

Influence of the Addition of Rosemary Essential Oil on the Volatiles Pattern of Porcine Frankfurters

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The effect of the addition of increasing levels of rosemary essential oil (150, 300, and 600 mg/kg) on the generation of volatile compounds in frankfurters from Iberian and white pigs was analyzed using solid-phase microextraction coupled to gas chromatography and mass spectrometry (SPME-GC-MS). Lipid-derived volatiles such as aldehydes (hexanal, octanal, nonanal) and alcohols (pentan-1-ol, hexan-1-ol, oct-1-en-3-ol) were the most abundant compounds in the headspace (HS) of porcine frankfurters. Frankfurters from different pig breeds presented different volatile profiles due to their different oxidation susceptibilities as a likely result of their fatty acid composition and vitamin E content. Rosemary essential oil showed a different effect on the generation of volatiles depending on the type of frankfurter in which they were added. In frankfurters from Iberian pigs, the antioxidant effect of the essential oil improved with increasing levels, showing the highest activity at 600 mg/kg. In contrast, 150 mg/kg of the essential oil improved the oxidative stability of frankfurters from white pigs, whereas higher levels led to no effect or a prooxidant effect. The activity of the essential oil could have been affected by the different fatty acid compositions and vitamin E contents between types of frankfurters. SPME successfully allowed the isolation and analysis of volatile terpenes from frankfurters with added rosemary essential oil including α -pinene, β -myrcene, *l*-limonene, (*E*)-caryophyllene, linalool, camphor, and 1,8-cineole, which might contribute to the aroma characteristics of frankfurters.

KEYWORDS: Frankfurters; lipid-derived volatiles; volatile terpenes; rosemary essential oil; vitamin E; fatty acid composition

INTRODUCTION

The analysis of volatiles in meat and meat products provides a large variety of information. For instance, the study of the volatile profile from a particular meat product allows the achievement of objective and valuable information regarding its aroma characteristics (1). In fact, close relationships have been established between volatile profiles and the aroma characteristics of different meat products, shedding light on the mechanisms of generation of volatile compounds (1–3). In addition, the analysis of the volatile components of a muscle food provides information about its deterioration during storage or manipulation because certain compounds are reliable indicators of particular enzymatic, microbial, or biochemical alteration processes (2, 4, 5). Lipid oxidation is one of the main causes of deterioration in the quality of meat products during storage and processing (6). Certain lipid-derived volatiles have been demonstrated to be potent odorants and contribute to the overall aroma of cooked and dry-cured meats (7, 8).

The use of herbs and spices has been widespread in recent years to inhibit the development of oxidative reactions in food

systems. Among the natural antioxidants, rosemary has been widely accepted as one of the spices with highest antioxidant activity (9–11). The antioxidant activity of rosemary essential oil is primarily related to two phenolic diterpenes: carnosic acid and carnosol (11). Essential oils and extracts from rosemary and other Labiatae herbs have been successfully used to reduce oxidative deterioration in a large variety of muscle foods (12–14). However, recent studies have described the complexity associated with the use of herbs or plant extracts as inhibitors of oxidative reactions (10, 15). The antioxidant activity of these substances is affected by many factors including the total number and location of hydroxyl groups on aromatic rings, the nature of the extracts, their concentration, and the characteristics of the system in which they are added (10, 15–17). Kähkönen et al. (10) suggested that the antioxidant activity of plant phenolics could be also affected by the oxidation conditions and lipid characteristics of the system, whereas Wong et al. (18) and Škerget et al. (17) reported that phenolic compounds from plants can interact with other substances such as tocopherols, leading to synergist effects. Furthermore, plant phenolics have shown unexpected prooxidant properties in biological materials and food systems (19, 20). However, most of these results have been reported in relatively simple model systems. Most of the studies

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carried out to evaluate the activity of rosemary essential oil in individual foods did not consider the effect of the compositional characteristics of the food. Meat and meat products from free-range-reared Iberian pigs and intensively reared white pigs are considerably different in terms of fatty acid composition and tocopherol contents, which could affect the activity of added rosemary essential oil, although this extent has never been elucidated.

The purpose of the present study was to evaluate the effect of increasing levels of added rosemary essential oil on the generation of volatile compounds in frankfurters from free-range-reared Iberian pigs and intensively reared white pigs using SPME-GC-MS.

MATERIALS AND METHODS

Animals, Feeds, and Sampling. Seven Iberian pigs were free-range-reared and fed on natural resources (grass and acorns) following traditional livestock farming procedures. The animals were slaughtered at ~150 kg live weight and an age of 14 months. Acorns (moisture, 46.10%; fat, 5.50%; protein, 4.31%) showed the following fatty acid profile (expressed as percentage of total fatty acids analyzed): palmitic acid (C16:0), 11.82%; stearic acid (C18:0), 0.56%; oleic acid (C18:1), 67.28%; linoleic acid (C18:2), 18.70%; linolenic acid (C18:3), 0.25%. The grass (moisture, 89.24%; fat, 6.26%; protein, 4.34%) fatty acid profile was as follows: C16:0, 13.95%; C18:0, 1.99%; C18:1, 5.24%; C18:2, 11.42%; C18:3, 57.80%.

Seven white pigs (Large White \times Landrace) were reared in an intensive livestock farm and fed a mixed diet. The analysis of the mixed diet (moisture, 10.42%; fat, 2.94%; protein, 18.28%) revealed the following fatty acid profile: C16:0, 18.70%; C18:0, 8.63%; C18:1, 32.84%; C18:2, 32.83%; C18:3, 2.45%. White pigs were slaughtered at ~85 kg live weight and 7 months of age.

After slaughter, adipose tissues and meat were removed from the carcasses, vacuum-packaged, and stored at -80°C until the manufacture of the frankfurters (<2 weeks).

