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Comparison of the Chemical Compositions and Antibacterial Activities of Two Iranian Mustard Essential Oils and Use of these Oils in Turkey Meats as Preservatives

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Abstract

Background and objective: Iranian mustard is cultivated in southern areas of Iran and used traditionally as natural preservative. Aims of the current study were identification and comparison of the chemical compositions and antibacterial activities of two Iranian mustard essential oils and assessment of these oils use for increasing the shelf life of turkey meats.

Material and methods: Chemical compositions of two Iranian mustard essential oils were identified using gas chromatography-mass spectrometry and antibacterial activities of these oils were assessed against *Salmonella typhimurium*, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Enterococcus faecalis* using disc diffusion and broth macrodilution assays. Inhibitory effects of the essential oils were assessed on growth of mesophilic psychrotrophic bacteria, yeasts and molds and sensory evaluation was carried out for the turkey meats.

Results and conclusion: Results of GC-MS showed presence of bioactive constituents, especially allyl isothiocyanate (75.87-80.07%). All the bacterial growth, especially for *Escherichia coli*, was inhibited with inhibition zones of greater than 20 mm and minimum inhibitory and bactericidal concentrations of 0.156 mg ml^{-1}. Treatment of turkey meat samples with the mustard essential oils significantly decreased the count of mesophilic psychrotrophic bacteria, yeasts and molds during 20 days of storage at 4°C ±1, compared to controls ($P \leq 0.05$). Over the time, the sensory score of the treated samples increased, compared to controls. Based on these findings, the Iranian mustard essential oils can be used as natural preservatives in foods.

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1. Introduction

In recent decades, the increasing consumer demands for natural antimicrobial agents in foods have led to further identification of plant metabolites, especially plant essential oils. Essential oils are volatile substances of plant origins, containing biologically active components. The ability of essential oils to inhibit growth of many spoilage bacteria and fungi in foods has well been documented [1-3]. The mustard plant is a member of Cruciferae family and belongs to Brassica genus, which includes three common species of yellow mustard (*Sinapis alba* L.), black mustard (*Brassica nigra*) and brown or oriental mustard (*B. juncea*) [4]. Mustard seeds are widely used in various regions as spicy, condiment, flavoring ingredient in types of sauce, salad dressing, ketchup and other products [2,4]. Various

phytochemical metabolites have been reported for Brassicaceae plants, including phenolic compounds and glucosinolates with nutraceutical, antibacterial, antioxidant and anticardiovascular properties. According to Crop Statistics of the FAO Report in 2017, production quantities and harvested areas of mustard seeds in Iran include five tons and seven hectares, respectively. Mustard (*B. juncea*) origin is minor Asia and Southern Iran and the center of origin is Middle East and China. For a long time, mustard has been cultivated in southern areas of Iran and widely used as a natural preservative to produce a traditional fermented fish sauce called mahyaveh for the extension of fish shelf life [5-7].

Nowadays, industrial production of turkey is developing worldwide due to its favorite growth characteristics such as weight gain, high growth rate, low feed conversion ratio and high nutritional value. In poultry breeding in Iran, turkey has the highest economic aspect ratio after chicken. According to the latest FAO Statistics on turkey meat production industries, Iran is ranked third in Asia. Due to its nature and composition, turkey meat is susceptible to growth of pathogen microorganisms and deterioration reactions which may lead to decrease of nutritional qualities, undesirable organoleptic changes and great economic losses even during cold storage. The extension of product shelf life of is an important challenge in poultry industries [8]. Therefore, the major aim of this study was to use of mustard (*B. juncea*) essential oils for preventing foodborne bacterial infections and assessment of these oils effects on shelf life and sensory properties of turkey meats. This is the first report on the bioactive components and antibacterial activities of Iranian mustard essential oils against foodborne bacteria.

2. Materials and methods

2. 1. Materials

The mustard (*B. juncea*) seeds with spherical, small and brown characteristics were collected in November, 2017, from two regions of Southern Iran, namely Larestan (Fars Province, N27° 40' 26.906", E54° 20' 8.824") and Hajiabad (Hormozgan Province, N28° 18' 39.594 ", E55° 54' 8.83"). The turkey meats (without skin and bone) were purchased from a local market in Karaj (Alborz Province, Iran) and transferred immediately to laboratories within Institute of Standard and Industrial Research of Iran (ISIRI) under hygienic and cold conditions. All the chemicals were of analytical grades (Merck, Germany). All the bacterial strains were provided by the American Type Culture Collection (ATCC).

2. 2. Extraction of the essential oils

Botanical identification of the samples was carried out by a botanist in Payame Noor University of Fars, Iran. Cleaned mustard seed samples were ground into the powder using blade-carbide grinding (Sanyo, Japan) for 2 min and passed through 40 mesh sieve (400 µm). Then, 200 g of the mustard powder, 600 ml of distilled water, 20 ml of buffer solution (pH 4.5) and 4 ml of ascorbic acid (1 mg ml^{-1}) were added to a 2000-ml stopper round flask and mixed well. Essential oils of the samples were extracted using hydrodistillation method and glass Clevenger apparatus (Jahan Shimi Gostar, Tehran, Iran) at 70°C for 3 h. To remove traces of water in the extracted essential oils, anhydrous sodium sulfate was used. The essential oils were stored in dark glass bottles at 4°C until use [9].

