New formulation of fermented sausages towards healthier and quality rectification by adding *Ferulago angulata* essential oil

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Abstract

The demand is improved for ready-to-eat meals by lifestyle changes and fermented sausages are popular meat products because of their flavor. Natural compositions are considered as substitutes of synthetic preservatives in products, which have been extensively employed. The aim of present research is to investigate the impact of *Ferulago angulata* Essential Oil (FAEO) as an antimicrobial and antioxidant factor for preserving of dry fermented sausages throughout storage. Initially, FAEO was extracted using microwave assisted hydrodistillation and its components were identified by gas chromatography-mass spectroscopy. Fermented sausages were treated by starter culture (Biobak K) and FAEO at various concentrations (0, 400, 800 and 1000 ppm). Afterwards, tests such as pH, moisture, thiobarbituric acid, texture, microbial growth, electron microscope images, and sensory evaluation were conducted during storage (28 d). The high levels of bioactive compositions such as limonene (30.71%) and α-pinene (19.02%) were indicated in FAEO. The results illustrated that pH and moisture of all fermented sausages were within the standard range during storage. At different concentrations, FAEO significantly decreased thiobarbituric acid of treated samples compared to control ($p<0.05$). Furthermore, FAEO was able to improve cohesiveness and elasticity of fermented sausages, which were also visible in electron microscope images. Antimicrobial feature of FAEO was distinguished by evaluating microbial attributes (total viable count, lactic acid bacteria and yeast) in fermented sausages. Ultimately, FAEO at 800 ppm concentration was detected as a promising and appropriate natural preservative during storage in fermented sausages.

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Introduction

Meat and its products are one of the most prominent food groups to synthesize protein structure. One of the most popular meat products is sausages, which is considered by million consumers all over the world.[1]

The manufacture process of fermented sausage includes cooking, fermentation and ripening, which provide an expanded storage by diminishing pH and water activity. The storage is expanded in mentioned products after preparing at low temperatures with atmospheric, vacuum or modified atmosphere packaging.[2] Uncooked fermented sausages are exposed to spoilage, pathogenic bacteria and lipid oxidation, so their storage is lowered.[3]

Food safety is considered as the main step in novel technology and also traditional and modern techniques are promoted in preserving foodstuff regarding to microbiological and physicochemical contaminants.[4,5]

In some issues, the use of synthetic antioxidants (such as butylated hydroxyanisole, butylated hydroxytoluene and propylene glycol) can cause toxic contamination that adversely influences consumer's health.[6,7] Therefore, a novel trend has attracted the most attention of consumers and meat processors for replacing artificial components with natural antioxidants.[3,8]

Healthy diet is considered as a strategically approach against non communicable diseases and indicates that the Mediterranean and vegetarian diets promote abundant health improvements such as a lesser cardiovascular disease risk.[9] *Ferulago angulata* (Schltdl.) Boiss (locally called "chavil") is recognized as an endemic medicinal plant in southwestern and western of Iran, which belongs to Apiaceae family. *Ferulago angulata* as a perennial plant grows 1.5 to 1.6 m height with thick stem, single and erect, sulcate or enor-

mous route.[10] The mentioned plant has yellowish, tiny and unspecific flowers with glabrous, basal, pale, bluish leaves; its seeds mature in September and flowering occurs between May and July.[1] The different parts of this plant are broadly applied as sedative, digestive, in the treatment of intestinal worms, as flavor yogurt, cheese and meat, tonic, antiparasitic, antibacterial, food preservative, as absorptive material for heavy metals such as cadmium, zinc, nickel, and as an antioxidant in metal toxicity.[11,12]

Many monoterpene hydrocarbons such as α-pinene, sabinene, (Z)-β-ocimene, p-cymene, α-phellandrene and β-phellandrene are detected in *Ferulago angulata* Essential Oil (FAEO).[13] The antimicrobial influence of FAEO was assessed on *Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes, Bacillus cereus, Streptococcus agalactiae*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella typhi, Campylobacter jejuni* and *Candida albicans*.[12,14,15]

In previous researches, antioxidant attributes of chestnut and grape seed extracts,[16] edible mushroom extract,[3] oregano and thyme essential oils,[17] rose polyphenols[18] and extract of pistachio hull[19] were assessed in fermented sausage. Consumer's preference is high to replace chemicals with natural substances, therefore natural compositions, such as FAEO with antioxidative and antimicrobial factors have been recommended. So far, several studies have been performed on fermented sausages, but FAEO has not been particularly investigated.

