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Article

# Analysis of Volatile Compounds in a Value-Added Jerky by Incorporating Ajwain and Thyme Essential Oils

Elaine Anit <sup>1</sup>, Helga Hernández <sup>1</sup>, Jan Banout <sup>1</sup> and Klára Urbanová <sup>1,\*</sup>

<sup>1</sup> Department of Sustainable Technologies, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6-Suchbát, 165 00, Czech Republic

\* Correspondence: urbanovak@ftz.czu.cz

**Abstract:** Ajwain essential oil (AEO) and Thyme essential oil (TEO) naturally contain bioactive compounds important for its growth and development. Various researchers have discovered that these compounds contribute biological benefits for living things such as humans and animals. Bioactive compounds found in essential oils, such as terpenes and terpenoids, possess antibacterial and flavouring qualities, making them promising natural preservatives in the food business. This study investigates the effect of essential oil treatment methods on their incorporation into dehydrated beef and its subsequent sensory acceptability. The meat samples underwent hot air blanching (HAB) and oil treatment (OT) with doses of 0.75 mL and 1.5 mL, respectively. Subsequently, the samples were dried at 55 °C for 6 hours after each treatment. The identification and quantification of volatile chemicals were performed using headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). Thymol,  $\gamma$ -terpinene, p-cymene, and  $\beta$ -pinene were the predominant compounds before and after the treatments. The findings revealed that the application of AEO and TEO treatments resulted in significant differences in the final concentration of the monoterpenes. However, the sensory evaluation indicated that the ajwain and thyme EO samples received similar overall ratings. Consequently, ajwain essential oil could be a suitable alternative to thyme in beef jerky.

**Keywords:** Thyme essential oil; Ajwain essential oil; HS-SPME-GC-MS; dried meat

## 1. Introduction

There has been a growing interest in the consumption of medicinal plants over the last two decades. The application of their extracts and essential oils (EOs) is an exciting trend in the food and pharmaceutical industries since they are considered a likely source of bioactive natural compounds [1–5]. One of the many plants used is thyme (*Thymus vulgaris*), an aromatic plant and herb belonging to the family Lamiaceae, with a grassy appearance that grows in many parts of the globe, is used for infusions, as a seasoning agent as well as a very valuable meat additive [6–9]. So far, the application of thyme essential oil (TEO) in meat products attracts food processors and consumers mainly due to its antimicrobial and flavouring properties.

Ajwain seeds, commonly used as a spice in Indian and Middle Eastern Asian dishes, are small, oval-shaped, and pale brown, with a bitter and spicy taste and aroma like thyme. It has been used as much for cooking as natural medicine, particularly for digestive ailments [10,11]. The application of ajwain (*Trachyspermum Ammi* L.) essential oil (AEO), a prominent fragrant herb belonging to the Apiaceae family, has been employed as a food preservative, antioxidant, flavouring agent, perfumery, and medicine [12]. In meat products, they could be used as a natural preservative and flavouring compound since they present very similar properties to thyme. There are some studies in meat using ajwain as a spice. The study tested different food preservative formulations to produce various shelf-stable and intermediate moisture meat products with hurdle technology [13]. One was a mix of spices, including ajwain seeds, to prepare shelf-stable buffalo meat chunks. Another recent study investigated the effects of chitosan coating containing AEO on the shelf life of chicken breasts

during refrigerated storage [14]. However, the application of ajwain essential oil has not been studied in other meat products.

Thyme usually contains between 1% and 2.5% essential oil, which is the cause of a moderate and pleasant aroma and often very marked balsamic and spicy taste [5,15]. Thymol and its phenol isomer, carvacrol, are frequently the significant components of extracted EO (representing around 30-50%). They are usually accompanied by p-cymene and  $\gamma$ -terpinene; these four monoterpenes are often biogenetically associated [16]. In the case of ajwain, studies have reported that the amount of essential oil extracted from the fruits was from 0.5% to 5%. Four different chemotypes are commonly determined in AEO: thymol, carvacrol, p-cymene, and  $\gamma$ -terpinene. The thymol chemotype could be accountable for 35 to 60% of the AEO. Nevertheless, these chemotypes vary according to different cultivation conditions, time of harvest, different extracting methods, and fruit storage conditions, among others [17]. Also, the main compounds in both EOs are reported as the cause of antioxidant, antimicrobial, fungicidal, antispasmodic, and expectorant, among other health benefits [4,5,9,18].

In this manuscript, the volatile headspace composition of the dried meat treated with different essential oils has been characterised using a headspace solid-phase microextraction (HS-SPME) method in combination with gas chromatography-mass spectrometry (GC-MS) for the final quantification of the volatile organic compounds (VOCs). To our knowledge, there is no defined technique for isolating volatile compounds from food. Fortunately, SPME extraction with GC-MS separation and identification is a valuable and reliable method for analysing the VOCs in meat products [19,20]. Some authors also suggested that a complete quantification of volatiles is difficult when using the SPME-GC-MS technique. Still, comparing the relative percentages of these compounds among samples is viable when the same analytical practice is implemented [5,21,22].