2. 3. Gas chromatography/mass spectrometry (GC-MS) analysis

Analysis of major active bioconstituents of the Iranian mustard seed essential oils was carried out using Agilent Gas Chromatography-Mass Spectrometry (Agilent Tecnologies, Santa Clara, CA, USA) coupled to a 5977A mass selective and triple-axis detector and a split-splitless injector (1:10 split ratio). The capillary column included a fused silica HP-5MS (5% phenyl methyl siloxane) type with length of 30 m, internal diameter of 0.250 mm and film thickness of 0.25 µm. Helium with a flow rate of 1.1 ml min^{-1} was used as the carrier gas. For the analysis, 1 µl of each mustard essential oil was injected to the device. The injector and column temperatures were set at 250°C. The column oven temperature was set at 65°C (2 min) and then increased to 170°C (5 min) with an increasing rate of 10°C min^{-1} and from 170 to 250°C (7 min) with an increasing rate of 25°C min^{-1}. Compounds were identified based on the mass spectral fragmentation, retention times and the retention time comparison with authentic constituent mass spectra of the device libraries [1,10].

2. 4. Antimicrobial activity of the essential oils

Antibacterial activity of the mustard essential oils was assessed against eight foodborne pathogens, including *Escherichia* (*E.*) *coli* ATCC 25922, *Salmonella (S.) typhimurium* ATCC 14028, *Citrobacter (C.) ferundii* ATCC 8090, *Pseudomonas* (*P.*) *aeruginosa* ATCC 27853, *Staphylococcus* (*S.*) *aureus* ATCC 25923, *Listeria* (*L.*) *monocytogenes* ATCC 13932, *Bacillus* (*B.*) *cereus* ATCC 11778 and *Enterococcus* (*E.*) *faecalis* ATCC 29212. The bacteria were cultured at 37°C for 24 h in brain heart infusion (BHI) broth. Turbidity of the suspensions for disc diffusion assay was adjusted to 0.5 McFarland turbidity standard [10].

2. 4. 1. Disc diffusion assay

Antibacterial activity of the mustard essential oils was assessed using standard disc diffusion assay. Briefly, 100 µl of a bacterial suspension of 0.5 McFarland standard (1.5×10^8 CFU ml^{-1}) were spread on surface of the sterile Mueller-Hinton (MH) Agar (Merck, Germany) plates using sterile swabs. A sterile paper disc with 6 mm of diameter containing 10 µl of the extracted essential oil was pressed slightly on the surface of the inoculated plates. Gentamicin (10 µg per disc) was used as positive control. After 24 h of incubation at 37°C, the inhibition zone diameters were measured using digital caliper and reported in millimeters [11-13].

2. 4. 2. Assessment of minimum inhibitory concentration (MIC)

The lowest concentration of each sample that inhibited visible growth of the foodborne bacteria was reported using broth macrodilution assay of Cui et al. [10] with modifications. The mustard essential oils were diluted using double-fold serial dilution by transferring 5 ml of the sterile mustard essential oil into 5 ml of the sterile MH broth. Each concentration was inoculated with 0.1 ml of the bacterial suspension (0.5 McFarland) in a separate sterile tube and incubated at 37°C for 24 h. Turbidity of the inoculated broth indicated the bacterial growth. Gentamicin used as positive control. The lowest concentration, at which no visible growth of the microorganisms was seen, was reported as MIC.

2.4.3. Assessment of minimum bactericidal concentration (MBC)

To assess MBC of the mustard essential oils, 0.1 ml of the inoculum from each tube with no bacterial growth was subcultured on MH agar plates in triplicate to investigate the inhibition was reversible or permanent. After incubation at 37°C for 24 h, the lowest essential oil concentration, corresponding to no bacterial growth, was considered as MBC [10,14].

2.4.4. Antimicrobial activity in turkey meat samples

Turkey meats were first washed with distilled water and then were drained and molded manually into pieces, weighing 30 g ±5. Samples were immersed for 2 min in the extracted mustard essential oils with a concentration of 1.5% under hygienic and sterile conditions. After draining sufficiently, the treated samples were dried in cold air for 45 min and packaged in sterile polypropylene trays at 4°C ±1. Samples were later analyzed on days 0, 5, 10, 15 and 20 for microbial specifications [15]. Then, 0.1 ml from each serial dilution of the turkey meat sample homogenates (0.1 and 0.01) was spread on the surface of plate count agar (PCA; Merck, Darmstadt, Germany). Inoculated plates were used for mesophilic total plate counts after incubation at 30°C for 72 h and for psychrotrophic bacteria after incubation at 7°C for ten days. Yeasts and molds were enumerated using Sabouraud dextrose agar and incubated at 25°C for three to ten days. Results were expressed as log CFU g^{-1} of samples [16].

2.5. Sensory evaluation of turkey meat samples

The sensory evaluation of prepared samples was carried out using quality index method by Yu et al. [17]. Turkey meat without any treatment was used as control. Important quality criteria of appearance, color, odor, texture (lack of slimes on surface of the samples) and overall acceptance were measured using a 9-point descriptive scale by 11 trained taste panel members. On this scale, scores between 7.0 and 9.0 indicated extremely like, scores between 4.0 and 6.9 indicated like and 3.9 was the limit of acceptability.

2. 6. Statistical analysis

All the experiments were carried out trice. The analysis of variance test was carried out using SPSS Software v.21.0 (SPSS Inc., Chicago, IL, USA) and results were expressed as the mean ±SD (standard deviation). Parametric data were analyzed using analysis of variance, while non-parametric data or sensory evaluation of the turkey meat samples was analyzed using Kruskal-Wallis test. The mean comparisons were carried out using the least significant difference and Duncan's multiple range tests at a 95% confidence level.