Materials and Methods

Material selection

Ferulago angulata was harvested and collected in Sepidan, Fars province of Iran in September 2020, which was recognized by traditional taxonomist. Beef without antibiotics was purchased from a slaughterhouse in Kermanshah with health protocols. Starter culture containing *Staphylococcus xylosus* (ATCC 35663), *Staphylococcus carnosus* (ATCC 51365), *Lactobacillus sake* (ATCC 15521), and *Pediococcus pentosaceus* (ATCC 25745) was achieved from Wiberg-Salzburg, Austria, which was identified as Biobak K. Mannitol Salt Agar, Sabouraud Dextrose Agar, Plate Count Agar, Violet Red Bile Glucose Agar, and De Man, Rogosa and Sharpe (MRS) Agar as well as chemical reagents were purchased from Merck Company (Darmstadt, Germany).

Microwave assisted hydrodistillation

Hydrodistillation was performed using Samsung domestic microwave oven (2450MHz, 900W). The solution was obtained by blending 2 g *Ferulago angulata* and 100 mL water. The solution was put into flask with round bottom and hydrodistillation of essential oil was carried out at 500 W microwave power for about 40 min. Afterwards, the achieved essential oil was collected and dehydrated with anhydrous Na_2SO_4, then it was maintained at 20°C. Hydrodistillation procedure was conducted at three replications and essential oil yield (%) was determined according to the following equation:[10]

$$\text{Essential oil yield (\%)} = \text{essential oil content (g)} / \text{plant amount (g)} \times 100$$

Gas chromatography–mass spectroscopy (GC-MS)

The A CP-3800 GC device (Varian, Walton-on-Thames, UK) was employed equipped with flame ionization detector, splitter injection port and Star Chromatography Workstation (Version 6.0). A Mega SE 54 (methyl phenyl polysiloxanes) was identified as a fused silica gas chromatographic capillary RTX column (25 m × 0.25 mm ID, 0.25 μm thickness) and conveyor gas (He) showed 50 kPa peak pressures with 99.99 % purity. In GC-MS, 1 μL essential oil was injected and oven temperature increased from 40 to 200°C at 10°C/min in all columns. The mass spectrometry was performed at temperature of surface (250°C) and the effect of 70 eV electron was observed using ionization capability. However, mass spectrometer was not operated and the gap time was 2 min in this method. GC-MS was performed in full scan model and measured by comparison of peak areas with standards.[20] The Excalibur software (Fisons) was applied to detect essential oil derivatives.

Manufacture of fermented sausage samples

In the first step, beef (75%), fat (25%), salt (14 g/kg), sodium nitrite (0.1 g/kg), pepper (36 g/kg), glucose (15 g/kg) were homogenized. In the second step, Biobak K starter culture (0.5 g/kg) and FAEO at different concentrations (0, 400, 800 and 1000 ppm) were added and mixed thoroughly. Afterwards, they were filled into casting to manufacture sausage dough and fermentation was performed with 90% relative humidity during 5 d at 25°C.[19] The present treatments were defined as Control samples (C), samples containing 400 ppm of FAEO (FE_{400}), samples containing 800 ppm of FAEO (FE_{800}) and samples containing 1000 ppm of FAEO (FE_{1000}).

