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Phytochemical Properties of Zarrin-Giah (*Dracocephalum Kotschyi*): Application as a Natural Antioxidant to Enhance Oxidative Stability of Cupcakes During Storage at low temperature

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ABSTRACT

Today, extensive research has been conducted on the use of natural antioxidants in food products as substitutes for synthetic antioxidants. In this study, the phytochemical components of the medicinal plant Zarrin-Giah (*Dracocephalum Kotschyi*) were identified and quantified using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). Additionally, the effects of various concentrations of Zarrin-Giah essential oil (0.02%, 0.05%, and 0.1%) on the oxidative stability, shelf life, and sensory characteristics of cupcake over a 4-month storage period were investigated and compared with the synthetic antioxidant tert-Butylhydroquinone (TBHQ). The results indicated that Zarrin-Giah essential oil contained 48 active compounds, with the most significant being a-Pinene, Geranial, Limonene, and Neral. Statistical analysis demonstrated that different concentrations of Zarrin-Giah essential oil and storage time had a significant impact on dependent variables pH, acid value, peroxide value, TOTOX value, p-Anisidine value, and antioxidant capacity of the samples. During the storage period, the pH of the cupcakes decreased, likely due to the breakdown of triglycerides and the production of free fatty acids. The results showed that with an increase in the concentration of Zarrin-Giah essential oil, the decrease in pH of the cupcakes during storage was less pronounced. The oxidative stability results of the cupcake oil content revealed that Zarrin-Giah essential oil had a greater capacity to maintain oxidative stability of the oil compared to the TBHQ. Despite the desirable properties of Zarrin-Giah essential oil in preserving the quality and oxidative stability of cupcake oil, sensory evaluation results indicated that this essential oil adversely affected the aroma and taste of the cupcakes. Considering the strong performance of Zarrin-Giah essential oil in maintaining the oxidative stability of cupcake oil, it seems that using lower amounts of this essential oil and incorporating natural flavoring agents could mitigate its adverse impact on the sensory properties of the cupcakes.

1. Introduction

Flour-based products are among the most widely consumed food products globally. Cakes, due to their desirable organoleptic properties, are especially popular with consumers. Flour, sugar, eggs, and fat serve as the primary components in cake production, each playing a crucial role in the structure and quality of the product [1]. In English, the word “cake” can refer to various meanings, but in its simplest sense, it denotes a small, baked piece of dough. Cakes, as a bakery product, typically have a shelf life of about a week in their fresh form and contain between 11% to 21% fat [2]. Moisture migration, staling, and the lack of soluble and insoluble fibers in cake flour are major challenges for this product, leading to irreversible changes in sensory and microbial properties, which ultimately shortens its shelf life [3]. The two main issues facing the cake industry are lipid oxidation and mold growth, which limit the shelf life of these products. Using antioxidants and preservatives, as well as storing cakes at low temperatures such as refrigeration, can significantly mitigate these issues. Plant essential oils, besides their antioxidant properties, also exhibit antibacterial, antifungal, anti-mold, and anticancer effects, making them promising candidates for use in bakery products [4]. Antioxidants are compounds that preserve food by slowing the spoilage, rancidity, or discoloration caused by oxidation, thus reducing the rate and severity of oxidation reactions [5]. The effectiveness of antioxidants depends on various factors, including processing, storage, and the surrounding environment of the food product. Antioxidants can be classified into synthetic and natural types. Synthetic antioxidants, such as Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), and tert-Butylhydroquinone (TBHQ), have long been used to maintain the oxidative quality of products containing oils and fats. However, recent reports have raised concerns regarding the potential health risks associated with these additives [6]. Consequently, the food industry, particularly the bakery companies, are now inclined toward natural antioxidant sources that are both effective and economical, as well as free from the health concerns related to synthetic antioxidants [7]. Amany, Shereen [8] added the essential oils of four medicinal plants (anise, black cumin, rosemary, and sage) in amounts ranging from 200 to 1000 ppm to cakes. They reported that, in addition to the positive impact of these essential oils on the sensory properties of cakes, the thermal stability and shelf life of this popular product were significantly enhanced. Similarly, Darughe, Barzegar [9] reported that adding coriander essential oil to cakes extended the shelf life of the product up to 60 days.

One of the most significant sources of natural antioxidants is medicinal plants. Due to their pleasant aroma, therapeutic properties, antimicrobial potential, and antioxidant characteristics, medicinal plants are ideal for use as natural antioxidant sources in food products. Numerous reports have documented the application of various medicinal plants in different forms—including essential oils, aqueous and alcoholic extracts, and direct use of powdered plant parts—in food products [1]. Plants are rich sources of natural antioxidants. The antioxidant compounds in plants generally include compounds such as tocopherols, carotenoids, phenolic acids, cinnamic acids, and flavonoids. Medicinal plants possess antibacterial, antifungal, anticancer, and antioxidant properties that can enhance the shelf life of food products [10]. The medicinal plant *Dracocephalum kotschyi*, known as "Zarrin-Giah" belongs to the Lamiaceae family, which includes 186 species worldwide and eight species in Iran,

where *Dracocephalum kotschyi* is endemic, meaning it grows only in certain regions of Iran [11]. In traditional medicine across different regions, this plant has been used to relieve pain and inflammation and to treat rheumatic conditions. The essential oil in its structure has medicinal value, exhibiting high antioxidant activity and antibacterial and antiseptic properties. It is also used to treat stomach pain and bloating. The leaves of this plant contain a compound known as Spinal-z, historically utilized in cancer treatment, and a flavonoid called xanthomicrol, which inhibits the proliferation of malignant cancer cells [12]. Additionally, Zarrin-Giah contains phenolic compounds that effectively act as hydrogen donors, making it a potent antioxidant. The antioxidant activity of flavonoids, which are specific phenolic compounds, is attributed to their ability to donate hydrogen. In another study, the total phenolic content of Zarrin-Giah was measured, revealing that glycosylated flavonoid content was higher than aglycone flavonoid levels. Various studies have indicated the antioxidant activity of this plant's extract, with an IC50 value close to that of BHT, suggesting it as a viable substitute for synthetic antioxidants [2]. To date, very few studies have been conducted on the various species of this plant, *especially D. kotschyi*, which grows in different regions of Iran, including Fars and Isfahan provinces. Given that Zarrin-Giah can serve as a rich source of antioxidant compounds, this study aims to investigate the bioactive compounds and antioxidant properties of this plant. The primary objectives of this research include identifying the phytochemical components of Zarrin-Giah extracts and essential oil, assessing the antioxidant potential of its essential oil, examining the effects of adding Zarrin-Giah essential oil on the physicochemical and quality properties of cupcakes, and monitoring the oxidative stability of the oil in cupcakes during refrigerated storage.

