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The effect of fermentation process on bioactive properties, essential oil composition and phenolic constituents of raw fresh and fermented sea fennel (*Crithmum maritimum* L.) leaves

Mehmet Musa Özcan*[,1,+], Nurhan Uslu[1], Gilles Figueredo[2], Fahad Al Juhaimi[3], Kashif Ghafoor[3], Elfadıl E Babiker[3], Omer N Alsawmahi[3], Mustafa Mete Özcan[4], Isam A & Mohamed Ahmed[3]

[1]Department of Food Engineering, Faculty of Agriculture, Selçuk University, 42031 Konya, Turkey

[2]Lexva Analytique 7 rue Henri Mondor Biopole Clermont-Limagne 63360 Saint beauzire France

[3]Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh-Saudi Arabia

[4]Karapınar Aydoğanlar High Vocational College, Selçuk University, Konya,Turkey

E-mail: [+]mozcan@selcuk.edu.tr; kghafoor@ksu.edu.sa

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The influence of fermentation on antioxidant activity, total phenol, total flavonoid and phenolic compounds of sea fennel and also volatile compounds of sea fennel essential oil was investigated and compared with fresh samples. Antioxidant activity, total fenolic and flavonoid contents decresed from 89.79 to 63.13%; from 259.58 to 77.92 mg/100 g; from 2114.67 to 390.50 mg/100 g, respectively. Twenty-six and thirty-three components of sea fennel oils were identified in raw and fermented sea fennel, accounting to about 99.99% and 99.44% of the total oil, respectively. The raw and fermented sea fennel leaves contained 22.31 and 1.32% sabinene, 12.08% and 7.45% limonene, 10.30% and 11.61% β-phellandrene, 8.59% and 9.17% (Z)-β-ocimene, 7.08% and 3.55% α-pinene, 28.36% and 42.05% γ-terpinene, 2.57% and 8.64% terpinene-4-ol, respectively. Dominant phenolic compounds were (+)-catechin, gallic acid, 3,4-dihydroxybenzoic acid and *p*-coumaric acid. Generally, all of the phenolic compounds reduced the effect of microorganisms during,. However, essential oil contents of sea fennel were not effected from fermentation process.

Keywords: Antioxidant activity, Brine oil constituents, Fermentation, Flavonoid, GC/MS, HPLC, Phenolic compounds, Sea fennel

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Sea fennel, known as maritime rock, deniz rezenesi (in Turkish) and rock samphire (*Crithmum maritimum* L.), belongs to the family of Apiaceae[1]. It is a salt-tolerant plant and widely grown inmaritime rocks and more rarely sandy beaches in Mediterranean countries and Atlantic coasts[2]. Sea fennel has several usage areas such as culinary, medicine and cosmetics because of the content of the nutrients and phytochemicals. Additionally, it has appetizer, tonic, carminative and diuretic effects[3]. The leaves of sea fennel contain various bioactive compounds which can be utilised aromatic, antimicrobial and insecticide purposes[4]. The aerial parts of sea fennel are generally used as a pickling herb[5,6]. In the Mediterranean region, fresh or fermented leaves are consumed as salad with yoğurt[7]. Sabinene, γ-terpinene, thymol methyl ether, dillapiol, α-pinen, p-cymol, apiole, cis-ß-ocimene and terpinen-4-ol are the key constituents of sea fennel leaves[8]. In recent years, Medicinal and aromatic plants have taken attention due to its bioactive substance. Limited studies were carried out on bioactive properrties and volatile compounds of fresh and fermented sea fennel[7,8]. The aim of current study was to determine the influence of fermentation on total phenol, antioxidant activity, phenolic constituents of sea fennel extract and volatile compounds of essential oil of sea fennel was investigated.

*Corresponding author

Material and methods

Materials

Sea fennels (aerial parts) collected from Muğla (Bodrum) province in Turkey were brought to laboratory in paper bags under cool conditions.

Methods

Fermentation process

Sea fennel (aerial parts) was fermented in 10% brine in a jar (1 L) at 24°C for 45 days.

Chemical properties

The titratable acidity , pH and salt contents of brine were carried out according to method of Sanchez et al.[9].

Essential oil content

Fresh and fermented sea fennels (leaves+stems) were cut into small pieces. The essential oil contents of samples were determined using Clevenger apparatus for 5 h.

Sample extraction process and identification of constituents

Aerial parts of sea fennel cut into small pieces were extracted according to method stated by Talhaoui et al.[10]. After methanol:water mixture (80/20, v/v) (10 mL) was added on the sample (0.5 g), the extracts concentrated by vacuum was completed to 25 mL by methanol,and filtered with 0.45 µm filter.

