Journal of
Food Safety

Journal of Food Safety ISSN 1745-4565

PROTEOLYSIS, LIPOLYSIS AND SENSORY CHARACTERISTICS OF A TUNISIAN DRY FERMENTED POULTRY MEAT SAUSAGE WITH OREGANO AND THYME ESSENTIAL OILS

SABRINE EL ADAB[1] and MNASSER HASSOUNA

Research Unity "Bio-Preservation and Valorization of Agricultural Products–UR 13 AGR02", High Food Industries School of Tunisia, 58 Avenue Alain Savary, Tunis El Khadra 1003, Tunisia

[1]Corresponding author.
TEL: +216-96-387-707;
FAX: +216-71-771-192;
EMAIL: eladabsabrina@gmail.com

Received for Publication December 14, 2014
Accepted for Publication June 15, 2015

doi: 10.1111/jfs.12209

ABSTRACT

The effects of the addition of oregano (0.25% v/v) and thyme (0.25% v/v) essential oils (EOs) on the microbiological, biochemical and sensory characteristics of a Tunisian dry fermented poultry meat sausage were investigated. The antimicrobial activity of oregano and thyme EOs improved the hygienic quality of dry fermented sausages by reducing the Enterobacteriaceae counts, total coliform counts and *Staphylococcus aureus* counts. Proteolysis was not significantly ($P > 0.05$) affected by the addition of EOs during ripening of sausages. In fact, the most abundant free amino acids detected in the final product were arginine, glycine, threonine, alanine, tyrosine, aspartic acid, glutamic acid and lysine. Regarding lipolysis and lipid oxidation, it can be deduced that the addition of oregano and thyme EOs decreased significantly the thiobarbituric acid values during the last two weeks of ripening, but lipolysis was not significantly affected. Finally, the addition of EOs did not significantly affect the sensory properties of sausages.

PRACTICAL APPLICATIONS

Poultry meat products provide a suitable environment for proliferation of meat spoilage microorganisms. The increase in resistance of food spoilage microorganisms to current preservatives, the consumer's demand for safe and minimally processed foods, and the hazards associated with the use of high doses of chemical preservatives have led to the need for finding safer alternatives in food preservation. Consumers have also become more critical about the use of synthetic additives to preserve food or enhance characteristics such as color and flavor. Hence, there is a growing trend toward minimally processed foods. As a consequence, antibacterial and antioxidant activities of essential oils have received considerable attention in food preservation. Nowadays, the need for safe products with standard and desirable technological properties has resulted in the use of essential oils for the production of the dry fermented sausages to inhibit the growth of undesirable microorganisms and extend the shelf life of the product.

INTRODUCTION

Poultry meat products are among the fastest growing food commodities on the markets in many parts of the world (Chouliara *et al.* 2007) due to their low cost of production, low fat content, high nutritional value and distinct flavor as compared to meat products as beef, lamb or pork (Barbut 2002; Ressurreccion 2004). However, because of its composition and high pH, poultry meat products present an ideal substrate supporting the growth of several spoilage and pathogenic bacteria (Mataragas *et al.* 2008). In spite of modern improvements in slaughter hygiene and food production techniques, food safety is an increasingly important public health issue (WHO 2014). Lately, much attention has been focused on essential oils (EOs), which have been used traditionally to improve the sensory characteristics and

extend the shelf life of foods (Botsoglou *et al.* 2003; Dussault *et al.* 2014). EOs are aromatic and volatile oily extracts obtained from aromatic and medicinal plant materials, including flowers, buds, roots, bark and leaves (Mastromatteo *et al.* 2009; Hyldgaard *et al.* 2012) by means of expression, fermentation, extraction or steam distillation (Burt 2004). EOs, which are called biopreservatives or green chemicals, are now being viewed as potential alternatives to chemical preservatives (Nychas 1995; Moreira *et al.* 2005; Bensid *et al.* 2014). In fact, these natural products have been shown to possess antibacterial and antifungal activities (Ouattara *et al.* 1997) against several microorganisms associated with meat, including gram-negative and gram-positive bacteria (Karabagias *et al.* 2011). Thyme and oregano EOs have increasingly gained the interest of researchers and food processors as potential natural antimicrobial and antioxidant agents (Bensid *et al.* 2014; Jouki *et al.* 2014). Thymol, carvacrol, *p*-cymene and γ-terpinene are the most active constituents of oregano and thyme EOs, with a wide spectrum of antimicrobial and antioxidant properties (Ultee and Smid 2001; Burt 2004; Rocha-Guzmán *et al.* 2007).

The aim of the present work was to study the effects of oregano and thyme EOs on the microbiological, biochemical and sensory characteristics of a Tunisian dry fermented poultry meat sausage.

MATERIALS AND METHODS

Preparation of Dry Fermented Sausages

The sausage formulation included 3.375 kg of poultry meat (75%), 1.125 kg of poultry fat (25%), 180 g of salt, 18 g of black pepper, 9 g of paprika, 45 g of glucose and 0.45 g of potassium nitrate. After chopping and mixing the ingredients, the mixture was divided into three batches (1.5 kg for each batch): batch 1, contained 0.25% (v/v) oregano EO (*Coridothymus capitatus*, Pharmacy Makni, Manouba, Tunisia) (Table 1); batch 2, contained 0.25% (v/v) thyme EO (*Thymus vulgaris*, Pharmacy Makni) (Table 1); and batch 3, control without EOs.

TABLE 1 MAJOR COMPOUNDS OF OREGANO AND THYME ESSENTIAL OILS

Plant species	Main compounds (area %)*
Coridothymus capitatus	Carvacrol (74,87), thymol (2,54), caryophyllene (2,07), *o*-cymene (9,97), γ-terpinene (4,16)
Thymus vulgaris	β-Linalool (79,17), thymol (6,58), caryophyllene (6,11), linalyl acetate (2,35), *p*-cymene (1,77)

* According to the data of the gas chromatography analysis of essential oils.

The mixture of each batch was stuffed into artificial casings, giving approximately 200 g as the final mass of each sausage and then placed in a fermentation chamber (BCR, CF 1 B, Antony, France). The sausages were fermented for 5 days at 24C and 80% relative humidity (RH). After 5 days of processing, the temperature was decreased to 14C for 23 days and the RH value was 80%. For sampling, three sausages of each batch at 0 day (mix before stuffing) and after 7, 14, 21 and 28 days of ripening were taken for the microbiological, physicochemical and textural analyses and each analysis was carried out in triplicate.

Microbiological Analysis

Sausage samples (10 g) of each batch were homogenized with 90 mL of sterile peptone water (Biolife, Milan, Italy) and decimal dilutions were prepared. Mesophilic lactic acid bacteria (LAB) were enumerated on MRS (de Man, Rogosa and Sharpe) agar (Biolife) after 48 h of incubation at 30C. The number of staphylococci was determined on mannitol salt agar (Biolife) after incubation at 37C for 48 h. Yeasts and molds were enumerated on Sabouraud dextrose agar (Biokar, Beauvais, France) at 28C for 4 days.

Total viable counts were determined on standard plate count agar (Biolife) at 30C for 48 h. Enterobacteriaceae were determined on violet red bile glucose (Biokar) at 37C for 24 h. The number of *Staphylococcus aureus* was determined on Baird–Parker egg yolk tellurite agar (Biolife, Milan, Italy) at 37C for 48 h. Total coliforms were determined on desoxycholate (0.1%) lactose agar (Biokar) at 37C for 24 h.

Physicochemical Analysis

pH, Moisture and Weight Loss. The pH values were measured in homogenates prepared by blending 10 g of sausage (Moulinex DPA141, Lyon, France) with 50 mL of distilled water for 2 min. Measurements were taken with a pH meter (microprocessor pH meter BT-500, Boeco, Hamburg, Germany). Water activity (a_w) was measured with water activity meter (HygroLab 3, Rotronic, Croissy Beaubourg, France). The moisture percentage was calculated by weight loss experimented by the sample (5 g) maintained in an oven (Memmert, UL 60, Schwabach, Germany) at 105C, until constant weight according to the ISO recommended method (ISO 1973). Weight loss was expressed as the percentage of the initial weight (Liaros *et al.* 2009).