We took a traditional meat snack product and enriched it with conventional essential oil, thyme, to add sensory and healthier properties. Moreover, we wanted to find an alternative EO from the folk culture that can potentially replace the TEO, namely, ajwain, for our value-added jerky. Bearing this in mind, after applying the EOs treatments and drying the product, we wanted to determine and be able to quantify the final monoterpene composition (which is responsible for most of the medicinal, preservative, and flavouring properties in EOs) on the final dried meat. Besides, to evaluate the impact of those treatments on the sensory properties of our jerky-type snack foods, as we intend to make them an option for consumption.

## 2. Materials and Methods

### 2.1. Essential Oil

Thyme essential oil (TEO) was obtained from Katyani Exports (New Delhi, India). According to the manufacturer's specifications, TEO was extracted by steam distillation from the entire flowering plant (herb) without the root. Ajwain essential oil (AEO) was purchased from the same supplier and was extracted from the crushed seeds through steam distillation. For the chemical analyses, TEO and AEO were diluted with hexane; 5  $\mu$ L EO was added to 1 mL of hexane. After 10 minutes of shaking, samples were analysed by GC-MS.

### 2.2. Meat Sample Preparation

For this study, fresh beef from biceps femoris was purchased from a local butchery in Prague, Czech Republic. The beef muscle surface was washed and stored at -6 °C for 1 day. After that, the frozen muscle was defrosted at ambient temperature ( $\pm$  25 °C) for 1 hour and was sliced into thick steaks of 1 cm by a meat cutter SILVER (Kalorik, Miami Gardens, FL, USA). Additionally, it was cut into 5 cm  $\times$  2.5 cm small rectangular samples. Meat slices were packaged in zipper plastic bags and stored at -6 °C in the freezer.

### 2.3. Preparation of Modified Blanching Treatments

Two modified blanching methods were used to prepare meat samples with EO: hot air blanching (HAB) and oil treatment (OT). The doses of EOs in the treatments were set at 0.75 ml and 1.5 ml.

Before each treatment, the meat sample was immersed for 10 minutes in a salt solution containing 12 grams of sodium chloride per litre (12 g NaCl/L). HAB involved saturating the filter paper with various amounts of EO and placing it in front of a fan in a dryer (Memmert dryer UFE 400, Germany) with two trays. Subsequently, the meat sample was placed in a dryer and dried for 10 minutes at 35 °C. Saturation of the filter paper led to the formation of vapours containing TEO and AEO and their incorporation into the meat samples. In the oil treatment, the sample was immersed in 20 ml of sunflower oil with each EO and the prescribed amount at room temperature for 10 minutes. All beef samples were subsequently dried in an oven (Memmert oven UFE 400, Germany) at 55 °C for 6 hours. After 3 hours of drying, the samples were turned over to ensure thorough drying of the entire surface.

#### 2.4. HS-SPME Extraction Method

The solid phase microextraction (SPME) technology was employed to extract volatile chemicals from the headspace (HS). The quantitative relationships between the primary volatile components of TEO and AEO have been identified. Before analysis, the fibre was conditioned at 250 °C for 60 minutes in the gas chromatograph inlet. After completing the drying process for each modified blanching treatment, a 4 mL vial sealed with PTFE-faced silicone septa was used to weigh 1±0.02 g of ground dried meat. The vial was then injected with an SPME fibre, which had a thickness of 50/30 µm and was coated by Divinylbenzene/Carboxen/Polydimethylsiloxane DVB/CAR/PDMS. The fibre was subjected to the gas phase above the sample at room temperature (± 25 °C) for 30 minutes. Following adsorption, the fibre was extracted from the vial and promptly placed into the gas chromatography inlet.

#### 2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis/Quantification

The volatile components of TEO and AEO were quantitatively analysed using gas chromatography-mass spectrometry (GC-MS) on samples subjected to various doses and treatments. The study used an Agilent 7890B GC equipped with an Agilent 5977 MSD mass spectrometer. Following the extraction procedure, the fibre was manually inserted into the injection port of the GC-MS. The temperature was set to 250 °C in splitless mode. The gas chromatography column used was HP/5MS 5% Phenyl methyl Siloxane with dimensions of 30m length, 250 µm inner diameter, and 0.25 µm film thickness. Helium was utilised as the carrier gas, maintaining a consistent flow rate of 1 mL/min. The temperature of the GC oven was increased in a controlled manner. It started at 45 °C and remained at this temperature for 5 minutes. Then, it was raised to 250 °C at 10 °C per minute. Once it reached 250 °C, it was further increased to 280 °C at a faster rate of 20 °C per minute. The temperature was then held at 280 °C for 5 minutes. The entire analysis process took a total of 32 minutes. Following each injection, the fibre was cleaned at a temperature of 250 °C for 10 minutes to guarantee its impeccable condition. The constituents' identification was based on comparing their retention indices, retention times, and spectra with the National Institute of Standards and Technology Library ver. 2.0.f (NIST, USA) and literature [23,24]. The RI was calculated for compounds separated by HP5-MS column using the retention times of n-alkanes series ranging from C8 to C40 for each EO analysed. The relative percentages of the components were calculated based on the gas chromatography peak areas, with only compounds exceeding 0.1 % being considered. The analyses were performed in triplicate.

