulting mixture was incubated in a water bath at 37^0 C for 30 minutes, resulting in the FRAP solution. Next, 810 microliters of the FRAP solution was mixed with the extract (90 microliters at a concentration of 1 mg/mL) and kept in the dark at room temperature for 30 minutes to allow for the formation of the ferric tripyridyltriazine complex. Subsequently, the absorbance of the solution was measured using a UV-Vis spectrophotometer at 595 nanometers. A Trolox (TE) standard curve was used to determine the FRAP values [19]. Finally, the antioxidant activity of the extracts in all three methods was compared at the same concentration with the standard antioxidant butylated hydroxyanisole (BHA).

2-7- Determination of phytochemical compounds of extracts

The phenolic compounds in the extracts were measured using high-performance liquid chromatography (HPLC). The device was equipped with a UV-Vis detector and a reverse phase (RP) C8 column with dimensions of 5 µm × 4.6 mm × 150 mm. The detector wavelength was set between 220 and 500 nanometers. The solvents used in this system included 90% aqueous acetic acid solution (0.1%) as solvent A and 10% acetonitrile as solvent B. The solvents were filtered using a 0.45-micron membrane filter. The injection volume and flow rate were 100 microliters and 1.2 mL/min, respectively. Phytochemical compounds present in the extracts were identified using an external standard method and reported in milligrams per kilogram (mg/kg) [20].

2.8- Statistical analysis of data

The experiments were conducted in triplicate, and the results were reported as mean ± standard deviation. The statistical design was a completely randomized design with a factorial arrangement (3 plants × 3 extraction methods). The data were analyzed using two-way analysis of variance (ANOVA). To indicate significant differences between treatments at a 95% probability level ($p < 0.05$), Duncan's multiple range test was employed. Graphs were constructed using Excel. The relationships between the total phenol content, total flavonoid content, and antioxidant activity of the extracts were analyzed using Pearson correlation and evaluated using SPSS software.

3- Results and Discussion

3-1- Total phenol and flavonoid content of extracts

Phenols and flavonoids are secondary metabolites produced in response to environmental stresses, exhibiting significant radical scavenging activities and being recognized as natural antioxidants. These compounds possess various health benefits, often characterized by strong antimicrobial and antioxidant activities [21]. The results of the total phenolic content analysis of extracts from dandelion flowers, olive leaves, and black hibiscus flowers extracted using percolation, ultrasound, and supercritical fluid methods (Table 1) indicated that the highest total phenolic content among the three plants studied was found in the olive leaf extracts, followed by the black hibiscus and dandelion flower extracts, respectively. Among the dandelion extracts, the highest total phenolic content was associated with the supercritical fluid extraction method, while for olive leaf and black hibiscus extracts, the ultrasound-extracted samples exhibited the highest total phenolic content. Overall, the total phenolic content of the extracts from dandelion flowers, olive leaves, and black hibiscus was found to be in the ranges of 17.85–24.60 mg/g DW, 33.54–39.84 mg/g DW, and 24.16–30.82 mg/g DW, respectively.

Table 1. Total phenol (TPC) and total flavonoid content (TFC) of the extracts

Plant	Extraction method	TPC (mg/g DW)	TFC (mg QE/g DW)
Taraxacum officinale L.	Percolation	17.85 ± 0.90 ^{h}	5.96 ± 0.11 ^{g}
	Ultrasound	20.22 ± 0.59 ^{g}	7.10 ± 0.16 ^{f}
	Supercritical fluid	24.60 ± 0.84 ^{f}	9.73 ± 0.09 ^{e}
Olea europaea L. leaves	Percolation	33.54 ± 0.80 ^{c}	12.32 ± 0.14 ^{d}
	Ultrasound	39.84 ± 0.97 ^{a}	15.31 ± 0.23 ^{c}
	Supercritical fluid	35.45 ± 0.66 ^{b}	16.05 ± 0.18 ^{b}
Althaea officinalis L.	Percolation	24.16 ± 0.79 ^{f}	15.86 ± 0.07 ^{b}
	Ultrasound	30.82 ± 0.74 ^{d}	18.34 ± 0.21 ^{a}
	Supercritical fluid	26.69 ± 0.92 ^{e}	18.57 ± 0.26 ^{a}

Values represent mean (n=3) ± SD. Different letters in each column represent statistical significant difference at 5% level.