Determination of Thiobarbituric Acid (TBA). This analysis was performed according to the method of Genot (1996). The TBA test is one of the most extensively used methods to detect oxidative deterioration of fat-containing foods. During lipid oxidation, malondialdehyde (MDA) is formed as a result of the degradation of polyunsaturated

fatty acids. In this assay, the MDA is reacted with TBA to form a pink MDA–TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm (Guillén-Sans and Guzmàn-Chozas 1998; Wang *et al.* 2002). Thus, 2 g of the sample was homogenized together with 16 mL of a 5% (w/v) aqueous solution of trichloroacetic acid (TCA) containing 100 μL of freshly prepared BHT (butylated hydroxytoluene) in ethanol (1 mg/mL) by using a homogenizer set at 20,000 rpm for 15 s. About 2 mL of supernatant, obtained by centrifugation at 10,000 × g for 10 min at 4C (Universal, 320, Tuttlingen, Germany), was mixed with 2 mL of TBA (20 M). Tubes were immersed in a 70C water bath (Memmert) for 30 min. After cooling, absorbance of the reaction solutions was read at 532 nm using a spectrophotometer (Jenway 6305, Chelmsford, England) against a blank containing 2 mL of TCA and 2 mL of TBA reagent. The results are expressed as mg MDA/kg of food sample using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} for malondialdehyde (Buedge and Aust 1978).

Color Measurement. Color measurements were carried out using a CR-300 colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan). Each sausage was cut and the color of the slices was measured three times for each analytical point L^*, a^* and b^* scale coordinates were obtained: L^* (lightness), a^* (redness) and b^* (yellowness). Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Free Amino Acid (FAA) Content. The content of FAA was determined by reverse phase high-performance liquid chromatography (HPLC). The amino acids were extracted after hydrolysis of meat proteins in the presence of concentrated hydrochloric acid. Thus, 5 g of dry fermented sausages was chopped and added to 4 mL of 37% HCl (6 M). The mixture was homogenized and then placed in an oven at 105C for 24 h. Hydrolysis was stopped by adding approximately 6 mL of NaOH (6 N). Then, the mixture was filtered through a syringe filter and the filtrate was stored at 4C until injection. The separation of the protein fraction of sausages was performed using HPLC Agilent L 100 system (Province, Canada), on a column C18 (250 mm × 4.6 mm dimensions of the column, 5 μm porosity). This system contains high pressure pump, an automatic injector fluorescence detector (FLD), FLD detector, and control software and acquisition of data (Chemstation). The separation was carried out for 30 min, the flow rate of the mobile phase is 1 mL/min. The excitation is at 340 nm and the emission is at 440 nm. The injection solution is composed of 2.5 μL of borate, buffer supplemented with 0.5 μL of the sample, 0.5 μL of H_2O and 0.5 μL of *o*-phthaldialdehyde solution. The whole is mixed with 3.5 μL of air. Then, everything is injected. The injection of reference amino acids allowed determining their retention times. To determine the concentration of amino acids in different samples, straight standards have been established relating the concentration of each reference amino acid to the area of the peak obtained.

Determination of Free Fatty Acids (FFAs). Lipids were extracted according to the Soxhlet method (ISO 1996). The FFA compositions were determined by means of gas chromatography coupled with mass spectrometry (GC-MS) after methyl esterification. The analysis was performed using an Agilent chromatograph (Agilent 5975 B) equipped with split injector and flame ionization detector, in an Agilent 19091S-933 column (1% phenyl(methylsiloxane), 30 m × 250 μm × 0.25 μm). Helium was the carrier gas. The oven temperature increased from 80 to 300C at the rate of 3C/ min and 10 min at 300C. The individual FFAs were identified in comparison with retention times of chemical standards by mass spectrometry. FFAs were expressed as percentage of total fatty acids.

Instrumental Texture Measurement. Instrumental texture analysis was performed with a texture analyzer (TA-XT2 stable Micro-systems, Haslemere, U.K.). Each sample of sausage was cut in a cylinder (3 cm × 3 cm). Each cylinder was compressed with a cylindrical probe at 5 mm/s speed and the level of compression was 60% of the thickness of the sample. The parameter determined from the force time curves was hardness. Hardness was defined by peak force during compression and expressed in grams. The sample elasticity was estimated by using the apparently Young's modulus which was calculated as the rate of strain (σ) (N/m^2) as a function of stress (ε) (%) in the straight line of the deformation curve. The apparent Young's modulus value (N/m^2) is higher as less elastic the sausage is.

Sensory Evaluation

The aim of the sensory evaluation was to determine whether the addition of oregano and thyme EOs affected the sensory quality of sausages, for which samples were evaluated by 30 assessors (aged between 20 and 32), none had specific training in sensory analysis of fermented poultry meat sausages. The analyses were performed under white fluorescent lights in individual booths constructed according to the specifications of the International Standards Organization (ISO 1985). A slice of each sample batch (5-mm thick approximately) was served to the assessors. Mineral water was provided to clean the palate between samples. A test was carried out using a nonstructured hedonic scale consisting of 10 levels (1: dislike extremely and 10: like extremely), in which the assessors evaluated different attributes: red color, odor, hardness, aftertaste, acidity

and overall acceptability. After the evaluation, mean values were calculated for each parameter.

Statistical Analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) procedure of SPSS 17.0 (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to determine any significant difference between mean values and evaluations were based on a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Microbiological Results

Figure 1 shows the evolution of LAB, staphylococci, yeasts and molds, Enterobacteriaceae, total viable counts, *S. aureus* and total coliforms during fermentation of control sausages and sausages added with EOs of oregano and thyme.

The number of total viable counts increased during the first 7 days of ripening and then decreased slightly to reach at the end of ripening levels of 7.39, 6.75 and 7.08 log cfu/g, respectively, for control sausages and sausages added with oregano and thyme EOs (Fig. 1). Our results showed that the addition of EOs affect significantly ($P < 0.05$) the quantitative evolution of total viable counts. Chouliara *et al.* (2007) showed that a concentration of 1% of oregano EO had an important antibacterial effect on the total viable counts than a concentration of 0.1%. Dzudie *et al.* (2004) reported that the addition of EOs reduced significantly the number of total viable counts.

The numbers of Enterobacteriaceae, *Staphylococcus aureus* and total coliforms decreased during the ripening phase of dry fermented sausages (Fig. 1). In fact, their numbers are significantly lower in sausages added with oregano and thyme EOs than those measured on control

