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Research Article

Effect of aromatic plants on the pork's, beef's, and sheep's fats quality

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Abstract
Foods rich in lipids, particularly meat and subcutaneous fat, are important macronutrient sources that can influence food quality and health. Lipids can unexpectedly oxidize due to several variables, including processing, storage conditions, and microbial deterioration. Antioxidants are one of the many methods employed to reduce lipid oxidation. Therefore, this study aimed to explore the effect of nine aromatic plants (oregano (*Origanum vulgare*), thymus (*Thymus vulgaris*), lemon balm (*Melissa officinalis*), nutmeg (*Myristica fragrans*), coriander seed (*Coriandrum sativum*), fennel seed (*Foeniculum vulgare*), rosemary (*Salvia rosmarinus*), basil (*Ocimum basilicum*), and mint (*Mentha spicata*)) on pork, beef, and sheep fats during a maturation and storage process lasting 210 days. The animals' fats lipolytic quality was monitored by analyzing the acidity index and degree, peroxidation and TBA index, and total fatty acid composition. The findings revealed that nutmeg, thymus, and rosemary protected against studied fats hydrolysis by showing minimum values of 27.7 mg, 32.5 mg, and 28.6 mg of KOH/g of fat, respectively. Despite hydrolysis, three plants, nutmeg, oregano, and coriander seed, had a substantial preventive effect against fat oxidation. Supplementation fats with these three aromatic plants had the lowest peroxidation and TBA indexes with values of 0.368 mg, 0.422 mg, and 0.806 mg of MDA/kg of fat, respectively. This was also represented in the percentage of polyunsaturated fatty acids. Adding aromatic plants had a significant effect on animals' fat quality. They reduced fat hydrolysis, protected fat against oxidation, and improved fat's total fatty acid composition, nutritional and health quality.

Keywords: Animals' fats, Aromatic plant, Fat sausage, Oxidative stability, Storage

INTRODUCTION

Like most Mediterranean countries, Morocco's diet is rich in fat and lipids, which, on average, represent 5% of the daily energy intake (Barich et al., 2020). Scientific evidence has shown that lipid intake's impact on human health is mainly associated with their content of saturated and unsaturated fatty acids (Munekata et al., 2022). In this field, animal fats still play a crucial role in both food industry and human health. Animal fats are widely considered an important energy source and contain essential fatty acids such as omega-3 and omega-6 and fat-soluble vitamins. Animal fats also enhance the texture and flavor of food and improve meat emulsions'

overall acceptability and stability (Khaldari and Ghiasi, 2022; Maleki et al., 2015). Due to these valuable contributions, animal fats have been used extensively in the food industry, especially in manufacturing cold cuts and processing meat products such as sausages. The evolution of food technologies and the diversification of the use of animal fats, the conservation of these fats remains a major issue in the industry. The transformation and storage processes of meat products can damage the fat composition and affect its stability (Li et al., 2017). Heat treatment and long shelf life of processed products can lead to the development of rancidity, which damages the flavor and odor of the products. Rancidity is caused by the degradation of fat and the peroxidation of free fatty acids, especially polyunsaturated ones (Zamuz et al., 2022). To overcome the peroxidation phenomenon, natural antioxidant agents are still the main option reported in many countries, and they are used in various agri-food processes. These antioxidants are isolated from aromatic plants, fruits, and vegetables, and have a high radical scavenging activity.

In this context, the present study was planned to evaluate the effect of nine aromatic plants from different regions of Morocco on the lipolytic phenomenon of pork, beef, and sheep fats to identify those that highlight the best protective effects and exhibit better quality for industrial purposes.

MATERIALS AND METHODS

Preparation of samples

Fresh beef and sheep fats were purchased from the local Tangier market, while pork fat was ordered from the Spanish market and were all stored at -20°C until used in manufacturing. Nine aromatic plants were used to evaluate their effects on animal fats: oregano (*Origanum vulgare*), thymus (*Thymus vulgaris*), lemon balm (*Melissa officinalis*), nutmeg (*Myristica fragrans*), coriander seed (*Coriandrum sativum*), fennel seed (*Foeniculum vulgare*), rosemary (*Salvia rosmarinus*), basil (*Ocimum basilicum*), and mint (*Mentha spicata*). All these aromatic plants were purchased from various local Moroccan markets (Tangier (35.76727, -5.79975), Beni Mellal (32.33699 -6.35649), Ouzoud (32.0152 -6.7189), El Kelâa des Sraghna (32.04216 -7.40499)), dried in the dark at room temperature, crushed and stored until use.

The pork backfat, abdominal beef, and sheep fats were stabilized at 4°C for 24 hours before manufacturing, then ground in a meat grinder (MG700, KNWOOD, China) with a 10 mm cutting plate. Each fat was distributed in eleven different batches. In 9 batches, fat was mixed with 1% oregano, 0.5% thymus, 2% lemon balm, 0.2% nutmeg, 9.7% coriander seed, 3.9% fennel seed, 0.8% rosemary, 1.1% basil, and 0.9% mint, respectively. In the 10th batch, fat remained intact (negative control), and in the 11th one, fat was mixed with 0.035% of ascorbic acid (positive control).

Afterwards, each sample was stuffed into a cow casing with a 35-40 mm diameter using the meat grinder (MG700, KNWOOD, China). The fat sausages were then subjected to a maturation process consisting of drying for 9 days at 6°C and 80% relative humidity, followed by 21 days at 12°C and 75% relative humidity, and finally stored at 4°C and 55-60% relative humidity for six months. Then, the dried fat sausages were placed in food-grade plastic bags with a size of 20 x 30 cm and frozen at -20°C until the analyses were carried out.

Determination of the acidity index and degree

Free acidity was determined according to the ISO R-937 procedure (ISO R-937, 1979). Acidity index is a colorimetric measurement of the total amount of mg of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fatty substances. Acidity degree corresponds to the amount of free fatty acids. It is expressed as a percentage of mass and often depends on the oleic acid quantity. The results of the acidity index and degree are expressed according to equations 1 and 2:

$$\text{Acidity index (mg of KOH / g of fat)} = \frac{V * 56.1 * 0.1}{P} \quad \text{Eq.1}$$

$$\text{Degree of acidity (\% of oleic Acid)} = \frac{V * 282 * 0.1}{10 * P} \quad \text{Eq.2}$$

V: volume of the potassium hydroxide solution (0.1 N).
P: weight of the sample.

Determination of the peroxidation index

The determination of peroxidation index was carried out according to the ISO, R-937 procedure (ISO R-937, 1979). The peroxidation index corresponds to the amount of oxygen chemically bound to fat in a peroxide form, particularly hydroperoxides (primary oxidation products). It is a determination method with an iodometric breakpoint. The results of the peroxidation index are expressed according to the equation 3:

$$\text{Peroxidation index (milliequivalents } O_2 \text{ / Kg of fat)} = \frac{V * 0.002 * 1000}{P} \quad \text{Eq. 3}$$

V: volume of sodium thiosulfate.
P: weight of the simple.

Determination of the TBA index

A thiobarbituric acid (TBA) test was used to determine the secondary oxidation products following the method proposed by IUPAC 2.531 (Paquot, 1982). This Spec-

trocolorimetric assay measures the malonaldehyde (MDA) amount, a bio-marker often used to estimate the final lipid peroxidation phases. The results were expressed as mg of MAD/kg of fat using a calibration range of 1,1,3,3-Tetramethoxypropane.