3. Results and discussion

3. 1. Essential oil extraction

Essential oils from the mustard seed samples included pale yellow liquids with pungent odor and the extraction yields included 0.5% ±0.03 (v w^{-1}). Results were similar to results of other studies. Khan et al. reported that the yield of brown mustard seed essential oils using hydrodistillation included 0.4% [18].

3. 2. Gas chromatography-mass spectrometry (GC-MS) analysis

Identified compounds, molecular formula and weights, retention times and measured peak areas for the samples B_1 and B_2 were shown in Table 1. Based on the results, 16 compounds were identified in mustard samples. These were divided into four major groups of saturated fatty acids (FA), unsaturated FAs and their associated esters, phenolic compounds and organosulfur compounds. The mass spectra and structural formulas of the identified antimicrobial components are shown in Fig. 1.

Results from GC-MS analysis revealed that the mustard essential oil samples had a significant quantity of allyl isothiocyanate (AITC) on a dry weight basis. This included 80.07% for the sample B_2; a relative advantage to that of the sample B_1 (75.87%). Major bioactive components of the mustard essential oil samples and their biological functions are listed in Table 2. Results for AITC were relatively comparable with the high contents of AITC (54.8-68.6%), reported in Chinese *B. Juncea* essential oil and 73.4% in Indian *Brassica* species. It is noteworthy that higher quantities of AITC were found in Iranian mustard essential oils, compared to previously reported values [6,19,20]. In general, chemical compositions of the essential oils from a single plant species of different geographical areas can differ significantly [21]. High quantity of AITC in Iranian mustard essential oils may occur due to the differences in genetic factors and climatic and geographical conditions (temperature, rainfall, altitude, hours of sunshine, contents of soil sulfur and nitrogen from fertilizers, harvest time and post-harvest conditions), which can affect the compound biological activities and pharmaceutical uses [22].