Physicochemical attributes

The pH was investigated by digital pH meter (Metrohm model) and moisture level was measured according to AOAC.[20,21] The 10 g samples were dried in petri dishes and heated in oven drying about to 150°C for 25 min (Memmert ULM500) to stable weight.[21]

Thiobarbituric acid reactive substances (TBARS) assay

The TBARS level was determined as mg malondialdehyde/g to assess lipid oxidation during storage of samples at different temperatures. The 20 mL trichloroacetic acid (4%) and 5 g sausages were blended during 30 min, and then filtered. The 5 mL sample was poured into an individual tube and mixed with TBARS solution at similar level (0.02 mol/L). The achieved solution was heated at 90°C for 40 min, placed in cold water (1 h) and then centrifuged (5 min). The 8 mL solution supernatant was blended with 5 mL trichloromethane and then preserved (20 min). The absorbance was calculated at 532 nm using an UV-visible spectrophotometer (Thermo Scientific, Madison, WI, USA).[22]

Texture profile analysis

This feature was evaluated through Brookfield device with TA25/1000 cylindrical probe. Sausage slices (1 cm × 1 cm × 2.5 cm) were compressed into small sizes at a crosshead speed (2 mm/sec) and load cell level was 5 kg. The time interval was adjusted at 30 sec between the first and the second cycle. Some features such as cohesiveness, springiness, hardness, chewiness, and gumminess were evaluated by compression probe with 17.25 cm^2 size of surface contact and the probe waited for 2 sec between compressions.[23]

Microbial assessment

After removal from casing, 10 g of each sample were aseptically weighted in a sterile plastic bag for assaying microbiological attributes. Then, they were homogenized by sterile solution of peptone water (90 mL, 0.1% w/v). After incubating, total viable counts were counted using Plate Count Agar at 30°C for 48 h, lactic acid bacteria by MRS Agar at 30°C for 48 h, staphylococci by Mannitol Salt Agar at 37°C for 37 h, yeasts by Sabouraud Dextrose Agar at 25°C for 5 d and Enterobacteriaceae by Violet Red Bile Glucose Agar at 37°C for 24 h; 25 to 250 colonies per plate[24] were used and

the microbiological data were converted into logarithms of Colony Forming Units (CFU)/g.[2]

Micromorphological aspects

The preparation of sample included fixing seven-day micellar disc (0.1 cm) with 2.5% glutaraldehyde solution into 0.1 M phosphate buffer about 2 h at 25°C; dehydration of ethanol solution for 30 min, drying about 1 h, and coating the sample with a gold layer. The prepared samples were investigated by a scanning electron microscope (SEM, JSMe6390 LV, Japan) at an accelerating voltage of 25 kV.[4]

Sensory investigation

Initially, samples were evaluated using hedonic procedure (5 points) with 30 semi trained assessors (10 males and 20 females) experienced in sensory investigation of various sausages. At starting of session, water was consumed to remove the remaining flavors and clean palates. After removing casting, 10 to 15 g sausages were prepared and cut into diameters about 5 mm thickness. Afterwards, panelists were asked to evaluate served sausages on white plastic containers at 25°C. The scores were considered as sensory indices and scales 1 (extremely undesirable) to 5 (extremely desirable) were devoted to texture, odor, taste, mouth feel, adhesiveness, and overall acceptance. Then, panelists added comments about samples on the questionnaire.[5]

Statistical analysis

The normality assessment was performed for all data and its investigation was done by ANOVA approach with all factors remaining constant in an entirely randomized design. Statistical analyses included effects of all treatments at different essential oil concentrations (0, 400, 800 and 1000 ppm), their interaction and storage (1, 7, 14, 21 and 28 d). The pH, moisture, TBARS assays and sensory evaluation were investigated on 1, 7, 14, 21 and 28 d as well as texture attribute and microbial function were evaluated on 1 and 28 d. All variations were assessed using a Duncan multiple comparison method and Minitab software (Version 19) was applied for statistical design. Each result was calculated according to mean of three replications ± standard deviation.

Results

Extraction efficiency and GC-MS of FAEO

Target plant was studied and the results demonstrated that extraction efficiency of FAEO was detected 3.02% according to the applied procedure. The FAEO composition was evaluated using preservation times, as portrayed in Table 1. The α-pinene (19.02%), ß-phellandrene (6.91%), limonene (30.71%), terpinolene (3.75%) and α-phellandrene (4.03%) are represented in FAEO composition.