The highest total flavonoid content among the three plants studied in this research was found in the black hibiscus extracts, followed by the olive leaf and dandelion flower extracts (Table 1). The total flavonoid content of the dandelion flower, olive leaf, and black hibiscus extracts was in the ranges of 5.96–9.73 mg QE/g DW, 12.32–16.05 mg QE/g DW, and 15.86–18.57 mg QE/g DW, respectively. Among the extracts of the three plants, the highest total flavonoid content was associated with the supercritical fluid extraction method, followed by ultrasound and percolation methods. However, for the black hibiscus plant, there was no statistically significant difference between the extracts obtained via supercritical fluid and ultrasound methods ($p < 0.05$). Overall, the extraction efficiency of phenolic compounds using modern supercritical fluid and ultrasound systems was higher than that of the traditional percolation method. The favorable and high efficiency of the ultrasound extraction system for phenolic compound extraction from plants, compared to conventional methods, is due to the phenomenon of cavitation induced by ultrasonic waves. This process creates high shear forces within the plant matrix, breaking the cell walls and allowing for greater solvent penetration into the plant tissue, thereby enhancing the release and diffusion of cellular contents into the surrounding environment [22]. Using ethanol as a solvent in combination with water facilitates the

formation of more bubbles during the ultrasound extraction process, increasing the destruction of plant tissues and improving the extraction of bioactive compounds. This effect is associated with the high vapor pressure of ethanol and its favorable viscosity when mixed with water [23]. The supercritical fluid extraction method also typically demonstrates high efficacy in extracting phenolic compounds from plants, and due to the absence of light and oxygen during the extraction process, it protects phenolic compounds from degradation and oxidation [24]. The superior efficiency of ultrasound in extracting active compounds from certain plants compared to supercritical fluid extraction may be attributed to the fact that ultrasound can extract a wide range of hydrophilic and hydrophobic compounds, while in supercritical fluid extraction, hydrophobic compounds are often effectively separated and the amount of hydrophilic phenolic compounds in some plants exceeds that of their hydrophobic counterparts [25]. In the study by Ahmad-Qasem et al. (2013), the favorable capability of the ultrasound method for extracting phenolic compounds from olive leaves was reported [26]. Similarly, in the study by Seyfollah et al. (2020), ultrasound exhibited higher efficiency for extracting phenolic compounds from Leptospermum petersonii compared to conventional extraction methods [13]. In the research by Mahdiania-Lechayi et al. (2018), there was no statistically significant difference in total phenolic and flavonoid content between the Metka plant extracts obtained by ultrasound and supercritical fluid methods, with both methods showing high efficiency in extracting the bioactive compounds from this plant [12].

3.2- Antioxidant activity of extracts

In this study, the antioxidant activity of extracts from dandelion flowers, olive leaves, and black hibiscus flowers was evaluated using DPPH radical scavenging, Ferric Reduction Antioxidant Power (FRAP), and ABTS radical inhibition methods. The results (Table 2) showed that all three plants demonstrated significant antioxidant activity, with the highest activity observed in olive leaves, followed by black hibiscus and dandelion flowers. The phenolic and flavonoid compounds present in various plants typically exhibit notable antioxidant activities. These compounds contain hydroxyl groups in their structure, enabling them to donate electrons or protons to free radicals, neutralize them, decompose peroxides, and counteract reactive oxygen species (both singlet and triplet states) [27]. Antioxidant activity in plants is largely dependent on the type and amount of bioactive compounds present, as well as their structural configurations [28]. The extraction method used for obtaining plant extracts is also a significant factor influencing the performance of these extracts. Modern methods such as ultrasound and supercritical fluid extraction often demonstrate higher efficiency in extracting bioactive compounds compared to traditional methods [29]. In ultrasound extraction, cavitation phenomena enhance mass transfer rates, improving solvent penetration into cellular tissues and increasing the extraction efficiency of phenolic compounds [30]. Similarly, during supercritical fluid extraction, bioactive plant compounds are well preserved against oxidation due to the ambient temperature and absence of light during the process [31]. Research by Exynos et al. (2012) also showed that supercritical fluid extraction effectively extracted active compounds (oleuropein) from olive leaves [32]. Dedic et al. (2022) observed that the antioxidant activity (DPPH and FRAP) of hydroalcoholic extracts of dandelion flowers obtained via ultrasound was significantly higher than that from conventional maceration and boiling methods [33]. Researchers have reported the presence of oleuropein, rutin, quercetin, luteolin, apigenin, and other compounds in olive leaves that contribute to their significant antioxidant activity [34]. Lama-Munoz et al. (2019) identified oleuropein as the most abundant active compound in olive leaves, attributing the

primary antioxidant activity to this compound [35]. Black hibiscus is rich in anthocyanins, flavonoids, and phenolic acids [36], which may explain the strong antioxidant activity of its extracts. Ahmad et al. (2016) reported the presence of triterpenes, saponins, flavonoids, alkaloids, phenolic acids, steroids, and tannins in black hibiscus extracts [37]. Researchers noted that chicoric acid is one of the main phenolic compounds found in various parts of the dandelion plant, exhibiting significant antioxidant activity. This compound belongs to the hydroxycinnamic acids and stabilizes radicals due to the presence of an acrylate group attached to the phenyl ring [38]. They also stated that the antioxidant activity of dandelion flowers is primarily attributed to their phenolic compounds, with flavonoids playing a comparatively lesser role.