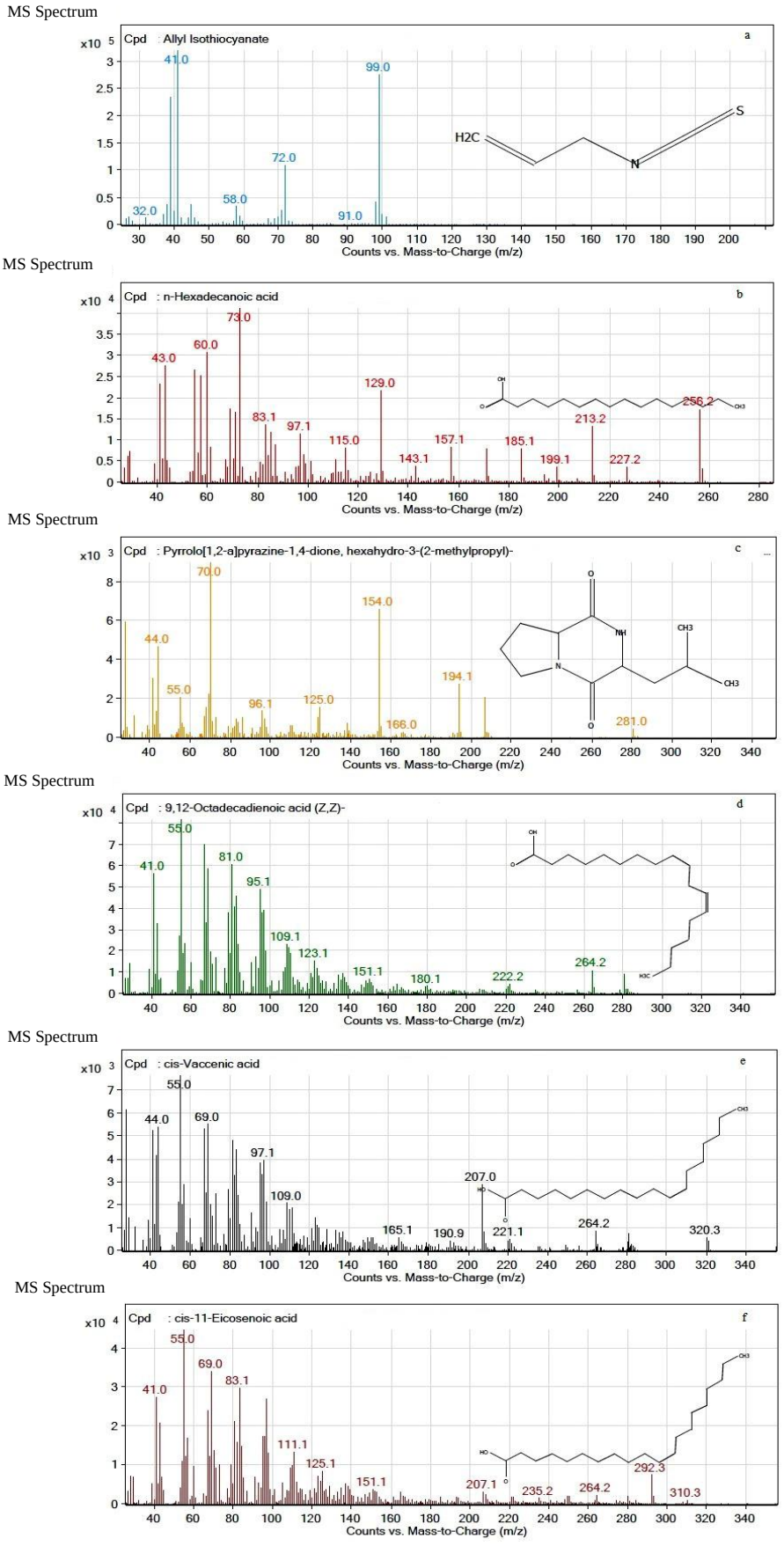


Figure 1. Mass spectra and structural formulas of compounds with antimicrobial activity in essential oils of the samples: a) retention time, 7.122 for allyl isothiocyanate; b) retention time, 23.025 for n-hexadecanoic acid; c) retention time, 24.376 for pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl); d) retention time, 25.741 for 9,12-octadecadienoic acid (Z,Z); e) retention time, 25.803 for cis-vaccenic acid; and f) retention time, 28.287 for cis-11-eicosenoic acid

Table 1. Chemical compositions of the Iranian mustard essential oils

No.	Compound[a]	MF[b]	MW[c]	RT[d]	B_1 (area%)	B_2	Identification method[e]
1	2-butenenitrile	C_4H_5N	67	3.162	0.789	-	1, 2
2	1,3,5-cycloheptatriene	C_7H_8	92.1	4.663	-	0.88	1, 2
3	Allyl isothiocyanate	C_4H_5NS	99	7.122	75.878	80.079	1, 2
4	Hexanoic acid,2-phenylethyl ester	$C_14H_{20}O_2$	220.1	7.305	0.100	-	1, 2
5	1,3,4-thiadiazole-2(3H)-thione,5-methyl	$C_3H4N_2S_2$	132	14.822	-	0.925	1, 2
6	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.2	23.025	1.853	-	1, 2
7	Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)	$C_{11}H_{18}N_2O_2$	210.1	24.376	-	1.063	1, 2
8	9,12-octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.2	25.741	7.122	0.954	1, 2
9	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282.3	25.803	2.647	4.658	1, 2
10	Cis-9-octadecenoic acid (oleic acid)	$C_{18}H_{34}O_2$	282.3	26.013	0.784	0.961	1, 2
11	Cis-11-eicosenoic acid	$C_{20}H_{38}O_2$	310.3	28.287	2.609	-	1, 2
12	Cis-13-eicosenoic acid	$C_{20}H_{38}O_2$	310.3	28.353	0.800	-	1, 2
13	9-octadecenoic acid (Z)-phenylmethyl ester	$C_{25}H_{40}O_2$	372.3	29.559	-	7.954	1, 2
14	(*Z*)-docos-13-enoic acid	$C_{22}H_{42}O_2$	338.3	31.016	5.350	-	1, 2
15	Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.3	30.872	-	1.024	1, 2
16	Trans-Z-alpha-bisabolene epoxide	$C_{30}H_{50}O_2$	442	33.369	-	1.504	1, 2
	Total				97.932	99.137	

[a] Compounds are listed in order of elution from HP-5MS column under the conditions listed in Section 2; [b] MF, molecular formula; [c] MW, molecular weight; [d] RT, retention time; [e] 1, retention index in HP-5MS column; [e] 2, GC/MS comparison with Wiley275.L, Wiley7n.L and NIST 14 MS libraries

Table 2. Major bioactive components of the Iranian mustard essential oils (samples B_1 and B_2) using GC-MS and the biological functions of these oils

Bioactive compound	Biological function	Ref.
Allyl isothiocyanate	Antimicrobial, fungicidal activity, anticancer, antitumor, controls soil-borne pathogens	[2,23,24]
n-hexadecanoic acid	Antibacterial, antioxidant, antiandrogenic, hypocholesterolemia, 5-alpha reductase inhibitor, anti-inflammatory	[25,26-28]
9,12-octadecadienoic acid	Antibacterial, fungicidal activity, anticancer, anti-inflammatory, medicinal benefits, antiandrogenic, antiarthritic, hypocholesterolemia	[2, 29].
11-cis-octadecenoic acid	Antimicrobial, dietary precursor of C9, T11 conjugated linoleic acid	[30, 31]
(Z)-9-octadecanoic acid	Antimicrobial, fungicidal activity, α-reductase inhibitor, antiautoimmune reducing blood pressure, preventing type 2 diabetes, anti-inflammatory	[2,23,24]
Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)	Antimicrobial, antioxidant, antitumor	[32]

GC-MS = Gas Chromatography/Mass Spectrometry

3. 3. Antibacterial activity of the essential oils

3. 3. 1. Disc diffusion assay

Results of the sample antibacterial activity against four Gram-negative and four Gram-positive bacteria based on the inhibitory diameter zone in mm are illustrated in Table 3. The positive control (gentamicin) produced zones of inhibition against all the microorganisms. The mustard essential oils were effective against all the bacterial species. The inhibition zones showed differences between the two samples and sample B_2 included a higher antibacterial activity, compared to that sample B_1 did. For both sample, inhibition zone diameters were greater than 20 mm for *E. coli* and 15-20 mm for *E. faecalis*. The lowest inhibition zone belonged to *L. monocytogenes* (< 10 mm). The most susceptible bacterial species were *E. coli* and *E. faecalis*,

followed by *B. cereus*, contrary to *L. monocytogenes*, *C. freundii* and *S. typhimurium* as the most resistant microorganisms.

