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ANTIOXIDANT CAPACITY AND FLAVOR PRESERVED HAZELNUT EXTRACT, MACERATION AND SC-CO_2 EXTRACTION AND COMPARISON OF EXTRACTION METHODS

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ABSTRACT

In this study, Tombul type hazelnuts (*Corylus avellana* L.) were extracted by supercritical carbondioxide (SC-CO_2) extraction and maceration method. The extraction yielded in 8.5% by SC-CO_2 extraction when 0.84% by maceration. Extracted hazelnuts were analyzed by Dynamic Headspace Analysis/Gas Chromatography-Mass Spectrometry and 24 volatile compounds were detected after SC-CO_2 method while 41 compounds were detected after maceration. Flavour and antioxidant value preserved Hazelnut extract by SC-CO_2 technique showed better radical scavenging ability in 2,2-Diphenyl-1-picrylhydrazyl (DPPH) than the one by maceration. Antioxidant activities of the extracts obtained by SC-CO_2 (12.35±0.06 µg/mL) and by maceration (8.79±0.23 µg/mL) had a medium impact compared with the synthetic antioxidant standards ($P< 0.05$). No significant difference was observed between the total phenolic contents of the hazelnut extracts obtained by SC-CO_2 method and by maceration ($P > 0.05$). As a result, SC-CO_2 technique for extraction of Tombul type hazelnuts was concluded to be more beneficial and suitable compared to maceration technique.

Keywords: Antioxidant capacity, dynamic headspace, hazelnut (*Corylus avellana* L.), SC-CO_2 extraction, volatile compound

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ANTİOKSİDAN AKTİVİTESİ VE AROMASI KORUNMUŞ FINDIK EKSTRAKTI ELDESİ, MASERASYON VE SC-CO_2 EKSTRAKSİYONU VE EKSTRAKSİYON YÖNTEMLERİNİN KIYASLANMASI

ÖZ

Bu çalışmada, Tombul tipi fındıklar (*Corylus avellana* L.) süperkritik karbondioksit (SC-CO_2) ekstraksiyonu ve maserasyon yöntemi ile ekstrakte edildi. Ekstraksiyon verimleri; maserasyon ile % 0.84 iken SC-CO_2 ekstraksiyonu ile % 8.5 olarak hesaplandı. Elde edilen ekstraktlarda uçucu bileşen analizleri Dinamik Headspace Analysis / Gaz Kromatografisi-Kütle Spektrometresi ile yapıldı ve 24 uçucu bileşik SC-CO_2 yöntemiyle elde edilen ekstraktta, 41 bileşik ise maserasyon ile elde edilen ekstraktta tepit edildi. SC-CO_2 tekniği ile elde edilen fındık ekstraktının 2,2-Dipenil-1-pikrilhidrazil (DPPH) yöntemiyle antioksidan kapasitesi incelendi buna göre; Süperkritik CO_2 yöntemi ile elde edilen fındık ekstraktının daha iyi radikal süpürme kabiliyeti gösterdiği görüldü. SC-CO_2 (12.35±0.06 µg / mL) ve maserasyon (8.79±0.23 µg / mL) yöntemi ile elde edilen ekstraktların antioksidan aktiviteleri için, sentetik antioksidan standartlarına kıyasla orta düzeyde bir etkiye sahip oldukları söylenebilir ($p < 0.05$). SC-CO_2 metodu ile elde edilen fındık ekstraktının toplam fenolik içeriği ile maserasyon ile elde edilen fındık ekstraktı arasında anlamlı bir fark gözlenmemiştir ($p < 0.05$). Sonuç olarak, Tombul tipi fındıkların ekstraksiyonu için SC-CO_2 tekniğinin, geleneksel bir yöntem olan maserasyon tekniğine kıyasla daha faydalı ve uygun olduğu sonucuna varılmıştır.