Table 2. Antioxidant activity of the extracts

Plant	Extraction method	DPPH (%)	ABTS (mg TE/g)	FRAP (mM TE)
Taraxacum officinale L.	Percolation	60.89 ± 0.52 ^{h}	13.62 ± 0.23 ^{h}	3.98 ± 0.11 ^{h}
	Ultrasound	65.99 ± 0.83 ^{g}	16.86 ± 0.10 ^{f}	4.67 ± 0.05 ^{f}
	Supercritical fluid	69.42 ± 0.76 ^{f}	17.03 ± 0.16 ^{f}	4.32 ± 0.16 ^{g}
Olea europaea L. leaves	Percolation	80.17 ± 1.20 ^{e}	23.95 ± 0.17 ^{c}	6.71 ± 0.10 ^{d}
	Ultrasound	89.14 ± 0.76 ^{a}	27.78 ± 0.15 ^{b}	8.85 ± 0.11 ^{a}
	Supercritical fluid	87.36 ± 0.94 ^{b}	28.60 ± 0.09 ^{a}	8.23 ± 0.15 ^{b}
Althaea officinalis L.	Percolation	79.96 ± 0.95 ^{e}	15.70 ± 0.21 ^{g}	6.03 ± 0.14 ^{e}
	Ultrasound	84.86 ± 1.11 ^{c}	20.33 ± 0.12 ^{d}	7.49 ± 0.07 ^{c}
	Supercritical fluid	82.50 ± 0.72 ^{d}	18.46 ± 0.18 ^{e}	6.81 ± 0.19 ^{d}
BHA		83.10 ± 0.83 ^{cd}	27.94 ± 0.16 ^{b}	6.24 ± 0.08 ^{c}

Values represent mean (n=3) ± SD. Different letters in each column represent statistical significant difference at 5% level.

3-3- Phytochemical compounds of extracts

The phytochemical constituents of extracts of three plants: dandelion, olive leaf, and black marshmallow were collected using ultrasound and characterized using HPLC. The following six phytochemical compounds were identified in dandelion extracts (Table 3) based on their concentrations in the extract: quercetin, 42.04 µg/mg; chicoric acid, 30.29 µg/mg; chlorogenic acid, 20.29 µg/mg; luteolin, 18.04 µg/mg; rutin, 11.21 µg/mg; kaempferol, 11.07 µg/mg. Nine phytochemical compounds were identified in the olive leaf extract (Table 4) as: Caffeic acid (103.66 µg/mg), oleuropein (91.90 µg/mg), vebascoside (39.63 µg/mg), quercetin (29.15 µg/mg), rutin (18.90 µg/mg), luteolin-7-glucoside (13.21 µg/mg), ferulic acid (10.01 µg/mg), hydroxytyrosol (4.98 µg/mg) and para-coumaric acid (4.55 µg/mg). Seven phytochemical compounds were identified in the extract of black marshmallow (Table 5). These include, in terms of concentration in the extract, myristicin (119.32 µg/mg), caffeic acid (68.08 µg/mg), para-coumaric acid (36.58 µg/mg), rutin (36.23 µg/mg), gallic acid (22.64 µg/mg), quercetin (18.36 µg/mg), and kaempferol (5.52 µg/mg). Thus, HPLC results demonstrated that presence of chicory acid, chlorogenic acid and quercetin, in dandelion, which are all hydroxy groups conjugated

structures, may also be significant in inhibition of free radicals and in the reducing capacity of this extract. oleuropein, caffeic acid and verbascoside in olive leaves, and hydroxytyrosol are the combination of phenolic structures with electron donating properties and stabilization of the free radicals. this is consistent with the high antioxidant activity of OELE. The great amount of myristicin, rutin and caffeic acid might also explain the pronounced antioxidant activity of this extract since these compounds scavenged radicals and reduced metal ions through their aromatic rings and numerous hydroxyl groups. Sánchez-Gutierrez et al. (2021) noted oleuropein, hydroxytyrosol, verbascoside, luteolin and apigenin in the ethanolic (50%) extract of olive leaves extracted using the microwave method. Prior studies reported hydroxycinnamic acids such as caffeic acid, chlorogenic acid, and chicoric acid as the most abundant phenolic compounds found in dandelion extract. According to Axo et al. (2017), important phytochemical compounds in dandelion flower extract are quercetin, chicoric acid, chlorogenic acid, and caffeic acid.