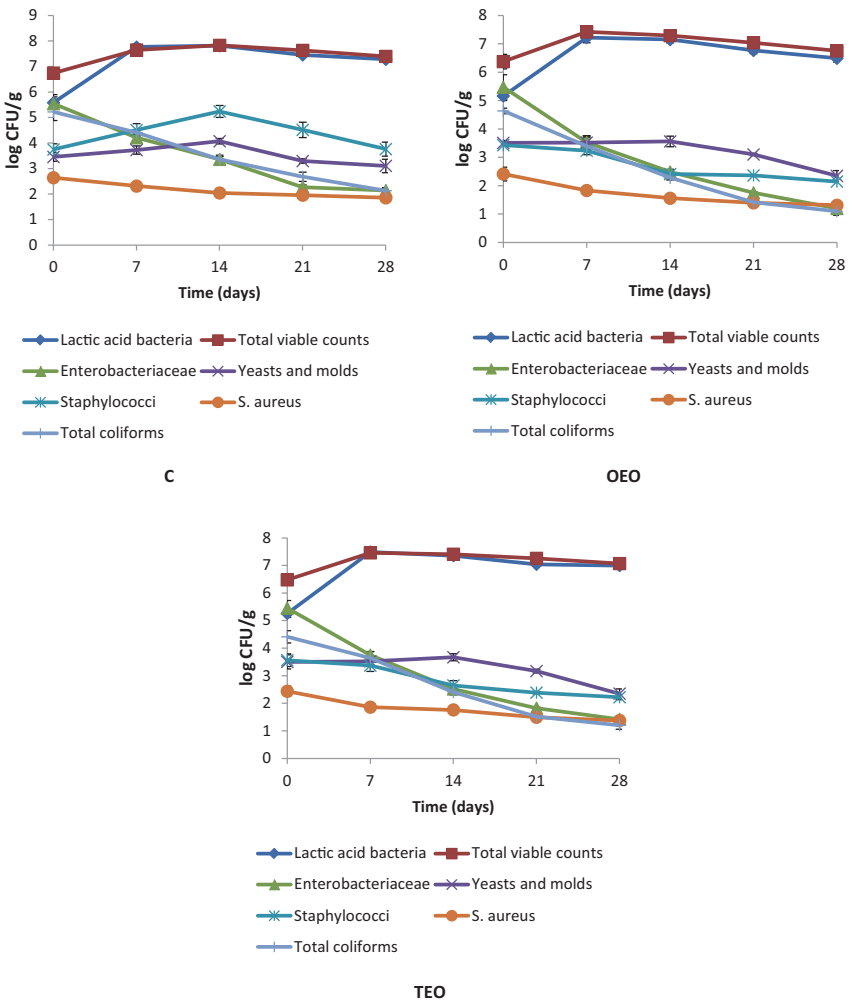

FIG. 1. EVOLUTION OF MICROBIAL POPULATION DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

samples. The reduction of the number of viable cells of these microorganisms is attributed first of all to the acidification activity of LAB that play an important role in the inhibition of spoilage and pathogenic microorganisms (Deumier and Collignan 2003) and second to the antimicrobial activity of EOs of oregano and thyme. In fact, carvacrol and thymol, the major components of oregano and thyme EOs, are mainly responsible for its antimicrobial activity (Juliano *et al.* 2000). The mode of action of carvacrol and thymol appears to have received the most attention from researchers. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. Due to their hydrophobic nature, carvacrol and thymol interact with the lipid bilayer of cytoplasmic membranes, causing loss of integrity and leakage of cellular material such as ions, ATP and nucleic acid (Mastromatteo *et al.* 2009). Our results are in agreement with many other studies (Deumier and Collignan 2003; Mastromatteo *et al.* 2009; Jouki *et al.* 2014).

The number of LAB increased during the first 7 days of ripening, reaching, at the end of the seventh day, maximum values of 7.22 and 7.487 log cfu/g for sausages added, respectively, with oregano and thyme EOs against 7.763 log cfu/g for the control ones (Fig. 1). Beyond the seventh day, the number of LAB decreased slightly, reaching, at the end of ripening, levels of 6.49 and 6.997 log cfu/g, respectively, for sausages added with oregano and thyme EOs. This slight decrease of LAB during ripening is probably due to the decrease of fermentable carbohydrates (Lorenzo and Franco 2012). LAB were the dominant microflora at the end of the ripening for all sausages; this result confirms the good adaptation of LAB to the meat environment and their faster growth rates during fermentation and ripening of sausages (Ferreiria *et al.* 2007). Our results showed that the addition of EOs did not affect significantly ($P > 0.05$) the quantitative evolution of LAB. Frangos *et al.* (2010) reported that the greater resistance of the LAB could be related to their better ability to deal with conditions of osmotic stress and respond more effectively to K^+ efflux caused by many of EOs. Holley and Patel (2005) also reported that the high tolerance of LAB toward the action of essential oils is attributed to their ability to generate ATP and to deal with conditions of osmotic stress. Our results are in agreement with those obtained by Kostaki *et al.* (2009) who reported that LAB are the most resistant bacteria among the gram-positive bacteria toward the antimicrobial action of EOs.

The number of yeasts and molds increased during the first seven days of maturation and this for all samples of sausages. Then, their concentrations decreased especially in the last two weeks of ripening to reach at 28th day values of 3.11, 2.34 and 2.35 log cfu/g, respectively, for control samples and sausages added with oregano and thyme EOs (Fig. 1). Our results showed that the addition of EOs did not affect significantly ($P > 0.05$) the quantitative evolution of yeasts and molds. Zdolec *et al.* (2008) found that the number of molds and yeasts in dry fermented sausages increased during the first days of ripening and then underwent a reduction to reach at the end of maturation values between 10^2 and 10^3 cfu/g.

In control sausages, the number of staphylococci increased during the first two weeks of ripening (Fig. 1). At the end of ripening, the number of staphylococci decreased to reach 3.76 log cfu/g. This decrease is due to the acidification of the medium by LAB, whereas in sausages added respectively with oregano and thyme EOs, the number of staphylococci decreased significantly, reaching 2.15 and 2.22 log cfu/g at the end of ripening. This decrease in the number of staphylococci is mainly due to the antimicrobial activity of EOs.

pH, Water Activity, Moisture and Weight Loss

The initial pH of all of sausages tested was 6.23. The pH decreased during the first seven days of ripening to reach values of 5.53, 5.52 and 5.5 at the seventh day, respectively, for control samples and sausages added with EOs of oregano and thyme ($P < 0.05$) (Fig. 2). The pH fall was presumably caused by an accumulation of organic acids, mainly lactic, present in this type of sausages as a result of carbohydrate breakdown during fermentation (Zaho *et al.* 2011). After seven days of ripening, the pH of control samples and sausages added with EOs of oregano and thyme increased gradually. This increase in pH is explained, first of all, by the reduction of the number of LAB due to the exhaustion of the sugar, and second, to proteolytic activity generated by microorganisms. Bacterial proteases induce proteolytic degradation, generating peptides, amino acids and amines which have a buffering effect on the organic acids produced by LAB during fermentation (Ruiz-Moyano *et al.* 2011). Our results are in agreement with many other studies (Bozkurt and Erkmen 2002; Kayaardı and Gök 2003; Martín-Sánchez *et al.* 2011).

The initial value of a_w was 0.93, which fell with time ($P < 0.05$) due to drying (Fig. 3), but differences were not significant ($P > 0.05$) between the control sausages and sausages added with EOs. At the end of the ripening period, the a_w values were close to 0.81–082.

The moisture content values decreased significantly ($P < 0.05$) from 57.33, 56.33 and 57%, respectively, for control sausages and sausages added with EOs of oregano and thyme, to reach values of 26.33, 28.47 and 26.47% at day 28 (Fig. 4). However, no significant difference ($P > 0.05$) was observed between treatments during all the ripening. This water loss is due, first of all, to the elevated

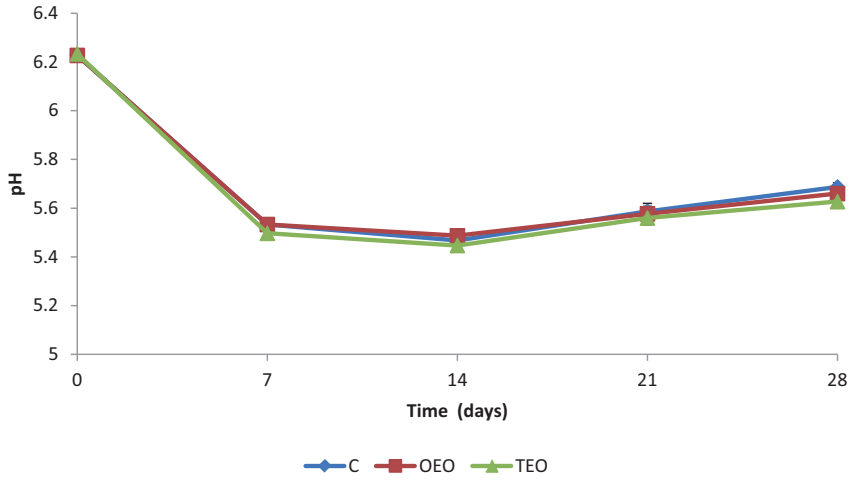

FIG. 2. EVOLUTION OF PH DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

temperature of fermentation (24C) that accelerates the drying of the product, and second, to the decrease of pH of sausages to their isoelectric pH which causes protein denaturation and thus a decrease in water retention capacity of myofibrillar proteins (Solignat 1999).