Physicochemical assessment

The pH levels are portrayed in Table 2; the results illustrate that time and essential oil had a significant influence on this factor, which decreased over time. On the other hand, pH increased by adding essential oil, but the concentration had no effect on this issue and it was diminished sharply for each sample from 1st to 7th

Table 1. Components of *Ferulago angulata* essential oil.

Components	RI	Percent
α-Thujene	812	2.11
α-Pinene	823	19.02
ß-Phellandrene	912	6.91
Limonene	1.11	30.71
ß-Ocimene	1.20	2.04
Linalool	1.31	3.22
Terpinolene	1.53	3.75
Thymol	1.15	3.14
Carvacrol	1.30	1.53
Longifolene	1.11	2.16
Germacrene B	1.65	1.82
Spathulenol	1.52	1.05
α-Phellandrene	1.63	4.03
Hexadecanol	1.21	1.52

Abbreviation: RI, retention indices. Retention indices of all components were measured using retention time compared withn-alkanes (C8–C26).

Table 2. Comparison results of mean interactions for distinct levels (essential oil and time) on pH, moisture and thiobarbituric acid of fermented sausage samples (mean±standard deviation)

pH Samples	1st d	7th d	14th d	21th d	28th d
C	6.20±0.05^{Aa}	5.96±0.07^{Ab}	5.89±0.10^{Ab}	5.69±0.02^{Ac}	5.66±0.05^{Ac}
FE_{400}	6.04±0.06^{Ba}	5.42±0.09^{Bb}	5.37±0.12^{Bb}	5.15±0.04^{Bc}	5.13±0.03^{Bc}
FE_{800}	6.06±0.02^{Ba}	5.50±0.08^{Bb}	5.28±0.10^{Bbc}	5.11±0.05^{Bc}	5.06±0.06^{Cc}
FE_{1000}	6.08±0.03^{Ba}	5.55±0.04^{Bb}	5.30±0.05^{Bbc}	5.10±0.10^{Bc}	5.00±0.07^{Cc}
Moisture (%) **Samples**	**1st d**	**7th d**	**14th d**	**21th d**	**28th d**
C	57.30±0.85^{Aa}	45.96±0.91^{Ab}	35.89±0.70^{Ac}	30.69±0.82^{Ad}	20.66±1.14^{Ae}
FE_{400}	61.04±1.81^{Ba}	48.43±0.20^{Bb}	40.31±1.42^{Bc}	38.14±1.64^{Bc}	25.10±0.35^{Bd}
FE_{800}	63.04±1.52^{Ba}	50.91±0.16^{Cb}	45.80±1.27^{Cc}	40.10±0.98^{Bcd}	39.64±1.61^{Cd}
FE_{1000}	66.17±1.15^{Ca}	51.44±0.36^{Cb}	47.00±1.02^{Cc}	45.61±1.71^{Cc}	40.00±0.85^{Cd}
Thiobarbituric acid (mg malondialdehyde/g) **Samples**	**1st d**	**7th d**	**14th d**	**21th d**	**28th d**
C	1.20±0.02^{Aa}	1.36±0.05^{Ab}	1.49±0.07^{Ac}	1.64±0.04^{Ad}	1.96±0.10^{Ae}
FE_{400}	1.17±0.08^{Ab}	1.00±0.10^{Ba}	1.11±0.12^{Bb}	1.29±0.06^{Bc}	1.45±0.05^{Bd}
FE_{800}	1.04±0.03^{Bb}	0.94±0.07^{Ba}	1.07±0.04^{Bb}	1.16±0.08^{Cbc}	1.30±0.11^{Bc}
FE_{1000}	1.00±0.06^{Bb}	0.83±0.02^{Ca}	1.01±0.03^{Bb}	1.11±0.01^{Dc}	1.17±0.05^{Cd}

$^{a-e}$Significant differences in each row; $^{A-D}$Significant differences in each column.

d. The pH of all samples remained constant from the 7th d to the end of 14th d ($p<0.05$) and pH changes were not noticeable after the 21st d. C represented the highest (6.20) on 1st d and FE_{1000} was the lowest (5.00) at the end of storage. Optimum pH of sausage was adjusted at 5.6 to 6.2 according to the standard and pH of all samples was observed in standard range.