Farhat et al. (2022) reported the presence of 5 phenolic acids: caffeic, synergic, gallic, trans-ferulic, and para-coumaric, and 8 flavonoids: catechin, chrysin, apigenin, kaempferol, quercetin, rutin, galangin, and genistein in the aqueous extract of marshmallow [41].

Table 3. Phytochemical composition of Taraxacum officinale L. extract

NO	Compounds	RT (min)	Peak area (%)	Concentration (µg/mg)
1	Rutin	4.04	7.58	11.21
2	Chlorogenic acid	7.21	22.59	29.20
3	Luteolin	10.81	13.83	18.04
4	Chicoric acid	14.23	21.26	30.29
5	Quercetin	14.43	26.62	42.04
6	Kaempherol	18.11	8.11	11.07

Table 4. Phytochemical composition of Olea europaea L. leaves extract

NO	Compounds	RT (min)	Peak area (%)	Concentration (µg/mg)
1	Caffeic acid	3.44	22.49	103.66
2	Ferulic acid	4.31	6.06	10.01
3	Vebascoside	5.26	13.57	39.63
4	Rutin	7.21	9.39	18.90
5	Hydroxytyrosol	9.67	4.68	4.98
6	*p*-Coumaric acid	10.18	4.72	4.55
7	Luteolin-7-glucoside	13.71	7.65	13.21
8	Quercetin	14.23	11.10	29.15
9	Oleuropein	15.19	20.32	91.90

Table 5. Phytochemical composition of Althaea officinalis L. extract

NO	Compounds	RT (min)	Peak area (%)	Concentration (µg/mg)

1	Gallic acid	3.44	8.99	22.64
2	Rutin	7.21	9.60	36.23
3	*p*-Coumaric acid	10.18	9.69	36.58
4	Myricetin	14.22	18.96	119.32
5	Caffeic acid	14.45	13.53	68.08
6	Quercetin	16.28	7.30	18.36
7	Kaempherol	18.11	4.38	5.52

3-4- Pearson correlation study of the relationships between total phenolic content, total flavonoids and antioxidant activity of extracts

The results of the Pearson correlation analysis between total phenolic content, total flavonoid content, and antioxidant activity of the extracts from dandelion flowers, olive leaves, and black hibiscus (using the DPPH radical scavenging method, ABTS+ radical scavenging method, and FRAP) are presented in Table 6. As shown in the table, there was a strong relationship among the parameters studied in this research, with higher total phenolic and flavonoid content correlating with increased radical scavenging activity and reducing capacity in the extracts.

The relationships among all five studied parameters were direct and significant. The Pearson correlation results indicated that there were strong positive correlations ($r > 0.85$, $p < 0.01$) between total phenolic content and antioxidant indices (DPPH, ABTS, and FRAP). In contrast, the correlation between total flavonoid content and the ABTS index was moderate ($r \approx 0.47$). This suggests that while flavonoids contribute to the antioxidant activity of the extracts, a significant portion of the observed changes in antioxidant response is likely dependent on other phenolic and phytochemical compounds.

Table 6. Pearson correlation of relationships between TPC, TFC and antioxidant activity of extracts

	TPC	TFC	DPPH	ABTS	FRAP
TPC	1				
TFC	0.623**	1			
DPPH	0.886**	0.903**	1		
ABTS	0.946**	0.471*	0.770**	1	
FRAP	0.959**	0.608**	0.867**	0.887**	1

** and * are significant correlations at the 1 and 5 percent confidence levels, respectively, TPC: total phenol content, TFC: total flavonoid content, FRAP: ferric reducing ability