2. Materials and Methods

The main ingredients for the cupcake preparation, including wheat flour (73% extraction rate, 12.3% protein, and 13.5% moisture), margarine, sunflower oil, whole eggs, sugar, vanilla, skimmed milk, salt, and baking powder, were purchased from a local store. The dried aerial parts of *Dracocephalum kotschyi* (harvested at the flowering stage, when the plant's essential oil content is highest) were procured from Ganj Darou Co. in Buin and Miandasht (Isfahan Province, Iran). Chemicals and solvents used in this experiment were of analytical grade and obtained from Sigma-Aldrich Company Ltd (Gillingham, UK).

2.1. Preparation of *Dracocephalum kotschyi*

Samples of *Dracocephalum kotschyi* flowers and leaves were collected in July, during the plant's flowering phase, from its cultivated fields in the Fereydan area of Isfahan. After cleaning, the samples were air-dried at room temperature (approximately 30°C) and away from light for five days until fully dried.

2.2. Essential Oil Extraction from *Dracocephalum kotschyi*

The dried samples of *Dracocephalum kotschyi* were subjected to essential oil extraction using a Clevenger-type apparatus by hydrodistillation for three hours following the sample's initial boiling. The essential oil yield was calculated as volume-to-weight (v/w) based on the dry weight of the samples. After dehydration with sodium sulfate, the extracted essential oils were stored in a freezer at -20°C until analysis by gas chromatography-mass spectrometry (GC-MS).

2.3. Extraction of Aqueous-Alcoholic Extract from *Dracocephalum kotschyi*

The aqueous-methanolic extract of *Dracocephalum kotschyi*, aimed at determining antioxidant activity and

identifying polyphenolic compounds, was prepared using a modified version of the method by Wojdyło, Oszmiański [13]. A total of 10 grams of dried *Dracocephalum kotschyi* powder was milled, and 100 mL of a methanol-water mixture (70:30) was added. The mixture was shaken at room temperature for 24 hours on a laboratory shaker and then centrifuged at 4032 ×g for 10 min. The supernatant was collected and stored in a laboratory freezer at -20°C. Phenolic compound extraction for identification was also performed using the method of Mišan, Mimica-Dukić [14] with slight modifications.

2.4. Identification and Quantification of Polyphenolic Compounds Using HPLC

The separation and quantification of polyphenolic compounds in the *Dracocephalum kotschyi* extract were conducted using an HPLC system (Agilent Technologies-1200 series). The setup included a C18 column (150 mm in length, 4.6 mm in internal diameter, and 5 µm particle size) and a DAD detector set to wavelengths of 320 and 280 nm. The oven temperature was maintained at 30°C. A gradient elution program with a flow rate of 1 mL/min and an injection volume of 20 µL was applied, using a mobile phase consisting of methanol and 1% formic acid.

For the identification of polyphenolic compounds, 20 µL of each sample extract was injected into the HPLC. To identify and calculate the relative percentage of each compound, standard solutions of each compound (HPLC grade, with a purity above 99%) were prepared at suitable concentrations (0–10,000 ppm) and injected into the system. Quantitative analysis was performed by comparing the retention time of each compound in the sample chromatogram with the corresponding standard, and the concentration of each compound in the samples was calculated based on the calibration curve generated.

2.5. Identification of *Dracocephalum kotschyi* Essential Oil Components by GC/MS

The composition of essential oils was analyzed using gas chromatography-mass spectrometry (GC/MS). The gas chromatograph was equipped with an HP-5-MS column (30 meters in length, 0.32 mm in diameter, and 0.25 µm film thickness). The temperature program started at 60°C and increased to 210°C at a rate of 4°C per min. The detector type was FID, set to 29°C, with nitrogen as the carrier gas at a flow rate of 0.5 mL per min. The MS detector was set to 280°C with helium as the carrier gas at 1 mL per min. The relative percentage of each essential oil component was determined by calculating the area under each GC peak and comparing it to the total area under all peaks. Compound identification was based on mass spectral databases, retention times, Kovats indices, and fragmentation pattern analysis compared to standard spectra and reputable sources [15].

2.6. Production of Cupcakes with *Dracocephalum kotschyi* Essential Oil

Dracocephalum kotschyi essential oil was added to the cupcake formulation at concentrations of 0.02%, 0.05%, and 0.1% of the oil weight. The cupcakes were prepared using a two-stage mixing method with a laboratory mixer. A fixed weight of batter was placed into molds and baked at 190°C for 15 min. After one hour of cooling, the cupcakes were packaged in polyethylene bags and stored at 5°C.

2.7. Physicochemical Properties of Cupcakes

2.7.1. Moisture Content

The moisture content was determined by gravimetric analysis using AACC Standard Method 44-15 (2000) [16].

2.7.2. Weight Loss

The weight loss percentage was calculated by comparing the cupcake weights immediately

after baking and at two and four months of storage.

2.8. Oil Extraction by Cold Method

Oil content was extracted using hexane in a 1:3 ratio through cold extraction. About 80 grams of homogenized sample was mixed with 250 mL of hexane, covering the sample completely. After stirring for 5 hours on a laboratory shaker, the mixture was allowed to settle for one hour to clarify the solvent phase, which was then filtered through filter paper and a vacuum pump to remove the solvent at room temperature.

2.9. Acid Value

The acid value was determined by titrating a set amount of oil with sodium hydroxide (NaOH), following Iranian national standard method 37. Approximately two grams of oil were weighed, combined with 30 mL of neutral ethanol, and titrated with 0.01% NaOH using phenolphthalein as an indicator:

$$Q = \frac{28.2 \times N \times V}{W} \quad (1)$$

where V is the volume of NaOH used, N is the normality of NaOH, W is the sample weight, and Q represents free fatty acids as oleic acid.