The weight of control sausages and sausages added with EOs decreased during the ripening period (Fig. 5). This loss in weight is due to the temperature of fermentation (24C) that accelerates the dehydration of the product. Our results are in agreement with other studies (Liaros *et al.* 2009; Jin *et al.* 2010) reporting that the weight loss of dry fermented sausage increases during the ripening period.

TBA

The TBA values increased gradually ($P < 0.05$) during the first two weeks of ripening from 0.21 to 0.73, 0.5 and 0.53 mg MDA/kg of sample in control sausages and sausages added with oregano and thyme EOs, respectively. Thereafter, TBA values decreased ($P < 0.05$) to reach values of 0.7, 0.42 and 0.44 mg MDA/kg of sample at the end of ripening (Fig. 6). Moreover, our results showed that TBA values were significantly affected by the addition of oregano and thyme EOs ($P < 0.05$). Similar results were reported by Jouki *et al.* (2014) and Radha Krishnan *et al.* (2014). Oregano and thyme EOs contain high level of phenolic compounds (Simitzis *et al.* 2008; Bensid *et al.* 2014). Several studies have reported on the relationship between phenolic content and antioxidant activity (Velioglu *et al.* 1998). The antioxidant activity of phenolic compounds is primarily attributable to their redox properties, which can perform an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides. This activity further stops the degradation to more active oxidizing forms, such as MDA (Radha Krishnan *et al.* 2014).

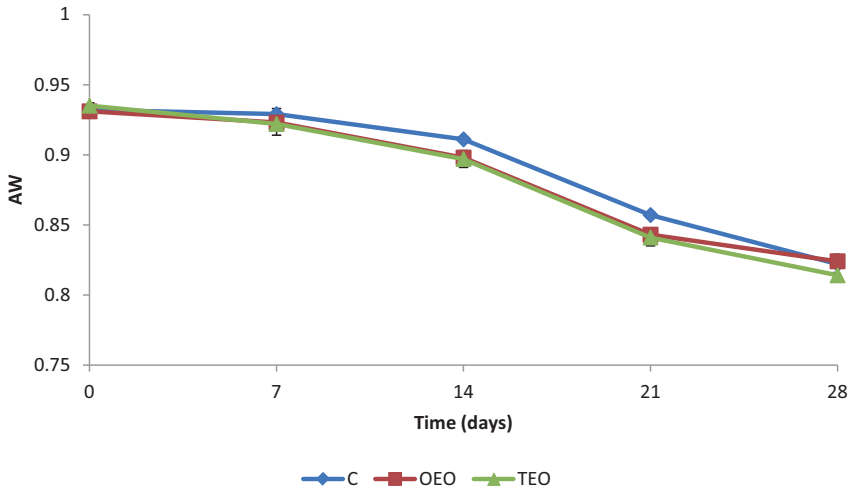

FIG. 3. EVOLUTION OF WATER ACTIVITY DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

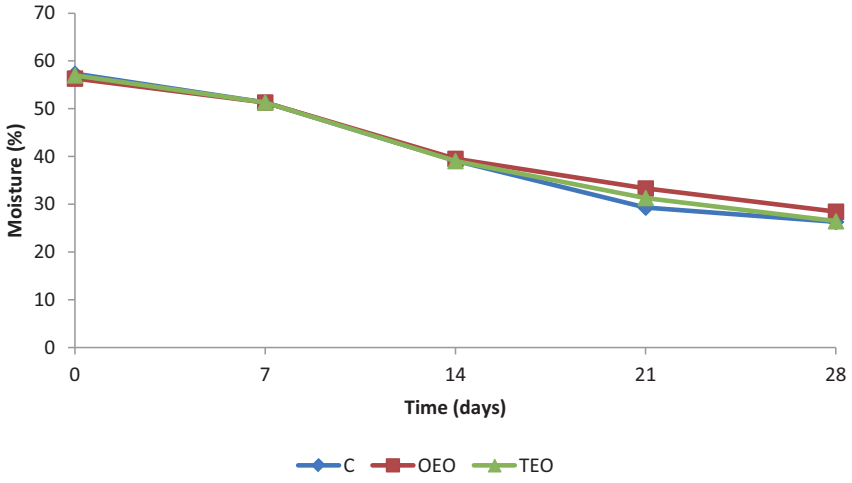

FIG. 4. EVOLUTION OF MOISTURE DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

Color Measurement

Color formation and stability are very important quality attributes of sausages (Gøtterup *et al.* 2008). Figure 7 shows the effect on color coordinates, lightness (L^*), redness (a^*) and yellowness (b^*) of adding oregano and thyme EOs to sausages. As can be seen, lightness decreased through ripening time of dry fermented sausages. The decrease in L^* values represented formation of dark color due to water loss (Sanabria *et al.* 2004). Similarly, Bozkurt (2007) found that L^* values generally decreased during the ripening time of dry fermented sausages. Our results show that lightness of sausages was significantly affected by the addition of oregano and thyme EOs ($P < 0.05$) and not by the ripening time of sausages ($P > 0.05$). With respect to a^* values, an increase was observed during the first three weeks of maturation, followed by a slight decrease (Fig. 7). The variation of the parameter color a^* during ripening of dry fermented sausages is linked to the formation of a small amount of nitrosomyoglobin pigment (pink-red). Our results are in agreement with those of Kayaardı and Gök (2003) who found that the a^* values of sausage increased during the first days of ripening and then decreased during the further ripening period. The possible reason for decreasing a^* values might be partial or total denaturation of nitrosomyoglobin because of the production of lactic acid (Perez-Alvarez *et al.* 1999). Our results showed that redness (a^*) and yellowness (b^*) of sausages were significantly affected by the ripening time of sausages ($P < 0.05$) and not by the addition of oregano and thyme EOs ($P > 0.05$). In relation to b^* values, a decrease was observed during the ripening period (Fig. 7). This decrease in b^* values could be due to oxygen consumption by microorganisms and therefore a decrease in oxymyoglobin that contributes to yellow color (Bozkurt 2007).