Anahtar kelimeler: Antioksidan kapasitesi, dinamik headspace, fındık (*Corylus avellana* L.), SC-CO_2 ekstrakisyonu, uçucu bileşenler

INTRODUCTION

Turkey is the largest producer country as well as the largest exporter for various species of hazelnuts in the world, contributing approximately 70% to the total global production, followed by Italy (12%), USA (4.5%) and Spain (2.5%) (Oliveira et al., 2008; Pelvan et al., 2012). Among 18 varieties of hazelnuts cultivated in Turkey, only Tombul (Round) hazelnut is classified as the premium quality due to its high oil content, distinctive taste and aroma. Its brown skin can be able to remove easily and quickly during roasting (Alasalvar et al., 2010; Alasalvar et al., 2012). Hazelnut provides a distinctive and unique flavour as a food ingredient. Hazelnut oily extracts are mainly composed of unsaturated fatty acids such as oleic (C18:1) and linoleic (C18:2) acids at high concentrations (80% and 12%, respectively) (Ozkal et al., 2005a; Bernardo-Gil et al., 2002). Taste and aroma of hazelnut extracts are caused by their taste-active components (free amino acids and phenolic acids, sugars, organic acids and condensed tannins) and aroma-active components (ketones, aldehydes, pyrazines, alcohols, aromatic hydrocarbons, furans, pyrroles, terpenes and acids) (Alasalvar et al., 2010). Hazelnuts can be consumed as raw and roasted. However, roasted hazelnuts have an advantage of improved desirable flavour in the extract which are caused by various components including Filbertone (5-methyl-(*E*)-2-hepten-4-one) and pyrazines (Saklar et al., 2001). Hazelnut extracts additionally contain antioxidant phenolics, vitamins (Vit E and Vit B complex), dietary fibres, minerals and phytosterols (Alasalvar et al., 2003a). These attributes make hazelnuts popular especially in Europe and Asia (Alasalvar et al., 2004). The antioxidant capacity of the hazelnut extract can be evaluated by considering their phenolic content, because phenolic substances are able to donate hydrogen atom to free radicals. Furthermore, antioxidants can increase the shelf life of products by retarding the process of lipid peroxidation when they are added to food products, especially to lipids and lipid containing foods (Singh et al, 2002).

Hazelnut extacts have a great potential for pharmaceutical and cosmetic purposes especially due to their fatty acid profile and high B vitamins, carotenoids, squalene and phytosterol content (Hotellier et al., 1972; Stuetz et al., 2017; Granata et al., 2017). Their chemoprotective potential have been reported to be caused by phenolic substances (Oliveira et al., 2008; Glei et al., 2018). Fatty acid content of hazelnut extracts may be

beneficial to prevent risk of coronary heart disease when tocopherol, squalene and phytosterol content provide cardioprotective effect (Maguire et al., 2004; Surra et al., 2013; Tey et al.; 2017).

Hazelnut extracts have antimicrobial, antimicotic and anticestodal effects (Oliveira et al., 2008; Eskandarian et al., 2018; Abuladze et al., 2015). Hazelnuts have also been recommended to use for their brain-protective activity and particularly reversing brain atrophy in Alzheimer's disease (Gorji et al., 2017).

Hazelnut extracts can be obtained by SC-CO_2 extraction and maceration methods (Alasalvar et al., 2010; Alasalvar et al., 2012; Alasalvar et al., 2004; Alasalvar et al., 2003b). Maceration technique presents some drawbacks like low efficiency and solvent residue in products. On the other hand, SC-CO_2 extraction which is an environmentally benign technique, is advantageous for providing higher efficiency by avoiding solvent residues and retaining organoleptic characteristics of a starting material. Various studies have been conducted on determination of efficiency, physicochemical characteristics and sensory properties of products obtained by using those 2 techniques, individually (Bernardo-Gil et al., 2002; Ozkal et al., 2005b; Farinelli et al., 2008**;** Ciurlia et al., 2009).

However, any comparison study on SC-CO_2 extraction and maceration method are not available in the literature for obtaining roasted hazelnut extracts. For the first time, it was aimed to compare antioxidant capacity, volatile compound contents of roasted hazelnut extracts obtained by using SC-CO_2 extraction and maceration methods in this study.

MATERIAL AND METHODS

Materials

Turkish Tombul variety of roasted hazelnuts were purchased from Ucuzcular Gıda (Istanbul, Turkey). Ethanol (EtOH, Merck), β-carotene (Sigma-Aldrich), butylated hydroxytoluene (BHT, Sigma-Aldrich), butylated hydroxyanisole (BHA, Sigma-Aldrich), α-tocopherol (Sigma-Aldrich) and Mili-Q water (TGI Pure Water System, USA) were provided from Aromsa A.S. (Kocaeli, Turkey). All other chemicals were of analytical grade.