Manufacture of the Frankfurters. The experimental frankfurters were manufactured in a pilot plant. Depending on the origin of the raw material, two types of frankfurters were produced: frankfurters from free-range-reared Iberian pigs (IF) and frankfurters from intensively reared white pigs (WF). Meat and adipose tissues from seven animals from each pig breed were used. The same formulation was used for all frankfurters except for the addition of a rosemary essential oil. The ingredients were as follows per 100 g of product: 50 g of meat, 10 g of adipose tissue, 37 g of distilled water, 2 g of sodium caseinate, and 1 g of potato starch. Sodium chloride (2%), sodium di- and triphosphates (0.5%) sodium ascorbate (0.05%), and sodium nitrite (0.03%) (all from ANVISA, Madrid, Spain) were also added. Rosemary essential oil (Soria Natural S.L., Soria, Spain) was added at 150 mg/kg (T#150), 300 mg/kg (T#300), and 600 mg/kg (T#600), giving four experimental groups within each pig breed including a control (CON) group with no added essential oil. The eight sets of frankfurters were independently produced in repeated manufacture processes. For the manufacture, the meat was first chopped into small cubes (1 cm³) and mixed with the sodium chloride and the nitrification mixture (sodium nitrite and ascorbate) 2 h before frankfurter's manufacture. Then, the meat was minced in a Foss Tecator homogenizer (model 2094) for 2 min together with the starch and 50% of the total amount of sodium caseinate, which was previously dissolved in water (75°C). After that, the adipose tissue was added together with the remaining dissolved sodium caseinate and minced for an additional 4 min until a homogeneous raw batter was obtained. Finally, the mixture was stuffed in 18-mm-diameter cellulose casings, handlinked at 10-cm intervals, and given the thermal treatment by immersion in a hot water bath (80°C/30 min). After that, frankfurters ($n = 5$ within each batch) were allowed to cool at 4°C for 24 h, after which time they were frozen at -80°C until analyses were carried out (<2 weeks).

Proximate Composition of Frankfurters. Moisture, total protein, and ash were determined using AOAC methods (21–23). The method

of Bligh and Dyer (24) was used for isolating and quantifying total lipids from frankfurters.

Iron Analysis. Total iron was determined following the procedure described by Miller et al. (25). The amount of iron was expressed as micrograms of iron per gram of frankfurter.

Tocopherols Content. α - and γ -tocopherols were extracted from frankfurters according to the method described by Rey et al. (26). The analysis was carried by reversed phase high-performance liquid chromatography (HPLC) (HP 1050, with a UV detector, HPIB 10) (Hewlett-Packard, Waldbronn, Germany).

Fatty Acid Composition. Fatty acid methyl esters (FAMES) were prepared by acidic esterification in the presence of sulfuric acid, following the method of López-Bote et al. (27). FAMES were analyzed using a Hewlett-Packard, model HP-5890A, gas chromatograph, equipped with a flame ionization detector (FID). The derivatives were separated on a FFAP-TPA fused-silica column (Hewlett-Packard, 30 m long, 0.53-mm i.d., and 1.0- μm film thickness). The injector and the detector temperature were held at 230°C. Oven temperature was maintained at 220°C. The flow rate of the carrier gas (N₂) was set at 1.8 mL min⁻¹. Identification of FAMES was based on retention times of reference compounds (Sigma). The quantification of fatty acids was carried out by using C13 as an internal standard. Results are expressed as grams of fatty acid per 100 g of total fatty acids analyzed.

SPME of Volatiles. The SPME fiber, coated with a divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS) 50/30 μm , was preconditioned prior to analysis at 220°C during 45 min. The HS sampling was performed following a method previously described (1). One gram of frankfurter was placed in a 2.5-mL vial, and the SPME fiber was exposed to the headspace of the frankfurter while the sample equilibrated during 30 min of immersion in water at 50°C. Analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass-selective detector (Agilent model 5973). Volatiles were separated using a 5% phenyl-95% dimethylpolysiloxane column (Restek) (30 m \times 0.25 mm i.d., 1.0-mm film thickness). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min⁻¹ at 40°C. The SPME fiber was desorbed and maintained in the injection port at 220°C during the whole chromatography run. The injector port was in the splitless mode. The temperature program was isothermal for 10 min at 40°C and then raised at the rate of 7°C min⁻¹ to 250°C and held for 5 min. The GC-MS transfer line temperature was 270°C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, a multiplier voltage of 1650 V, and a data collection rate of 1 scan s⁻¹ over a range of m/z 40–300. Volatile compounds were either positively identified by comparing their linear retention indexes (LRI) with those from standard compounds (Sigma-Aldrich, Steinheim, Germany) or tentatively identified by comparing their mass spectra with those contained in the Wiley library and by comparison of their LRI with those reported in the scientific literature (28). Chromatographic areas from MS are provided as area units (AU).

Data Analysis. Means and deviations from five measurements within a batch were obtained for all analytical experiments. The proximate compositions, tocopherols contents, and fatty acid compositions of frankfurters from Iberian and white pigs were compared using a Student's *t* test for independent variables from the SPSS software (11.0 version). Chromatographic areas of all tentatively identified peaks were used as variables. To determine the effect of the frankfurter origin (Iberian and white pigs) and the addition of rosemary essential oil (control, 150, 300, and 600 mg/kg) on the generation of volatiles, an analysis of variance (ANOVA) for a four (rosemary) \times two (origins) together with the interaction was used. Tukey's tests were used when ANOVA found significance differences between treatments. Significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate and Fatty Acid Composition of Frankfurters. No significant differences were detected between frankfurters concerning their proximate composition because they had similar moisture, fat, protein, and ash contents (Table 1). IF had, however, a significantly higher amount of iron (16.3 $\mu\text{g/g}$ of frankfurter) than WF (11.7 $\mu\text{g/g}$ of frankfurter). This result was

Table 1. Proximate, Vitamin E, and Fatty Acid Composition of Frankfurters from White and Iberian Pigs