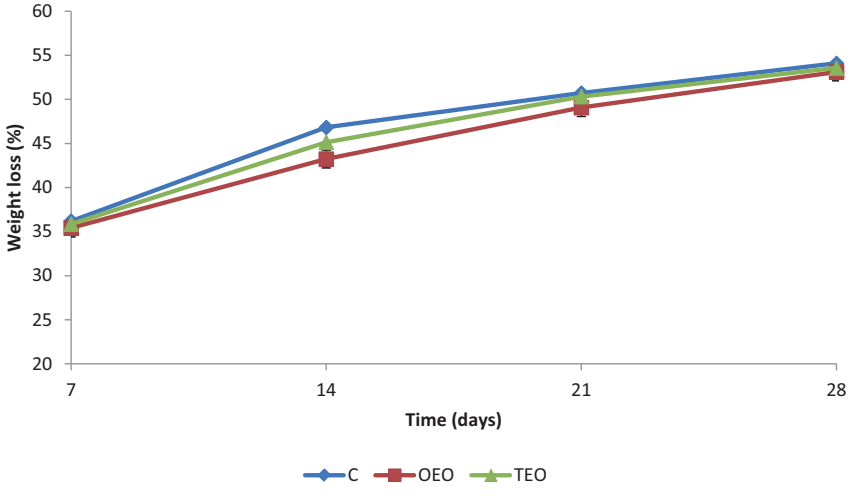

FIG. 5. EVOLUTION OF WEIGHT LOSS DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

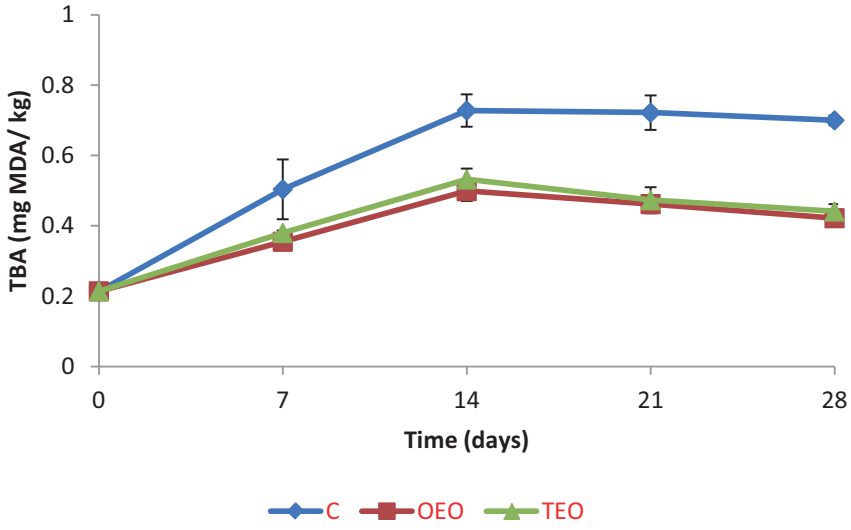

FIG. 6. CHANGES IN THIOBARBITURIC ACID (TBA) VALUES DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

FAA Content

To evaluate the effect of the addition of oregano and thyme EOs on proteolysis, FAAs were determined through ripening using a chromatographic approach. The concentrations of total FAAs during ripening are shown in Table 2. The total FAA content of sausages increased significantly from 263.36 mg/100 g on day 0 to 338.12, 302.75 and 329.19 mg/

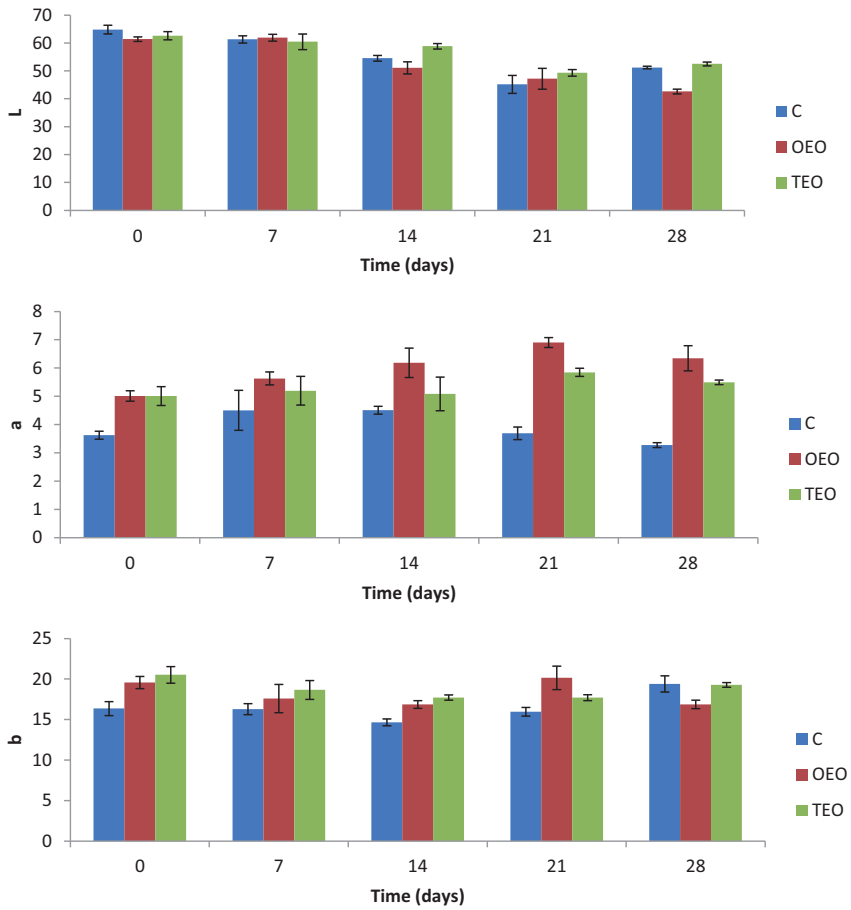

FIG. 7. EVOLUTION OF *L**, *A** AND *B** VALUES DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

TABLE 2 FREE AMINO ACID CONTENT (MG AMINO ACIDS/100 G OF SAUSAGE) DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIALS OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

	Time (days)							
	0	14			28			
Amino acids (mg amino acids/100 g of sausage)	C	C	OEO	TEO	C	OEO	TEO
Aspartic acid	14.07 ± 0.31^{d}	43.81 ± 11.95^{bcd}	27.155 ± 4.02^{b}	27.195 ± 0.76^{d}	19.62 ± 3.03^{abc}	24.96 ± 0.36^{e}	24.785 ± 0.06^{e}
Glutamic acid	27.625 ± 0.12^{g}	51.55 ± 0.05^{cd}	43.625 ± 1.34^{e}	27.195 ± 0.76^{d}	19.62 ± 3.03^{abc}	24.96 ± 0.36^{e}	28.8 ± 3.19^{f}
Serine + Glutamine + Histidine	6.12 ± 0.73^{b}	13.8 ± 2.08^{a}	8.68 ± 1.11^{a}	7.805 ± 1.09^{ab}	11.18 ± 4.26^{ab}	8.485 ± 0.22^{b}	9.96 ± 0.48^{b}
Arginine + Threonine + Glycine	82.285 ± 0.14^{i}	101.025 ± 2.43^{e}	72.985 ± 4.08^{f}	75.22 ± 0.66^{f}	104.005 ± 10.5^{e}	73.265 ± 1.45^{i}	84.475 ± 1.6^{i}
Alanine	23.935 ± 0.01^{f}	30.99 ± 16.8^{abc}	36.37 ± 1.97^{c}	35.725 ± 2.19^{e}	34.095 ± 8.82^{c}	20.455 ± 0.39^{d}	21.32 ± 0.24^{d}
Tyrosine	43.865 ± 0.39^{h}	60.045 ± 2.15^{d}	68.03 ± 10.16^{f}	71.15 ± 5.23^{f}	65.995 ± 7.13^{d}	49.3945 ± 0.13^{h}	51.62 ± 0.04^{i}
Valine + Methionine	7.17 ± 0.54^{b}	23.445 ± 8.73^{ab}	11.795 ± 1.56^{a}	$12.5550.32^{bc}$	10.595 ± 1.61^{a}	12.305 ± 1.28^{c}	12.165 ± 1.3^{c}
Phenylalanine	3.58 ± 0.32^{a}	11.185 ± 3.29^{a}	6.51 ± 1.54^{a}	6.13 ± 0.23^{a}	5.295 ± 0.97^{a}	5.215 ± 0.56^{a}	5.305 ± 0.45^{a}
Leucine	22.2 ± 0.38^{e}	27.985 ± 0.13^{abc}	37.54 ± 4.95^{cd}	38.26 ± 0.26^{e}	28.565 ± 4.45^{c}	31.59 ± 3.45^{f}	32.555 ± 2.12^{g}
Isoleucine	8.88 ± 0.06^{c}	11.22 ± 1.06^{a}	13.085 ± 0.96^{a}	13.935 ± 0.23^{c}	11.635 ± 2.23^{ab}	12.23 ± 0.56^{c}	12.975 ± 1.04^{bc}
Lysine	23.63 ± 0.33^{f}	23.585 ± 0.06^{ab}	42.905 ± 5.41^{de}	41.23 ± 0.97^{e}	27.51 ± 1.79^{bc}	39.885 ± 0.99^{g}	45.225 ± 0.36^{h}
Total	263.36	398.64	368.68	356.4	338.115	302.7445	329.158

Note: Averages with different superscript letters in the same row are different ($P < 0.05$).