Sample Preparation

Roasted hazelnuts were chopped in a planetary grinder. Particle size fraction (1,5 mm mesh size) of the coarse powder was obtained by standard Retsch sieves (Germany) and stored at 0-4 °C until the extraction process.

SC-CO_2 Method

SC-CO_2 extraction was performed using a Waters SFE 1000 supercritical fluid extraction system (U.S.A) equipped with an 1000 mL extractor under 450 bar pressure and 125 g_{CO2}/min CO_2 mass flow rate. Parameters were optimized including pressure, temperature, CO_2 flow rate, time, feed amount, particle size, co-solvent which are critical and valuable for the extraction process affecting yield. For this purpose, one parameter was changed in each experiment when the others were kept constant. This study was replicated 3 times.

Maceration Method

A maceration method reported by Contini *et al.* was modified for extraction of hazelnuts (Yilmaz, O. 2009). Chopped hazelnuts were dispersed in EtOH (80%, v/v) with a solid to solvent ratio of 1:1 (w/v). The dispersion was kept at the room temperature for 24 h until filtration process under vacuum at 50 bar (Büchi, Switzerland) and the supernatant was collected. The residue was macerated for three times under the same conditions (50 bar). EtOH content of the supernatants was evaporated by a rotary evaporator (Büchi, Switzerland) until 65°Bx under vacuum at 40 °C. All extracts are evaporated until 65°Bx because the extracts used in food industry, the extracts microbiological load is so important. It is known that If the extract is evaporated until 65°Bx, the extracts is got rid of their microbiological loads. Flavour and antioxidant value preserved Hazelnut extracts obtained were stored at 0-4 °C.

Determination of the Total Phenolic Content

The determination of the total phenolic content of the extracts were determined by Folin Ciocalteu method (Contini et al., 2008; Slinkard et al., 1977). The extracts were dissolved in EtOH (1000 ppm) and, 500 µL and 1000 µL samples were taken from the extracts. 0.1 mL 2N Folin-Ciocalteu reagent was added to each sample. After 3 min, 0.3 mL anhydrous sodium carbonate solution was added and they were diluted to 5 mL with distilled water. Solutions obtained were kept in the dark for reaction for 2 h and absorbance values were determined at 760 nm (Pgeneral T80+double beam UV/VIS Spectrometer, U.K.). Gallic acid was initially used to construct the standard curve (0-200 mg/ml. Results were expressed as mg of gallic acid equivalents/100g of extract (GAEs). This study replicated 3 times.

Antioxidant Capacity of the Extracts

Determination of Radical Scavenging Capacity Using DPPH Method

DPPH method was used for verifying the antioxidant capacity of the extracts (Contini et al., 2008; Slinkard et al., 1977). The extracts were kept in the dark and ambient conditions for 30 min. Absorbance values were measured spectrophotometrically at 517 nm and radical scavenging capacity (RSA) - inhibition percent was calculated. This study was repeated for each extract and synthetic antioxidant reagent (BHT, BHA, α-tocopherol) by replicating 3 times.

ß-Carotene-Linoleic Acid System Model

Antioxidant capacity of the extracts was measured by β-carotene-linoleic acid system model (Oliveira et al., 2008). A mixture prepared as above without β-carotene served as the control sample. Antioxidant capacity of the extracts was determined. This study was replicated 3 times for each extract and synthetic antioxidant reagent (BHT, BHA, α-tocopherol).

Dynamic Headspace/Gas Chromatography-Mass Spectrometry

Volatile compounds in hazelnut extracts were analyzed by DHA/GC-MS. Total ion chromatogram of the volatiles were obtained using the Gerstel DHS System (Germany) connected to an Agilent 7890A GC and an Agilent 5975C MS (Inert MSD with Triple Axis Detector, ABD). Preparation of the internal standard was based on the method described by Alasalvar (Alasalvar et al., 2003b). 1g hazelnut extract was weighed in a 20 mL standart headspace vial and 0.2 g 5 ppm 2,4,6-trimethyl-prydine solution was added. It was placed in a tray of Gerstel Multi Purpose Sampler. Desorbed compounds were injected automatically into an INNOWAX GC column (CP-WAX 52, 60 m x 0.25 µm film thickness x 0.25 mm inner diameter). The flow rate of the helium carrier gas was 1.2 mL/min. Samples were injected in the splitless mode. The GC oven temperature was programmed to a range from 40 °C to 240 °C at 5 °C/min heating rate.