Akkoyun et al. [33] reported that all their investigated bacteria were inhibited by mustard extracts with inhibition zone diameters of 10 mm against *P. aeruginosa*, 10 mm for *E. coli*, 15 mm against *E. faecalis* and 15 mm against *S. aureus.* Differences in antibacterial activity of mustard essential oils could be attributed to differences in their bioactive compounds, especially AITC. Based on the results of GC-MS, the major antimicrobial component of Iranian mustard essential oils is AITC, which is further effective against Gram-negative bacteria, especially *E. coli* [21].

3.3.2. Assessment of minimum inhibitory concentration (MIC)

The MIC of the samples was different for each strain, varied from 0.156 to 0.625mg ml^{-1}. For both samples, the lowest MIC belonged to *E. coli* with 0.156 mg ml^{-1} and the highest to *L. monocytogenes* with 0.625 mg ml^{-1} (Table 4). Furthermore, the inhibitory activity of Sample B_2 was higher than that of Sample B_1. The MIC results revealed data from the disc diffusion assay (Table 3).

Due to limited changes in sensory and organoleptic characteristics, use of these compounds at low concentrations is desirable for the food industries [34]. Based on the results from GC-MS analysis, antimicrobial activity against the assessed bacteria can be linked to the presence of antimicrobial compounds and the higher antibacterial activity of sample B_2 was possibly due to the existence of higher quantities of the major antibacterial component (AITC). The AITC, is one of the most important phytochemicals with antimicrobial properties [2-4,6,35].

Table 3. Antimicrobial activity of the mustard essential oils (samples B_1 and B_2) and gentamicin using disc diffusion and broth macrodilution assays against the highlighted bacteria

Bacterial strain	Inhibition zone (mm)		
	B_1	B_2	GM_{10}
S. typhimurium ATCC 14028	+	++	++++
E. coli ATCC 25922	++++	++++	++++
C. ferundii ATCC 8090	+	+	+++
P. aeruginosa ATCC 27853	+	+	++++
S. aureus ATCC 25923	++	++	+++
L. monocytogenes ATCC 13932	+	+	++++
B. cereus ATCC 10876	++	+++	++++
E. faecalis ATCC 29212	+++	+++	+++

+: radius of clear zone less than 10 mm; ++: radius of clear zone 5.0–10.0 mm; +++: radius of clear zone 15.0–20.0 mm; ++++: radius of clear zone more than 20 mm; GM_{10}: gentamicin
S. typhimurium = Salmonella typhimurium, E. coli= Escherichia coli, C. ferundii= Citrobacter freundii, P. aeruginosa= Pseudomonas aeruginosa, S. aureus= Staphylococcus aureus, L. monocytogenes= Listeria monocytogenes, B. cereus = Bacillus cereus, E. faecalis= Enterococcus faecalis

Table 4. Antibacterial activity (MIC and MBC) of the mustard essential oils (samples B_1 and B_2) and gentamicin using broth macrodilution assay

Bacterial strain	MIC (mg ml^{-1})			MBC (mg ml^{-1})		
	B_1	B_2	GM_{10}	B_1	B_2	GM_{10}
S. typhimurium ATCC 14028	0.312	0.156	0.078	0.625	0.625	0.156
E. coli ATCC 25922	0.156	0.156	0.078	0.312	0.156	0.156
C. ferundii ATCC 8090	0.625	0.625	0.625	1.250	0.625	0.312
P. aeruginosa ATCC 27853	0.312	0.312	0.078	0.625	0.625	0.312
S. aureus ATCC 25923	0.312	0.312	0.078	0.625	0.625	0.156
L. monocytogenes ATCC	0.625	0.625	0.078	1.250	1.250	1.156
B. cereus ATCC 10876	0.312	0.156	0.078	0.625	0.625	0.156
E. faecalis ATCC 29212	0.156	0.156	0.078	0.312	0.312	0.156

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; GM_{10}: gentamicin,
S. typhimurium = Salmonella typhimurium, E. coli= Escherichia coli, C. ferundii= Citrobacter freundii, P. aeruginosa= Pseudomonas aeruginosa, S. aureus= Staphylococcus aureus, L. monocytogenes=Listeria monocytogenes, B. cereus = Bacillus cereus, E. faecalis= Enterococcus faecalis

It affects the entire bacterial cell membrane. Mechanisms such as prevention of oxygen absorption, alteration of proteins by oxidative cleavage of disulfide bonds and blocking of intracellular enzyme activity and increase of the lag phase of bacteria have been reported [1,19]. The action mechanism of AITC against *E. coli* O157:H7 is based on the interaction of AITC with sulfhydryl groups of thioredoxin reductase enzyme which could affect the bacterial DNA and RNA syntheses. Moreover, AITC can affect sulfhydryl groups of the acetate kinase enzyme and inhibit the bacterial energy metabolism. However, a bacterial myrosinase-like action mechanism has been reported in *E. coli* O157:H7, which can help degradation of glucosinolate [36]. The GC-MS analysis revealed that other compounds in the essential oil samples included antimicrobial properties (Fig. 1). The major target of FAs, similar to essential oils, is the bacterial membrane [37]. The shape and especially the -OH group of FA carboxyl groups affect their antimicrobial activities. The cis free fatty acids include a higher antibacterial activity than trans free fatty acids [29,35]. Furthermore, it seems that the identified phenolic compounds such as pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl) may contribute to the antimicrobial activity of mustard against microorganisms through the damage of their cytoplasmic membrane, leak of intracellular constituents and disruption of cell wall peptidoglycan, which can result in loss of cellular structural integrity [35,36].