	Iberian	white	SEM ^a	p value ^b
moisture ^c	63.44	62.33	0.39	0.161
fat ^c	18.38	18.69	0.19	0.444
protein ^c	11.43	10.88	0.17	0.096
ash ^c	1.28	1.36	0.05	0.448
iron ^d	16.3	11.7	0.88	0.000
α -tocopherol ^d	3.72	1.31	0.41	0.000
γ -tocopherol ^d	0.23	0.05	0.03	0.000
fatty acids ^e				
C14:0	1.27	1.39	0.02	0.038
C16:0	20.41	24.05	0.58	0.004
C18:0	9.17	14.17	0.81	0.000
Σ SFA ^f	31.56	40.66	1.46	0.000
C16:1	2.63	2.68	0.01	0.358
C18:1	54.48	43.91	1.77	0.006
C20:1	1.43	1.08	0.06	0.017
Σ MUFA ^f	58.85	48.14	1.80	0.010
C18:2	8.95	10.99	0.33	0.001
C18:3	0.72	0.73	0.00	0.559
C20:2	0.52	0.55	0.01	0.097
C20:4	0.42	0.45	0.01	0.108
Σ PUFA ^f	11.29	13.51	0.35	0.002

^a SEM, standard error of the mean. ^b Statistical significance in Student's *t* test for independent variables. ^c Grams per 100 g of raw material. ^d Micrograms per gram of raw material. ^e Grams of fatty acid per 100 g of total fatty acids analyzed. ^f SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

expected because it is generally known that meat from Iberian pigs contains higher amounts of iron than that from white pigs due to the higher concentration of myoglobin pigments (29). The addition of rosemary essential oil did not affect the proximate composition of frankfurters (data not shown).

Large differences were found between types of frankfurters for most of the fatty acids analyzed. IF had significantly smaller amounts of myristic (1.3 vs 1.4 g/100 g), palmitic (20.0 vs 24.1 g/100 g), stearic (9.2 vs 14.2 g/100 g), and total saturated fatty acids (SFA) (31.6 vs 40.7 g/100 g) than WF. Compared to WF, IF contained significantly higher amounts of oleic (54.5 vs 43.9 g/100 g) and total monounsaturated fatty acids (MUFA) (58.9 vs 48.1 g/100 g). WF had, conversely, larger amounts of polyunsaturated fatty acids (PUFA) (13.5 vs 11.3 g/100 g) such as linoleic (11.0 vs 9.0 g/100 g) acid. The fatty acid composition of frankfurters and other composite meat products reflects the fatty acid composition of the ingredients, mainly meat and adipose tissue used for their elaboration (29). The differences reported between frankfurters from Iberian and white pigs are mainly explained by the different fatty acid compositions of the feeds given to the animals during the fattening period, and, therefore, meat and adipose tissues from Iberian pigs reflected the fatty acid composition of the acorns, which had high levels of oleic acid (29). On the other hand, white pigs were fed on commercial mixed diets with relatively high amounts of PUFA, which would explain the high levels of such fatty acids in their tissues and, consequently, in the elaborated frankfurters.

Tocopherol Content of Frankfurters. Results from the quantification of tocopherols in frankfurters are shown in **Table 1**. IF presented higher levels of α - and γ -tocopherol compared to those from WF (3.7 vs 1.3 $\mu\text{g/g}$ and 0.23 vs 0.05 $\mu\text{g/g}$, respectively), which is consistent with previously reported data regarding tocopherol contents in the tissues (meat and adipose tissue) from free-range-reared Iberian pigs and white pigs reared indoors (29, 30). The α - and γ -tocopherol contents in animal tissues reflect the tocopherol concentration of the diets (30), and, therefore, the high levels of tocopherols in the grass and

acorns with which Iberian pigs were fed explain the high levels of such substances in their tissues and frankfurters. The relatively small amounts of tocopherols in WF were expected because the white pigs were fed on a nonsupplemented mixed diet with no access to fresh materials. The high content of tocopherols in tissues and meat products from free-range-reared Iberian pigs has been profusely described in previous works and considered to be one of the most appreciated quality traits (29, 30) as long as tocopherols enhance the oxidation stability of the meats and meat products, improving their nutritional and technological properties (6).

Analysis of Volatiles from Frankfurters. From the total of volatile compounds detected in the HS, 92 of them are shown in **Tables 2** and **3**. According to Chevance and Farmer (3) volatile components of the HS of frankfurters are derived from the main ingredients (meat and adipose tissue) and from the addition of spices and other minority additives. The generation of volatile compounds in frankfurters will be discussed according to their apparent origin.

Analysis of Volatiles Generated from Ingredients. Considering volatile compounds generated from main ingredients (meat and adipose tissue), lipid-derived volatiles such as aldehydes (hexanal, heptanal, octanal, nonanal, decanal, dodecanal), ketones (heptan-2-one, 1-phenyl-propanone), and alcohols (oct-1-en-3-ol) were the most abundant compounds in the HS of frankfurters. Relatively high amounts of esters and aliphatic hydrocarbons were also detected, whereas acids and furans were minor components. Most of the volatile compounds detected in the present study have been previously described as components of the HS of cooked pork and beef (5, 8). The similarity between the volatile profiles from cooked meats and frankfurters was expected because pork and porcine back fat were the major ingredients. Ahn et al. (31) and Jo and Ahn (32) described lipid-derived volatiles such as hydrocarbons, ketones, alcohols, and aldehydes as the most abundant volatile compounds in porcine cooked sausages. Similarly, Chevance and Farmer (3) reported that the HS of frankfurters without spices and smoke was dominated by volatiles generated from lipid oxidation. The production of frankfurters involves meat handling, mincing, and cooking, which greatly enhance the development of oxidative reactions (6). In addition, the high levels of fat and iron could explain the high levels of lipid-derived volatiles in the HS of frankfurters. Some of these compounds such as hexanal are useful indicators of lipid decomposition and have been commonly used to assess oxidative changes in meat, meat products, and several food systems (1, 4, 33). In addition, some of the lipid-derived volatiles described in the present work are recognized odorants commonly isolated from frankfurters and other cooked meats. Hexanal is responsible for "green" odors in frankfurters (3), although other authors have associated this volatile compound with rancidity and warmed-over flavors (4, 5, 34). Oct-1-en-3-ol contributes to "mushroom" odor notes, whereas unsaturated aldehydes derived from PUFA degradation are thought to contribute with "unpleasant, stale, oily" odors (3).

