

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

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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 phenolic and flavonoid contents decreased 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 Apiaceae¹. It is a salt-tolerant plant and widely grown in maritime rocks and more rarely sandy beaches in Mediterranean countries and Atlantic coasts². 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 effects³. The leaves of sea fennel contain various bioactive compounds which can be utilised aromatic, antimicrobial and insecticide purposes⁴. 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 yogurt⁷. Sabinene, γ -terpinene, thymol methyl ether, dillapiol, α -pinene, *p*-cymol, apiole, cis- β -ocimene and terpinene-4-ol are the key constituents of sea fennel leaves⁸. In recent years, Medicinal and

aromatic plants have taken attention due to its bioactive substance. Limited studies were carried out on bioactive properties 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.

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.

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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/min, 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 analysed 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 variance¹⁴.

Results and discussion

The bioactive properties of fresh and fermented sea fennel are presented in Table 1. Antioxidant activity, total phenolic and total flavonoid 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 fermentation. 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 phenolics¹⁵; (iii) the liberation of free forms resulting in the hydrolysis of phenolic glycosides¹⁶. In contrast, Othman *et al.*¹⁷ found that phenolic compounds were degraded and antioxidant activity decreased after fermentation. Therefore, phenolic, flavonoid 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 process¹⁵. 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, respectively⁴. 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 contents 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 fennel²³. 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, respectively²⁴. 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%, respectively²⁵. Özcan²⁶ 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 found²¹. 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 -pinene¹⁸. 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

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 ether | 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 each row with different letters are significantly different ($p < 0.05$)

****nonidentified

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.

Limonene, terpinene and sabinene were the main compounds of sea fennel essential oil²⁸. It was observed that the major essential oil components compared to literature 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. Fermentation process generally caused the degradation 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 changes¹⁵. 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 fennel²³. Epicatechin amounts

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 |
| <i>p</i> -Coumaric Acid | 15.77±0.72a | 0.29±0.04b |
| <i>trans</i> -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 |
| <i>trans</i> -cinnamic acid | 0.55±0.06b | 1.18±0.09a |
| Naringenin | 2.71±0.53a | 1.49±0.08b |
| Kaempferol | 4.07±0.74a | 3.78±0.47b |
| Isorhamnetin | 2.19±0.37b | 3.62±0.78a |

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 fermentation seem to be reason for the differences observed in the phenolic composition of sea fennel.

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. Fermentation process generally caused the degradation of phenolic compounds.

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