#### 2.6. Chemical Composition of TEO and AEO (GC-MS)

The chemical content of both essential oils was determined using GC-MS. The essential oil was diluted in hexane at a 5 µm/mL concentration for sample preparation. The sample was injected into the GC-MS Agilent 7890B GC port in split mode, with a split ratio of 10:1. The injection temperature was set to 250 °C, and the carrier gas used was Helium, flowing at a constant rate of 1 mL/min. An identical column to the previous GC-MS quantification method was utilised. The initial oven temperature was set to 45°C and maintained for 5 minutes. It was then increased to 250°C at a rate of 10 °C/min. Finally, the temperature was further increased to 280°C at a rate of 20 °C/min and kept for

5 minutes. The total run duration of the oven was 45.5 minutes. The constituents' identification was based on comparing their retention indices, retention times, and spectra with the National Institute of Standards and Technology Library ver. 2.0.f (NIST, USA) and literature [23,24]. The RI was calculated for compounds separated by HP5-MS column using the retention times of n-alkanes series ranging from C8 to C40 for each EO analysed. The relative percentages of the components were calculated based on the gas chromatography peak areas, with only compounds exceeding 0.1% being considered. The analyses were performed in triplicate.

### 2.7. Statistical Analysis

The statistical study was conducted using Statistica Vs.13.5.0.17 software on Windows 10. A total of 60 samples were utilised in this investigation, each consisting of 2 dosages, 2 essential oils, and 2 blanching treatments. Additionally, each sample was duplicated, resulting in a total of 2 controls per replication. Furthermore, three independent experiments, or replications, were carried out. The normal distribution was assessed using the Kolmogorov-Smirnov and Lilliefors tests, while variance homogeneity was verified using the Levene test. The chemical composition (expressed as a percentage of the total area) was analysed using analysis of variance (ANOVA) to determine the major effects of each treatment type. The experimental design included each composition (expressed as a percentage of the total area) as a dependent variable. The categorical parameters in this study were samples, dosages, and repetition, all of which were considered simultaneously. The key interaction we utilised in the analysis was the combination of the sample and dosage variables. A Tukey correction was performed for doing multiple mean comparisons. Statistical significance is defined as  $p < 0.05$ .

Additionally, to analyse the sensory data, we accomplished ANOVA main effects and Tukey's HSD post hoc test to separate mean differences. In the model, each evaluated trait was a dependent variable, and the categorical factor was the sample type. Afterwards, a generalised Procrustes analysis (GPA) was performed using XLSTAT software (Addinsoft, France) to reduce the differences between five scored traits significantly affected by the sample type (ANOVA previously assessed), establish a consensus, and summarise the results in an attribute and samples biplot map [25].

### 2.8. Sensory Analysis

Sensory analysis was performed in a specialised laboratory for sensory analysis. A panel of 15 assessors, consisting of students and staff from the Faculty of Tropical AgriSciences at the Czech University of Life Sciences Prague, was assembled to conduct a descriptive product assessment. The assessors underwent training on how to assess the sensory characteristics and how to complete the evaluation framework. A non-structured graphical 100 mm scale, composed of straight lines aligned with descriptions at both ends, was employed for sensory evaluation and rating. The samples were prepared one day in advance and stored in sealed plastic bags to preserve the thyme and ajwain flavour. In addition, during the salting process, the meat sample was immersed in a salt solution (12 grams of NaCl per litre for ten minutes). The meat was then dried using the same method as before to prepare the modified blanching treatments (at 55 °C for 6 hours).

The samples were encoded using randomly generated three-digit numbers. Each panellist was provided with six samples. 1. Control + salt 2. Ajwain OT 0.75 mL 3. Thyme HAB 0.75 mL 4. Ajwain HAB 0.75 mL 5. Thyme OT 1.5 mL and 6. Ajwain OT 1.5 mL. To cleanse the assessor's taste, unsalted bread, water, and Ethanol 30% were provided after each sample.

The factors assessed were the visual appearance, aroma, colour, texture, taste, intensity of individual flavours, and overall evaluation of the jerky samples (Table 1).