2.10. Peroxide Value

The peroxide value was measured spectrophotometrically by mixing 0.1 g of oil with 9.8 mL of a chloroform-methanol mixture, followed by adding 50 µL of ammonium thiocyanate and 50 µL of iron (II) solution. Absorbance was read at 500 nm after 5 min in a dark room:

$$\mathrm{PV} = \frac{(A_s - A_b) \times m}{55.84 \times \mathrm{W} \times 2} \quad (2)$$

where A_s and A_b are the sample and blank absorbances, m is the slope from the calibration curve, and W is the oil sample weight.

2.11. p-Anisidine Value

The p-anisidine value was measured spectrophotometrically at 350 nm. A 0.5 g oil sample was dissolved in 25 mL of iso-octane, and the absorbance was measured. Then, 1 mL of p-anisidine solution was added to 5 mL of the initial solution and left for 10 min in the dark before reading at 350 nm:

$$PA = \frac{10 \times 1.2 \times (\mathrm{Eb} - \mathrm{Ea})}{\mathrm{W}} \quad (3)$$

where PA is the p-anisidine value, E_b and E_a are absorbances of the mixed and initial solutions, and W is the sample weight.

2.12. TOTOX Value

The TOTOX value, representing total oxidation, was calculated as [17]:

$$\mathrm{TOTOX} = 2\mathrm{PV} + \mathrm{PA} \quad (4)$$

2.13. Antioxidant Activity

The antioxidant activity was measured via spectrophotometric analysis of DPPH reduction in a methanolic solution dissolved in n-hexane [18].

2.14. Sensory Evaluation

Ten trained panelists evaluated cupcake characteristics one day after preparation using a 5-point hedonic scale (1 = poorest, 5 = best). Attributes evaluated included crust color, appearance, freshness, crumb color, porosity, aroma, taste, texture firmness, mouthfeel, and overall acceptability.

2.15. Statistical Analysis

A factorial experiment with two factors (essential oil concentration at three levels: 0.02%, 0.05%, and 0.1%, a control without additives, and a sample with TBHQ) and storage time at three levels (0, 2, and 4 months) was conducted using a completely randomized design. Data were analyzed using JMP software (version 8.0) with Tukey's test at a 95% confidence level for mean comparisons.

3. Results and Discussion

3.1. Identification of *Dracocephalum kotschyi* Essential Oil Components by GC/MS

Following the injection of *Dracocephalum kotschyi* essential oil into the GC/MS device, the components were analyzed both qualitatively and quantitatively using retention time, retention Kovats index, mass

spectra, and comparisons with standards and GC/MS library data. A total of 48 compounds were identified in the essential oil, accounting for 97.98% of the total composition (Table 1). Major compounds included α-Pinene (25.50%), Geranial (14.01%), Limonene (12.39%), Neral (11.07%), Geranyl acetate (7.39%), Methyl geranate (5.70%), Geraniol (3.28%), 6-methyl-5-hepten-2-one (2.02%), α-Campholenal (1.57%), Myrcene (1.38%), Linalool (1.35%), and trans-Carveol (1.14%). Essential oils generally have low molecular weights and high volatility, leading to significant loss during high-heat processes such as baking or frying. Encapsulation within heat-resistant structures or dissolving the essential oil in the oil phase prior to baking can minimize these losses [19]. Sultan, Butt [20] reported that dissolving essential oils in the oil phase of baked products increased their stability during baking by more than 85%.

Table 1. Chemical compounds identified in *Dracocephalum kotschyi* essential oil that used in this research

No.	Compounds	RI	Percentage	No.	Compounds	RI	Percentage
1	a-Pinene	*932*	25.50	25	Citronellal	*1153*	0.59
2	Camphene	*951*	0.15	26	cis-Chrysanthenol	*1162*	0.61
3	Thuja-2,4(10)-diene	*956*	0.20	27	p-Mentha-1,5-dien-8-ol	*1167*	0.50
4	Sabinene	*975*	0.48	28	Terpinen-4-ol	*1178*	0.90
5	b-Pinene	*979*	0.44	29	p-Cymen-8-ol	*1186*	0.35
6	6-methyl-5-Hepten-2-one	*989*	2.02	30	a-Terpineol	*1190*	0.19
7	Myrcene	*991*	1.38	31	Myrtenol	*1195*	0.62
8	a-Phellandrene	*1006*	0.15	32	Verbenone	*1201*	0.36
9	a-Terpinene	*1018*	0.14	33	trans-Carveol	*1220*	1.14
10	p-Cymene	*1026*	0.48	34	Nerol	*1230*	0.41
11	Limonene	*1028*	12.39	35	Neral	*1238*	11.07
12	1,8-Cineole	*1031*	0.64	36	Carvone	*1244*	0.29
13	(Z)-b-Ocimene	*1033*	0.40	37	Geraniol	*1255*	3.28
14	(E)-b-Ocimene	*1047*	0.06	38	Geranial	*1268*	14.01
15	Bergamal	*1054*	0.10	39	Bornyl acetate	*1287*	0.21
16	g-Terpinene	*1059*	0.27	40	Carvacrol	*1298*	0.15
17	cis-Sabinene hydrate	*1070*	0.09	41	Methyl geranate	*1326*	5.70
18	trans-Linalool oxide	*1076*	0.24	42	Eugenol	*1361*	0.15
19	Terpinolene	*1090*	0.24	43	Neryl acetate	*1364*	0.31
20	Linalool	*1098*	1.35	44	Geranyl acetate	*1384*	7.39
21	trans-p-Mentha-2,8-dien-1-ol	*1120*	0.22	45	(E)-Caryophyllene	*1420*	0.08
22	a-Campholenal	*1127*	1.57	46	allo-Aromadendrene	*1461*	0.05
23	cis-Verbenol	*1139*	0.16	47	Germacrene D	*1483*	0.10
24	trans-Verbenol	*1143*	0.77	48	Spathulenol	*1576*	0.05
	Total				97.98%		

RI: The retention Kovats index were determined on HP-5 capillary column.
Components less than 0.05% was not reported.