Moisture changes of fermented sausages are depicted in Table 2. Time and essential oil had a significant influence on moisture level, which was diminished over time and elevated by adding essential oil. The lowest (20.66%) and highest (66.17%) levels of C and FE_{1000} were detected on 28th and 1st d of storage, respectively.

Biological attributes

The TBARS level increased remarkably ($p<0.05$) during storage (Table 2). The results depicted that mentioned factor increased with a significant difference in C during each period time. In fact, FAEO at three concentrations considerably reduced TBARS level in fermented samples during fermentation and storage ($p<0.05$). This result demonstrated that FAEO has been able to maintain oxidation in samples because of high antioxidant capacity. The highest and lowest levels of TBARS belonged to C at the end of storage (1.96 mg malondialdehyde/g) and FE_{1000} on the 7th d (0.83 mg malondialdehyde/g), respectively.

Texture investigation

The features of this evaluation such as cohesiveness, elasticity, hardness, chewability and gummy texture is represented in samples on the 1st and 28th d of fermentation, as outlined in Table 3. C had the highest hardness (18.04 kg) and chewability (30.51 kg × mm) with significant differences at the end of storage. The high cohesiveness (0.50) and gummy texture (9.94 kg) were detected in FE_{1000} and C at the beginning and last day of storage, respectively.

Microbial contamination

The number of microorganisms is demonstrated in samples during fermentation and storage (Table 3). Because *Staphylococcus* spp. and Enterobacteriaceae were not observed in the samples, the obtained results have not been shown. The total viable counts were high in each sample during fermentation, but the lowest level (4.32 CFU/mL) was detected in FE_{1000} on 28th d. Of course, this factor was visible without significant differences and changes were due to the impact of time and essential oil. Total viable counts were 10^5 CFU/mL and all samples had standard microbial quality even at the end of storage (except C), which were free of essential oil or preservative. A high number of lactic acid bacteria was detected significantly during fermentation ($p<0.05$), which this number was more in FE_{800} compared to other samples. In the present study, the survival of lactic acid bacteria was improved by concentration of essential oil, which was higher in FE_{800} (4.86 CFU/mL) compared to others.

The total counts of yeast increased during fermentation ($p<0.05$), the lowest and highest levels were recorded in FE_{1000} (5.39 CFU/mL) and control (7.53 CFU/mL) on 28th d. The FAEO had a remarkably influence on reducing yeast counts during storage, but the adverse impact of time was more obvious on this factor.

Micromorphological aspects

The structural network of fermented sausage dough is illustrated for control in Figure 1a. It indicated that myofibrils and swollen proteins could be found as an adhesive composition, which gathered particles of meat and fat in sausage dough. In Figures 1 (b, c and d), the images of treated samples outlined that essential oil presence led to new connections with various parts of fat in sausages, which indicated morphological changes. These changes were considerably correlated to essential oil level and intra-and intermolecular crosslinks. The results also portrayed that a more uniform and cohesive structure was visible in treated samples.

Sensory perception

The effect of FAEO was evaluated on sensory attributes in fermented sausages according to the results of evaluators as a general assessment (Figure 2). The results of sensory evaluation illustrated that presence of target essential oil had the greatest influence on taste factor and treated samples had unfavorable taste for evaluators. The second factor is texture, which indicated a similar trend to first parameter with less intensity. Better results were observed in treated

Table 3. Comparison results of mean interactions for distinct levels (essential oil and time) on texture and microorganisms of fermented sausage samples (mean±standard deviation).