**Table 1.** Sensory traits and their scale orientations.

Sensory trait	Scale orientation	
	left = 0 %	right = 100 %
<i>Appearance:</i>		
general appearance	very bad	excellent
<i>Smell:</i>		
general pleasantness of the smell	very bad	excellent
intensity of thymol smell	imperceptible	very strong
<i>Colour:</i>		
general likableness of the colour	dislike	like
general intensity of the colour	extremely light	extremely dark
<i>Texture:</i>		
general pleasantness of the texture	very bad	excellent
juiciness	dry	juicy
chewiness	difficult	easy
<i>Taste:</i>		
general pleasantness of the taste	very bad	excellent
general intensity of the taste	imperceptible	very strong
<i>Intensity of partial tastes:</i>		
Thymol taste	imperceptible	very strong
salty	imperceptible	very strong
bitter	imperceptible	very strong
astringent	imperceptible	very strong
pungent	imperceptible	very strong
<i>Overall evaluation of the sample:</i>	very bad	excellent

### 3. Results

#### 3.1. Chemical Analysis of TEO and AEO

The results of the chemical composition of the analysed TEO and AEO are shown in Table 2. Ten components were analysed in pure TEO, which accounted for 95.15% of the total area. The primary constituent was thymol, accounting for 46.98%. It was accompanied by three monoterpene hydrocarbons:  $\gamma$ -terpinene (26.78%), p-cymene (18.32%), and  $\beta$ -pinene (1.89%). Simultaneously, 11 compounds were found for AEO, accounting for 99.99% of the total area. Same as in the TEO, the AEO contained thymol (37.03%), followed by  $\gamma$ -terpinene (35.75%), p-cymene (23.67%), and  $\beta$ -pinene (2.24%).

**Table 2.** Chemical composition of ajwain and thyme essential oils.

Compounds	<sup>a</sup> RI		<sup>b</sup> Content [%]	
	Obs.	Lit.	AEO	TEO
$\alpha$ -Thujene	924	931	0.20	0.18
$\alpha$ -Pinene	930	939	0.30	0.26
$\beta$ -Pinene	975	980	2.24	1.89
Myrcene	990	991	0.33	0.32
$\alpha$ -Terpinene	1016	1018	0.27	0.27

p-Cymene	1027	1026	23.67	18.32
$\gamma$ -Terpinene	1062	1062	35.75	26.78
Isoterpinolene	1090	1086	0.04	–
p-Cymenene	1093	1090	0.07	0.08
Limonene oxide	1172	1184	0.09	–
Carvone	1259	1242	–	0.07
Thymol	1315	1290	37.03	46.98
Total identified			99.99	95.15

<sup>a</sup>RI = retention indices. Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C<sub>8</sub>–C<sub>40</sub>) on the HP-5MS column. Lit. = literature RI values [23,24]. <sup>b</sup>Relative peak area percentage as the mean of three measurements.

Subsequently, the chemical composition of the headspace of dried meat, treated with the HAB and OT methods with ajwan and thyme EOs, was analysed. With this, it was observed how individual chemical components were incorporated into dried meat samples. For this study,  $\beta$ -pinene, p-cymene,  $\gamma$ -terpinene, and thymol were selected as the main components since they were still presented as significant after applying the EOs treatments.

The results in Table 3 revealed significant differences between the composition of treated samples.

**Table 3.** Headspace analysis of the main components of EOs in dried meat samples.

Compound	TEO	TEO	TEO	TEO	AEO	AEO	AEO	AEO
	HAB	HAB	OT	OT	HAB	HAB	OT	OT
	0.75	1.50	0.75	1.50	0.75	1.50	0.75	1.50
	Content [%] (SD)							
$\beta$ -Pinene	0.22 (0.12) <sup>a</sup>	0.63 (0.13) <sup>ab</sup>	2.29 (1.44) <sup>cd</sup>	2.66 (1.19) <sup>a</sup>	0.46 (0.09) <sup>abc</sup>	1.14 (0.55) <sup>abc</sup>	1.75 (0.63) <sup>bcd</sup>	2.79 (0.38) <sup>d</sup>
p-Cymene	11.55 (6.28) <sup>ab</sup>	25.09 (5.42) <sup>c</sup>	24.53 (8.75) <sup>c</sup>	19.94 (6.04) <sup>bc</sup>	20.67 (5.15) <sup>bc</sup>	40.84 (6.91) <sup>d</sup>	20.82 (2.48) <sup>bc</sup>	27.47 (5.84) <sup>c</sup>
$\gamma$ -Terpinene	4.28 (2.55) <sup>ab</sup>	11.73 (3.33) <sup>bcd</sup>	9.45 (4.85) <sup>abcd</sup>	7.08 (1.95) <sup>abc</sup>	9.59 (2.48) <sup>abcd</sup>	16.15 (7.66) <sup>d</sup>	8.84 (2.98) <sup>abcd</sup>	12.52 (4.56) <sup>cd</sup>
Thymol	12.87 (3.80) <sup>c</sup>	11.89 (3.91) <sup>de</sup>	5.93 (1.53) <sup>bc</sup>	11.63 (5.41) <sup>de</sup>	6.66 (1.72) <sup>bc</sup>	7.81 (0.85) <sup>bc</sup>	5.26 (1.3) <sup>b</sup>	8.82 (2.19) <sup>cd</sup>