Determination of total fatty acid composition

Samples were methylated using the method of Shehata (Shehata et al., 1970) and then analyzed using a Varian CP-3800 gas chromatography system equipped with a 3800 Channel FID/T° 260°C flame ionization detector, a capillary column of 100 m in length and 0.25 mm in diameter, and a Varion PAL autosampler.

The column was operated at 140°C for 5 minutes, followed by a temperature program of 4°C/min until reaching 240°C, using helium as the carrier gas at a 1 ml/min flow rate.

Methylated fatty acids were identified by comparing their retention time with those of the methylated C4-C24 standard. The results of the fatty acid analysis were expressed as the percentage of the area occupied by each fatty acid methyl ester peak relative to the total peak area.

Statistical analysis

All statistical analyses were performed using Statgraphics centurion 19.1.1 software (Statgraphics Technologies Inc, The Plains, Virginia, USA). The significant difference between all samples and the effect of aromatic plants were determined using the analysis of variance test "ANOVA" with a significance level of 5% ($p < 0.05$).

RESULTS

Acidity index

Acidity indexes of the three fats supplemented with different aromatic plants and their respective negative and positive controls are reported in Fig. 1, and results clearly showed that fat hydrolysis was variable with the animal species.

For the pork fat, all samples supplemented with aromatic plants showed an increased acidity index compared to the control. For these samples, the acidity index ranged from 27.7 mg of KOH/g of fat obtained with nutmeg to 41.1 mg of KOH/g of fat obtained with coriander seed. Of interest, supplementation with oregano, thymus, coriander seed, rosemary and mint showed high acidity index as compared to the positive control ($p < 0.001$), with 39.4, 33.6, 41.1, 31.4 and 34.7 mg of KOH/g of fat, respectively (Fig. 1A).

For the beef, plain fat showed a higher acidity index (45 mg of KOH/g of fat) that was maintained in the presence of mint (45.9 mg of KOH/g of fat) but significantly decreased in the presence of the other aromatic plants, as well as the ascorbic acid used as positive control ($p < 0.001$) (Fig. 1B). For these samples, acidity index ranged from 32.5 mg of KOH/g of fat obtained with thymus to 40.0 mg of KOH/g of fat obtained with basil and lemon balm.

The plain sheep fat also showed a high acidity index reaching 43.0 mg of KOH/g of fat (Fig. 2C). The acidity index was increased when the fat was mixed with basil (47.3 mg of KOH/g of fat) and thymus (44.6 mg of KOH/g of fat) and decreased significantly when the fat was mixed with nutmeg (37.8 mg of KOH/g of fat), lemon balm (37.7 mg of KOH/g of fat), mint (31.8 mg of KOH/g of fat), and rosemary (28.6 mg of KOH/g of fat).

Degree of acidity

The acidity degree of the three fats supplemented with different aromatic plants, as well as their respective negative and positive controls, are reported in Fig. 2. The percentages of oleic acid released had the same distribution as the total amount of mg of potassium hydroxide detected (acidity index).

For the pork fat, all samples supplemented with aromatic plants released more substantial oleic acid than the control ($p < 0.001$). The total amount of oleic acid released ranged from 13.9% obtained with nutmeg to 20.7% obtained with coriander seed (Fig. 2A).

For the beef fat, the percentage of oleic acid released decreased significantly in the presence of aromatic plants except mint, ranging from 16.3% obtained with thymus to 20.1% obtained with lemon balm (Fig. 2B).

On the other hand, the addition of nutmeg, lemon balm, mint and rosemary decrease significantly the acidity degree of sheep fat with 19.0%, 18.9%, 16.0%, and 14.4% of oleic acid, respectively (Fig. 2C).

Peroxidation index

The peroxidation indexes of the three fats mixed with the nine aromatic plants and their corresponding positive and negative controls are reported in Fig. 3. The results revealed that the amount of hydroperoxides produced varied depending on the fat origin.

For pork fat, control showed a higher peroxidation index (77.8 milliequivalents O_2/kg of fat) maintained in basil (80.6 milliequivalents O_2/kg of fat). The peroxidation index was increased in the presence of lemon balm (126.7 milliequivalents O_2/kg of fat) and ascorbic acid (120.9 milliequivalents O_2/kg of fat) and decreased in the presence of other aromatic plants ($p < 0.001$). For these samples, peroxidation index ranged between 12.6 milliequivalents O_2/kg of fat reached with coriander seeds to 53.1 milliequivalents O_2/kg of fat reached with rosemary (Fig. 3A).

For beef fat, supplementation with thymus did not affect the peroxidation index maintained as plain fat. For the other aromatic plants, supplemented showed an increased peroxidation index with values ranging from 7.9 milliequivalents (nutmeg) to 13.9 milliequivalents