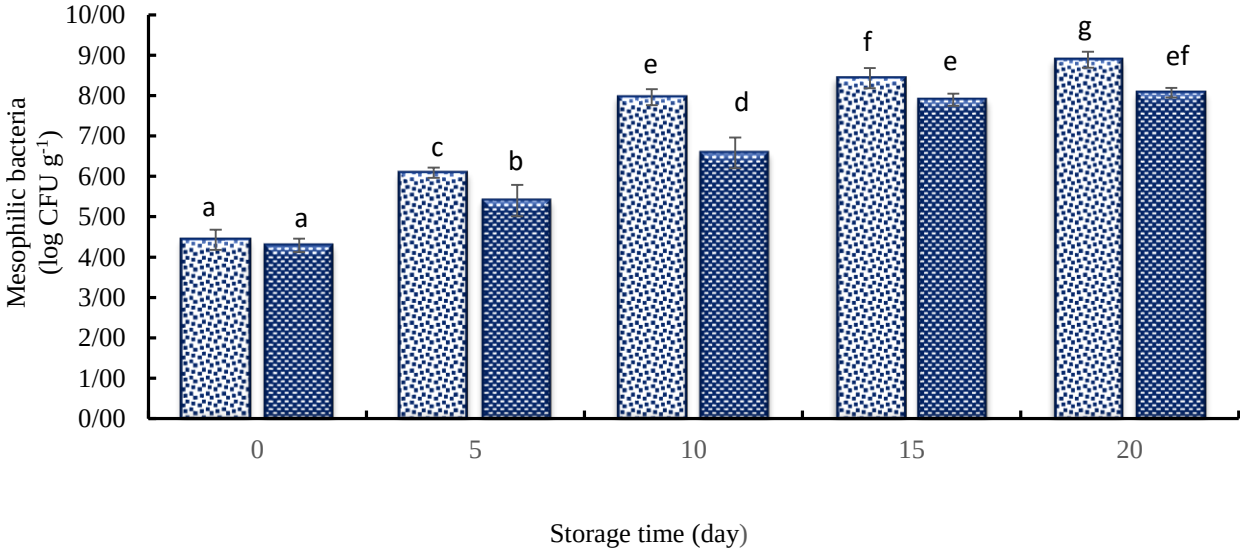
3.3.3. Assessment of minimum bactericidal concentration (MBC)

Results of MBC were different for each bacteria, varied from 0.156 to 1.250 mg ml^{-1}. The lowest MBC belonged to *E. coli* with 0.312 mg ml^{-1} for sample B_1 and 0.156 mg ml^{-1} for sample B_2. The highest MBC belonged to *L. monocytogenes* with 1.250 mg ml^{-1} for both samples (Table 4). Results from MBC supported results from MIC and disc diffusion assay.

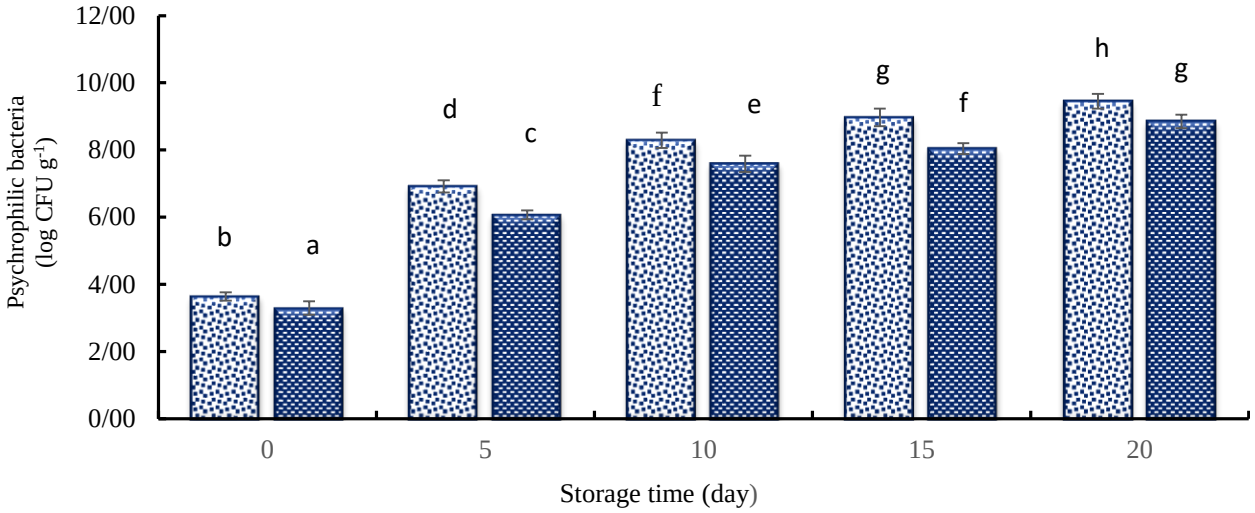
3.3.4. Antimicrobial activity in turkey meat samples

Since the essential oil from sample B_2 showed a further antimicrobial activity, compared to that the essential oil from sample B_1 did ($P \leq 0.05$), supplementary assays were carried out in foods using 1.5% (w w) concentrations of the mustard essential oils and their antimicrobial activities were assessed in turkey meat samples over a 20-day storage period at 4°C ±1. Changes in total activities of mesophilic (a spoilage indicator of poultry meats packed under aerobic conditions) and psychrotrophic (a spoilage indicator of poultry meats stored under refrigeration conditions) bacteria, yeasts and molds are illustrated in Fig. 2.