Data Treatment and Statistics

The statistical analyzes were managed using one-way analysis of variance (ANOVA) and Tukey's multiple range tests to identify significant differences among data obtained from antioxdant capacity analysis of the extracts. The results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Process Yield of SC-CO_2 Extraction: Optimization of Parameters

Co-Solvent

Co-solvent use may be often required for optimization of parameters affecting yield of SC-CO_2 extraction process. Different co-solvents ratios and various CO_2 flow rates were found to result in extracts having polyphenols at differing ratios in a recent study (Manna et al., 2015). Thus, it should be decided whether to use a co-solvent or not. Parameters like pressure, CO_2 flow rate, extraction temperature and hazelnut feed amount must be kept constant in the case of co-solvent use. In this study, experiments were performed with and without co-solvent. The extract yield of extraction without a co-solvent was observed to be higher than one with co-solvent. This can be attributed to higher solubility of fats in CO_2 compared to EtOH and combination of EtOH and CO_2 (Kabouche et al., 2007). However, flavouring part of the extract, which is expressed as the Filbertone, could not be extracted without

co-solvent according to the dynamic headspace results (Table 1). Because Filbertone is freely soluble in EtOH. Therefore, EtOH was decided to be used as a co-solvent for SC-CO_2 extraction process.

Table 1. Optimization of Process Parameters for SC-CO_2 extraction process

Optimization	Test	EtOH (%)	P (Bar)	T (°C)	t (min)	Feed (g)	M_{CO2} (g/min)	Yield (%)	Filbertone (%)
Co-Solvent Usage	I	0	450	60	380	200±2	40	40.2	0
	II	5	450	60	380	200±2	40	19.1	1.85
Co-Solvent Ratio	I	2	350	50	45	150±2	125	9.2	0.89
	II	3	350	50	45	150±2	125	10.3	4.63
	III	5	350	50	45	150±2	125	8.4	5.22
	IV	7	350	50	45	150±2	125	11	2.42
	V	10	350	50	45	150±2	125	6	0
Process Time	I	5	350	50	45	255±2	125	6.4	4.85
	II	5	350	50	90	255±2	125	1.8	0.5
	III	5	350	50	180	255±2	125	8.9	0.79
	IV	5	350	50	420	255±2	125	3.9	0.38
	V	5	350	50	495	255±2	125	6.5	0.14
Feed Amount	I	5	350	50	45	150±2	125	9.7	4.84
	II	5	350	50	45	200±2	125	6.4	4.36
	III	5	350	50	45	335±2	125	4.9	3.29
CO_2 Flow Rate	I	5	350	50	45	150±2	40	1.5	3.6
	II	5	350	50	45	150±2	50	5	0.13
	III	5	350	50	45	150±2	75	4.8	0.91
	IV	5	350	50	45	150±2	100	5.6	4.87
	V	5	350	50	45	150±2	125	7.7	5.38
Pressure	I	5	150	50	45	150±2	125	2.5	3.97
	II	5	300	50	45	150±2	125	3.2	3.91
	III	5	350	50	45	150±2	125	8	4.98
	IV	5	400	50	45	150±2	125	6.5	4.21
	V	5	450	50	45	150±2	125	6.9	3.36
Temperature	I	5	350	40	45	150±2	125	2.9	2.65
	II	5	350	50	45	150±2	125	8.3	5.64
	III	5	350	60	45	150±2	125	7.5	3.46
	IV	5	350	70	45	150±2	125	5	1.41