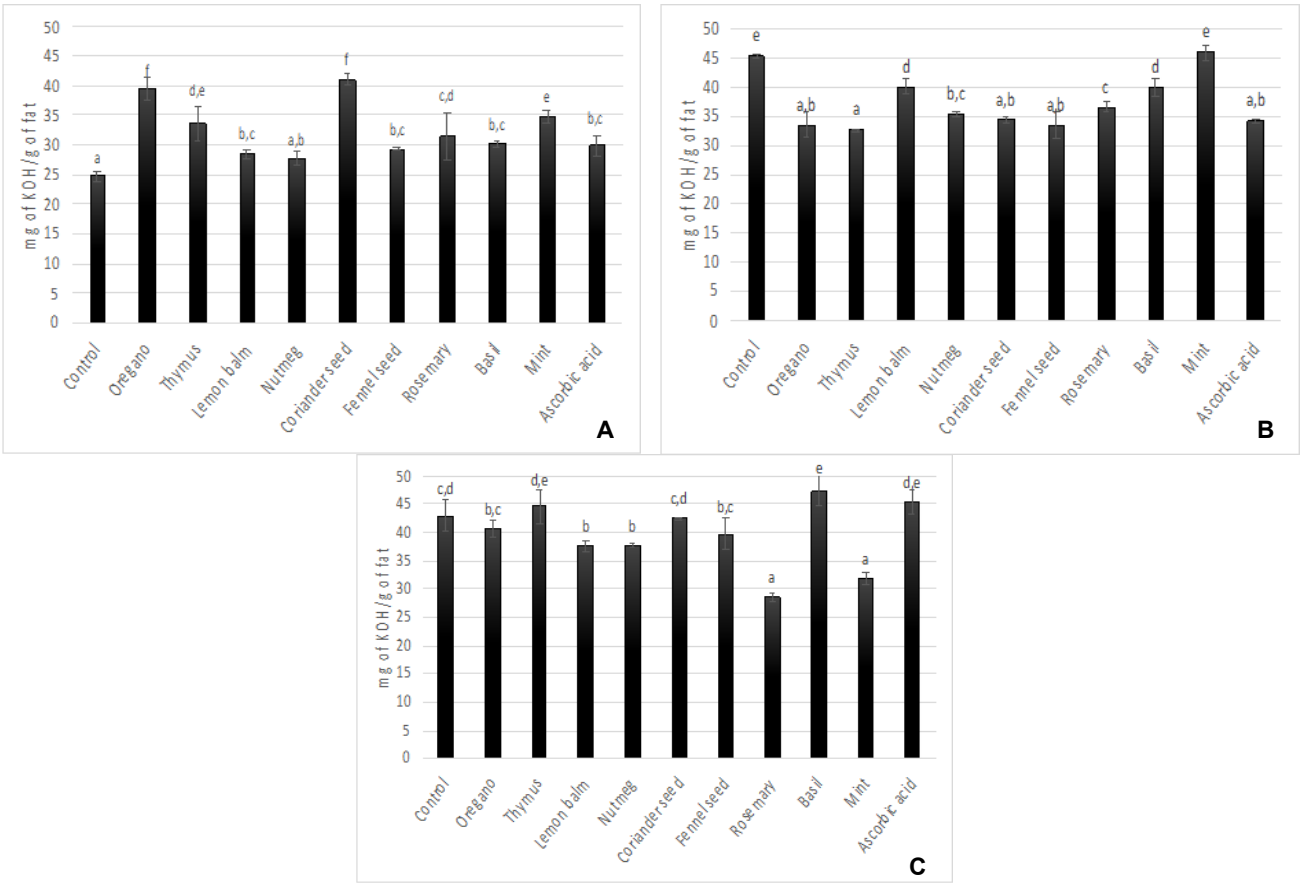

Fig. 1. *Influence of aromatic plants on the acidity index values of pork (A), beef (B) and sheep (C) fats*

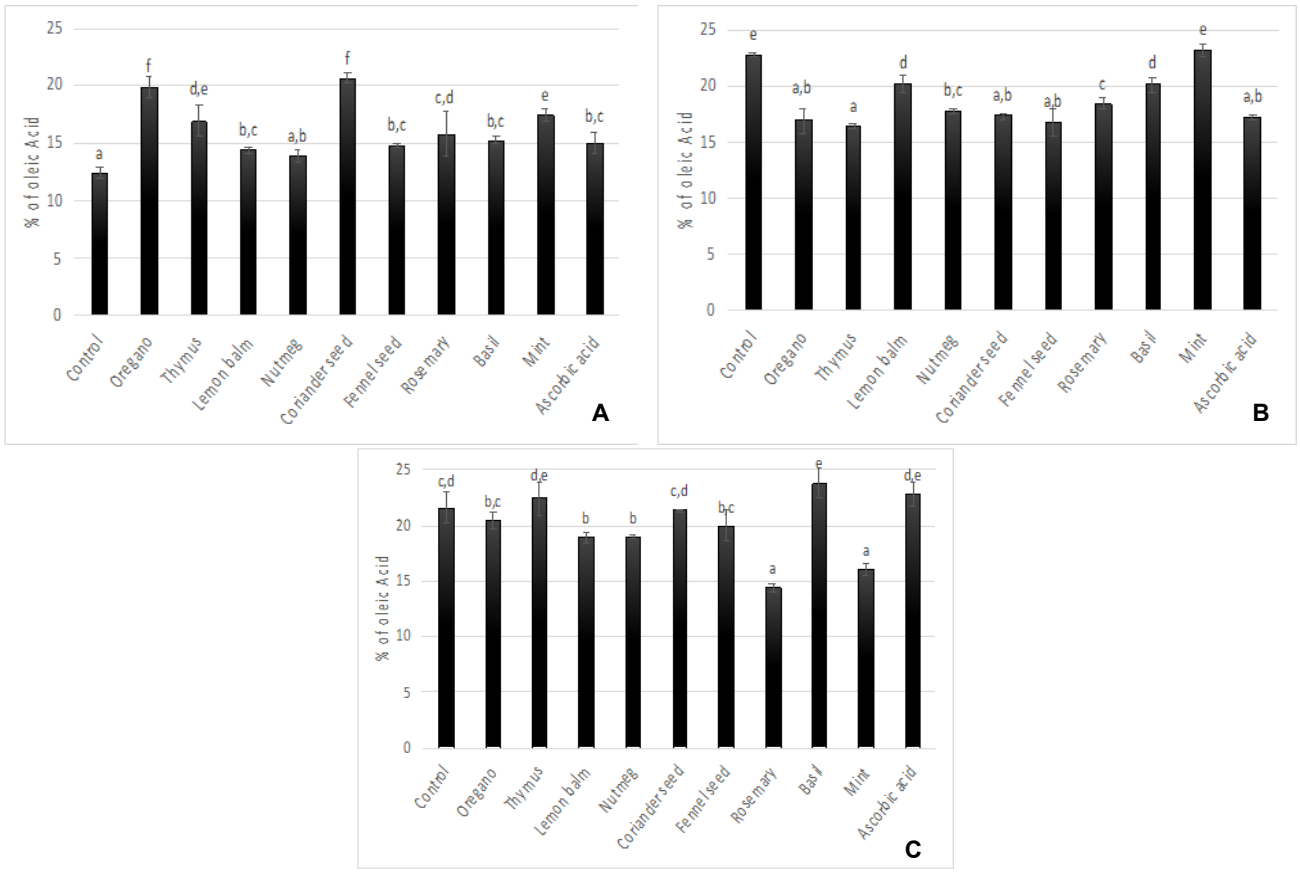

Fig. 2. *Influence of aromatic plants on the degree of acidity values of pork (A), beef (B) and sheep (C) fats*