3.2. Identification of Polyphenolic Compounds in *Dracocephalum kotschyi* Extract by HPLC

The HPLC analysis of *Dracocephalum kotschyi* extract revealed 11 major polyphenolic compounds, as presented in Table 2**.**

Table 2. Polyphenol constituents identified by HPLC in *Dracocephalum kotschyi* extract

Compound name	Content (mg/g dry weight)	Retention time (min)	Wavelength (nm)
Gallic Acid	0.115	3.3	280
Catechin	Nd.	8.3	280
Chloregenic acid	Nd.	10.5	320
Caffeic acid	Nd.	11.6	280
Rutin	Nd.	12.4	280
Vanilin	Tr.	13.5	280
P-cumaric acid	Tr.	15.6	280
Trans-ferulic acid	Nd.	16.3	280
Sinapic acid	0.238	16.5	280
Cumarin	0.387	17.4	280
Ellagic acid	2.104	19.02	280
Rosmarinic acid	1.591	19.2	280
Quercetin	3.233	21.6	280
Eugenol	0.591	23.7	280
Carvacrol	2.914	28.4	280
Thymol	Nd.	28.9	280

Tr.: Trace amount Nd: not detected nm: nanometer

3.3.Moisture Content of Cupcakes

The results showed that the independent effects of storage time, essential oil concentration, and their interactions significantly influenced the moisture content of cupcakes ($p<0.05$). Moisture content in all samples decreased during the first two months of storage (Table 3). Although moisture content continued to decrease with extended storage, the rate of decrease was less pronounced after two months. The highest moisture loss was observed in the control sample and the sample containing TBHQ, while the samples with 0.1% and 0.05% *Dracocephalum kotschyi* essential oil had the least moisture loss. Despite their hydrophobic nature, essential oils can form emulsions in the presence of surfactants (emulsifiers), improving moisture retention in cakes.

Table 3. Physicochemical properties of cupcake at different concentration of D. *kotschyi* essential oil.

Treatments	Storage time (month)	Weight loss (%)	Moisture content (%)
Control	0	10.80±0.26 ^{f}	19.77±0.38 ^{b}
	2	16.13±0.11 ^{cd}	18.80±0.36 ^{cd}
	4	19.13±0.31 ^{b}	17.24±0.10 ^{g}
DKEO 0.02%	0	11.17±0.18 ^{ef}	20.52±0.09 ^{a}
	2	15.23±0.15 ^{d}	18.71±0.22 ^{cd}
	4	18.40±0.10 ^{ab}	17.84±0.11 ^{g}
DKEO 0.05%	0	11.43±0.20 ^{ef}	20.43±0.23 ^{a}
	2	15.70±0.30 ^{cd}	19.72±0.11 ^{cd}
	4	17.60±0.10 ^{a}	18.01±0.10 ^{ef}
DKEO 0.1%	0	11.73±0.16 ^{e}	20.32±0.14 ^{a}
	2	15.63±0.60 ^{d}	19.37±0.12 ^{de}
	4	17.83±0.15 ^{ab}	18.81±0.07 ^{g}
TBHQ 0.02%	0	10.63±0.26 ^{f}	20.80±0.38 ^{a}
	2	16.77±0.11 ^{d}	18.97±0.36 ^{c}
	4	19.90±0.31 ^{bc}	18.51±0.10 ^{de}

Different superscript lowercase letters in the same column indicates significant differences ($p < 0.05$).

3.4. Weight Loss of Cupcakes

Variance analysis indicated that storage time, essential oil concentration, and their interaction had a significant effect on weight loss in cupcakes ($p < 0.05$). The weight loss trend was similar to moisture loss, with the highest weight loss occurring during the first two months of storage at 4°C, followed by a slower rate of decrease from the second to the fourth month (Table 3). Weight loss was highest in the control and TBHQ samples, while the 0.1% *Dracocephalum kotschyi* essential oil sample had the lowest weight loss.

3.5. Oxidative Stability of Cake Oil during Storage

3.5.1. pH

Variance analysis showed that storage time, essential oil concentration, and their interaction had a significant effect on pH changes ($p < 0.05$). The pH of all cupcake samples decreased significantly with extended storage time, though there was no significant change in pH from the second to the fourth month. The lowest pH was observed in the control sample after two months of storage, whereas the highest pH was found in the sample containing 0.02% *Dracocephalum kotschyi* essential oil after baking. The observed pH changes could be attributed to the production of free fatty acids and buffering characteristics of the food formulation components [10]. The TBHQ sample exhibited the smallest pH change after two months, while samples containing 0.05% and 0.1% *Dracocephalum kotschyi* essential oil showed the least pH reduction after four months of storage (Figure 1). As storage time increases, triglycerides can break down, releasing fatty acids. Hematian Sourki, Ghani [10] reported that cookies containing lemon verbena essential oil had a slower pH reduction than the control, suggesting that lemon verbena essential oil reduces the rate of lipid degradation and fatty acid formation.

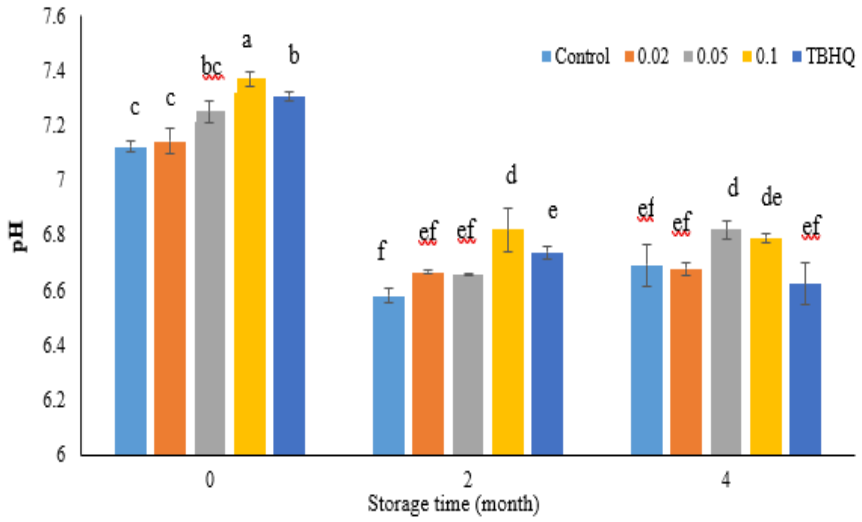

Figure 1. pH changes of cupcake at different concentration of D. *kotschyi* essential oil.