P, pressure; T, temperature; t, time; Mco_2, CO_2 flow rate

Optimization of co-solvent ratio which is critical for affecting extraction yield and selectivity, is required for achievement of extraction process. Following the "like-dissolves-like" principle, EtOH was specified as the co-solvent since the extracted chemical compound was a ketone and could be dissolved in alcohol (Kabouche et al., 2007) 2, 3, 5, 7 and 10% co-solvent ratios (v/v) were studied at constant extraction temperature, pressure, CO_2 flow rate, process time and feed amount, then extracts were analysed sensually and with dynamic headspace. 5% EtOH ratio was

confirmed to be optimum since maximum amount of Filbertone and one of the highest yields were obtained at this ratio (Table 1). Yield of extract obtained with SC-CO_2 extraction was 10 fold higher compared to the maceration method due to the lower diffusion coefficient of supercritical fluids and high mass transfer capability of the liquid CO_2 (Helena et al., 1995). In the case of Filbertone yields, similar results were obtained since EtOH getting contact with the material was renewed continuously as the principle of SC-CO_2 extraction method.

Process Time

It was reported that the extraction yield was enhanced as the process time was increased (Kabouche et al., 2007). Pressure, extraction temperature, feed amount, rate of CO_2 flow and EtOH ratio were kept constant while different process times (45, 90, 180, 420 and 495 min.) were performed in each test for observing the effect of the process time. The extracts were then analysed both sensually and with dynamic headspace. It was seen that the maximum yield (8.9%) was achieved with a process time of 180 min. 0.79 % Filbertone was confirmed to be extracted during 180 min (Table 1). On the other hand, 45 min provided the highest filbertone rate (4.85%). Extract yield was 6.4% for 45 min SC-CO_2 extraction because of this reason, "45 min" was chosen as the optimum process time. SC-CO_2 extraction method introduces several advantages over traditional methods like maceration. When maceration is employed at least 24 hours, SC-CO_2 extraction method can be completed in a few hours for yielding purer extracts with higher quality compared to traditional methods. This is the biggest advantage of SC-CO_2 extraction method for large-scale production in food industry.

Hazelnut Feed Amount

Particle size of the feed material is very critical for extraction process. When the particle size is very small, the caking problem occurs, which is the tendency of the powders to form lumps, comes out in the extractor; on the other hand, when the particle size is very large, it results in channeling which can decrease the extraction yield (Manna et al., 2015). In a study, yield has been reported to enhance and caking not to observe when particle size of the feed material was between 0.85 mm and 2 mm (Ozkal et al., 2005). In this study, we used particle fraction of roasted hazelnut between 1 mm and 2 mm mesh sieves. To determine the amount of hazelnut in the extractor feed tank, pressure, extraction temperature, CO_2 flow rate, EtOH ratio and process time were kept constant while 150, 200 and 335 g hazelnut feed amounts were tested. Optimum hazelnut feed amount was chosen as "150 ± 2 g" since the maximum yield (9.7%) and the highest Filbertone percentage (4.84%) were observed with this amount (Table 1).

CO_2 Flow Rate

The yield of an extraction process increases with increasing flow rate. When the flow rate is comparably high, the mass transfer resistance around the feed particles decreases and the yield enhances (Doker et al., 2004). For the purpose of optimizing of the flow rate; pressure, extraction temperature, process time, feed amount and EtOH ratio were kept constant while 40, 50, 75, 100 and 125 g_{CO2}/min flow rates were applied on to hazelnuts. Optimum CO_2 flow rate was specified as "125 g_{CO2}/min" because the maximum yield (7.7%) and the highest Filbertone amount (5.38%) were obtained with 125 g_{CO2}/min of CO_2 flow rate (Table 1).

Process Pressure

Pressure affects the yield and the selectivity of the extraction process. The solubility is increased and the volume of the fluid is decreased by increasing pressure at a constant extraction temperature (Kabouche et al., 2007). Temperature, CO_2 flow rate, EtOH ratio, process time and feed amount were kept constant while 150, 300, 350, 400 and 450 Bar pressures were tried during the extraction process for optimizing. The pressure was optimized as "350 bar" since the maximum yield (8.0%) and the highest Filbertone amount (4.98%) were observed at this pressure (Table 1).

Temperature

When temperature is increased at a constant pressure, density of a supercritical fluid is

decreased and its solubility is affected (Manna et al., 2015). However, the process temperature is directly related to the raw material and the flavouring part which is wanted to be extracted. While optimizing the temperature, heat sensitive raw materials should be considered. SC-CO_2 extraction of hazelnuts were replicated at various temperatures (40, 50, 60 and 70 °C) while keeping the other process conditions constant. 50 °C was confirmed to be the most suitable temperature since maximum yield and the highest Filbertone amount were gained (Table 1).