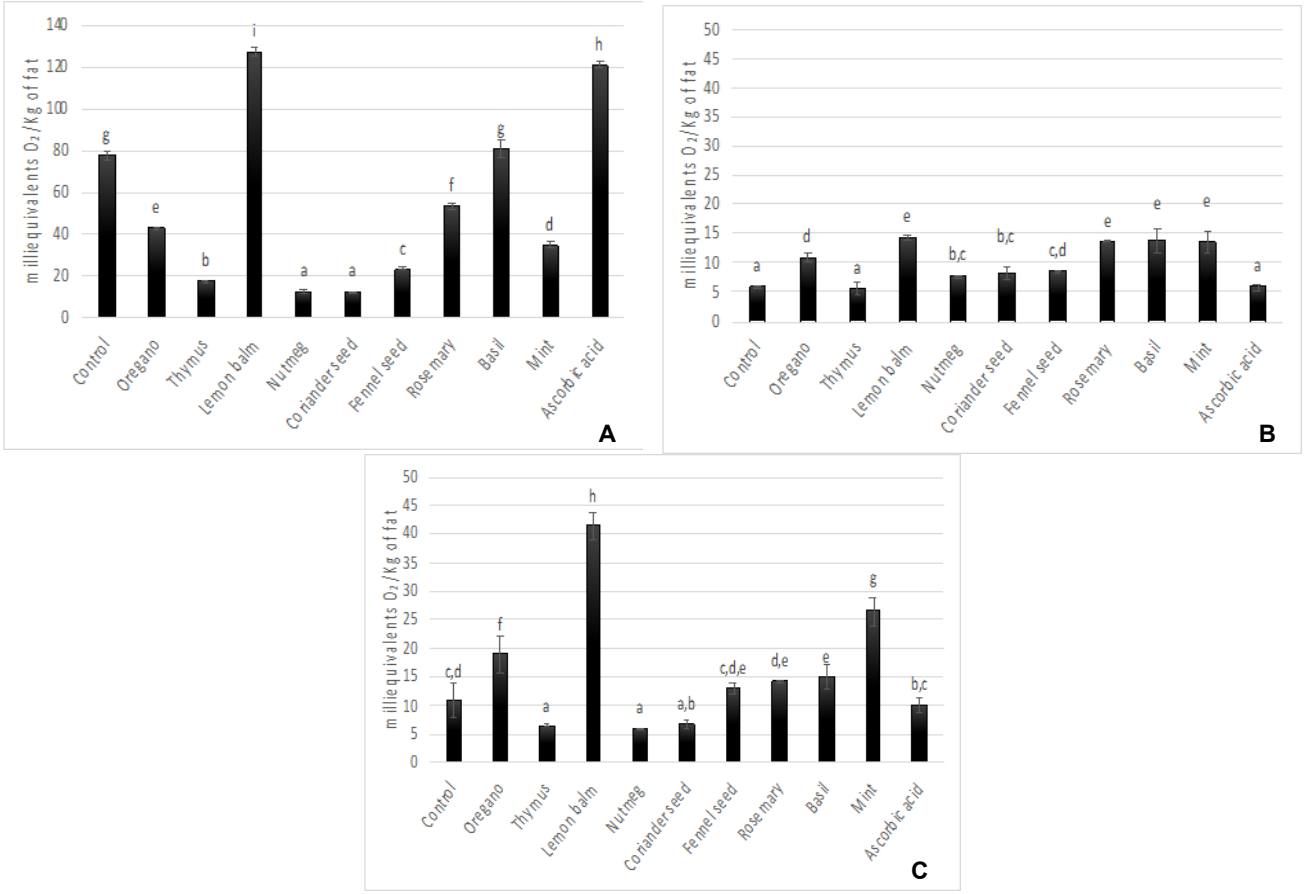

Fig. 3. *Effect of aromatic plants on peroxidation index values of pork (A), beef (B) and sheep (C) fats*

O_2/kg of fat (basil) (Fig. 3B).

For sheep fat, significant increase of the peroxidation index was obtained with oregano, basil and mint with 19.1 milliequivalents, 41.4 milliequivalents, 14.9 milliequivalents, and 26.5 milliequivalents O_2/kg of fat, respectively, and the best increase was obtained with lemon balm that exhibited a peroxidation index of 41.4 milliequivalents O_2/kg of fat (Fig. 3C). However, no significant effect was obtained with rosemary and a significant decrease of the peroxidation index was obtained in the presence of thymus (6.5 milliequivalents O_2/kg of fat), nutmeg (6.0 milliequivalents O_2/kg of fat), and coriander seeds (6.8 milliequivalents O_2/kg of fat).

TBA index

The TBA indexes of the three fats mixed with the nine aromatic plants and their corresponding positive and negative controls are reported in Fig. 4. The results revealed that the amount of MDA produced varied depending on the fat origin.

For pork fat, plain fat showed a higher TBA index (1.458 mg of MDA/kg of fat) maintained in fennel seeds (1.399 mg of MDA/kg of fat) and significantly decreased in the presence of other aromatic plants. For these samples, TBA index ranged between 0.806 mg of MDA/kg of fat obtained with thymus and 1.250 mg of MDA/kg of fat obtained with oregano (Fig. 4A).

For beef fat, only oregano (0.422 mg of MDA/kg of fat) and rosemary (0.410 mg of MDA/kg of fat) decreased the TBA index compared to the control. Nutmeg (0.518 mg of MDA/kg of fat) and basil (0.521 mg of MDA/kg of fat) maintained the control TBA index. The other aromatic plants showed an increased TBA index, ranging from 0.543 mg of MDA/kg of fat (coriander seeds) to 1.537 mg of MDA/kg of fat (fennel seeds). Substantial increase as compared to the positive control was also obtained with thymus, lemon balm and mint that have exhibited TBA indexes of 1.018 mg, 0.641 mg, 1.537 mg, and 0.626 mg of MDA/kg of fat, respectively (Fig. 4B).

All samples mixed with aromatic plants for sheep fat showed an increased TBA index compared to the control. For these samples, TBA index ranged from 0.351 mg of MDA/kg of fat obtained with oregano to 0.912 mg of MDA/kg of fat obtained with fennel seeds. Supplementation with fennel seeds and lemon balm showed a higher TBA index than the positive control, with 0.912 mg and 0.835 mg of MDA/kg of fat, respectively (Fig. 4C).

Total fatty acid composition

The proximate fatty acid composition of the three fats supplemented with different aromatic plants and their respective negative and positive controls are reported in Table 1.

The fatty acid composition of the three fats studied represents the same major fatty acids: palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) but with a different distribution for each fat. For pork fat, oleic acid prevails, representing 47% of total fatty acids, followed by palmitic acid with 24% and stearic acid with 12%. Beef fat shows the same distribution, with oleic acid representing 36% of the general composition, palmitic acid with 28%, and stearic acid with 23%. However, stearic acid comes first for sheep fat, occupying 32% of the composition, followed by oleic acid with 30%, and palmitic acid with 23%.

The aromatic plants' protective effect of fatty acid composition was variable according to the fat origin. For pork fat, all samples supplemented with aromatic plants retained more unsaturated fatty acid (UNFA) with a value between 61.70% (fennel seeds) and 63.47% (nutmeg). Thus, the oleic acid variation rate (UNFA/SAFA) and the nutritional quality coefficient (PUFA/SAFA) significantly increase compared to the control. Except basil, which had less UNFA (60.02%), decreased the oleic acid variation rates by 0.04 and maintained the nutritional quality coefficient as a control. In terms of human health effect coefficients ((C18+C18:1)/C16 and UNFA+C18), only oregano, nutmeg, and coriander seeds showed significantly increased coefficients, with 2.51 and 73.55%, 2.52 and 73.62%, and 2.51 and 73.62%, respectively. However, lemon balm, fennel seeds, rosemary, and mint maintain the control human health effect coefficients (2.46 and 72.97%) and thymus and basil show a decreased coefficient.

For beef fat, the percentage of UNFA and the oleic acid variation rate was maintained in the presence of coriander seeds, rosemary, and mint and more retained in the presence of the other aromatic plants, with a value between 43.26% and 0.76 respectively obtained with thymus and 45.19% and 0.82 obtained with lemon balm. All samples mixed with aromatic plants showed increased human health effect coefficients. For these samples, coefficients ranged from 65.42% and 2.06 to 67.88% and 2.34. The nutritional quality coefficient increased only when fat was mixed with coriander seeds (0.06) and maintained in the presence of the other aromatic plants.