3.5.2. Acid Value

Variance analysis of the acid value data revealed that the independent and interactive effects of storage time and *Dracocephalum kotschyi* essential oil concentration on acid value changes were significant ($p < 0.05$). Results showed that acid value increased during the first two months of storage but subsequently decreased across all samples by the fourth month. The acid value reflects the level of free fatty acids in the cupcakes. During the initial two months, higher moisture content in the cupcakes facilitated the hydrolysis of triglycerides; however, with further storage and decreasing moisture, the rate of triglyceride hydrolysis reduced. Additionally, some fatty acids may have volatilized or reacted with other formulation components, reducing their acidity and consequently lowering the acid value [5]. Similar trends in acid value changes were reported by Hematian Sourki, Ghani [10], and Badei, El-Akel [21], in cookies containing lemon verbena essential oil, and cookies with cardamom and cinnamon powders, respectively. The highest acid values were observed in the control and 0.02% essential oil samples after two months, which were lower than values reported by Badei, El-Akel [21], and Reddy, Urooj [22] for cookies and biscuits with herbal essential oils.

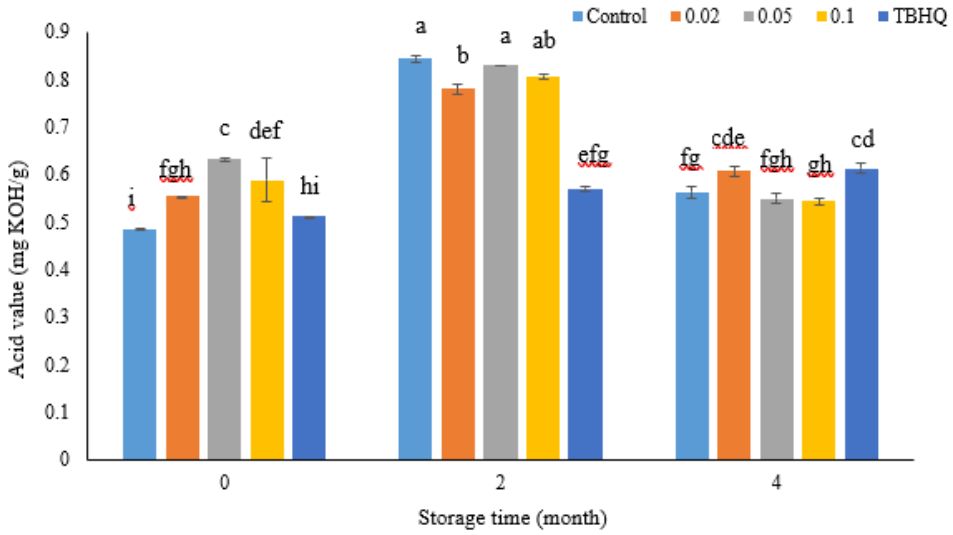

Figure 2. The effect of storage time and D. *kotschyi* essential oil on acid value of cupcakes

3.5.3. Peroxide Value

The peroxide value trends of the extracted oil from various cupcake samples are illustrated in Figure 3. Variance analysis showed that storage time, essential oil concentration, and their interaction significantly affected peroxide values ($p< 0.05$). Peroxide values increased significantly for all samples during the first two months of storage at 4°C, then decreased from the second to the fourth month (Figure 3). Peroxide values reflect the level of hydroperoxides formed due to lipid oxidation. Given the instability of primary oxidation products, hydroperoxides rapidly convert to secondary oxidation products (such as ketones, aliphatic aldehydes, alcohols, and hydrocarbons), leading to a reduction in peroxide values over time. Peroxide levels between 10 and 20 meq/kg fat indicate rancidity, although the product may still be acceptable for sensory qualities. Levels above 20 meq/kg indicate rancidity and consumer rejection [23]. In this study, peroxide values remained below 1 meq/kg in all samples, indicating a high antioxidant potential for *Dracocephalum kotschyi* essential oil**.**

Due to the instability of primary oxidation compounds, peroxide value alone cannot fully represent the oxidation state of fats. Therefore, additional indicators like the p-anisidine value and the TOTOX value should also be determined to assess oil quality [7].

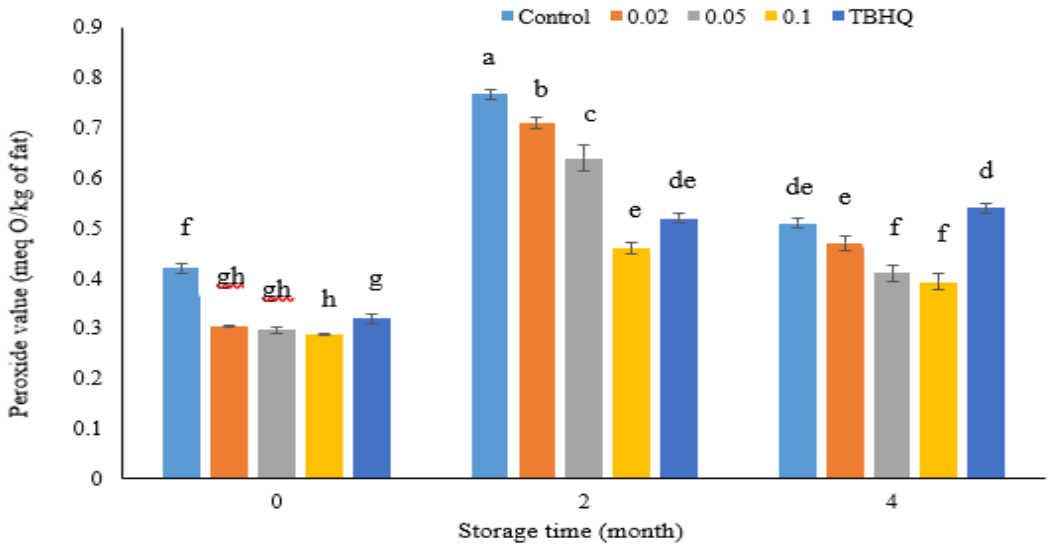

Figure 3. The effect of storage time and D. *kotschyi* essential oil on peroxide value of cupcakes.