Volatiles derived from other chemical reactions were also detected. Strecker aldehydes (2- and 3-methylbutanal, benzaldehyde) and alcohols (2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol) were isolated from the HS of frankfurters. These compounds are common components of cooked meats and meat products contributing desirable "almond-like", "toasted" aroma notes (7). The presence of sulfur and nitrogen volatile compounds derived from Maillard reactions was highly restricted, which is in disagreement with the results obtained

Table 2. Volatile Compounds (AU × 10⁶) Derived from the Main Ingredients of Frankfurters from Iberian and White Pigs with 150, 300, and 600 mg/kg of Added Rosemary Essential Oil

compound	LRI ^b	Iberian				white				SEM ^c	p value ^a			rel ^d
		control	T#150	T#300	T#600	control	T#150	T#300	T#600		O	A	O × A	
ethanol	527	20.6	7.31	19.7	17.5	5.90	6.31	10.5	10.4	1.00	0.000	0.000	0.000	MS + LRI
2-methylpropan-1-ol	552	1.07	0.56	0.82	0.77	1.39	0.28	0.41	0.39	0.10	0.000	0.000	0.000	MS + Iri
2-methylpentane	569	2.16	0.92	3.17	1.17	1.44	0.62	2.43	2.27	0.16	0.408	0.000	0.006	MS + Iri
acetic acid ethyl ester	613	0.41	0.53	0.00	0.00	0.58	0.59	0.67	1.07	0.06	0.000	0.007	0.000	MS + Iri
but-(E)-2-enal	624	0.30	0.20	0.21	0.08	0.33	0.25	0.22	0.61	0.02	0.000	0.000	0.000	MS + LRI
2-methylprop-2-en-1-ol	633	3.47	0.33	0.81	1.49	0.33	0.33	1.22	0.90	0.16	0.000	0.000	0.000	MS + Iri
3-methylbutanal	662	2.22	0.43	0.46	0.75	0.42	0.39	0.38	0.36	0.10	0.000	0.000	0.000	MS + LRI
2-methylbutanal	674	1.03	0.50	0.56	0.60	0.47	0.45	0.53	0.42	0.03	0.000	0.000	0.000	MS + LRI
2-methylbut-(E)-2-enal	679	0.00	0.00	0.00	0.00	0.70	0.00	0.55	1.33	0.08	0.000	0.000	0.000	MS + Iri
pentanal	691	0.56	0.36	0.56	0.41	0.93	0.65	0.70	0.84	0.05	0.003	0.383	0.754	MS + LRI
heptane	700	0.66	0.43	0.58	0.61	0.37	0.51	0.69	0.75	0.03	0.817	0.002	0.001	MS + LRI
N,N-diethylethanamine	711	4.74	2.83	2.70	2.89	1.36	4.03	1.41	3.77	0.25	0.096	0.058	0.000	MS + Iri
pentan-2-ol	730	0.53	0.30	0.29	0.14	0.45	0.26	0.56	0.67	0.03	0.000	0.000	0.000	MS + Iri
hex-2-enal	746	0.00	0.00	0.00	0.00	0.34	0.00	0.69	0.47	0.04	0.000	0.000	0.000	MS + Iri
3-methylbutan-1-ol	756	3.63	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.20	0.000	0.000	0.000	MS + Iri
2-methylbutan-1-ol	761	1.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.001	0.000	0.000	MS + Iri
pyridine	769	1.89	0.55	0.41	0.26	1.64	0.70	0.81	0.64	0.10	0.110	0.000	0.115	MS + LRI
methylbenzene	784	1.54	1.37	1.46	1.40	0.96	0.85	1.07	1.46	0.05	0.000	0.064	0.036	MS + Iri
hexan-1-ol	824	0.00	0.00	0.00	0.00	0.46	0.35	0.32	0.87	0.05	0.000	0.000	0.000	MS + LRI
hexanal	814	14.9	6.68	7.51	5.31	22.1	11.0	18.1	22.2	1.18	0.000	0.000	0.012	MS + LRI
pent-4-enal	820	0.41	0.29	0.29	0.17	0.37	0.32	0.29	0.32	0.01	0.017	0.000	0.000	MS + Iri
ethylbenzene	879	6.57	2.94	2.71	2.82	4.71	1.20	3.64	4.35	0.29	0.448	0.000	0.003	MS + Iri
1,2-dimethylbenzene	886	6.90	4.26	4.13	3.39	15.8	1.60	1.20	0.94	0.79	0.727	0.000	0.000	MS + Iri
heptan-2-one	905	0.53	0.30	0.23	0.27	0.37	0.22	0.38	0.52	0.02	0.204	0.001	0.000	MS + LRI