For sheep fat, the percentage of UNFA and the oleic acid variation rate was significantly increased in the presence of oregano (37.65% and 0.60 respectively), thymus (37.89% and 0.61 respectively), and fennel seeds (38.40% and 0.62 respectively), maintained in the presence of lemon balm, coriander seeds, and rosemary, and decreased in the presence of nutmeg (34.95% and 0.54 respectively), basil (35.59% and 0.55 respectively), and mint (35.69% and 0.56 respectively). The mix with fennel seeds significantly increased the

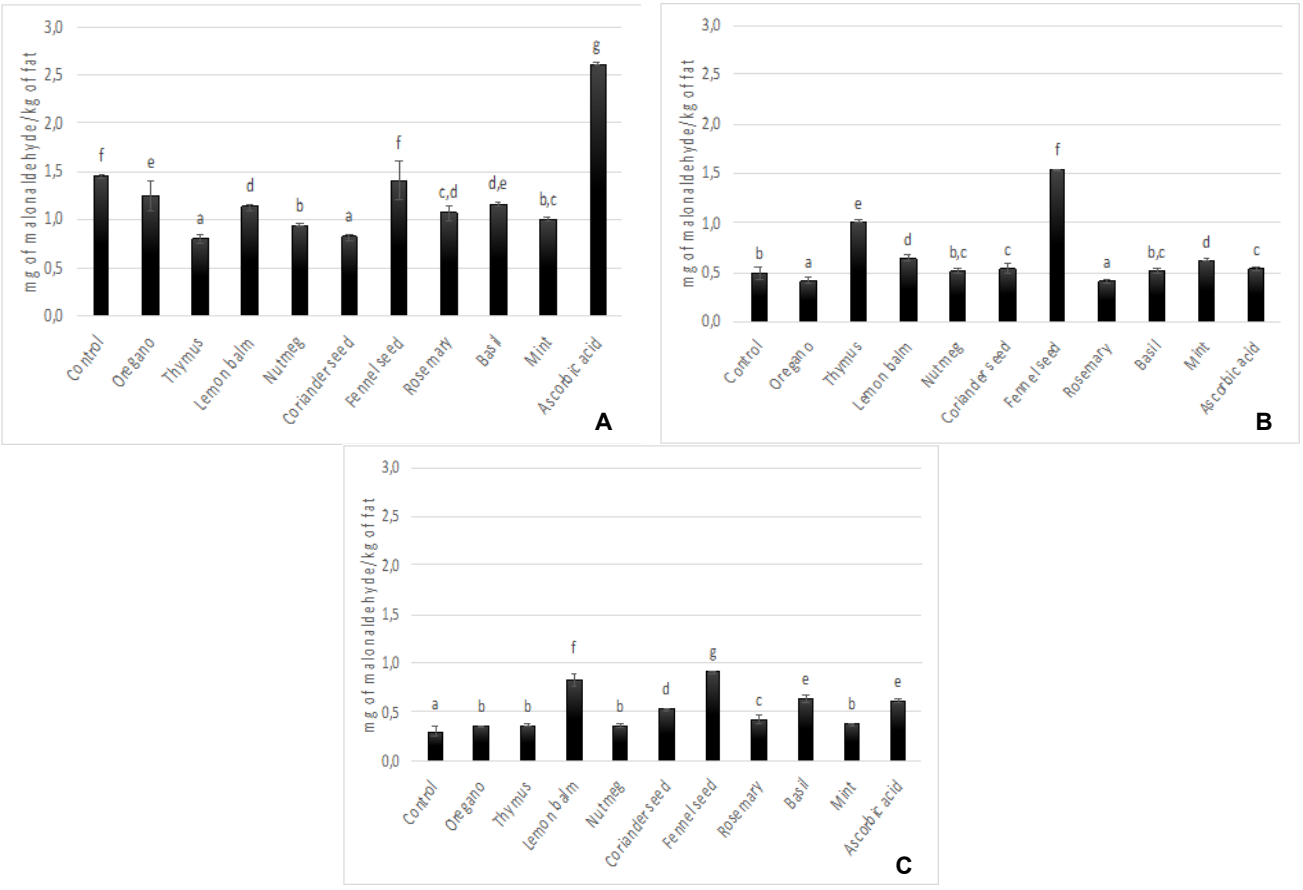

Fig. 4. *Effect of aromatic plants on TBA values of pork (A), beef (B) and sheep (C) fats*