The essential oil were analysed on a AGILENT gas chromatograph Model 7890, coupled to a AGILENT MS model 5975, equipped with a DB5 MS column (20 m X 0,18 mm, 0,18 µm), programming from 50°C (3.2 min) to 300°C at 8°C/mn, 5 min hold.

Total phenolic content

The total phenol content (as mg gallic acid equivalent (GAE)/100 g) of samples was determined by using the Folin-Ciocalteu (FC) reagent according to method stated by Yoo *et al.*[11].

Total flavonoid content

Total flavonoid contents were analsed by using colorimetric method according to method stated by Hogan *et al.*,[12]. The absorbance was recorded at 510 nm with a spectrophotometer.

Antioxidant activity

The free radical scavenging activities of extracts were determined at 517 nm in a spectrophotometer using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to method stated by Lee *et al.*[13].

Phenolic compounds

The phenolic compounds were determined by HPLC (Shimadzu-HPLC), equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6×250 mm) column.

Statistical Analyses

Results were analysed for mean±standard deviation (MSTAT C) and statistical significance by analysis of variance[14].

Results and discussion

The bioactive properties of fresh and fermented sea fennel are presented in Table 1. Antioxidant activity, total phenolic and total flavanoid contents were decreased from 89.79 to 63.13%; from 259.58 to 77.92 mg/100 g and from 2114.67 to 390.50 mg/100 g respectively with fermentation process ($p<0.05$). After fermentation, titratable acidity, pH and salt content of sea fennel brine were determined as 0.13%, 4.78 and 5.53 g/100 mL, respectively. A major reduction in the contents of antioxidant, phenolic and flavonoid can be due to fermantation. In previous studies, fermentation increases the antioxidant activity of foods, which is explained by (i) the enzymatic cleavage of the cell wall and facilitation of the extraction of antioxidants; (ii) depolymerization high molecular weight phenolic compounds by lactic acid bacteria and releasing simple phenolics[15]; (iii) the liberation of free forms resulting in the hydrolysis of phenolic glycosides[16]. In contrast, Othman *et al.*[17] found that phenolic compounds were degraded and antioxidant activity decreased after fermentation. Therefore, phenolic, flavanoid and antioxidant contents vary depending on many factors such as activity of microorganisms, temperature, pH, fermentation time, food, cultivation environment, presence of inhibitor and aerobic conditions with fermentation process[15]. Total polyphenol and flavonoid contents of sea fennel ranged from 7.16 mg GAE/g; 4.77 mg CE/g in vegetative period to 8.27 mg GAE/g; 3.45 mg CE/g in flowering period, respectively[4]. Aerial parts of raw and fermented sea fennel (*Crithmum maritimum* L.) collected from Muğla (Bodrum) province contained 0.079% and 0.090% essential oil, respectively. The essential oil contentes of sea fennel changed between 0.17% and 0.85% [2, 18-22]. An important change was not observed in essential oil content with fermentation process. Leaves (61.8%) and flowers (61.0%) of sea fennel exhibited better antioxidant activity than stems (13.0%)[3]. The amount of total phenolics varied between 12.4-12.8 mg GAE/200 mL in stems;

Table 1 — Some physicochemical properties of fresh and fermented sea fennel

	Fresh sea fennel	Fermented sea fennel
Antioxidant activity (%)	89.79 ± 0.00*a	63.13±0.01b
Total phenolic content (mgGAE /100g)	259.58 ±0.03a**	77.92±0.02b
Total flavonoid content (mg/100g)	2114.67±0.01a	390.50±0.01b
Essential oil content (%)	0.09±0.05a	0.08±0.03a

33.7-35.3 mg GAE/200 mL in leaves; 21.2-22.6 mg GAE/200 mL in flowers of sea fennel, while total flavonoid contents were found between 22.9-27.1 mg RE/200 mL in stems; 54.4-57.2 mg RE/200 mL in leaves; 48.0-40.7 mg RE/200 mL in flowers of sea fennel[23]. In another study, total polyphenol and flavonoid contents ranged between 4.1 and 14.1 mg GAE/g; 2.9 and 6.7 mg CE/g, respectively[24]. In addition, titratable acidity, pH and salt content in brine of sea fennel were between 0.17-0.18 (5, lactic acid), 4.47-4.50 and 8.65-9.18%, respectively[25]. Özcan[26] reported that fresh leaves and stems of sea fennel in 8% salt and 8% salt+1% yogurt+1% sugar (saccharose) were fermented for 25 days, and the 8% salt+1% yogurt+1% sugar samples were superior to the 8% salt brines samples for final product quality.