Process Yield

The process yield of the hazelnut extraction is related with the extraction efficiency. In SC-CO_2 method, detected optimum process parameters were used and extraction yield was calculated as 8.5% while in maceration, it was 0.84%. The higher efficiency of SC-CO_2 extraction is the result of its process conditions. Thus, SC-CO_2 technique can be appreciated as a preferable method to maceration for hazelnut extraction.

Volatile Compounds (DHA/GC-MS) in the Extracts

24 and 41 compounds were detected in hazelnuts extracted by SC-CO_2 and maceration method, respectively (Table 2). It was observed that rates of the characteristic flavouring components, Filbertone (5-methyl-(*E*)-2-hepten-4-one and 2,5-dimethyl pyrazine) were higher in the extract obtained by SC-CO_2 than by maceration. 5-methyl-(*E*)-2-hepten-4-one and 2,5-dimethyl pyrazine are particularly desired volatile compounds from flavour and antioxidant value preserved hazelnut extracts for food industry in all over the world. 5-methyl-(*E*)-2-hepten-4-one was obtained at higher concentrations by SC-CO_2 method than maceration. SC-CO_2 extraction method was also confirmed to be a selective technique for obtaining hazelnut extract. However, 2-ethyl-5-methyl pyrazine concentration was higher in the extract obtained by maceration than by SC-CO_2.

Table 2. Comparison of Volatile Compounds

Peak	Compound	RT (min)	RI*	SC-CO_2 extraction (ng/g)	Maceration (ng/g)
1	Ethyl acetate	5.837	907	nd	12670±1558
2	EtOH	6.322	929	946±69	11700±1663
3	Hexanal	8.980	1084	110±7	721±80
4	n-Undecane	9.067	1100	nd	263±13
5	n-2-pentanol	9.611	1091	nd	126±21
6	3(*E*)-penten-2-one	10.102	1122	nd	137±11
7	Heptanal	11.333	1186	49±7	Nd
8	n-Dodecane	11.367	1200	155±10	212±38
9	3-methyl-1-butanol	11.705	1184	nd	324±37
10	2-pentyl furan	12.319	1229	nd	220±34
11	Ethyl hexanoate	12.406	1223	nd	186±21
12	n-pentan-1-ol	12.800	1213	383±10	592±37
13	p-cymene	13.400	1272	57±5	201±35
14	n-Octanal	13.935	1278	260±11	148±11
15	Tridecane	13.885	1312	263±10	228±39
16	5-methyl-(E)-2-hepten-4-one	14.054	1288	855±25	564±12
17	3-penten-2-one	14.371	1123	nd	60±7
18	2,5-dimethyl pyrazine	15.098	1306	536±15	325±13

Table 2. continuation

19	Ethyl lactate	15.312	1358	nd	546±12
20	1-Hexanol	15.457	1345	485±13	663±78
21	Butan-2-ol	15.876	975	nd	130±11
22	Tetradecane	16.450	1400	nd	352±6
23	IS (2,4,6-trimethyl pridine)	16.498	1360	-	-
24	Nonanal	16.617	1382	336±13	Nd
25	2-ethyl-5-methyl pyrazine	16.785	1386	172±5	269±10
26	3-octene-2-one	17.126	1388	nd	52±6
27	5-methyltetradecane	17.611	1444	97±9	Nd
28	Trans-2-octenal	17.710	1427	232±20	Nd
29	4-methyl tetradecane	17.786	1460	175±12	Nd
30	Furfural	18.675	1455	nd	201±17
31	2-ethyl-1-hexanol	18.902	1485	472±6	379±43
32	Ethyl 3-hydroxy butyrate	19.842	1521	nd	91±9
33	Propanoic acid	20.187	1523	nd	430±71
34	Butanal	20.284	832	nd	227±41
35	1-Octanol	20.597	1519	775±21	Nd
36	Isobutyric acid	20.868	1558	nd	242±55
37	Hexadecane	21.410	1600	nd	103±7
38	Butanoic acid	22.334	1619	nd	192±9
39	Butyrolactone gamma	22.983	1632	319±10	318±58
40	Furfuryl alcohol	23.206	1199	173±14	154±17
41	2-methyl butanoic acid	23.286	1665	nd	456±56
42	Benzyl acetate	24.842	1697	nd	156±17
43	Pentanoic acid	24.845	1720	172±3	Nd
44	Hexanoic acid	27.176	1872	659±2	621±4
45	Phenyl Ethyl alcohol	28.889	1859	nd	104±7
46	Octanoic acid	31.521	2083	1089±8	323±7
47	Decanoic acid	35.472	2361	778±21	169± 14
48	Benzoic acid	39.092	1624	nd	81±3
49	Lauric acid	42.757	2517	nd	235±13
	Total Volatiles			9548±326	35171±4201