Table 1. Fatty acid composition of animals' fats with aromatic plants

Animal fat		Control	Oregano	Thymus	Lemon balm	Nutmeg	Coriander seed	Fennel seed	Rosemary	Basil	Mint	Ascorbic acid	SD
Pork fat	C16:0	$24.12^{b.c}$	23.39^{a}	25.07^{d}	24.06^{b}	23.28^{a}	23.46^{a}	24.06^{b}	24.06^{b}	25.68^{e}	24.41^{c}	25.54^{e}	0.20
	C18:0	12.05^{h}	10.61^{c}	9.25^{a}	$10.63^{c.d}$	10.15^{b}	$10.70^{c.d}$	11.29^{f}	$10.75^{d.e}$	10.86^{e}	$10.65^{c.d}$	11.57^{g}	0.09
	C18:1	$47.23^{c.d}$	48.08^{f}	$47.48^{d.e}$	$47.75^{e.f}$	48.54^{g}	$48.14^{f.g}$	$46.92^{b.c}$	$47.09^{b.c.d}$	45.84^{a}	$46.89^{b.c}$	46.72^{b}	0.30
	Σ SAFA	39.08^{f}	37.06^{b}	$37.47^{b.c}$	$37.84^{c.d}$	36.53^{a}	37.08^{b}	38.30^{e}	$37.87^{c.d.e}$	39.73^{g}	$38.00^{d.e}$	39.98^{g}	0.30
	Σ UNFA	60.92^{b}	62.94^{f}	$62.53^{e.f}$	$62.16^{d.e}$	63.47^{g}	62.92^{f}	61.70^{c}	$62.13^{c.d.e}$	60.27^{a}	$62.00^{c.d}$	60.02^{a}	0.30
	Σ MUFA	$50.64^{b.c.d}$	$51.63^{e.f.g}$	$51.04^{d.e}$	$51.35^{e.f}$	52.14^{g}	$51.67^{f.g}$	$50.33^{b.c}$	$50.71^{c.d}$	49.37^{a}	$50.30^{b.c}$	50.10^{b}	0.43
	Σ PUFA	10.28^{a}	11.31^{d}	$11.49^{d.e}$	10.81^{b}	$11.33^{d.e}$	$11.25^{c.d}$	$11.37^{d.e}$	$11.42^{d.e}$	$10.89^{b.c}$	11.70^{e}	9.92^{a}	0.29
	PUFA/SAFA	0.26^{b}	$0.31^{d.e}$	$0.31^{d.e}$	0.29^{c}	0.31^{e}	$0.30^{d.e}$	$0.30^{c.d}$	$0.30^{d.e}$	0.27^{b}	$0.31^{d.e}$	0.25^{a}	0.01
	(C18+C18:1)/C16	2.46^{e}	2.51^{f}	2.26^{b}	$2.43^{d.e}$	2.52^{f}	2.51^{f}	$2.42^{d.e}$	2.40^{d}	2.21^{a}	2.36^{c}	2.28^{b}	0.03
	UNFA/SAFA	1.56^{b}	1.70^{f}	1.67^{e}	1.64^{d}	1.74^{g}	$1.70^{e.f}$	1.61^{c}	1.64^{d}	1.52^{a}	$1.63^{c.d}$	1.50^{a}	0.02
	UNFA+C18	72.97^{c}	73.55^{d}	71.79^{b}	72.78^{c}	73.62^{d}	73.62^{d}	72.99^{c}	72.88^{c}	71.13^{a}	72.65^{c}	71.59^{b}	0.25
Beef fat	C16:0	28.52^{d}	28.05^{c}	$28.43^{c.d}$	$28.27^{c.d}$	27.40^{b}	27.28^{b}	27.32^{b}	26.80^{a}	26.66^{a}	27.33^{b}	27.45^{b}	0.34
	C18:0	$23.07^{b.c.d}$	$22.64^{b.c}$	22.16^{b}	20.42^{a}	$22.66^{b.c}$	$24.18^{d.e}$	$23.66^{c.d}$	25.82^{f}	$22.73^{b.c}$	$25.38^{e.f}$	$24.25^{d.e}$	0.70
	C18:1	35.50^{a}	$37.06^{c.d.e}$	$36.45^{b.c}$	38.78^{g}	$37.82^{e.f}$	$36.70^{b.c.d}$	$37.35^{d.e}$	$36.93^{c.d}$	$38.33^{f.g}$	$36.05^{a.b}$	$36.79^{b.c.d}$	0.55
	Σ SAFA	$58.16^{f.g}$	$56.64^{c.d}$	$56.74^{c.d.e}$	54.81^{a}	$56.26^{b.c}$	$57.20^{c.d.e.f}$	$56.58^{c.d}$	$57.94^{e.f.g}$	$55.23^{a.b}$	58.65^{g}	$57.66^{d.e.f.g}$	0.85
	Σ UNFA	$41.84^{a.b}$	$43.36^{d.e}$	$43.26^{c.d.e}$	45.19^{g}	$43.74^{e.f}$	$42.80^{b.c.d.e}$	$43.42^{d.e}$	$42.06^{a.b.c}$	$44.77^{f.g}$	41.35^{a}	$42.34^{a.b.c.d}$	0.85
	Σ MUFA	$39.10^{a.b}$	$40.41^{c.d}$	$40.18^{b.c.d}$	42.35^{f}	$40.81^{d.e}$	$39.55^{a.b.c}$	$40.34^{c.d}$	$39.52^{a.b.c}$	$41.82^{e.f}$	38.87^{a}	$39.69^{a.b.c}$	0.74
	Σ PUFA	$2.73^{a.b.c}$	$2.95^{c.d}$	$3.08^{d.e}$	$2.84^{b.c.d}$	$2.93^{b.c.d}$	3.26^{e}	$3.08^{d.e}$	2.54^{a}	$2.96^{c.d}$	2.48^{a}	$2.66^{a.b}$	0.16
	PUFA/SAFA	$0.05^{a.b}$	0.05^{b}	$0.05^{b.c}$	0.05^{b}	0.05^{b}	0.06^{c}	$0.05^{b.c}$	0.04^{a}	0.05^{b}	0.04^{a}	0.05^{b}	0.00
	(C18+C18:1)/C16	2.05^{a}	2.13^{b}	2.06^{a}	$2.09^{a.b}$	2.21^{c}	2.23^{c}	2.23^{c}	2.34^{e}	2.29^{d}	$2.25^{c.d}$	2.22^{c}	0.04
	UNFA/SAFA	$0.72^{a.b}$	$0.77^{c.d.e}$	$0.76^{c.d.e}$	0.82^{g}	$0.78^{e.f}$	$0.75^{b.c.d.e}$	$0.77^{d.e}$	$0.73^{a.b.c}$	$0.81^{f.g}$	0.71^{a}	$0.73^{a.b.c.d}$	0.03
	UNFA+C18	64.91^{a}	$66.00^{c.d}$	65.42^{b}	$65.61^{b.c}$	$66.40^{d.e}$	$66.99^{f.g}$	$67.08^{g.h}$	67.88^{i}	$67.50^{h.i}$	$66.73^{e.f.g}$	$66.60^{e.f}$	0.35
Sheep fat	C16:0	$23.54^{a.b}$	$23.90^{b.c}$	23.35^{a}	24.43^{d}	24.99^{e}	$23.90^{b.c}$	24.48^{d}	$23.90^{b.c}$	25.51^{f}	$24.08^{c.d}$	25.10^{e}	0.30
	C18:0	31.42^{g}	29.32^{c}	30.54^{e}	29.08^{b}	30.53^{e}	30.88^{f}	27.23^{a}	31.08^{f}	28.87^{b}	31.70^{h}	30.30^{d}	0.15
	C18:1	30.55^{c}	$31.05^{c.d}$	31.53^{d}	30.58^{c}	$29.01^{a.b}$	30.42^{c}	31.31^{d}	30.56^{c}	$29.15^{a.b}$	29.55^{b}	28.63^{a}	0.48
	Σ SAFA	63.22^{d}	$62.35^{b.c}$	$62.11^{a.b}$	$62.91^{c.d}$	65.05^{e}	63.32^{d}	61.60^{a}	63.47^{d}	64.41^{e}	64.31^{e}	64.97^{e}	0.58
	Σ UNFA	36.78^{b}	$37.65^{c.d}$	$37.89^{d.e}$	$37.09^{b.c}$	34.95^{a}	36.68^{b}	38.40^{e}	36.53^{b}	35.59^{a}	35.69^{a}	35.03^{a}	0.58
	Σ MUFA	33.62^{c}	$34.32^{d.e}$	34.69^{e}	$33.75^{c.d}$	$32.02^{a.b}$	33.61^{c}	34.53^{e}	33.45^{c}	$32.21^{a.b}$	32.44^{b}	31.69^{a}	0.42
	Σ PUFA	$3.16^{a.b.c}$	$3.33^{b.c}$	$3.19^{a.b.c}$	$3.35^{b.c}$	2.93^{a}	$3.07^{a.b}$	3.87^{d}	$3.09^{a.b.c}$	3.38^{c}	$3.25^{b.c}$	$3.34^{b.c}$	0.21
	PUFA/SAFA	$0.05^{a.b}$	$0.05^{a.b}$	$0.05^{a.b}$	$0.05^{a.b}$	0.05^{a}	0.05^{a}	0.06^{c}	$0.05^{a.b}$	0.05^{b}	$0.05^{a.b}$	0.05^{b}	0.00
	(C18+C18:1)/C16	$2.63^{e.f}$	2.53^{d}	2.66^{f}	2.44^{c}	$2.38^{b.c}$	2.56^{d}	$2.39^{b.c}$	$2.58^{d.e}$	2.28^{a}	2.54^{d}	2.35^{b}	0.04
	UNFA/SAFA	0.58^{c}	$0.60^{d.e}$	$0.61^{e.f}$	$0.59^{c.d}$	0.54^{a}	0.58^{c}	0.62^{f}	0.58^{c}	$0.55^{a.b}$	0.56^{b}	$0.54^{a.b}$	0.01
	UNFA+C18	$68.20^{f.g}$	66.97^{d}	68.42^{g}	66.17^{c}	65.48^{b}	$67.57^{d.e}$	$65.63^{b.c}$	$67.62^{e.f}$	64.47^{a}	$67.39^{d.e}$	65.33^{b}	0.46

$^{a.b.c}$: The values in the same row that have different letters mean they are significantly different ;SD: standard deviation, ND: not determined ;SAFA: saturated fatty acid, UNFA: unsaturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

nutritional quality coefficient, unlike the other aromatic plants, which maintained the control nutritional quality coefficient. The human health effect coefficients were maintained in the presence of thymus (68.42% and 2.66) and significantly decreased in the presence of the other aromatic plants.

DISCUSSION

Supplemented fat with aromatic plants in the formulation of dried fat sausages could enhance the product's nutritional value by increasing the antioxidant capacity and the overall content of unsaturated and polyunsaturated fatty acids. This innovative approach will allow the production of original products that will satisfy consumers' requirements by offering acceptable, functional and healthier products.