100 g, respectively, for control sausages and sausages added with oregano and thyme EOs. Many other studies reported an increase in total amino acid content during ripening of dry fermented sausages (Bolumar *et al.* 2001; AroAro *et al.* 2010; Lorenzo and Franco 2012; Essid and Hassouna 2013). The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and FAAs; this degradation can be produced by endogenous and microbial enzymes (Hughes *et al.* 2002). Moreover, similar amino acids were detected during the ripening of the three types of sausages. In fact, the pH of the control sausages and sausages added with oregano and thyme EOs was around 5 and thus the activity of endogenous proteases would have led to amino acidic profiles identical in the three types of sausages (Casaburi *et al.* 2008). Our results showed that the addition of EOs did not significantly affect ($P > 0.05$) the FAA content during ripening of sausages. In addition, the results showed that the highest enzymatic activity took place at the beginning of the process in coincidence with the fermentation stage. This increase has been attributed to the higher temperature applied during fermentation compared with the low temperature applied during drying (Essid and Hassouna 2013). A decrease in amino acid content was noted during the two last weeks of ripening for all samples of sausages; this decrease may indicate their metabolism by bacteria (Sekikawa *et al.* 2003; Essid and Hassouna 2013).

The most abundant FAAs detected in the final product were arginine, glycine, threonine, alanine, tyrosine, aspartic acid, glutamic acid and lysine. The sensory properties of FAA have been established: glutamic acid and aspartic acid showing pleasantly fresh taste; glycine, alanine and serine being sweet; arginine, leucine, isoleucine, valine, phenylalanine, methionine and histidine being bitter; lysine and proline contributing sweet and bitter tastes; and others showing sour or salty taste (Lorenzo and Franco 2012). It is important to note that the metabolic reactions driven by meat microbiota will be affected by the time and temperature of ripening, which may affect specific amino acids, making it difficult to compare samples in different studies (Essid and Hassouna 2013).

FFA Content

The concentrations of saturated, monounsaturated and polyunsaturated fatty acids during ripening of the three types of sausages are reported in Table 3. The results showed that monounsaturated fatty acids displayed concentrations higher than saturated and polyunsaturated in all the samples analyzed during ripening. Our results are similar to those of Martín-Sánchez *et al.* (2011) and Pereira *et al.* (2000). On the other hand, Essid and Hassouna (2013) and Lorenzo and Franco (2012) found that saturated fatty acids (SFAs) dominate in sausages. High variability between

TABLE 3 FREE FATTY ACID COMPOSITION (%) DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIALS OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

Fatty acid composition (%)	Time (days)						
	0	14			28		
	C	C	OEO	TEO	C	OEO	TEO
C14:0	0.31 ± 0.01^{a}	0.355 ± 0.05^{a}	0.295 ± 0.01^{a}	0.33 ± 0.02^{a}	0.325 ± 0.01^{a}	0.3 ± 0.02^{a}	0.36 ± 0.02^{a}
C16:0	28.255 ± 0.56^{d}	32.325 ± 1.39^{d}	28.435 ± 0.79^{e}	31.435 ± 2.38^{d}	32.575 ± 0.27^{d}	28.43 ± 0.25^{e}	31.81 ± 0.27^{e}
C16:1	4.605 ± 0.01^{b}	4.42 ± 0.07^{b}	4.155 ± 0.02^{b}	5.48 ± 0.38^{b}	4.42 ± 0.02^{b}	4.22 ± 0.11^{b}	4.24 ± 0.04^{b}
C18:0	5.45 ± 0.16^{b}	6.79 ± 0.4^{bc}	5.975 ± 0.15^{c}	7.02 ± 0.15^{b}	7.17 ± 0.01^{c}	5.985 ± 0.16^{c}	6.495 ± 0.05^{c}
C18:1	49.18 ± 0.36^{e}	46.895 ± 1.09^{e}	46.86 ± 0.53^{f}	44.225 ± 2.11^{e}	48.42 ± 0.19^{e}	46.57 ± 0.17^{f}	45.86 ± 0.06^{f}
C18:2	12.2 ± 0.05^{c}	9.21 ± 0.02^{c}	14.27 ± 0.11^{d}	11.507 ± 0.79^{c}	7.085 ± 0.06^{c}	14.495 ± 0.16^{d}	11.235 ± 0.14^{d}
ΣSFA	34.015 ± 0.73	39.47 ± 1.84	34.705 ± 0.95	38.455 ± 2.55	40.07 ± 0.29	34.715 ± 0.43	38.665 ± 0.34
ΣMUFA	53.785 ± 0.37	51.315 ± 1.16	50.725 ± 0.55	49.705 ± 2.49	52.84 ± 0.21	50.79 ± 0.28	50.1 ± 0.1
ΣPUFA	12.2 ± 0.05	9.21 ± 0.02	14.27 ± 0.11	11.507 ± 0.79	7.085 ± 0.06	14.495 ± 0.16	11.235 ± 0.14
SFA/UFA	0.516	0.652	0.534	0.628	0.668	0.532	0.631

Note: Averages with different superscript letters in the same row are different ($P < 0.05$).
MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

sausages is normal since the concentration of FFAs in the fat depends on many factors, such as the raw materials used to prepare the sausages, the length of the process and the starter cultures (Lorenzo and Franco 2012). Furthermore, control sausages and sausages added with oregano and thyme EOs showed the same FFA composition, considering that the major release of these latter was due to endogenous lipases. Muscle and fat tissue lipases are very important when the pH in the sausages is around their optimal pH (4.5–5.5) (Martín-Sánchez *et al.* 2011). Our results showed that lipolysis was not significantly affected by the addition of oregano and thyme EOs ($P > 0.05$).

As Table 2 shows, the SFA/UFA ratio increased during the ripening process. Our results are in agreement with those of Lizaso *et al.* (1999) who reported that the values of the SFA/UFA ratio increased during fermentation, as a result of the greater susceptibility of UFAs to oxidation. However, Martín-Sánchez *et al.* (2011) and Navarro *et al.* (1997) observed a decrease in this ratio under different processing conditions, probably due to the preferred hydrolysis of phospholipids as the main FFA source. Moreover, the acid present in greater percentage was oleic acid followed by palmitic acid, linoleic acid and stearic acid. The same result was observed by Pereira *et al.* (2000) and Martín-Sánchez *et al.* (2011).