Note: TEO HAB 0.75 = EO: Thyme, treatment method: Hot air blanching; the dose of EO: 0.75; TEO HAB 1.5 = EO: Thyme, treatment method: Hot air blanching; the dose of EO: 1.5; TEO OT 0.75 = EO: Thyme, treatment method: Oil treatment; the dose of EO: 0.75; TEO OT 1.5 = EO: Thyme, treatment method: Oil treatment; the dose of EO: 1.5; AEO HAB 0.75 = EO: Ajwain, treatment method: Hot air blanching; the dose of EO: 0.75; AEO HAB 1.5 = EO: Ajwain, treatment method: Hot air blanching; the dose of EO: 1.5; AEO OT 0.75 = EO: Ajwain, method of treatment: Oil treatment; the dose of EO: 0.75; AEO OT 1.5 = EO: Ajwain, method of treatment: Oil treatment; the dose of EO: 1.5. Values are given as mean  $\pm$  SD (n=12). Values represent the means of three replicates/trials. Values in the same line followed by different high-case letters are significantly different at  $p < 0.05$ .

For  $\beta$ -Pinene, OT samples in the higher dose were reported higher ( $P < 0.05$ ) than the HAB samples.  $\beta$ -pinene content was the highest in OT 1.5 mL (2.66%, 2.75 %) in both essential oils. p-Cymene was recorded significantly higher in ajwain HAB 1.5 mL (40.84%) than the control and OT samples. Interestingly, p-cymene content is almost double that of pure AEO, representing 23.67%. The share for  $\gamma$ -terpinene showed ajwain HAB 1.5 mL (16.15%), thyme HAB 1.5 mL (11.73%), and ajwain OT 1.5 mL (as the primary samples significantly higher ( $P < 0.05$ ) than the control. Moreover,

the  $\gamma$ -terpinene portion was lower than the initial share in thyme and ajwain essential oils, 26.78 and 35.75%, respectively. Additionally, thymol content was superior in OT and HAB samples with the higher dose (1.5 mL). It is notably higher in thyme HAB 0.75 mL (12.87%) and, in the case of ajwain with OT 1.5 mL (8.82%).

### 3.2. Sensory Analysis

Hence, to approach dried meat's main sensory properties, we evaluated 16 traits (Table 1). The ANOVA main effects analysis disclosed significant differences for 5 of 16 assessed sensory attributes.

The means for appearance, smell, colour, and texture attributes are shown in Table 4. Considering the differences between the means among samples, panellists evaluated ajwain OT 0.75 mL as superior to the control in general pleasantness of the smell and more intensive in thyme smell than the control ( $P < 0.05$ ). Regarding the likableness of the colour, samples treated with OT got superior scores compared to the HAB samples and the control. Concurrently, the results obtained from general appearance presented the best score samples as ajwain OT 0.75 mL and thyme OT 1.5 mL.

In general, texture and flavour are usually identified as the most important descriptors of beef quality. Regarding texture, the ajwain OT 1.5 mL sample was evaluated as superior to the other samples in general pleasantness of the texture, the juiciest and moderated chewable. Ajwain OT 0.75 mL and thyme OT 1.5 mL were acceptable regarding the general pleasantness of the texture compared to other samples and the control, and they were rated as not tough and less intensive for juiciness.

**Table 4.** Mean [%] (standard deviation) sensory trait scores of dried meat sample.

Sensory Attribute	Control + salt	Thyme HAB 0.75 mL	Ajwain HAB 0.75 mL	Ajwain OT 0.75 mL	Thyme OT 1.5 mL	Ajwain OT 1.5 mL
General Appearance	51.07 (20.32) <sup>a</sup>	51.33 (23.17) <sup>a</sup>	53.00 (22.95) <sup>a</sup>	59.80 (27.45) <sup>a</sup>	63.73 (19.78) <sup>a</sup>	52.77 (22.68) <sup>a</sup>
General pleasantness of the smell	51.90 (10.26) <sup>a</sup>	50.60 (19.90) <sup>a</sup>	44.03 (14.84) <sup>a</sup>	56.00 (20.20) <sup>a</sup>	55.00 (24.47) <sup>a</sup>	48.93 (24.57) <sup>a</sup>
Intensity of the thymol smell	19.13 (16.08) <sup>a</sup>	45.13 (18.94) <sup>b</sup>	30.27 (26.96) <sup>ab</sup>	50.47 (17.83) <sup>b</sup>	49.90 (23.64) <sup>b</sup>	45.07 (21.11) <sup>b</sup>
General likableness of the colour	51.13 (22.81) <sup>a</sup>	54.67 (22.93) <sup>a</sup>	45.10 (29.90) <sup>a</sup>	66.53 (24.82) <sup>a</sup>	67.47 (24.68) <sup>a</sup>	61.67 (24.97) <sup>a</sup>
General intensity of the colour	45.47 (12.59) <sup>a</sup>	55.07 (17.66) <sup>ab</sup>	51.37 (24.43) <sup>ab</sup>	66.07 (18.90) <sup>b</sup>	66.13 (16.70) <sup>b</sup>	49.60 (22.37) <sup>ab</sup>
General pleasantness of the texture	46.23 (17.33) <sup>a</sup>	45.53 (20.32) <sup>a</sup>	49.00 (25.28) <sup>a</sup>	50.30 (19.80) <sup>a</sup>	49.40 (25.87) <sup>a</sup>	56.80 (19.84) <sup>a</sup>
Juiciness	27.93 (20.35) <sup>a</sup>	33.20 (23.64) <sup>a</sup>	29.80 (23.73) <sup>a</sup>	35.10 (21.43) <sup>a</sup>	39.33 (30.01) <sup>a</sup>	42.87 (22.32) <sup>a</sup>
Chewiness	51.60 (20.66) <sup>a</sup>	52.77 (19.91) <sup>a</sup>	44.27 (19.24) <sup>a</sup>	58.33 (21.13) <sup>a</sup>	54.20 (27.45) <sup>a</sup>	60.60 (21.24) <sup>a</sup>