Texture attributes	Storage (d)	C	FE_{400}	FE_{800}	FE_{1000}
Hardness (kg)	1	15.13 ± 0.07^{Aa}	13.41 ± 0.12^{Ba}	11.00 ± 0.73^{Ca}	9.87 ± 0.95^{Ca}
	28	18.04 ± 0.16^{Ab}	16.20 ± 0.10^{Bb}	14.20 ± 0.54^{Ab}	13.05 ± 0.63^{Ab}
Cohesiveness	1	0.41 ± 0.06^{Aa}	0.46 ± 0.05^{ABa}	0.48 ± 0.04^{ABa}	0.50 ± 0.07^{Ba}
	28	0.30 ± 0.05^{Ab}	0.35 ± 0.04^{Ab}	0.38 ± 0.03^{ABb}	0.40 ± 0.02^{Bb}
Elasticity (mm)	1	3.06 ± 0.14^{Aa}	3.23 ± 0.32^{ABa}	3.41 ± 0.21^{Ba}	3.50 ± 0.43^{Ba}
	28	2.40 ± 0.22^{Ab}	2.71 ± 0.42^{ABb}	2.93 ± 0.52^{Ba}	3.10 ± 0.40^{Ba}
Gummy (kg)	1	9.10 ± 0.61^{Aa}	7.36 ± 0.12^{Ba}	6.00 ± 0.35^{Ca}	5.08 ± 0.23^{Da}
	28	9.94 ± 0.44^{Ab}	8.20 ± 0.09^{Bb}	7.11 ± 0.51^{Cb}	6.15 ± 0.60^{Cb}
Chewability (kg × mm)	1	20.09 ± 1.82^{Aa}	16.00 ± 1.02^{Ba}	13.32 ± 1.42^{Ca}	11.04 ± 1.66^{Da}
	28	30.51 ± 1.06^{Ab}	25.10 ± 1.09^{Bb}	17.07 ± 1.73^{Cb}	16.20 ± 1.52^{Cb}
Microbial contamination (Log CFU/mL)					
Overall count	1	4.70 ± 0.03^{Aa}	4.31 ± 0.06^{Ba}	4.29 ± 0.02^{Ba}	4.30 ± 0.04^{Ba}
	28	6.00 ± 0.11^{Ab}	5.01 ± 0.06^{Bb}	4.87 ± 0.10^{Cb}	4.32 ± 0.03^{Da}
Lactic acid bacteria	1	3.57 ± 0.04^{Aa}	4.30 ± 0.05^{Ba}	4.28 ± 0.03^{Ba}	4.29 ± 0.06^{Ba}
	28	3.96 ± 0.02^{Ab}	4.65 ± 0.01^{Cb}	4.86 ± 0.05^{Db}	4.55 ± 0.04^{Bb}
Yeast	1	6.04 ± 0.01^{Aa}	5.67 ± 0.03^{Aa}	5.41 ± 0.07^{Aa}	5.20 ± 0.01^{Aa}
	28	7.53 ± 0.02^{Aa}	5.82 ± 0.05^{Aa}	5.60 ± 0.02^{Aa}	5.39 ± 0.06^{Aa}

$^{a-b}$Significant differences in each row; $^{A-D}$Significant differences in each column.

samples compared to control and odor had the lowest changes among all evaluation factors regarding to alternations of adhesion agent. FE_{800} and control samples had similar results and also a significant negative influence was not observed on sensory features by essential oil. However, FE_{1000} indicated the lowest score in terms of taste factor, so the consumption was not suggested.

Discussion

The essential oils in aerial parts of *F. angulata* (from northeast of Iran, Khorasan) were Z-β-ocimene, bornyl acetate, germacrene D, α-pinene, myrcene, limonene, γ-terpinene, and cymene. Limonene, α-pinene and ß-myrcene were detected as the chief compositions[25] and major components such as bornyl acetate, Z-ß-ocimene, α-pinene, trans-ocimene and germacrene D were reported in mentioned plant.[14] Microwave-assisted hydrodistillation was employed for extracting essential oils and main components such as ß-phellandrene, α-phellandrene, limonene, α-pinene and terpinolene were distinguished using hydrodistillation.[10] The major constituents such as o-cymene (7.83%), 2-hexanal (7.01%), bornyl acetate (37.91%), α-pinene (3.64%), camphene (5.57%) were reported in *Ferulago macrocarpa* extracts from Lorestan, Iran[26] and also, Z-ß-ocimene (14.22%) and α-pinene (12.61%) were considered as the most components.[12] Isoflavones and associated components bind to the estrogen receptors with epigenetic and cytotoxic feature in *F. angulata* essential oil and are applied in postmenopausal women, the selective treatment of tumors, having also a dose- and time-dependent inhibitory influence on lymphoma proliferation.[9,27] The previous results of researches showed that 0.1 % FAEO developed the growth of broilers and their carcass traits and also affected lipid metabolism and intestinal microflora.[28]