Texture Measurements

The results concerning the instrumental texture showed that no significant differences were observed between control sausages and sausages added with oregano and thyme EOs; however, hardness and elasticity were affected by time of ripening. Hardness (Fig. 8) and Young's modulus (Fig. 9) increased significantly through ripening time for all samples of sausages. The increase of hardness and elasticity through the ripening of sausages is mostly due to water loss. These results match with those found by Kargozari *et al.* (2014), who reported that higher hardness in sausages is

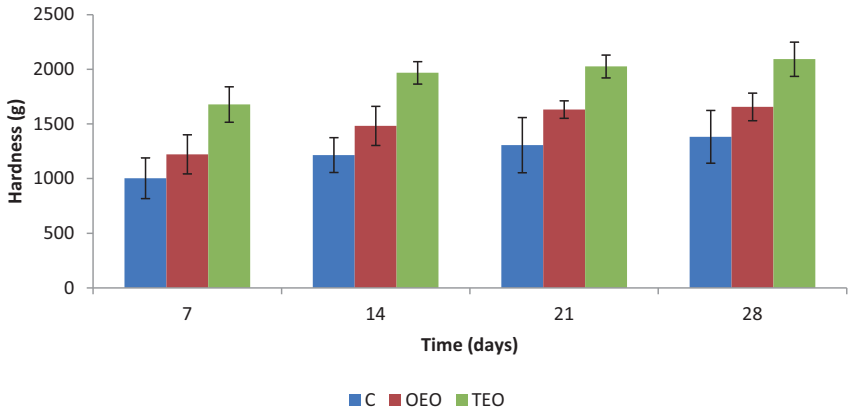

FIG. 8. EVOLUTION OF HARDNESS DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

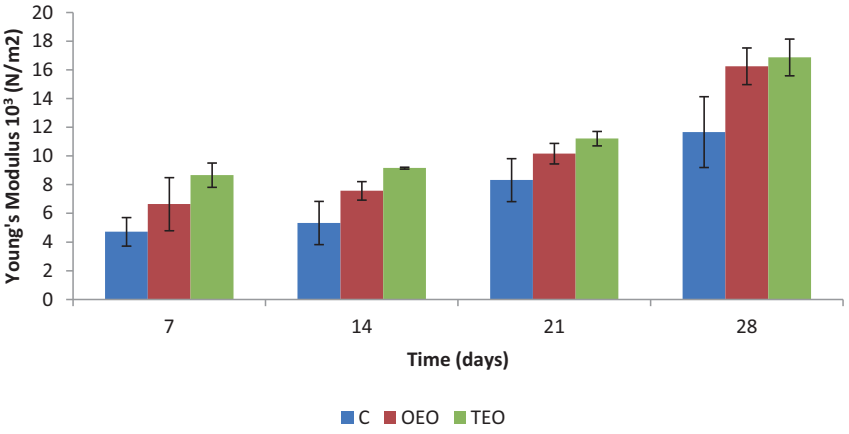

FIG. 9. EVOLUTION OF YOUNG'S MODULUS DURING THE RIPENING OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

probably due to much higher weight loss. Generally, the major changes in fermented sausage structure take place during fermentation when the pH declines and the myofibrillar proteins aggregate to from a gel. After fermentation, drying is a major factor affecting rheological properties (Gonzalez-Fernandez *et al.* 2006).

Sensory Evaluation

Figure 10 shows the results of a sensorial evaluation of the different samples assayed. The statistical analysis of the sensory results showed that the addition of oregano and thyme EOs had no significant ($P > 0.05$) negative effect on the organoleptic acceptability of the sausage. The same result was confirmed by Jouki *et al.* (2014). Sausages with oregano and thyme EOs showed a more pronounced red color when compared to control ones. Similar results were obtained with colorimeter. Acid taste obtained similar values in all the samples analyzed. Hardness values of sausages were also similar between panelists' appreciation and instrumental measurements. Hardness could arise from microbiological and physiochemical process, such as enhanced acidification and proteolysis. It should be noted that despite the marked odor of oregano and thyme EOs, this was not found unpleasant by the panelists who scored the sausages containing these EOs higher than the control samples. As regards the aftertaste of sausages, it was similar for all treatments ($P > 0.05$). Aftertaste of sausages is related to compounds (fatty acids, amino acids, aldehydes, esters, etc.) released by endogenous proteases and lipases through ripening of sausages.

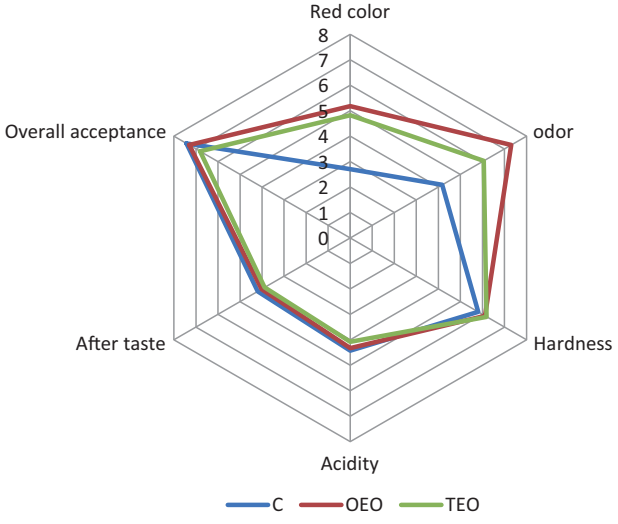

FIG. 10. SENSORY EVALUATION OF CONTROL SAUSAGES AND SAUSAGES ADDED WITH ESSENTIAL OILS (EOs): C (CONTROL SAUSAGE), OEO (SAUSAGE ADDED WITH OREGANO EO), TEO (SAUSAGE ADDED WITH THYME EO)

CONCLUSION

The addition of oregano and thyme EOs seems to be a viable alternative for the production of dry sausages since they did not negatively affect the sensory properties and have desirable effects with regard to oxidative stability. Moreover, the use of EOs could be useful for maintaining hygienic quality of sausages by inhibition of spoilage and pathogenic microorganisms, which allows a good preservation of sausages and consequently improved their shelf life. Proteolysis, water activity and textural parameters (hardness and elasticity) were not significantly ($P > 0.05$) affected by the addition of EOs during ripening of the sausages.

ACKNOWLEDGMENTS

The authors thank the National Institute of Research and Physicochemical Analysis (Sidi Thabet, Ariana, Tunisia) and the National Center for Nuclear Sciences and Technologies (Sidi Thabet, Ariana, Tunisia) for providing part of the equipment used in this work.

REFERENCES

AROARO, J.M., NYAM-OSOR, P., TSUJI, K., SHIMADA, K.I., FUKUSHIMA, M. and SEKIKAWA, M. 2010. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chem. *119*, 279–285.

BARBUT, S. 2002. *Poultry Products Processing. An Industry Guide*, 1st Ed., CRC Press, London, U.K.

BENSID, A., UCAR, Y., BENDEDDOUCHE, B. and ÖZOGUL, F. 2014. Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. Food Chem. *145*, 681–686.

BOLUMAR, T., NIETO, P. and FLORES, J. 2001. Acidity, proteolysis and lipolysis changes in rapid-cured fermented sausage dried at different temperatures. Food Sci. Technol. Int. *7*, 269–276.

BOTSOGLOU, N.A., GRIGOROPOULOU, S.M., BOTSOGLOU, E., GOVARIS, A. and PAPAGEORGIOU, G. 2003. The effects of dietary oregano essential oil and α-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. Meat Sci. *65*, 1193–1200.

BOZKURT, H. 2007. Comparison of the effects of sesame and *Thymbra spicata* oil during the manufacturing of Turkish dry-fermented sausage. Food Control *18*, 149–156.

BOZKURT, H. and ERKMEN, O. 2002. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). Meat Sci. *61*, 149–156.

BUEDGE, J.A. and AUST, S.D. 1978. Microsomal lipid peroxidation. In *Biomembranes (Part C: Biological Oxidation) Methods in Enzymology*, Vol. *52* (S.F. Fleisher and L. Packer, eds.) pp. 302–309, Academic Press, London, U.K.

BURT, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol. *94*, 223–253.

CASABURI, A., DI MONACO, R., CAVELLA, S., TOLDRA, F., ERCOLINI, D. and VILLANI, F. 2008. Proteolytic and lipolytic starter cultures and their effect on traditional fermented sausages ripening and sensory traits. Food Microbiol. *25*, 335–347.

CHOULIARA, E., KARATAPANIS, A., SAVVAIDIS, I. and KONTOMINAS, M.G. 2007. Combined effect of oregano oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. Food Microbiol. *24*, 607–617.