1,3-dimethylbenzene	920	10.4	4.02	3.23	3.12	3.70	1.06	3.05	21.0	1.00	0.000	0.000	0.000	MS + Iri
heptanal	933	7.04	4.89	4.86	3.64	8.04	5.65	9.49	11.6	0.46	0.000	0.004	0.000	MS + LRI
2,5-dihydrofuran	967	0.00	0.00	0.00	0.00	0.56	0.00	0.44	1.07	0.06	0.000	0.000	0.000	MS + Iri
1-phenylethanone	975	0.70	0.00	0.00	0.00	0.53	0.00	0.00	0.00	0.05	0.185	0.000	0.160	MS + Iri
hexanoic acid	981	0.00	0.00	0.00	0.00	0.99	0.00	0.44	0.34	0.06	0.000	0.000	0.000	MS + Iri
benzaldehyde	986	3.24	1.65	2.50	2.04	2.03	1.41	1.54	1.10	0.12	0.000	0.000	0.102	MS + Iri
hexane-2,5-dione	995	0.00	0.00	0.00	0.00	0.42	0.35	0.38	0.42	0.03	0.000	0.119	0.119	MS + LRI
oct-1-en-3-ol	999	2.34	1.32	1.87	0.40	3.12	2.06	3.01	5.60	0.24	0.000	0.000	0.000	MS + LRI
hexanoic acid ethyl ester	1010	7.06	4.81	7.05	5.60	5.05	3.27	5.25	3.64	0.28	0.000	0.001	0.977	MS + Iri
2-methylnonane	1014	14.9	2.80	2.78	2.83	0.96	1.87	1.41	1.90	0.69	0.000	0.000	0.000	MS + LRI
octanal	1018	11.7	8.79	8.79	6.17	11.8	9.55	8.19	13.5	0.46	0.006	0.007	0.000	MS + LRI
1-methoxy-2-methylbenzene	1032	0.00	0.41	1.12	1.71	0.00	0.45	1.08	1.75	0.11	0.738	0.000	0.794	MS
nitric acid hexyl ester	1043	0.00	0.30	0.55	0.30	1.21	1.79	1.81	1.59	0.12	0.000	0.001	0.712	MS
heptanoic acid	1078	0.00	0.00	0.00	0.00	1.73	0.00	1.59	0.94	0.15	0.000	0.029	0.029	MS + Iri
octan-1-ol	1084	1.04	0.00	0.00	0.00	0.95	0.00	1.59	1.84	0.12	0.000	0.000	0.000	MS + LRI
1-phenylpropanone	1096	2.28	4.00	6.62	9.05	0.78	4.10	2.76	4.88	0.44	0.000	0.000	0.004	MS + Iri
undecane	1100	5.34	0.00	0.00	0.00	3.09	0.00	0.00	0.00	0.32	0.019	0.000	0.002	MS + LRI
1-methyl-3-(1-methylethyl)benzene	1105	1.24	1.15	1.06	1.95	0.00	0.57	1.30	1.59	0.09	0.000	0.000	0.000	MS
octan-2-one	1110	0.00	0.00	0.00	0.00	0.31	0.00	0.41	0.71	0.04	0.000	0.000	0.000	MS + LRI
heptanoic acid ethyl ester	1113	1.64	1.60	2.43	2.38	2.64	1.21	2.23	1.91	0.14	0.970	0.082	0.179	MS + Iri
nonanal	1128	36.1	34.8	24.9	24.5	36.4	38.1	40.3	38.8	1.41	0.074	0.005	0.044	MS + LRI
octanoic acid	1183	2.07	0.00	0.00	0.00	5.20	0.00	5.88	8.05	0.53	0.000	0.000	0.000	MS + Iri
dodecane	1200	2.32	5.71	9.84	7.77	1.94	6.43	7.95	16.3	0.81	0.081	0.000	0.003	MS + LRI
octanoic acid ethyl ester	1213	5.28	7.85	9.16	9.90	5.45	7.42	11.1	18.6	0.74	0.004	0.000	0.002	MS + Iri
decanal	1219	2.75	1.77	1.19	0.85	3.81	2.75	4.38	4.87	0.25	0.000	0.098	0.001	MS + LRI
nitric acid nonyl ester	1232	0.89	2.15	3.24	3.57	0.81	1.76	1.92	2.35	0.19	0.006	0.000	0.250	MS
nonanoic acid	1240	1.97	0.00	0.00	0.00	7.70	0.00	4.77	6.49	0.57	0.000	0.000	0.008	MS + Iri
nonanoic acid ethyl ester	1259	1.76	4.22	6.31	6.42	1.89	2.21	4.84	4.08	0.30	0.000	0.000	0.001	MS + Iri
dec-(E)-2-enal	1290	0.00	0.00	0.00	0.00	0.63	0.00	1.43	1.37	0.10	0.000	0.000	0.000	MS + LRI
tridecane	1300	0.98	1.28	1.63	1.38	0.72	0.93	1.34	1.18	0.05	0.000	0.000	0.811	MS + LRI
decanoic acid ethyl ester	1416	2.20	3.00	4.15	4.13	2.55	2.67	4.50	3.38	0.15	0.552	0.000	0.056	MS + Iri
dodecanal	1440	2.30	2.09	5.10	3.02	2.45	1.40	4.69	5.86	0.29	0.181	0.000	0.004	MS + Iri
N,N-dimethyl-1-dodecamine	1529	3.46	4.83	14.9	12.8	7.19	2.58	17.7	5.83	1.01	0.570	0.000	0.013	MS + Iri