The chemical composition of fresh and fermented sea fennel (*C. maritimum* L.) essential oil are given in Table 2. It was observed important changes in the constituents of fresh and fermented sea fennel oils. Twenty-six and thirty-three components were identified in fresh and fermented sea fennel, accounting to about 99.99% and 99.44% of the total oil, respectively (Table 2). The fresh and fermented sea fennel leaves contained 22.31 and 1.32% sabinene, 12.08% and 7.45% limonene, 10.30% and 11.61% β-phellandrene, 8.59% and 9.17% (Z)-β-ocimene, 7.08% and 3.55% α-pinene, 28.36% and 42.05% γ-terpinene, 2.57% and 8.64% terpinene-4-ol, respectively ($p<0.05$). During fermentation, while sabinene, limonene, α-pinene contents of sea fennel leaves are decreased, β-phellandrene, (Z)-β-ocimene, γ-terpinene, carvacrol methyl ether and terpinene-4-ol contents had increased. These increases and decreases in essential oil components of the sea fennel are probably due to biochemical activities during fermentation. In sea fennel leaves collected from two different location in Turkey 32 and 36% γ-terpinene, 21 and 22% β-phellandrene and 9 and 13% sabinene were found[21]. The essential oil of sea fennel was characterized as sabinene (26.9%), limonene (24.2%) and γ-terpinene (19.3%)[20]. Barroso et al.[27] reported that sea fennel (*Crithmum maritimum* L.) oil collected from Costa de Caparica in Portugal contained 7-42% sabinene and 26-55% γ-terpinene. Sea fennel oil contained 37% γ-terpinene, 29% methyl thymol, 10% p-cymene and 8% β-pinene[18]. In another investigation, the main components of the sea fennel aerial parts were γ-terpinene (39.3%), methylcarvacrol (21.6%) and p-cymene (11.8%), while the major compounds of roots oil were terpinolene (36.9%), dillapiole (26.8%) and γ-terpinene (21.9%)[19]. Jallali *et al.*[24] observed that essential oil yield of *C. maritimum* varied from 0.07% to 0.34%. Houta et al.[2] reported that the essential oil of sea fennel mainly contained dillapiole (2.39-41.35%), thymyl methyl ether (20.13-34.75%), p-cymene (4.83-22.08%) and γ-terpinene (22.54-43.29%). According to Mekinic *et al.*[3], essential oil yields of flowers, leaves and stems of sea fennel were 2.44, 0.55 and 0.19%, respectively.

Table 2 — Chemical composition of fresh and fermented sea fennel (*C.maritimum* L.) oil*(%)

RT	Constituents	Fresh sea fennel	Fermented sea fennel
11.86	α-thujene	0.24 ± **0.03b	0.30 ± 0.01a
12.17	α-pinene	7.08 ± 0.01a***	3.51 ± 0.01b
12.78	Camphene	0.06 ± 0.01a	0.05 ± 0.00a
13.61	Sabinene	22.30 ± 0.03a	1.32 ± 0.01b
13.80	β-pinene	0.40 ± 0.01a	0.19 ± 0.00b
14.16	Myrcene	1.22 ± 0.05a	1.02 ± 0.03b
14.68	Octanal	-****	0.06 ± 0.01
14.73	p-mentha-1(7),8-diene	0.10 ± 0.01a	0.05 ± 0.00b
14.82	α-phellandrene	0.28 ± 0.01b	0.35 ± 0.04a
14.89	δ-3-carene	0.04 ± 0.01b	0.07 ± 0.01a
15.20	α-terpine	0.53 ± 0.03b	1.09 ± 0.0a
15.50	p-cymene	2.53 ± 0.05b	5.68 ± 0.07a
15.68	Limonene	12.08 ± 0.09a	7.45 ± 0.05b
15.76	β-phellandrene	10.39 ± 0.03b	11.61 ± 0.09a
15.84	(Z)-β-ocimene	8.59 ± 0.05b	9.17 ± 0.07a
16.18	(E)-β-ocimene	0.34 ± 0.03b	0.38 ± 0.05a
16.74	γ-terpinene	28.36 ± 0.09b	42.05 ± 0.11a
17.56	Terpinolene	0.17 ± 0.01b	0.40 ± 0.03a
18.90	Allo-ocimene	0.47 ± 0.05b	0.77 ± 0.09a
20.71	Terpinene-4-ol	1.22 ± 0.07b	2.81 ± 0.05a
21.14	α-terpineol	0.01 ± 0.00b	0.06 ± 0.01a
21.19	Estragol	-	0.13 ± 0.01
21.89	Thymol methyl ether	0.21 ± 0.01b	0.39 ± 0.03a
22.10	Carvacrol methyl ethe r	2.56 ± 0.03b	8.64 ± 0.07a
25.09	δ-elemene	0.05 ± 0.01b	0.11 ± 0.01a
26.34	α-copaene	-	0.02 ± 0.00
26.66	β-elemene	-	0.02 ± 0.00
27.56	β-caryophyllene	0.02 ± 0.00b	0.04 ± 0.01a
29.14	Germacrene-D	0.36 ± 0.03b	1.00 ± 0.03a
29.50	Bicyclogermacrene	0.37 ± 0.01b	0.52 ± 0.03a
29.98	δ-cadinene	-	0.03 ± 0.00
30.46	α-cadinene	-	0.03 ± 0.00
31.54	Spathulenol	-	0.12 ± 0.01
Total		99.99	99.44