Note: Means in the same row followed by different superscript letters are significantly different ( $P < 0.05$ ). For the evaluation, unstructured graphical 100 mm linear scales were used. The overall evaluation and the general pleasantness of the taste (0% = very bad and 100% = excellent). General intensity of taste and intensity of partial tastes: thyme taste, salty, bitter, astringent, and pungent (0% = imperceptible and 100% = very strong).

In terms of taste and intensity of partial tastes, the results are revealed in Table 5. Ajwain OT 0.75 mL was scored superior to the control and other samples concerning the general pleasantness and general intensity of the taste. At the same time, it was ranked as the most intense in thymol taste compared to the control, which matches our previous results concerning smell intensity (Table 4). Regarding the salty taste (Table 5), the samples did not show significant differences and were ranked as bland in saltiness. The control was perceived as the saltiest; very likely, the lack of thymol allows other partial tastes to overcome.

**Table 5.** Mean [%] (standard deviation) sensory trait scores for taste of dried meat sample.

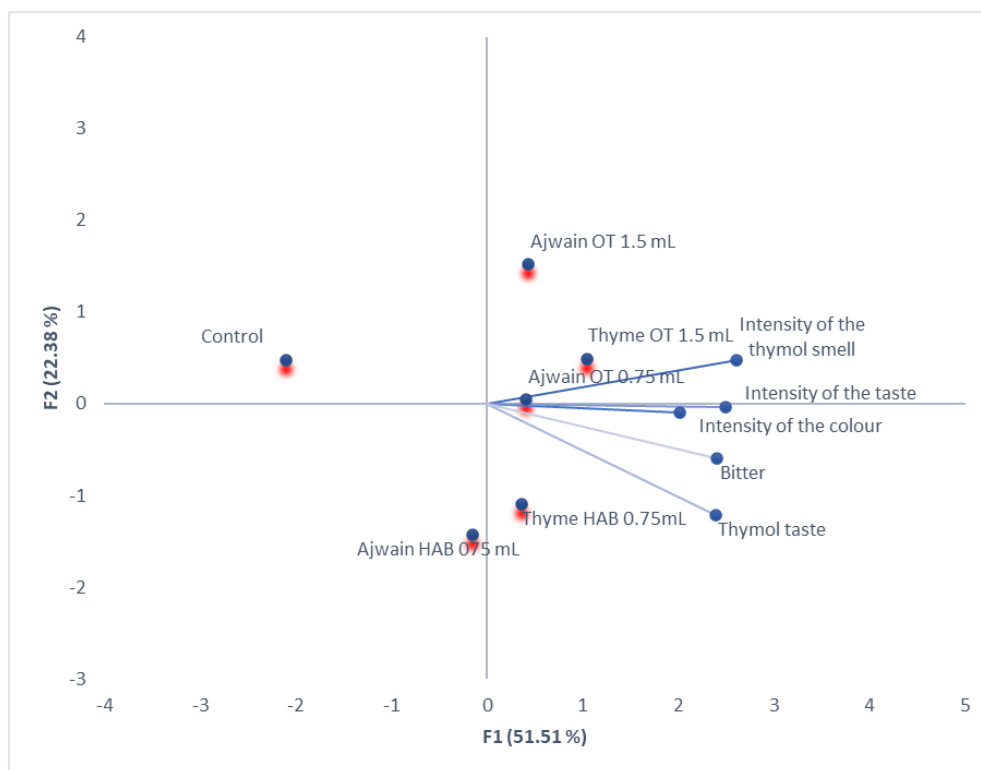
Sensory Attribute	Control + salt	Thyme HAB 0.75 mL	Ajwain HAB 0.75 mL	Ajwain OT 0.75 mL	Thyme OT 1.5 mL	Ajwain OT 1.5 mL
General pleasantness of the taste	48.33 (17.48) <sup>a</sup>	42.53 (23.82) <sup>a</sup>	44.47 (22.11) <sup>a</sup>	56.53 (19.59) <sup>a</sup>	48.53 (26.02) <sup>a</sup>	50.53 (22.38) <sup>a</sup>
General intensity of the taste	39.73 (19.98) <sup>a</sup>	52.40 (16.96) <sup>ab</sup>	56.33 (17.74) <sup>ab</sup>	62.40 (16.43) <sup>b</sup>	58.73 (15.62) <sup>b</sup>	57.33 (15.62) <sup>ab</sup>
Thymol taste	23.40 (19.79) <sup>a</sup>	55.27 (16.68) <sup>b</sup>	56.27 (19.69) <sup>b</sup>	57.33 (17.83) <sup>b</sup>	51.97 (17.22) <sup>b</sup>	47.00 (16.96) <sup>b</sup>
Salty	41.40 (16.72) <sup>a</sup>	29.13 (16.15) <sup>a</sup>	31.93 (22.18) <sup>a</sup>	34.53 (19.04) <sup>a</sup>	37.13 (12.51) <sup>a</sup>	35.30 (17.48) <sup>a</sup>
Bitter	12.00 (15.15) <sup>a</sup>	31.60 (27.23) <sup>ab</sup>	33.00 (23.03) <sup>ab</sup>	23.33 (21.47) <sup>ab</sup>	39.20 (28.27) <sup>b</sup>	29.63 (24.12) <sup>ab</sup>
Astringent	18.40 (16.37) <sup>a</sup>	28.20 (25.72) <sup>a</sup>	29.23 (27.14) <sup>a</sup>	21.73 (20.38) <sup>a</sup>	39.33 (30.32) <sup>a</sup>	28.73 (26.26) <sup>a</sup>
Pungent	18.73 (15.16) <sup>a</sup>	28.53 (24.27) <sup>a</sup>	28.06 (27.06) <sup>a</sup>	26.23 (21.84) <sup>a</sup>	27.43 (24.85) <sup>a</sup>	23.33 (21.66) <sup>a</sup>
Overall evaluation of the sample	50.70 (17.19) <sup>a</sup>	46.60 (19.60) <sup>a</sup>	45.07 (24.51) <sup>a</sup>	52.80 (17.92) <sup>a</sup>	48.80 (25.15) <sup>a</sup>	54.33 (17.55) <sup>a</sup>