^a Statistical significance: O, effect of the origin of the raw material; A, effect of the addition of rosemary; O × A, interaction between origin and addition of rosemary.

^b Linear retention index. ^c Standard error of the mean. ^d Reliability of identification: LRI, volatiles identified comparing their LRI with standard compounds; Iri, volatiles tentatively identified by comparing their LRI with those reported in the literature; MS, volatiles tentatively identified by mass spectrometry.

by Chevance and Farmer (3), who described a large variety of those compounds in porcine frankfurters. Maillard products such as pyrazines and thiophenes are potent odorants that have been linked to desirable "roasted meat" flavors. Although those authors suggested that the Maillard compounds isolated from the commercial frankfurters were generated from the main ingredients (meat and back fat), it is more probable that those could be added as volatile components of flavorings to enhance

consumer's acceptability. In fact, these compounds were not detected when the addition of spices and smoking flavors in frankfurters was avoided. The strategy of improving the aroma characteristics of a foodstuff through the addition of particular volatile compounds has been recently described in liver products (35). On the other hand, Chevance and Farmer (3) analyzed the volatile components of frankfurters using either static or dynamic HS coupled to GC and olfactometry, which certainly

Table 3. Volatile Compounds (AU $\times 10^6$) Derived from the Addition of Rosemary Essential Oil (150, 300, and 600 mg/kg) in Frankfurters from Iberian and White Pigs

compound	LRI ^b	Iberian				white				SEM ^c	<i>p</i> value ^a			
		control	T#150	T#300	T#600	control	T#150	T#300	T#600		O	A	O \times A	rel ^d
α -thujene	946	0.00	7.86	13.6	19.3	0.00	7.48	10.4	16.7	1.12	0.046	0.000	0.349	MS + Iri
α -pinene	956	0.54	505	828	1282	0.00	475	770	1365	78.0	0.978	0.000	0.272	MS + Iri
camphene	970	0.00	173	285	448	0.00	162	268	458	26.7	0.599	0.000	0.693	MS + Iri
β -1-pinene	1002	0.00	229	297	455	0.00	165	276	457	26.7	0.020	0.000	0.035	MS + Iri
β -myrcene	1006	0.00	148	279	396	0.00	147	259	379	23.3	0.158	0.000	0.609	MS + Iri
δ -3-carene	1020	0.00	1.07	2.38	3.09	0.00	1.34	1.98	3.83	0.21	0.125	0.000	0.002	MS + Iri
β -2-pinene	1029	0.00	4.18	6.47	8.83	0.00	3.54	5.67	8.30	0.51	0.005	0.000	0.363	MS + Iri
β -terpinene	1036	0.00	5.60	8.04	11.2	0.00	4.74	7.34	11.3	0.66	0.085	0.000	0.309	MS + Iri
α -terpinene	1048	0.00	1.64	5.29	7.08	0.00	1.64	5.23	7.55	0.47	0.513	0.000	0.590	MS + Iri
1-methyl-4-(1-methylethyl)-benzene	1050	0.00	231	380	536	0.76	212	360	534	31.8	0.277	0.000	0.788	MS + Iri
l-limonene	1055	1.95	375	575	803	0.78	345	641	820	49.0	0.428	0.000	0.214	MS + Iri
1,8-cineole	1061	0.00	1661	2120	2816	0.00	1287	2038	2901	169	0.043	0.000	0.005	MS + Iri
γ -terpinene	1063	0.00	6.31	8.46	18.6	0.00	4.39	10.3	12.9	0.99	0.014	0.000	0.000	MS + Iri
(<i>E</i>)-ocimene	1067	0.00	2.96	6.30	8.66	0.00	2.31	5.27	6.69	0.49	0.001	0.000	0.060	MS + Iri
β -ocimene	1090	0.00	24.1	40.1	56.1	0.00	21.9	36.9	54.4	3.29	0.075	0.000	0.700	MS + Iri
α -terpinolene	1119	0.00	15.0	27.1	36.7	0.00	15.2	24.8	35.0	2.16	0.193	0.000	0.544	MS + Iri
linalool	1121	1.59	75.0	152	231	0.00	69.7	164	217	13.7	0.601	0.000	0.242	MS + Iri
α -fenchene	1132	0.00	28.3	48.0	74.6	0.00	27.3	44.7	77.8	4.50	0.868	0.000	0.541	MS + Iri
<i>p</i> -menth-3-en-1-ol	1167	0.00	4.80	6.72	11.4	0.00	3.31	6.95	9.64	0.63	0.002	0.000	0.005	MS + Iri
1-methyl-4-(1-methylethenyl)-cyclohexanol	1179	0.00	7.32	12.8	19.9	0.00	5.58	12.6	16.1	1.10	0.001	0.000	0.004	MS + Iri
camphor	1189	0.00	783	1058	1642	0.00	563	996	1641	96.4	0.015	0.000	0.027	MS + Iri
α -terpineol	1205	0.00	8.69	25.2	38.0	0.00	9.41	20.6	30.5	2.18	0.009	0.000	0.025	MS + Iri
endoborneol	1210	0.00	18.8	58.8	56.3	0.00	4.84	11.0	18.0	1.12	0.116	0.000	0.018	MS + Iri
linalyl propionate	1215	0.00	69.2	131	223	0.00	63.3	156	281	15.4	0.000	0.000	0.000	MS + Iri
isoterpinolene	1224	0.00	16.29	29.23	43.2	0.00	11.3	25.5	31.8	2.38	0.000	0.000	0.012	MS + Iri
linalyl acetate	1248	0.00	11.1	15.7	27.6	0.00	6.64	14.2	22.6	1.57	0.016	0.000	0.314	MS + Iri
endobornyl acetate	1265	0.00	83.8	134	214	0.00	67.4	142	203	12.5	0.294	0.000	0.216	MS + Iri
α -cubebene	1366	0.00	1.09	2.03	3.34	0.00	0.81	2.12	3.36	0.20	0.364	0.000	0.035	MS + Iri
α -copaene	1427	0.00	0.94	1.61	2.48	0.00	0.70	1.77	2.50	0.15	0.644	0.000	0.002	MS + Iri
geranyl propionate	1430	0.00	2.86	5.25	9.06	0.00	2.54	6.05	8.75	0.54	0.730	0.000	0.007	MS + Iri
(<i>Z</i>)-caryophyllene	1469	0.00	2.56	2.93	4.76	0.00	4.20	7.30	12.1	0.66	0.000	0.000	0.001	MS + Iri
(<i>E</i>)-caryophyllene	1486	0.00	40.7	78.2	133	0.00	34.3	84.0	113	7.52	0.025	0.000	0.002	MS + Iri
β -selinene	1520	0.00	3.54	8.09	13.6	0.00	3.89	9.57	12.6	0.81	0.386	0.000	0.002	MS + Iri
α -elemene	1538	0.00	1.29	3.08	4.46	0.00	1.30	2.80	4.08	0.26	0.034	0.000	0.168	MS + Iri
δ -cadinene	1580	0.00	1.05	2.83	3.95	0.00	1.02	2.82	4.11	0.26	0.827	0.000	0.950	MS + Iri

^a Statistical significance: O, effect of the origin of the raw material; A, effect of the addition of rosemary; O \times A, interaction between origin and addition of rosemary.