*Kovats Retention Indices

Determination of Antioxidant Capacity of the Extracts

When scavenging capacity of the extracts on DPPH radicals was compared, hazelnut extract obtained by SC-CO_2 extraction was determined to be better than extract obtained by maceration (Table 3). DPPH radical capacity of the hazelnut extract obtained by SC-CO_2 extraction was found to be 1.5 fold higher than maceration. Total phenolic contents and % inhibition of hazelnut extracts obtained by SC-CO_2 method was insignificantly higher compared to maceration ($p < 0.05$). However, both of the methods were seen to show lower scavenging activities than the synthetic antioxidants (BHT, BHA and α-tocopherol). In addition, both methods showed no significant difference in β-carotene assay ($p < 0.05$). β-carotene-linoleic acid results proved that synthetic antioxidant standards were superior to both extraction methods. It could be concluded that antioxidant activities of the hazelnut extract obtained by SC-CO_2 and by maceration have a medium impact compared with the synthetic antioxidant standards. As we expected, substances dissolved in the lipophilic extract drifted with CO_2 flow. This process resulted in higher antioxidant amounts in the extracts.

Table 3. Antioxidant capacity results of hazelnut extracts

	DPPH	TPC	β-Carotene-Linoleic Acid
Extraction Model	Inhibition %	(mg of GAE/100 g)	Inhibition%
SC-CO_2 Extraction	12.35±0.06	149.38±0.92	44.36±0.33
Maceration	8.79±0.23	146.16±0.91	42.08±0.60
BHT	45.50±0.13		84.73±0.56
BHA	67.46±0.17		90.56±0.57
α-Tocopherol	59.46±0.23		67.47±0.51

[a]Data are expressed as mean ± SD (n=3, P <0.05).

CONCLUSION

The aim of the present study was to detect the volatile compounds of the extracts of roasted Tombul hazelnuts obtained by SC-CO_2 and maceration methods, furthermore to compare the yields, antioxidant activities and volatile molecules by DHA/GC-MS analysis of these two techniques. The yield of SC-CO_2 extraction (8.5 %) was higher to the yield of maceration (0.84 %) as a result of the low temperature and high pressure process conditions of SC-CO_2 method. Extract is obtained from SC-CO_2, yield is 8,5%. SC-CO2 conditions are detected and the optimum conditions are 5% EtOH used as a co-solvent, 350 Bar (pressure), 50°C (Temperature), 125 gCO2/min. (flow rate) and 45 minutes (process time). 24 volatile compounds in SC-CO_2 extraction and 41 compounds in maceration were detected by DHA/GC-MS. 5-methyl-(*E*)-2-hepten-4-one and 2,5-dimethyl pyrazine are particularly desired volatile compounds from flavour preserved hazelnut extracts for food industry in all over the world. 5-methyl-(*E*)-2-hepten-4-one was obtained at higher concentrations by SC-CO_2 method than maceration. DPPH radical capacity of the hazelnut extract obtained by SC-CO_2 extraction was found to be 1,5 fold higher than maceration. Total phenolic contents and % inhibiton of hazelnut extracts obtained by SC-CO_2 method was insignficantly higher compared to maceration.

CONFLICT OF INTEREST

Asli Barla Demirkoz declares that she has no conflict of interest. Melis Karakas declares that she has no conflict of interest. Pelin Bayramoğlu declares that she has no conflict of interest. Melike Üner declares that she has no conflict of interest.

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