Note: Means in the same row followed by different superscript letters are significantly different ( $P < 0.05$ ). For the evaluation, unstructured graphical 100 mm linear scales were used. The overall evaluation and the general pleasantness of the taste (0% = very bad and 100% = excellent). General intensity of taste and intensity of partial tastes: thyme taste, salty, bitter, astringent, and pungent (0% = imperceptible and 100% = very strong).

As for bitterness, there were significant differences among the samples. Thyme OT 1.5 mL was the bitterest compared to the control. The analysis shows no significant differences between meat samples (Table 5) regarding astringent and pungent descriptors. Even though ajwain OT 0.75 mL and 1.5 mL were not significantly different regarding the intensity of the taste, the trend shows that increasing the dose of EO also increases the magnitude of the taste and is often associated with a more bitter taste.

We run a Generalised Procrustes Analysis (PCA) to reduce the scale effects and obtain a consensus among panellists among the scores given to some critical sensory attributes [26]. We selected five sensory attributes as the criteria according to the results previously assessed by ANOVA, considering the sample effect. F1 and F2 dimensions obtained from the GPA explained 73.89% of the total variability (Figure 1). According to these results, the thymol taste characterised thyme HAB 0.75mL, the intensity of the taste and intensity of the colour to ajwain OT 0.75 mL, and the intensity of the thymol smell to thyme and ajwain OT 1.5 mL; At the same time, the whole criteria have very low effect on the control and thyme HAB 0.75 mL samples.

In the end, the ajwain OT 0.75 mL and 1.5 mL samples scored superior in the overall evaluation of the sample.



**Figure 1.** GPA biplot for attributes and samples. The sensory attributes are represented with blue dots. Treatments are represented with blue and red circles.

## 4. Discussion

### 4.1. Chemical Analysis of EOs

Thymol, p-cymene and  $\gamma$ -terpinene are important components of the essential oils of *Thymus vulgaris* and *Trachyspermum ammi* L [11,27,28]. The monoterpenes found in TEO are consistent with those documented by Viuda-Martos et al. [29], where the essential oil of *T.vulgaris* from Egypt collected from the whole plant including stems, leaves and flowers contains thymol (32.23%),  $\gamma$ -terpinene (21.19%) and p-cymene (20.27%) as its primary constituents. Furthermore, these findings are consistent with results previously documented in Romania [30], which identified the primary components of *T.vulgaris* essential oil (obtained from the aerial part of the plant) as thymol (47.59%),  $\gamma$ -terpinene (30.90%) and p-cymene (8.41%)

The findings of our study on the composition of *Trachyspermum ammi* L were consistent with the findings of Zarshenas [18] from Torbat-e Heydaryeh, Iran. In both studies, thymol was the most significant component identified, which comprised 35.04% of the composition. The  $\gamma$ -terpinene content was 23.11%, while the p-cymene content was 31.80%. While other research indicated that  $\gamma$ -terpinene and p-cymene had higher thymol content in northern Sinai, Egypt, it was concluded that the plant was grown in soil with a higher saline concentration [31].