The variation of pH was shown as a pivotal factor for evaluating and monitoring the main stages (fermentation and ripening) in sausage manufacture.[2] The reduction in pH is attributed to breakdown of carbohydrates and organic acid accumulations through lactic acid bacteria of starter culture in the 1st week of fermentation.[3] The impact of thyme-chitosan and chitosan-rosemary essential oils indicated initial pH levels between 4.99 and 6.95 in fermented sausages. This factor of fermented sausages was improved considerably by essential oils after the 1st month, but no significant difference was found among fermented sausages with fungi during storage.[2] The value of pH varied from 6.0 to 5.4 for Portuguese fermented sausages (recognized as alheira),[29] which was in line with present research.

Moisture declined noticeably during fermentation stage ($p<0.05$); the reduction trend corresponded to moisture loss at less relative humidity and high temperature during 28th d according to previous research.[30] Moisture loss did not change considerably in each sample during storage due to influence of packaging.[31] Table 2 shows that moisture level is increased by FAEO for FE_{1000} during fermentation. Natural essential oils were applied to improve moisture level of fermented sausages, which was expressed by several research.[16,30]

Several previous research expressed that TBARS was diminished by adding grape seed and chestnut,[16] rose polyphenols,[18] pistachio hull extract[19] and green tea extract[32] in fermented sausages during storage.

The preservation induced higher hardness and chewability due to gradual product moisture loss, so that it became harder during storage. However, treatments including essential oil induced less hardness. Previous research indicated that reduction in hardness was caused by natural essential oils in fermented sausages.[16,30] However, more cohesiveness degree was observed in treatment with the highest tested concentration of essential oil of present study, which exhibited inverse correlation of this factor with hardness. The present study indicates a positive correlation between hardness and chewability, which was lowered by adding essential oil and is in line with previous results showing that natural essential oils lessened chewiness.[16] No significant influence was induced by green tea extract on texture factors in fish sausage.[32] Linseed oleogels including *y*-oryzanol, *β*-sitosterol and bees wax affected texture evolution in dry cured sausages.[23] The pistachio hull extract showed no negative effect on texture at lower concen-

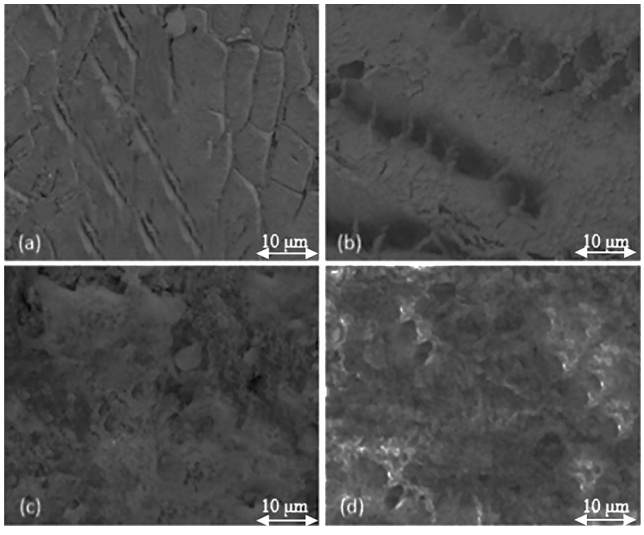
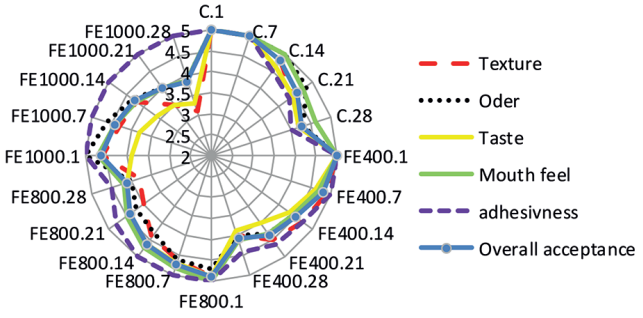