During the manufacturing process, it was noticed that all three fats underwent a strong hydrolysis ($p < 0.001$), which was reflected by an increase in the acidity index and the percentage of oleic acid released. Moreover, a significant difference was obtained across samples supplemented with aromatic plants, such as pork fat, where all aromatic plants exceeded the control values ($p < 0.05$) (Fig. 1 (A)). These results clearly showed that studied aromatic plants exert a promoting effect of hydrolysis by releasing more substantial oleic acid, as compared to the pork fat control ($p < 0.001$), which ranged from more than 1.5% obtained with nutmeg to 8.2% obtained with coriander seeds (Fig. 2 (A)). On the other hand, aromatic plants have either ineffective or protective effects against hydrolysis for beef and sheep fat. The acidity index decreased significantly when the beef fat was mixed with all aromatic plants, especially with thymus (32.5 mg KOH/g fat) (Fig. 1 (B)) and when the sheep fat was mixed with rosemary (28.6 mg KOH/g fat), mint (31.8 mg of KOH/g of fat), lemon balm (37.7 mg of KOH/g of fat), and nutmeg (37.8 mg of KOH/g of fat) ($p < 0.05$) (Fig. 1 (C)). These results are in agreement with those reported by Zeng *et al. (2017)*, who noted that the supplementation with natural antioxidants, such as rosemary, reduces the acidity index values of Cantonese sausage, highlighting the useful role of natural antioxidants in delaying the oxidation of fats. This may also be due to the antibacterial effect that some aromatic plants, widely reported and discussed (Bagheri et al., 2020; Ghazanfari et al., 2020), and showing the crucial role of bacterial hydrolysis in generating digestive enzymes primarily involved in dietary fat hydrolysis (Li et al., 2017; Mejri et al., 2017). Accordingly, the antibacterial effect of these aromatic plants will decrease bacterial levels, consequently decreasing the amount of digestive enzyme and thus partially inhibiting fat hydrolysis.

Since the hydrolysis phenomenon is never completely inhibited, oxidation will take place due to the presence of unsaturated fatty acids. Once in contact with atmospheric oxygen, oxidative rancidity is triggered. Of note, light and temperature are widely considered as accelerating factors but are not necessary and sufficient elements to trigger oxidation phenomena (Judde, 2004).The oxidative alterations resulting from this phenomenon were studied by examining the primary (peroxidation index) and secondary (TBA index) oxidation products. Accordingly, the rate of oxidative changes occurring in fat was determined by various factors, including the composition of fatty acids, the presence of pro-oxidants and antioxidants, and the storage conditions (Bilska et al., 2019; Zamuz et al., 2022; Zeng et al., 2017). In present study, the supplementation with thymus, nutmeg, and coriander seeds has shown a significant reducing effect ($p < 0.05$) towards the primary oxidation products generation in the three fats studied, as compared to the negative control, with 5.6 milliequivalents (Fig. 3 (B)), 6.0 milliequivalents (Fig. 3 (C)), and 12.6 milliequivalents O_2/kg of fat (Fig. 3 (A)), respectively which is lower than the level specified by the Codex Alimentarius Commission (15 milliequivalents O_2/kg of fat) (FAO, 2023). These results are in agreement with previously reported data confirming that the use of natural antioxidants from aromatic plants can slow fat oxidation and could have a similar effect as obtained with synthetic antioxidants (Bianchin et al., 2017, 2020; Bilska et al., 2019; Gahruie et al., 2017). However, supplemented pork fat with oregano, lemon balm, rosemary, basil, and mint (Fig. 3 (A)) and supplemented sheep fat with lemon balm (Fig. 3 (C)) exceeded the limits "30 milliequivalents O_2/kg" to be considered safe for human consumption (Sbihi et al., 2013).

By evaluating the secondary oxidation products, we observed that thymus mixed to beef fat and nutmeg mixed to sheep fat does not exhibit a slowdown effect on the production of MDA. Conversely, oregano and rosemary inhibited beef fat by reducing 0.070 mg and 0.082 mg of MDA/kg of fat, respectively (Fig. 4 (B)). For sheep fat, no aromatic plants have been found to slow down MDA production (Fig. 4 (C)). This change can be explained by the non-stability of the primary oxidation products and their rapid transformation into secondary products (MDA), meaning that the low peroxidation index values first detected were due only to their conversion to secondary products (Li et al., 2017). Thus, oregano and rosemary are aromatic plants that provide a protective effect against beef fat oxidation. Nevertheless, all supplemented animals' fats with aromatic plants showed satisfactory results without exceeding the 2 mg of malonaldehyde/kg of fat limit (Mejri et al., 2017) (Fig. 4).

The supplementation with aromatic plants in this study significantly influenced the saturated and unsaturated fatty acid composition ($p < 0.05$), especially the polyun-

saturated fatty acids of the three fats studied, because they are the most sensitive to lipid peroxidation (Kang et al., 2005). The differences in the percentages of polyunsaturated fatty acids observed compared to the negative controls, which varied between 0.34% and 1.41%, were due to aromatic plants' protective effect against oxidation (Table 1). This is why even the three fats underwent hydrolysis; some plants have been able to differentiate significantly by keeping a higher percentage of unsaturated fatty acids than controls, thereby proving their power to slow down oxidative stress. The results of the ratios of polyunsaturated fatty acids are consistent with the peroxidation and TBA index values revealed; for example, it was found that the thymus, which revealed the minimum quantity of secondary volatile products of pork fat, has one of the highest percentages of unsaturated and polyunsaturated fatty acids.

The variation in percentages of unsaturated fatty acids influence significantly ($p < 0.05$) the other coefficients (variation of oleic acid (UNFA/SAFA), effect on human health coefficients ((C18+C18:1)/C16 and UNFA+C18) and nutritional quality rate (PUFA/SAFA)) (Table 1). More aromatic plants preserve a maximum of unoxidized unsaturated fatty acids. Higher values of these coefficients are observed, which improve the fat quality and reduce its harmful effect on human health. Given that, several studies have shown that a decrease in the percentage of saturated fatty acids and an increase in the percentage of unsaturated fatty acids will generate a low risk of cardiovascular diseases developing (Hoenselaar, 2012; Mestre Prates and Branquinho Bessa, 2008). Moreover, increasing the level of unsaturated fatty acids in the diet can reduce cholesterol levels. This effect is generally linked to the decrease in saturated fatty acids in the diet. According to the recommendations of the Federal Office for Public Health of Switzerland (Federal Department of Home Affairs, 2007), it was noted that palmitic acid (C16:0) causes an increase in cholesterol levels, and oleic acid (C18:1) plays the opposite effect by lowering blood cholesterol levels, while stearic acid (C18:0) has no effect on cholesterol status. Adding aromatic plants to pork, beef, and sheep fats improved their lipolytic quality, providing better quality than that control fat by reducing the oxidation level and limiting the formation of secondary metabolites to be safe and beneficial for human consumption.

The present study is very informative and clearly shows the interest in mixing animal fats with aromatic plants to preserve lipids for nutritional benefit. However, the study's main limitation is the limited number of tested aromatic plants. In fact, several aromatic plants are commonly used in Moroccan culinary preparations and widely reported for their medicinal properties and as sources of many bioactive compounds. Moreover, it would be interesting to evaluate the dose-dependent effect of each plant and the combined effect of two or more aromatic plants, as Moroccan recipes generally use several complementary ingredients and an assortment of aromatic plants.