This study used the primary components  $\beta$ -pinene, p-cymene,  $\gamma$ -terpinene and thymol for SPME analysis because they remained the predominant components in dried meat after EOs application. Higher doses of OT samples containing  $\beta$ -pinene were reported to be higher ( $P < 0.05$ ) than HAB samples. Ahmed et al. (2019) [34] also found the same high concentration of  $\beta$ -pinene as this study result showed.  $\beta$ -pinene was observed to be most significant in OT 1.5 mL of both essential oils; this could be because soaking in sunflower oil increased the proportion of some monoterpene hydrocarbons and, at the same time, accelerated the oxidation process [32].

HAB-treated samples containing 1.5 ml of EOs contained a higher concentration of p-cymene than OT samples. This may be because p-cymene is generated by isomerisation and oxidation from monoterpene hydrocarbons, and its amount potentially increases during heat treatment [33]. The  $\gamma$ -

terpinene content was comparably lower than the original thyme and ajwain essential oil ratio. This result could be attributed to the higher volatility of antibacterial compounds in EO [1].

In addition, the content of phenols, specifically thymol, which indicates the quality of ajwain and thyme oil, was higher in the OT and HAB samples administered at the higher dose. Thymol concentrations in the various treatments were significantly lower than in purified essential oils. One possible explanation is that the concentrations of  $\gamma$ -terpinene and p-cymene, precursors of thymol biosynthesis, increased while thymol concentrations decreased [16,34].

#### 4.2. Sensory Analysis

Visual appearance and the perception of taste and texture in the mouth are among the most critical sensory properties influencing consumer perception of meat products [35–37]. Regarding colour preference, OT-treated samples scored higher than HAB and control samples. The increased colour preference of the OT-treated samples is associated with their intense dark reddish colour and polished appearance. Font-i-Furnols Maria and Guerrero Luis [38] found that consumers associate red-purple shades with freshness and brown colours with a lack of brightness.

Texture and flavour are considered primary indicators of beef quality. No significant differences were found between the samples in terms of texture. Consumers are likely to expect and tolerate a certain level of toughness in dried meat instead of freshly prepared meat. The evaluation of texture attributes appears more challenging than the evaluation of aroma and taste concerning these specific items [39].

In this study, smell, colour, and taste were the attributes that influenced the overall evaluation score, unlike in other studies, where the differences in texture might affect the overall experienced quality [40,41].

### 5. Conclusions

In conclusion, ajwain essential oil has great potential to improve the quality of dried meat in terms of bioactive composition and sensory properties. The solvent-free HS-SPME technique combined with GC-MS analysis is a valuable alternative for the extraction, identification and quantification of volatile compounds. Four major monoterpene compounds were identified in ajwain essential oil and thyme essential oil: thymol,  $\gamma$ -terpinene, p-cymene, and  $\beta$ -pinene. These bioactive compounds are responsible for antimicrobial, antioxidant and other medicinal properties and are valuable as a natural preservative for cured meats. Dried meat samples treated with HAB and OT showed different trends based on each volatile compound observed.

OT samples had higher levels of  $\beta$ -pinene than HAB samples. Regarding p-cymene content, ajwain HAB 1.5 ml sample showed the highest amount. The  $\gamma$ -terpinene content was highest in ajwain HAB 1.5 mL. Thymol content was found to be as high in the HAB thyme samples as in the OT 1.5 ml and ajwain OT 1.5 ml samples. Since the European Commission and the US Food and Drug Administration (FDA) consider thymol a safe flavouring agent, AEO can be used as a food preservative in value-added meat products.

In the sensory analysis, the panellists determined ajwain OT 0.75 ml as the best rated for a general pleasant aroma and more intense thyme aroma. Regarding colour, the OT samples performed better than the HAB and control samples. Ajwain OT 0.75ml and Thyme OT 1.5ml were rated best in overall appearance. Moreover, ajwain OT 0.75 ml and 1.5 ml were the highest in the overall ranking as they were preferred for their aroma, colour and taste.

For future research, a larger number of trained sensory assessors would be appropriate, supporting the results in determining the best treatment of dried jerky with essential oils. In addition, it would be advisable for research to continue with shelf-life tests to determine the antimicrobial activity of applied essential oils on dried jerky and to determine the appropriate and consumer-acceptable dose of essential oils.

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