Figure 1. Scanning electron micrograph of fermented sausage samples. Control samples without essential oil (a); samples containing 400 (ppm) of *F. angulata* essential oil (b); samples containing 800 (ppm) of *F. angulata* essential oil (c); samples containing 1000 (ppm) of *F. angulata* essential oil (d). Scale bar: 10 µm magnification.



C_1 to C_{28}: samples without essential oil, $FE400_1$ to $FE400_{28}$: samples containing 400 (ppm) of *F. angulata* essential oil, $FE800_1$ to $FE800_{28}$: samples containing 800 (ppm) of *F. angulata* essential oil, $FE1000_1$ to $FE1000_{28}$: samples containing 1000 (ppm) of *F. angulata* essential oil on 1, 7, 14, 21 and 28 d of storage.

Figure 2. Comparison of mean interactions for distinct levels (essential oil and time) on sensory evaluation of fermented sausage samples.

trations, but hardness increased significantly at 1000 ppm levels.[19] No considerable differences were observed in texture of fermented sausage with inoculated lactic acid bacteria compared to control.[33]

The natural antioxidants of previous studies were shown to reduce total viable counts in fermented sausage incorporated rose polyphenols[18] and Spanish dry cured sausage loaded with tea, chestnut, grape seed and beer extracts,[30] which were consistent with the results of overall count reduction for samples in the present study. The extract of rose polyphenols[18] and pistachio hull[19] were applied to extend the survival of lactic acid bacteria in fermented sausages and maximum levels of these extracts delayed growth of this bacteria; however higher growth of bacteria was observed at lower concentrations. The count of yeast and molds was noticeably lowered from 5.39 to 2.51 log CFU/g ($p<0.05$) by immersing sausages into potassium sorbate.[2] A reduction of yeast and molds was found by adding basil and caraway essentials in fermented sausages.[4] Phenolic compositions inhibited enzymatic activity by reacting with sulfhydryl groups or nonspecific interactions with proteins, which indicated antimicrobial resistance. Polyphenols were also able to form heavy soluble complexes with proteins, thereby binding to bacteria and destroying receptors on surface of bacterial cell.[6,34] Quercetin and its derivatives also prevented bacterial growth by inhibiting the DNA gyrase enzyme.[35] The antibacterial activity was observed due to the presence of α-pinene, thymol, and terpinolene constituents in studied plant.[26]

The SEM results of previous research indicated that chitosan and gelatin nanofibers (1:6) including 40% thyme essential oil detected a suitable diameter and stability[36] as well as supplemented of electrospun chitosan based nanofiber mats with garcine extract caused to an enhancement in average diameter of fiber from 205 to 251 nm[37] that were in line with our results.

Oleogelation and substitution of pork back fat showed significant differences in general acceptability of fermented sausages.[23] The effect of *Kitaibelia vitifolia* extract[31] and green tea extract[32] were not detected on sensory attributes in fermented sausages. Sensory features were considerably influenced by adding lactic acid bacteria at the lowest utilized levels in control.[33]

Conclusions

Consumer demand is increased to substitute chemicals with natural antimicrobials. FAEO as a natural antimicrobial and antioxidant agent affected the quality promotion of fermented sausages during storage in present study. The best approach was adding essential oil (800 ppm) in present research according to physicochemical, microbial and sensory attributes. However, the knowledge of technologies, raw materials, quality attributes of fermented meat commodities and industrial process are speedily expanded and functional meat starter cultures are detectable.

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