Conclusion

Supplementation with aromatic plants significantly influenced the lipolytic characteristic of studied animals' fats by reducing fat hydrolysis, preserving lipids from oxidation and enhancing the fatty acid profile. Above all, it is recommended that pork fat should be supplemented with nutmeg and coriander seeds, beef fat should be supplemented with oregano and thymus, and sheep fat should be supplemented by thymus.

Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Bagheri, L., Khodaei, N., Salmieri, S., Karboune, S. & Lacroix, M. (2020). Correlation between chemical composition and antimicrobial properties of essential oils against most common food pathogens and spoilers: In-vitro efficacy and predictive modelling. *Microbial Pathogenesis*, *147*. https://doi.org/10.1016/j.micpath.2020.104212
2. Barich, F., Laamiri, F. Z., Mehdad, S., Benaich, S., Rami, A., Idrissi, M., … & Aguenaou, H. (2020). Energy and macronutrients intakes among childbearing age women living in the urban area of Morocco: A Cross-sectional study. *Journal of Nutrition and Metabolism*, *2020*, 1–10. https://doi.org/10.1155/2020/2685809
3. Bianchin, M., Pereira, D., Almeida, J. de F., de Moura, C., Pinheiro, R. S., Heldt, L. F. S., … & Carpes, S. T. (2020). Antioxidant properties of pyophilized rosemary and sage extracts and its effect to prevent lipid oxidation in poultry pate. *Molecules*, *25*(21). https://doi.org/10.3390/MOLECULES25215160
4. Bianchin, M., Pereira, D., dos Reis, A. S., Almeida, J. de F., Silva, L. D. da, Moura, C. de & Carpes, S. T. (2017). Rosemary essential oil and lyophilized extract as natural antioxidant source to prevent lipid oxidation in pork sausage. *Advance Journal of Food Science and Technology*, *13*(5). https://doi.org/10.19026/ajfst.13.5070
5. Bilska, A., Kobus-Cisowska, J., Kmiecik, D., Danyluk, B., Kowalski, R., Szymanowska, D., … & Szczepaniak, O. (2019). Cholinesterase inhibitory activity, antioxidative potential and microbial stability of innovative liver pâté fortified with rosemary extract (Rosmarinus officinalis).

Electronic Journal of Biotechnology, *40*. https://doi.org/10.1016/j.ejbt.2019.03.007
6. FAO. (2023). *Standard for edible fats and oils not covered by individual standards*.
7. Federal Department of Home Affairs FDHA, Federal Office of Public Health OFSP & Management Unit Consumer Protection. (2007). Fat in our diet recommendations of the federal office of public health.
8. Gahruie, H. H., Hosseini, S. M. H., Taghavifard, M. H., Eskandari, M. H., Golmakani, M. T. & Shad, E. (2017). Lipid oxidation, color changes, and microbiological quality of frozen beef burgers incorporated with shirazi thyme, cinnamon, and rosemary extracts. *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/6350156
9. Ghazanfari, N., Mortazavi, S. A., Yazdi, F. T. & Mohammadi, M. (2020). Microwave-assisted hydrodistillation extraction of essential oil from coriander seeds and evaluation of their composition, antioxidant and antimicrobial activity. *Heliyon*, *6*(9). https://doi.org/10.1016/j.heliyon.2020.e0 4893
10. Hoenselaar, R. (2012). Saturated fat and cardiovascular disease: The discrepancy between the scientific literature and dietary advice. *Nutrition*. https://doi.org/10.1016/j.nut.2011.08.017
11. ISO R-937. (1979). *International standard ISO R-936, according to the official method of analysis for meat and meat products. Presidency of the government*.
12. Judde, A. (2004). Prevention of fatty acid oxidation in a cosmetic product: mechanisms, consequences, means of measurement, which antioxidants for which applications? *Oléagineux, Corps Gras, Lipides*, *11*(6). https://doi.org/10.1051/ocl.2004.0414
13. Kang, M. J., Shin, M. S., Park, J. N. & Lee, S. S. (2005). The effects of polyunsaturated:saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats. *British Journal of Nutrition*, *94*(4), 526–532. https://doi.org/10.1079/bjn20051523
14. Khaldari, M. & Ghiasi, H. (2022). Fatty acids composition and health indices in different fat and muscle locations of lambs from crossbreeding between Lori-Bakhtiari and Romanov sheep breeds. *Small Ruminant Research*, 106786. https://doi.org/10.1016/j.smallrumres.2022.106786
15. Li, Y., Li, Y. B. & Liu, C. J. (2017). Changes in lipid oxidation and fatty acids in Altay sheep fat during a long time of low temperature storage. *Journal of Oleo Science*, *66*(4), 321–327. https://doi.org/10.5650/jos.ess16139
16. Maleki, E., Kafilzadeh, F., Meng, G. Y., Rajion, M. A. & Ebrahimi, M. (2015). The effect of breed on fatty acid composition of subcutaneous adipose tissues in fat-tailed sheep under identical feeding conditions. *South African Journal of Animal Sciences*, *45*(1). https://doi.org/10.4314/sajas.v45i1.2
17. Mejri, L., Ziadi, A., El Adab, S., Boulares, M., Essid, I. & Hassouna, M. (2017). Effect of commercial starter cultures on physicochemical, microbiological and textural characteristics of a traditional dry fermented sausage reformulated with camel meat and hump fat. *Journal of Food Measurement and Characterization*, *11*(2), 758–767. https://doi.org/10.1007/s11694-016-9445-6
18. Mestre Prates, J. & Branquinho Bessa, R. (2008). Trans and n- 3 fatty Acids. In *Handbook of Muscle Foods Analysis*. https://doi.org/10.1201/9781420045307.ch23
19. Munekata, P. E. S., Domínguez, R., Pateiro, M., Echegaray, N. & Lorenzo, J. M. (2022). Animal source: Meat, subcutaneous fat, milk, and dairy products. In *Food Lipids* (pp. 19–50). Elsevier. https://doi.org/10.1016/B978-0-12-823371-9.00004-6
20. Paquot, C. (1982). Standard methods for the analysis of oils, fats and derivatives. *Pure and Applied Chemistry*, *54* (1). https://doi.org/10.1351/pac198254010233
21. Sbihi, H. M., Nehdi, I. A. & Al-Resayes, S. I. (2013). Characterization of Hachi (Camelus dromedarius) fat extracted from the hump. *Food Chemistry*, *139*(1–4), 649–54. https://doi.org/10.1016/j.foodchem.2013.01.118
22. Shehata, A. Y., deMan, J. M. & Alexander, J. C. (1970). A simple and rapid method for the preparation of methyl esters of fats in milligram amounts for gas chromatography. *Canadian Institute of Food Technology Journal*, *3* (3), 85–89. https://doi.org/10.1016/s0008-3860(70)74286-4
23. Zamuz, S., Bohrer, B. M., Campagnol, P. C. B., Domínguez, R., Pateiro, M., Santos, E. M. & Lorenzo, J. M. (2022). Lipid oxidation of animal fat. In *Food Lipids: Sources, Health Implications, and Future Trends* (pp. 89–103). Academic Press. https://doi.org/10.1016/B978-0-12-823371-9.00002-2
24. Zeng, X., Bai, W., Lu, C. & Dong, H. (2017). Effects of composite natural antioxidants on the fat oxidation, textural and sensory properties of cantonese sausages during storage. *Journal of Food Processing and Preservation*, *41* (2). https://doi.org/10.1111/jfpp.13010