^b Linear retention index. ^c Standard error of the mean. ^d Reliability of identification: Iri, volatiles tentatively identified by comparing their LRI with those reported in the literature; MS, volatiles tentatively identified by mass spectrometry.

provide a greater sensitivity than the SPME-GC-MS used in the present study, particularly for the detection of volatiles with low odor thresholds such as Maillard compounds.

Regardless of the addition of the rosemary essential oil, frankfurters from white pigs ("control" group) had, compared to those from Iberian pigs, a higher number of lipid-derived volatiles because hexanoic and heptanoic acids, hex-2-enal, dec-(*E*)-2-enal, 2-methylbut-(*E*)-2-enal, 2,5-dihydrofuran, hexane-2,4-dione, and octan-2-one were not detected in the HS of IF. Furthermore, WF showed significantly ($p < 0.05$) higher chromatographic areas of certain compounds closely related to lipid oxidation and off-flavors such as octanoic (5.2 vs 2.1 AU) and nonanoic acids (7.7 vs 2.0 AU), pentanal (0.93 vs 0.56 AU), and heptan-2-one (0.53 vs 0.37 AU). Differences between types of frankfurters were also significant on hexanal (white, 22.1 AU; Iberian, 14.9 AU; $p < 0.05$), which has been widely used in meat products as an indicator of lipid oxidation (1, 5). These results are in agreement with those obtained in previous works in which the oxidative stabilities of meat and meat products from Iberian and white pigs were evaluated (1, 36). The significantly higher amount of iron in IF compared to that in WF could have played a prooxidant role because that metal is considered to be the most potent oxidation promoter in muscle foods (6). The present results and those from previous studies suggest that other circumstances should be considered to fully

comprehend the considerably high oxidative stability of meats from Iberian pigs. A higher proportion of MUFA and lower proportion of PUFA (more prone to be oxidized) and the presence of significantly ($p < 0.05$) higher amounts of tocopherols in IF, compared to those from white pigs, could partly explain those results. More recently, some authors (37) have suggested the possibility that other substances with antioxidant activity such as plant phenolics could be accumulated in Iberian pig's tissues as a result of the intake of natural resources and, hence, contribute to the inhibition of oxidative reactions in meat and muscle foods from free-range-reared Iberian pigs.

On the other hand, the large differences between types of frankfurters in terms of fatty acid composition could affect the aromatic characteristics of frankfurters as long as the pathways for the generation of volatile compounds from lipid oxidation are fairly specific for each fatty acid. Oleic acid-derived volatiles are associated with pleasant notes, described as "floral" and "sweet" (38), whereas the aromatic notes of linoleic and PUFA-derived volatiles have been described as intense "grass-like" and related to rancidity in cooked meat and other food systems (4, 34). IF had significantly higher amounts of oleic and MUFA than WF, which contained significantly higher amounts of linoleic and PUFA and, accordingly, the ratio between oleic-derived volatiles (octanal, nonanal, octan-1-ol) and linoleic-derived volatiles [hexanal, hex-2-enal, dec-(*E*)-2-enal] was

significantly higher in IF than in WF (Iberian, 3.28; white, 2.12; $p < 0.05$). The high content of oleic acid and its oxidation-derived aldehydes in meat products from Iberian pigs has been related to essential quality traits (1, 29). In addition, significantly higher amounts of Strecker aldehydes (2- and 3-methylbutanal, benzaldehyde) and alcohols (2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol) were detected in IF compared to those in WF, which could contribute also to define different aromatic profiles between types of frankfurters. Strecker volatiles have been described as quality indicators in Iberian dry-cured products in which they contribute desirable "almond-like", "toasted" aroma notes (7). Finally, IF contained also significantly higher amounts of certain aliphatic and aromatic hydrocarbons [heptane, 2-methylnonane, undecane, methylbenzene, 1,3-dimethylbenzene, 1-methyl-3-(1-methylethyl)benzene] and volatile terpenes (α -pinene, l-limonene, linalool). These compounds have been previously reported as volatile components of frankfurter-type sausages (3) and are likely to have derived from the direct deposition in animal tissues from grass, which would explain the significantly higher amounts in IF. Anyway, certain benzene derivative compounds could be contaminants derived from the workup procedure.

In general, the addition of rosemary essential oil had a significant effect on the generation of major volatile compounds, but this effect was different depending on the amount of essential oil and the type of frankfurter in which it was added. In fact, the interaction between "origin of frankfurter" and "rosemary" was significant for most volatiles (Table 2), suggesting that the effect of the addition of rosemary was influenced by the type of frankfurter. In agreement with previous research on several meats and meat products (13, 18) the addition of rosemary essential oil had an antioxidant effect on frankfurters from Iberian pigs because the generation of lipid-derived volatiles was inhibited as the amount of added essential oil increased. The addition of 150 mg/kg of essential oil significantly inhibited the generation of certain lipid-derived volatiles such as octanoic and nonanoic acids, pentan-2-ol, octan-1-ol, and pent-4-enal. Higher antioxidant effects were achieved with higher rosemary levels with the highest antioxidant effect being detected at 600 mg/kg. Compared to the control ones, frankfurters with 600 mg/kg of rosemary essential oil had significantly smaller amounts of octanoic and nonanoic acids, pentan-2-ol, oct-1-en-3-ol, octan-1-ol, hexanal, pent-4-enal, but-(*E*)-2-enal, heptanal, octanal, and decanal. The rosemary essential oil also inhibited the generation of Strecker volatiles and certain hydrocarbons in IF.