*Compound listed in the order of elution from a HP-5MS column
**mean±standard deviation
***Values in eachrow with different letters are significantly different ($p<0.05$)
****nonidentified

Limonene, terpinene and sabinene were the main compounds of sea fennel essential oil[28]. It was observed that the major essential oil components compared to litereture were mostly the same while the amounts were different. The highest increasing was observed in γ-terpinene and carvacrol methyl ether during fermentation.

Phenolic compositions of fresh and fermented sea fennel samples are given Table 3. The main flavonoids were (+)-catechin and quercetin in fresh sample. Fermentatiton process generally caused the degredation of phenolic compounds. The highest decrease was observed in catechin content (from 31.58 to 15.47 mg/100 g), followed by p-coumaric acid (from 15.77 to 0.29 mg/100 g) ($p<0.05$). While 1,2-Dihydroxybenzene, *trans*-cinnamic acid and isorhamnetin contents increase during fermentation, other constituents decreased. In fermentation process, the amount and structure of phenolic compounds showed changes based on especially pH changes[15]. The quercetin content of sea fennel decreased from 13.48 to 5.37 mg/100 g. Moreover, the reduction in gallic (23.71 to 21.38 mg/100 g), 3,4-dihydroxybenzoic (21.73 to 16.22 mg/100 g), syringic (11.04 to 7.24 mg/100 g) and caffeic (10.33 to 7.23 mg/100 g) acids were determined during fermentation. Only 1,2-dihydroxybenzene amount of sea fennel increased from 13.83 to 24.62 mg/100 g with fermentation process. Coumaric and ferulic acid contents were 0.09-0.12 and 0.15-0.18 mg/g in stems, 0.36-0.38 and 0.57-0.77 mg/g in leaves, 0.29-0.31 and 0.23-0.28 mg/g in flowers of sea fennel[23]. Epicatechin amounts of stems, leaves and flowers were found as 0-0.35; 0.84-1.16 and 0.26-0.35 mg/g, respectively. The extraction process, plant parts, harvest time, climatic factors and fermantation seem to be reason for the differnces observed in the phenolic composition of sea fennel.

Table 3 — Phenolic compounds of fresh and fermented sea fennel

Phenolic compounds (mg/100 g)	Fresh sea fennel	Fermented sea fennel
Gallic Acid	23.71±0.69*a	21.38±1.25b
3,4-Dihydroxybenzoic Acid	21.73±1.34a**	16.22±0.58b
(+)-Catechin	31.58±1.98a	15.47±0.00b
1,2-Dihydroxybenzene	13.83±1.41b	24.62±0.23a
Syringic Acid	11.04±0.11a	7.24±0.02b
Caffeic Acid	10.33±0.04a	7.23±0.35b
Rutin trihydrate	8.41±1.80a	2.16±0.44b
p-Coumaric Acid	15.77±0.72a	0.29±0.04b
trans-Ferulic Acid	6.92±0.97a	2.19±0.66b
Apigenin 7 glucoside	6.31±0.73a	1.88±0.79b
Resveratrol	2.03±0.35a	0.46±0.06b
Quercetin	13.48±0.66a	5.37±0.46b
trans-cinnamic acid	0.55±0.06b	1.18±0.09a
Naringenin	2.71±0.53a	1.49±0.08b
Kaempherol	4.07±0.74a	3.78±0.47b
Isorhamnetin	2.19±0.37b	3.62±0.78a

Conclusion

Sea fennel is a significant source of antioxidants, phenolic and flavonoid compounds. The fermentation caused a major reduction in antioxidant, phenolic and flavonoid values. The highest increasing was observed in γ-terpinene and carvacrol methyl ether during fermentation. Gallic acid, 3,4-dihydroxybenzoic acid and *p*-coumaric acid were the major phenolic acids in fresh sea fennel. While 1,2-dihydroxybenzene, *trans*-cinnamic acid and isorhamnetin contents increase during fermentation, other constituents decreased. In addition, the main flavonoids were (+)-catechin and quercetin in fresh sample. Fermentatiton process generally caused the degredation of phenolic compounds.

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