4- Conclusion

In this study, the results demonstrated that both modern methods of ultrasound and supercritical fluid extraction significantly increased the total phenolic and flavonoid content as well as antioxidant activity indices compared to the traditional solvent extraction method. Among the plants examined, olive leaf extracts exhibited the highest total phenolic content and antioxidant activity, while the black hibiscus extracts contained the highest total flavonoid content. Strong and significant correlations were found between total phenolic content and various antioxidant activity indices, as well as between total flavonoids and some antioxidant tests, indicating the important role of phenolic compounds, especially flavonoids, in the

antioxidant response of the extracts. The HPLC results for the ultrasound extracts revealed that quercetin, chicoric acid, and chlorogenic acid were dominant in dandelion flowers. In olive leaves, oleuropein, caffeic acid, and verbascoside were present in significant amounts, while myristin, rutin, quercetin, gallic acid, and caffeic acid were the primary compounds in black hibiscus. This phytochemical profile aligns with the observed antioxidant activity in the extracts and may largely explain the differences in antioxidant indices among the plants and extraction methods. Given that olive leaves, black hibiscus, and dandelion flowers produced extracts with high phenolic content and acceptable antioxidant activity under the studied conditions, these plants can be considered as potential suitable sources for obtaining natural antioxidant extracts for use in food and possibly pharmaceutical formulations. However, directly generalizing the results of antioxidant chemical tests to the performance of these extracts in real food systems requires further investigation. Therefore, it is recommended that future research uses statistical optimization approaches to determine the operational conditions for each extraction method to maximize yield and bioactive compound content. Additionally, a comprehensive examination of the phytochemical profile in other extraction methods should be carried out, and the antioxidant performance of the extracts in real food systems (such as fats and oils, emulsions, or meat products) should be assessed during processing and storage. It is also advisable to evaluate sensory effects, consumer acceptance, and safety aspects of using these extracts at practical levels in food products to facilitate their practical recommendation as natural alternatives to synthetic antioxidants.

Data Availability: "Data will be available upon request.

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5-References

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مقاله علمی_پژوهشی

بهینه‌سازی فرمولاسیون بیسکویت پروبیوتیک حاوی آرد ذرت، آرد باکویت و اینولین به روش طرح مخلوط و ارزیابی خواص عملکردی و کیفی

آیناز قره باشی[۱]، راحیل رضایی[۱*]، حمید بخش آبادی[۲]

۱-گروه علوم و صنایع غذایی، واحد گنبد کاووس و مینودشت، دانشگاه آزاد اسلامی، گنبد کاووس، ایران.

۲-گروه کشاورزی، مرکز آموزش عالی میناب، دانشگاه هرمزگان، بندرعباس، ایران.

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* مسئول مکاتبات:

rezaei.rahil@yahoo.com

چکیده

غذاهای عملگرا، علاوه بر تأمین نیازهای تغذیه‌ای بدن، نقش مهمی در ارتقای سلامت و کاهش خطر ابتلا به بیماری‌ها دارند. در همین راستا این مطالعه با هدف بهینه‌یابی فرمولاسیون بیسکویت پروبیوتیک بدون گلوتن صورت پذیرفت. در این پژوهش اثرات سه غلظت آرد باکویت (۰ تا ۱۰۰ درصد)، آرد ذرت (۰ تا ۱۰۰ درصد) و اینولین در محدوده‌ی ۰ تا ۳ درصد با کمک طرح آماری مخلوط بر پارامترهای ظرفیت نگهداری آب، مدول ذخیره و افت، سختی، حجم، شاخص L^*، تعداد باکتری‌های پروبیوتیک و پذیرش کلی آرد، خمیر و بیسکویت‌های تولیدی بررسی گردید. بر اساس نتایج این پژوهش کلیه ویژگی‌های مورد آزمایش تحت تأثیر اجزای مخلوط بود ($p<0/05$). نتایج به‌دست آمده نشان داد که برای دستیابی به بیسکویتی با بیشترین تعداد باکتری‌های پروبیوتیک، شاخص L^*، حجم، پذیرش کلی و کمترین میزان سفتی بایستی به‌ترتیب از ۲۳/۸۳، ۷۴/۱۶ و ۲/۰۲ درصد آرد باکویت، ذرت و اینولین در فرمولاسیون بیسکویت استفاده نمود تا مطلوبیت ۰/۷۶۹ حاصل گردد. از طرفی مشخص شد که در این شرایط میزان ظرفیت نگهداری آب ۹/۰ گرم بر گرم، مدول G' ۱/۳۹ $\times 10^6$ پاسکال، مدول G'' ۴۱۴۰۹۰ پاسکال، سختی ۱۳/۳۹ نیوتن، حجم ۴/۱۶ سانتی متر مکعب، تعداد باکتری‌های پروبیوتیک ۹/۷۷ log cfu/g، شاخص L^* ۳۹/۸۵ و پذیرش کلی ۳/۹۱ بود. در نهایت می‌توان بیان داشت که فرمولاسیون بهینه پیشنهادی می‌تواند به‌عنوان یک محصول عملگرای بدون گلوتن با ویژگی‌های تکنولوژیکی مطلوب و پتانسیل بالای پروبیوتیکی معرفی گردد.