3.5.4. p-Anisidine Value

Variance analysis indicated that storage time, essential oil concentration, and their interactions significantly influenced changes in p-anisidine values ($p<0.05$). The p-anisidine values in all cupcake samples increased significantly over time ($p<0.01$). As the p-anisidine index measures secondary oxidation products, its highest increase in all samples was observed from the second month onward, aligning with the decrease in peroxide values (Figure 4). The lowest p-anisidine values were observed in the cupcakes containing 0.1% *Dracocephalum kotschyi* essential oil and TBHQ after baking, while the control sample showed the highest values after four months of storage. Hematian Sourki, Ghani [10] reported a 14% reduction in p-anisidine values in cookies with lemon verbena essential oil compared to the control after six months of storage. Lean and Mohamed [24] reported p-anisidine values of 3.15, 7.2, and 25.03 for cupcakes with turmeric, lemongrass, and *Garcinia atroviridis* essential oils, respectively, after four weeks of storage. They observed a decline in p-anisidine values after two weeks due to the conversion of aldehydes into more stable malonaldehydes [24].

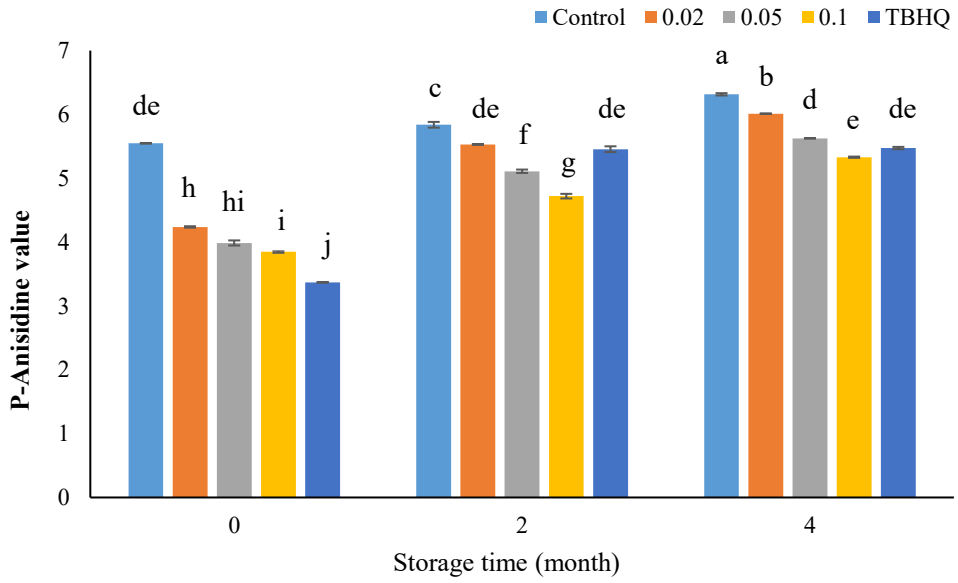

Figure 4. The effect of storage time and D. *kotschyi* essential oil on P-Anisidine value of cupcakes.

3.5.5. TOTOX Value

The peroxide and p-anisidine indices represent the extent of oxidation during the initial and final stages of lipid oxidation, respectively. The TOTOX value, which combines both hydroperoxide formation and subsequent decomposition products, is a more comprehensive indicator of fat oxidation throughout storage [25]. Specifically, TOTOX value reflects the total carbonyl compounds formed in fats, as well as other oxidative products developing over time [26]. As shown in Figure 5, TOTOX value changes over time mirrored the trends seen in peroxide and p-anisidine values across different cupcake formulations. The TOTOX value increased steeply during the first two months, then the rate of increase slowed. The highest TOTOX values were observed in the control samples after four months, while the lowest values were found in the samples containing 0.1% *Dracocephalum kotschyi* essential oil and TBHQ. Lean and Mohamed [24] reported TOTOX values of 69.27, 23.55, and 32.20 for cakes containing turmeric, lemongrass, and *Garcinia atroviridis* essential oils, respectively, after four weeks of storage, which were significantly higher than those observed in this study.

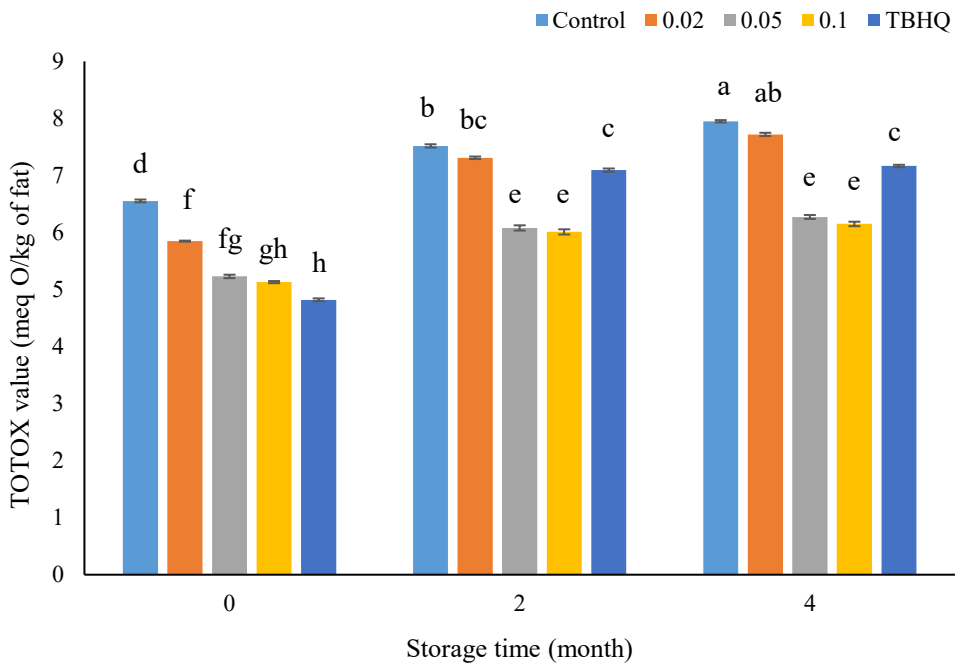

Figure 5. The effect of storage time and D. *kotschyi* essential oil on TOTOX value of cupcakes.