In contrast, the addition of rosemary essential oil in WF had a different effect that changed with the amount of essential oil added. The addition of 150 mg/kg showed, in general, an antioxidant effect, significantly decreasing the amount of certain lipid-derived volatiles such as pentanal, hex-2-enal, hexanal, dec-(*E*)-2-enal, 2,5-dihydrofuran, heptan-2-one, 1-phenylethanone, 1-phenylpropanone, and octan-2-one in WF. Rosemary essential oil added at 300 mg/kg had no effect on the generation of the major lipid-derived volatiles, whereas 600 mg/kg addition levels resulted in clear prooxidant effects, significantly increasing the production of a large variety of volatile compounds from lipid decomposition such as octanoic acid, hexan-1-ol, pentan-2-ol, oct-1-en-3-ol, octan-1-ol, but-(*E*)-2-enal, heptanal, dec-(*E*)-2-enal, dodecanal, 2,5-dihydrofuran, 1-phenylpropanone, and octan-2-one.

Although the antioxidant activity of plant phenolics is generally recognized (39), the prooxidant properties of these substances have also been described, being able to generate reactive oxygen species and damage lipids, proteins, and other

cellular components (19, 20). Results from the present work suggest that the activity of the rosemary essential oil was dependent on the compositional characteristics of the food matrix. In fact, the effect of plant phenolics has been considered to be influenced by the compositional characteristics of the food system and the presence of other active substances (20, 39). Food systems, and particularly comminuted meat products such as frankfurters, are very complex in the number and type of chemicals in the mixture, and a particular combination of these compounds might behave differently from the individual components. In this sense, Wong et al. (18) and Fang and Wada (40) reported possible interactions between phenolic compounds from sage and rosemary essential oils and vitamin E, resulting in different activities depending on the individual amounts of these substances in the food system. In the present work, significant differences ($p < 0.05$) were found between frankfurters from Iberian and white pigs regarding tocopherols contents (4.0 vs 1.4 $\mu\text{g/g}$ of frankfurter). Therefore, the presence of a certain amount of endogenous antioxidants (tocopherols) in the raw material and manufactured product might influence the activity of exogenous active extracts, leading to antioxidant or prooxidant effects. In addition, the different fatty acid compositions between frankfurters from Iberian and white pigs could have also been an influence. In accordance with Huang and Frankel (16), whether phenolic compounds act as antioxidants or prooxidants appears to be dependent on the lipid characteristics of the model system. These authors reported antioxidant activities of tea catechins in corn oil triglycerides, whereas in oil-in-water emulsions, these compounds were all prooxidants. Moreover, the prooxidant activity was stronger with higher concentrations, which is in agreement with the results from the present study. The different fatty acid compositions between frankfurters affect the physical state of the lipids that could have affected the dispersion and antioxidant activity of the rosemary essential oil, leading to different effects.

Finally, the activity of the rosemary essential oil could have been affected by the initial oxidation state of the frankfurter in which it was added. In systems with higher oxidative instability, the activity of plant phenolics could be reduced because phenolic compounds can be oxidized and the oxidation products could act as prooxidants promoting oxidative reactions (16). These would explain the prooxidant activity of the rosemary essential oil in frankfurters from white pigs, with higher oxidative instability than in those from Iberian pigs. Furthermore, the oxidation of phenolics in IF could have been inhibited by the presence of high levels of tocopherols with which plant phenolics interact, leading to regeneration and synergist effects (9, 18). The results obtained in the present work are in agreement with those obtained in a previous study in which sage and rosemary essential oils (1000 mg/kg) showed an antioxidant effect when added to liver pâtés from Iberian pigs and exhibited the opposite (prooxidant) effect in liver pâtés from white pigs (1). The differences between liver pâtés from Iberian and white pigs reported in that study in terms of fatty acid composition and tocopherol contents are consistent with those reported in the present study, which supports the hypothesis and mechanisms suggested.

Analysis of Volatiles from Added Rosemary Essential Oil.

The highest chromatographic areas detected were due to volatile terpenes derived from the addition of the rosemary essential oil (Table 3). SPME allowed the isolation and analysis of 33 volatile terpenes including monoterpene hydrocarbons such as α -pinene, camphene, β -myrcene, and l-limonene, sesquiterpene hydrocarbons such as α -cubene and (*E*)-caryophyllene, and

oxygen derivative terpenes such as alcohols (linalool, endoborneol, terpinene-4-ol), esters (linalyl acetate, linalyl propionate), carbonyls (camphor), and ethers (1,8-cineole). Most of these compounds have been previously reported as volatile components of sage and rosemary essential oils and isolated in the HS of several spiced foods (1, 3). In fact, Chevance and Farmer (3) reported that the most abundant headspace compounds from frankfurters were terpenes originated from spices, with smaller quantities of volatiles derived from meat, fat, and other ingredients. The rosemary essential oil also contributed large quantities of aromatic hydrocarbons and alcohols such as 1-methyl-4-(1-methylethyl)benzene, 1-methyl-4-(1-methylethenyl)cyclohexanol, and 1-methoxy-2-methylbenzene. The chromatographic areas of these compounds enlarged with increasing levels of the added rosemary essential oil.

As expected, no differences were detected between treated IF and WF within each level of added essential oil as long as the same formulation was used for all of them. Several of the volatile terpenes detected are recognized odorants and are commonly used in the food industry as flavor and fragrance ingredients (12). Volatile terpenes such as α -pinene, 1,8-cineole, and linalool have been related to "spices, pine needles", "medicinal, cough syrup" and "flowers, carnation" odors, respectively, and have been reported as contributors to the aroma of spiced cooked sausages (3). In the absence of olfactometry or sensory assessment of frankfurters, the contribution of these compounds to the overall aroma of frankfurters remains unknown, and, therefore, the attitude of consumers toward frankfurters with odor notes referred to such aromatic herbs would be a future work of interest. On the other hand, using deodorized extracts of these plants would be also an interesting option to achieve antioxidant effects in meat and fat products without including unexpected aroma components (41). The present results suggest that further research would be needed to establish the optimal level of added essential oil to achieve antioxidant effects and pleasant aromatic characteristics considering the individuality of the food system in terms of fatty acid composition and endogenous antioxidant content.

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