(a)



(b)



(c)

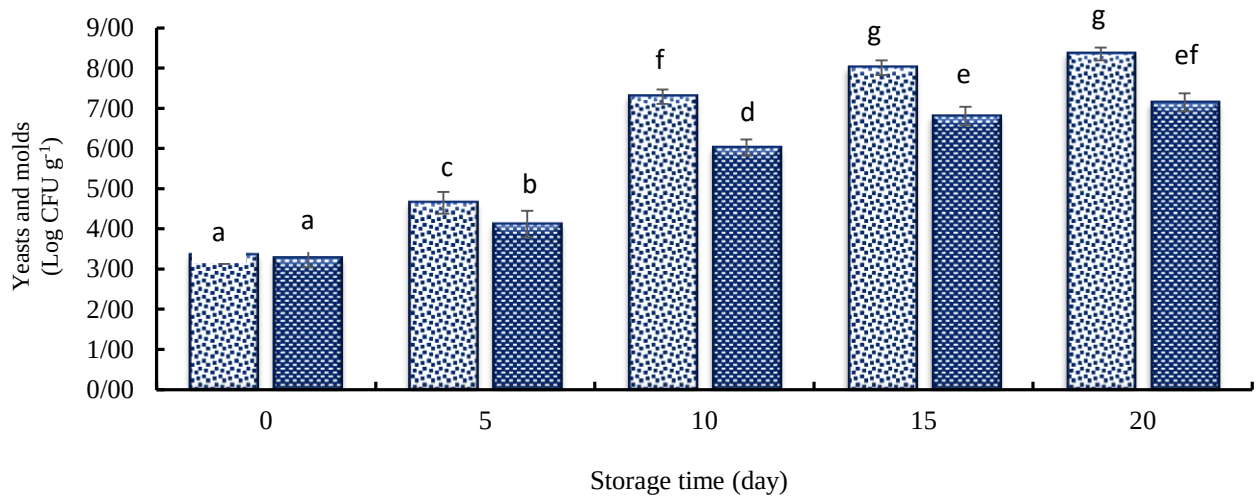


Figure 2. Antimicrobial activity of mustard essential oils in turkey meat samples during 0, 5, 10 and 20 days of refrigerated storage at 4°C ±1: a) mesophilic bacteria; b) psychrotrophic bacteria; and c) yeasts and molds. Values followed by different letters were significantly different ($P \leq 0.05$)

Recommended microbiological criteria for fresh and refrigerated commercial poultry meats include 6 log CFU g^{-1} for total aerobic, mesophilic bacteria and 7-8 log CFU g^{-1} for psychrotrophic bacteria. When the bacterial count reaches this limit, off-odor, surface slim and fat lipolysis appear. On day 0, the total mesophilic bacteria included 4.43 log CFU g^{-1} in control and 4.36 log CFU g^{-1} in treated samples with essential oils. After 5 days, the number of bacteria reached approximately 6.09 log CFU g^{-1} in control samples while the number of bacteria reached 6.58 log CFU g^{-1} in treated samples after 10 days. The lowest population of total mesophilic bacteria was observed in treated samples during the storage. Similar results were seen for psychrotrophic bacteria as well as yeasts and molds. Based on the results, number of the microorganisms increased in

all samples during the storage; however, this increase was significantly lower in sample treated with mustard essential oils, compared to that in controls ($P \leq 0.05$). These results indicated that use of mustard essential oils included inhibitory effects against growth of the three microbial groups and could delay spoilage of turkey meats during storage. The present results are similar to previous results from antimicrobial activity assessments of mustard in various foods such as mayonnaise, salad dressing, hotdog and burger [2,6,7]. Mustard essential oils are classified as "generally recognized as safe" [19,38].

3.4. Sensory evaluation

Results from sensory evaluation of the turkey meat samples treated by mustard essential oils during refrigerated storage are shown in Table 5.

Based on the statistical analysis and mean values of results for sensory evaluation, significant differences were reported between the treatment and storage time ($P \leq 0.05$). Except for the odor, no significant differences were seen between the color, appearance, texture and overall acceptance values of treated and control samples before day 5 of storage. The lower odor score of the treated samples at the beginning of storage was possibly due to the pungent sulphur odor of the mustard essential oils, which resulted in dissatisfaction of the assessed group. Furthermore, the low-fat content in turkey meat samples possibly made the smell of mustard essential oils more noticeable due to their less dissolution in the product fatty phase. However, sensory score of the treated samples increased over time, compared to controls. This probably occurred due to the preservative compounds of mustard essential oils and their effects in inhibition of spoilage microorganism growth, breakdown of peptides and decrease of oxidation changes, which lead to formation of undesirable aromatic compounds such as ammonia, dimethylamine and trimethylamines, compared to controls. However, treated samples included higher overall acceptability scores after five days of storage ($P \leq 0.05$). Based on the current results, odor, texture and overall acceptability of control sample oils received unacceptable scores at day 10, whereas samples treated with essential oils received unacceptable scores at day 15. Similar results have been reported for the adverse effects on sensory properties of various foods after use of high concentrations of mustard seeds [37,39].