3.6.Antioxidant Activity

The results of the analysis of variance indicated that the independent effects of time and essential oil concentration, as well as their interaction effects, on the antioxidant capacity of cupcakes containing *Dracocephalum Kotschyi* essential oil were significant ($p<0.005$). The highest antioxidant activity was observed immediately after baking in samples containing 0.1% and 0.05% of *Dracocephalum Kotschyi* essential oil (Figure 6). The lowest antioxidant activity was found after 4 months of storage in cakes containing TBHQ. The antioxidant activity of the TBHQ-containing sample remained acceptable for two months after the storage of the cake; however, it sharply declined thereafter, nearly equal to the control sample. These results indicate the higher stability of *Dracocephalum Kotschyi* essential oil compared to the synthetic antioxidant TBHQ during storage. The results demonstrated that over time, the antioxidant capacity of all cake samples decreased. The volatility of essential oils and the loss of a portion of them during storage may account for the reduced antioxidant capacity of the cupcake samples. Vergara-Valencia, Granados-Pérez [27] reported that the antioxidant activity of cookies and bread containing mango fiber decreased during baking and storage. Jiménez-Escrig, Jiménez-Jiménez [28] suggested that the antioxidant activity of processed foods is highly dependent on the levels of phenolic compounds, and thermal processes can reduce their antioxidant capacity by degrading phenolic compounds.

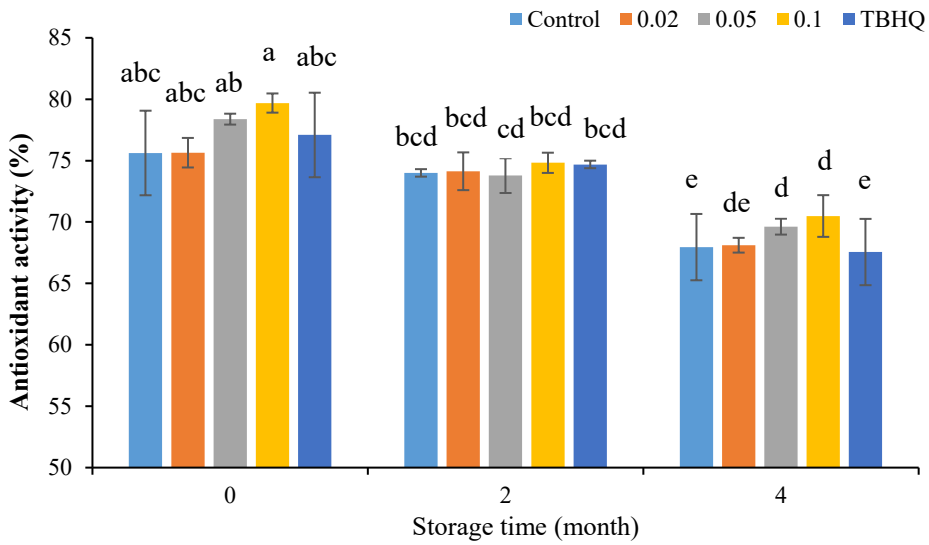

Figure 6. The effect of storage time and D. *kotschyi* essential oil on antioxidant capacity of cupcake

3.7.Sensory Evaluation

The results of the sensory evaluation of cupcakes containing various concentrations of *Dracocephalum Kotschyi* essential oil compared to the control and TBHQ samples are presented in Table 4. The analysis of variance indicated that the addition of *Dracocephalum Kotschyi* essential oil had a significant effect on the aroma, flavor, mouthfeel, and overall acceptance of the cupcakes ($p<0.05$). Conversely, the addition of *Dracocephalum Kotschyi* essential oil did not significantly affect sensory attributes such as crust color, appearance characteristics, freshness, porosity, texture color, texture firmness, and cake hardness ($p<0.05$). Ibrahium, Abd El-Ghany [29] reported that the addition of clove essential oil significantly affected only the aroma, flavor, and overall acceptance of the cake and had no significant effect on other sensory attributes, including appearance, color, and texture characteristics. Essential oils contain numerous aromatic and volatile compounds, which can exert either positive or negative effects on the aroma and flavor characteristics of food products. The scores for all sensory attributes of the cupcakes containing different concentrations of *Dracocephalum Kotschyi* essential oil are shown in Table 4. The results indicated that the highest sensory attribute score for aroma was observed in the control sample. This result suggests that the *Dracocephalum Kotschyi* essential oil at the concentrations used in this study did not have a favorable effect on the cake's aroma from the consumer's perspective. The results also revealed that the scores for other sensory attributes of the cupcakes that showed statistically significant differences (flavor, mouthfeel, and overall acceptance) decreased with increasing concentrations of *Dracocephalum Kotschyi* essential oil. According to Table 1, the main significant compounds in *Dracocephalum Kotschyi* essential oil are α-Pinene (25.50%) and Geranial (14.01%), which likely contributed to the unfavorable effects on the aroma and flavor characteristics of the cupcakes.

Despite the improvement in antioxidant properties and thermal stability of the cupcake oil due to *Dracocephalum Kotschyi* essential oil, the lack of favorable acceptance scores from consumers suggests that the use of these concentrations of this essential oil in cupcakes is not recommended. Badei, El-Akel [21] reported that while the use of cardamom, cinnamon, and clove essential oils in small amounts increased consumer acceptance in cookies, increasing the concentrations of these essential oils above threshold levels resulted in decreased consumer acceptance. They reported that the perception threshold levels for cardamom, cinnamon, and clove essential oils were 0.05%, 0.05%, and 0.075%, respectively. Ibrahium, Abd El-Ghany [29] noted that increasing clove essential oil concentrations from 400 to 800 ppm resulted in decreased sensory scores of the cake, with the cake containing 800 ppm of clove essential oil being unacceptable to consumers**.**

Table 4. Organoleptic properties scores of cupcakes contain different concentration of D. *kotschyi* essential oil.