4. Conclusion

In conclusion, differences in antimicrobial activities of the essential oils were resulted from the nature of antimicrobial compounds, which vary significantly from a plant species to another one. Mustard essential oil was effective in prevention of foodborne pathogens and suppressing microbial spoilage of fresh turkey meats at 4°C ±1 due to various antimicrobial compounds, especially AITC. It is suggested that Iranian mustard essential oils can be consider as a natural antimicrobial agent to control foodborne pathogens and replace chemical additives in food industries. However, use of these essential oils in foods as preservatives may be limited due to the oil high volatility and strong pungent flavor, especially at high concentrations. Therefore, it is recommended to use novel techniques to improve sensory properties of these antimicrobial agents and enhance capability and functionality of mustard essential oils in foods.

Table 5. Sensory evaluation of the turkey meat samples treated by mustard essential oils during refrigerated storage

Sensory attribute		Storage time (day)				
		0	5	10	15	20
Color	Control	8.72±0.46^{a}	6.90±0.94^{b}	4.81±0.75^{c}	3.27±1.19^{de}	1.81±0.87^{c}
	MEO	8.72±0.64^{a}	7.00±0.63^{b}	5.09±1.44^{c}	4.00±1.26^{d}	2.90±0.83^{e}
Odor	Control	8.72±0.46^{a}	6.18±1.25^{c}	2.09±0.94^{e}	1.09±0.30^{e}	1.00±0.00^{f}
	MEO	7.90±0.83^{b}	6.00±0.44^{c}	3.00± 0.63^{d}	2.09±0.70^{e}	1.09±0.83^{f}
Appearance	Control	8.72±0.46^{a}	7.81±0.98^{b}	4.90±1.13^{c}	2.54±0.93e^{f}	2.27±0.78^{f}
	MEO	8.72±0.46^{a}	8.00±0.89^{b}	5.45±0.52^{c}	4.27±0.46^{d}	3.09±0.30^{e}
Texture	Control	9.00±0.00^{a}	8.90±0.30^{a}	3.27±1.00^{c}	1.27±0.46^{e}	1.00±0.00^{e}
	MEO	9.00±0.00^{a}	8.81±0.40^{a}	5.63±1.36^{b}	3.00±1.09^{c}	2.27±0.46^{cd}
Overall acceptance	Control	8.72±0.46^{a}	5.81±0.98^{b}	2.54±1.03^{d}	1.45±0.68^{ef}	1.09±0.30^{f}
	MEO	8.63±0.50^{a}	6.09±0.53^{b}	4.91±0.53^{c}	2.81±0.60^{d}	1.90±0.70^{e}

*Different small letters show significant differences in each column ($P \leq 0.05$); MEO: mustard essential oil

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6. Conflict of interest

The authors report no conflicts of interest.

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مقایسه ترکیب شیمیایی و خواص ضد باکتریایی دو اسانس خردل ایرانی و کاربرد آن در گوشت بوقلمون به عنوان نگهدارنده

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چکیده

سابقه و هدف: خردل ایرانی در مناطق جنوبی ایران کشت می شود و به طور سنتی به عنوان نگهدارنده ای طبیعی مورد استفاده قرار می گیرد. هدف مطالعه حاضر، شناسایی و مقایسه ترکیبات شیمیایی، فعالیت ضد میکربی اسانس روغنی دو رقم خردل ایرانی و ارزیابی کاربرد آنها برای افزایش عمر انباری گوشت بوقلمون بوده است.

مواد و روش ها: ترکیبات شیمیایی اسانس های روغنی دو رقم خردل ایرانی با استفاده از کروماتوگرافی گازی-طیف سنجی جرمی شناسایی شد و فعالیت ضدباکتریایی آنها در مقابل *سالمونلا تیفی موریوم، اشریشیا کلی، سیتروباکتر فروندی، سودوموناس آئروژینوزا، استافیلوکوکوس اورئوس، لیستریا مونو سیتوژنز، با سیلوس سرئوس، انتروکوکس فکالیس* با استفاده از روش های انتشار دیسک و رقت سازی لوله ای ارزیابی شد. اثرات مهارکنندگی اسانس های روغنی بر رشد باکتری های میان‌دمادوست و سرما دوست[۱] مخمرها و کپک ها و همچنین خواص حسی در گوشت بوقلمون بررسی شد.

یافته‌ها و نتیجه‌گیری: نتایج GC-MS، وجود ترکیبات زیست فعال به ویژه آلیل ایزوتیوسیانات (۷۵/۸۷ - ۸۰/۰۷ درصد) را نشان دادند رشد تمام باکتری ها، به ویژه *اشریشیا کلی*، با هاله مهار رشدی بزرگتر از ۲۰ میلی متر مهار شد و حداقل غلظت مهارکنندگی و باکتری کشی $0/156\ mg\ ml^{-1}$ بود. تیمار نمونه های گوشت بوقلمون با اسانس خردل منجر به کاهش معنی‌دار باکتری های میان‌دمادو ست و سرما دو ست، مخمرها و کپک ها در طی ۲۰روز نگهداری در دمای۱±۴ درجه سلسیوس در مقایسه با نمونه های کنترل شد ($p\leq 0/05$). با گذشت زمان، امتیاز حسی نمونه های تیمار شده در مقایسه با نمونه های شاهد افزایش یافت. بر اساس یافته های این یافته ها، اسانس های روغنی خردل ایرانی می توانند به عنوان یک نگهدارنده های طبیعی در مواد غذایی مورد استفاده قرار گیرند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- آلیل ایزوتیوسیانات
- فعالیت ضدمیکروبی
- باکتری‌های بیماری‌زای غذایی
- اسانس خردل
- گوشت بوقلمون

***نویسنده مسئول**

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