		Treatments				
		Control	0.02%	0.05%	0.10%	TBHQ
Organoleptic properties	crust color	4.2 ns	4.0 ns	4.1 ns	4.3 ns	4.3 ns
	appearance	4.3 ns	4.1 ns	4.0 ns	4.4 ns	4.2 ns
	freshness	4.2 ns	4.1 ns	4.0 ns	4.1 ns	4.1 ns
	porosity	4.5 ns	3.9 ns	4.0 ns	3.9 ns	4.2 ns
	crumb color	4.5 ns	4.0 ns	4.0 ns	4.1 ns	4.3 ns
	flavor	4.9 a	2.9 c	2.6 c	2.8 c	4.6 b
	taste	3.7 a	2.2 c	2.1 c	2.7 b	4.1 a
	texture strength	4.4 ns	3.8 ns	3.9 ns	3.5 ns	4.3 ns
	hardness	4.3 ns	4.0 ns	3.8 ns	3.7 ns	4.3 ns
	mouthfeel	4.1 a	2.9 b	2.4 c	2.6 c	4.3 a
	overall acceptability	4.2 a	3.2 b	2.8 b	3.0 b	4.3 a

Note: Different lowercase letters in the same row denote significant differences (p < .05). (ns: Not significant)

4. Conclusion

The addition of *Dracocephalum Kotschyi* essential oil at various concentrations to high-fat baked goods, including cakes, can significantly reduce the rate of fat oxidation due to the antioxidant properties of its active compounds. Our results indicated that enriching cupcakes with varying concentrations of *Dracocephalum Kotschyi* essential oil could significantly enhance the oxidative stability of cake oils. The findings obtained from GC/MS and HPLC analyses, as well as phytochemical tests, showed that the main active compounds present in *Dracocephalum Kotschyi* essential oil and extract possess radical scavenging capabilities and can halt or significantly delay the chain oxidation of fats by inactivating these compounds; thus, they can compete with synthetic antioxidants as natural antioxidants. During storage at low temperature, it was observed that the acidity, peroxide value, p-anisidine value, and TOTOX value, which are considered oxidation indicators, increased at a much slower rate in the samples containing *Dracocephalum Kotschyi* essential oil compared to the control sample. The only drawback of using *Dracocephalum Kotschyi* essential oil in the cakes was its negative impact on the sensory properties. Two possible reasons can be considered for this result. The first reason might be the presence of a novel aroma that the panelists were unaccustomed to, which is somewhat typical for new products. The second reason could be the presence of pungent aromatic compounds like α-Pinene or Nerol in *Dracocephalum Kotschyi* essential oil, which significantly reduced the scores for aroma, flavor, and overall acceptance of the cake as the concentration increased in the cake formulation. Since no previous studies have investigated the use of this essential oil in food products, especially cakes, determining an appropriate concentration was not feasible, and the results of this research can be utilized in future studies. The findings of this research demonstrate that despite the high antioxidant capacity and positive effects of a food additive in enhancing the quality and shelf life of a product, the most important and influential factor for consumer acceptance is the sensory attributes. A food product with high nutritional properties will not be acceptable to consumers if it lacks desirable sensory attributes. Therefore, despite the positive effects of higher concentrations of *Dracocephalum Kotschyi* essential oil on the oxidative stability of cupcakes over time, and considering the importance of sensory evaluation and product acceptance by consumers, it is recommended to use *Dracocephalum Kotschyi* essential oil at a concentration of 0.02% in the formulation of cupcakes. It is suggested that future research could explore methods such as fractionating the components of *Dracocephalum Kotschyi* essential oil to remove undesirable aromatic and flavor compounds or encapsulating this essential oil to mitigate its negative effects on the sensory characteristics of food products, allowing its use as a natural antioxidant in various food products.

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بررسی خصوصیات فیتوشیمیایی زرین‌گیاه (*Dracocephalum kotschyi*): کاربرد آن به عنوان آنتی‌اکسیدان طبیعی در افزایش پایداری اکسیداتیو کیک فنجانی در طول نگهداری در دمای پایین

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چکیده

امروزه تحقیقات زیادی در زمینه استفاده از آنتی‌اکسیدان‌های طبیعی در مواد غذایی به جای آنتی‌اکسیدان‌های مصنوعی انجام شده‌است. در این پژوهش، اجزای فیتوشیمیایی گیاه دارویی زرین‌گیاه با استفاده از کروماتوگرافی مایع با کارایی بالا (HPLC) و کروماتوگرافی گازی/جرمی (GC/MS) شناسایی و تعیین مقدار شد. همچنین اثر غلظت‌های مختلف اسانس زرین‌گیاه (صفر به عنوان شاهد، ۰/۰۲، ۰/۰۵ و ۰/۱ درصد وزن روغن فرمولاسیون) بر پایداری اکسیداتیو، ماندگاری و ویژگی‌های حسی کیک فنجانی در طول ٤ ماه ماندگاری در دمای پایین (چهار درجه سانتی‌گراد) بررسی و با آنتی‌اکسیدان مصنوعی TBHQ مقایسه شد. نتایج نشان داد اسانس زرین گیاه حاوی ٤۸ ترکیب معطر بود که مهمترین آنها شامل a-Pinene، Geranial، Limonene و Neral بود. نتایج آنالیز آماری نشان داد اثر غلظت‌های مختلف اسانس زرین گیاه و زمان نگهداری کیک فنجانی تاثیر معنی‌داری بر متغیرهای وابسته شامل pH، عدد اسیدی، عدد پراکسید، عدد توتوکس، عدد پی-آنیسیدین و قدرت آنتی‌اکسیدانی نمونه‌ها داشت. در طول نگهداری کیک فنجانی، میزان pH کاهش یافت که احتمالا به دلیل شکستن تری گلیسریدها و تولید اسیدهای چرب آزاد بود. نتایج نشان داد با افزایش غلظت اسانس زرین گیاه، کاهش pH کیک در طول نگهداری کمتر بود. نتایج پایداری اکسیداتیو محتوی روغن کیک فنجانی نشان داد که اسانس زرین گیاه نسبت به آنتی اکسیدان مصنوعی TBHQ توانایی بیشتری در حفظ پایداری اکسیداتیو روغن کیک داشت. علی‌رغم ویژگی‌های مطلوب اسانس زرین گیاه در حفظ کیفیت و پایداری اکسیداتیو روغن کیک فنجانی، نتایج ارزیابی حسی نشان داد که از نظر مصرف کنندگان، این اسانس تاثیر نامطلوبی بر عطر و طعم کیک فنجانی داشت. با توجه به عملکرد اسانس زرین گیاه بر حفظ پایداری اکسیداتیو روغن کیک فنجانی به نظر می‌رسد درصورت استفاده از مقادیر کمتر این اسانس و استفاده از مواد عطر و طعم دهنده طبیعی، تاثیر نامطلوب آن بر ویژگی‌های حسی کیک روغنی رفع شود.