DEUMIER, F. and COLLIGNAN, A. 2003. The effects of sodium lactate and starter cultures on pH, lactic acid bacteria, *Listeria monocytogenes* and *Salmonella spp.* levels in pure chicken dry fermented sausage. Meat Sci. *65*, 1165–1174.

DUSSAULT, D., VU, K.D. and LACROIX, M. 2014. In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. Meat Sci. *96*, 514–520.

DZUDIE, T., KOUEBOU, C.P., ESSIA-NGANG, J.J. and MBOFUNG, C.M.F. 2004. Lipid sources and essential oils effects on quality and stability of beef patties. J. Food Eng. *65*, 67–72.

ESSID, I. and HASSOUNA, M. 2013. Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. Food Control *32*, 707–714.

FERREIRIA, V., BARBOSA, J., SILVA, J., VENDEIRO, S., MOTA, A., SILVA, F., MONTEIRO, M.J., HOGG, T., GIBBS, P. and TEIXEIRA, P. 2007. Chemical and microbiological characterization of "Salpicao de Vinhais" and "Chourica de Vinhais": Traditional dry sausages produced in the North of Portugal. Food Microbiol. *24*, 618–623.

FRANGOS, L., PYRGOTOU, N., GIATRAKOU, V., NTZIMANI, A. and SAVVAIDIS, I.N. 2010. Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiol. *27*, 115–121.

GENOT, C. 1996. Some factors influencing TBA test. Report of diet-ox project (AIRIII-CT-92-1577).

GONZALEZ-FERNANDEZ, C., SANTOS, E.M., ROVIRA, J. and JAIME, I. 2006. The effect of sugar concentration and starter culture on instrumental and sensory textural properties of chorizo-Spanish dry cured sausage. Meat Sci. *74*, 467–475.

GØTTERUP, J., OLSEN, K., KNØCHEL, S., TJENER, K., STAHNKE, L.H. and MØLLER, J.K.S. 2008. Colour formation in fermented sausages by meat-associated staphylococci with different nitrite and nitrate reductase activities. Meat Sci. *78*, 492–501.

GUILLÉN-SANS, R. and GUZMÀN-CHOZAS, M. 1998. The thiobarbituric acid (TBA) reaction in foods: A review. Crit. Rev. Food Sci. Nutr. *38*, 315–330.

HOLLEY, R.A. and PATEL, D. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol. *22*, 273–292.

HUGHES, M.C., KERRY, J.P., ARENDT, E.K., KENNEALLY, P.M., MCSWEENEY, P.L.H. and O'NEILL, E.E. 2002. Characterization of proteolysis during the ripening of semi-dry fermented sausages. Meat Sci. *62*, 205–216.

HYLDGAARD, M., MYGIND, T. and MEYER, R.L. 2012. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Front Microbiol. *3*, 1–24.

ISO. 1973. Meat and meat products – determination of moisture. ISO, 1442-1973 (E).

ISO. 1985. Sensory analysis. Methodology. General guidance (ISO-DP 6658). ISO.

ISO. 1996. Meat and meat products – determination of free fatty acids. ISO, 1444: 1996 (F).

JIN, G., ZHANG, J., YU, X., ZHANG, Y., LEI, Y. and WANG, J. 2010. Lipolysis and lipid oxidation in bacon during curing and drying-ripening. Food Chem. *123*, 465–471.

JOUKI, M., YASDI, F.T., MORTAZAVI, S.A., KOOCHEKI, A. and KHAZAEI, N. 2014. Int. J. Food Microbiol. *174*, 88–97.

JULIANO, C., MATTANA, A. and USAI, M. 2000. Composition and in vitro antimicrobial activity of the essential oil of

Thymus herba-barona Loisel growing wild in Sardinia. J. Essent. Oil Res. *12*, 516–522.

KARABAGIAS, I., BADEKA, A. and KONTOMINAS, M.G. 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci. *88*, 109–116.

KARGOZARI, M., MOINI, S., BASTI, A.A., EMAM-DJOMEH, Z., GHASEMLOU, M., MARTIN, I.R., HASSAN, G., CARBONELL-BARRACHINA, Á.A. and SZUMNY, A. 2014. Development of Turkish dry-fermented sausage (sucuk) reformulated with camel meat and hump fat and evaluation of physicochemical, textural, fatty acid and volatile compound profiles during ripening. LWT – Food Sci. Technol. *59*, 849–858.

KAYAARDI, S. and GÖK, V. 2003. Effect of replacing beef fat with olive oil on quality characteristics of Turkish soudjouk (sucuk). Meat Sci. *66*, 249–257.

KOSTAKI, M., GIATRAKOU, V., SAVVAIDIS, I.N. and KONTOMINAS, M.G. 2009. Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. Food Microbiol. *26*, 475–482.

LIAROS, N.G., KATSANIDIS, E. and BLOUKAS, J.G. 2009. Effect of ripening time under vacuum and packaging film permeability on processing and quality characteristics of low fermented sausages. Meat Sci. *83*, 589–598.

LIZASO, G., CHASCO, J. and BERIAIN, J. 1999. Microbiological and biochemical changes during ripening of salchichon, a Spanish dry cured sausage. Food Microbiol. *16*, 219–228.

LORENZO, J.M. and FRANCO, D. 2012. Fat effect on physico-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage. Lipolysis, proteolysis and sensory properties. Meat Sci. *92*, 704–714.

MARTÍN-SÁNCHEZ, A.M., CHAVES-LÓPEZ, C., SENDRA, E., SAYAS, E., FENÁNDEZ-LÓPEZ, J. and PÉREZ-ÁLVAREZ, J.Á. 2011. Lipolysis, proteolysis and sensory characteristics of a Spanish fermented dry-cured meat product (salchichón) with oregano essential oil used as surface mold inhibitor. Meat Sci. *89*, 35–44.

MASTROMATTEO, M., LUCERA, A., SINIGAGLIA, M. and CORBO, M.R. 2009. Combined effects of thymol, carvacrol and temperature on the quality of non conventional poultry patties. Meat Sci. *83*, 246–254.

MATARAGAS, M., SKANDAMIS, P.N. and DROSINOS, E.H. 2008. Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. Food Microbiol. *126*, 1–12.

MOREIRA, M.R., PONCE, A.G., DEL VALLE, C.E. and ROURA, S.I. 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT – Food Sci. Technol. *38*, 565–570.

NAVARRO, J.L., NADAL, M.I., IZQUIERDO, L. and FLORES, J. 1997. Lipolysis in dry cured sausages as affected by processing conditions. Meat Sci. *45*, 161–168.

NYCHAS, G.J.E. 1995. Natural antimicrobials from plants. In *New Methods of Food Preservation* (G.W. Gould, ed.) pp. 58–89, Blackie Academic and Professional, London, U.K.

OUATTARA, B., SIMARD, R.E., HOLLEY, R.A., PIETTE, G.J.P. and BEGIN, A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. *37*, 155–162.

PEREIRA, N.R., TARLEY, C.R.T., MATSUSHITA, M. and DE SOUZA, N.E. 2000. Proximate composition and fatty acid profile in Brazilian poultry sausages. J. Food Compost. Anal. *13*, 915–920.

PEREZ-ALVAREZ, J.A., SAYES-BARBARE, M.E., FERNANDEZ-LOPEZ, J. and ARANDA-CATALA, V. 1999. Physicochemical characteristics of Spanish type dry-cured sausage. Food Res. Int. *32*, 599–607.

RADHA KRISHNAN, K., BABUSKIN, S., AZHAGU SARAVANA BABU, P., SASIKALA, M., SABINA, K., ARCHANA, G., SIVARAJAN, M. and SUKUMAR, M. 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. Int. J. Food Microbiol. *171*, 32–40.

RESSURRECCION, A.V.A. 2004. Sensory aspects of consumer choices for meat and meat products. Meat Sci. *66*, 11–20.

ROCHA-GUZMÁN, N.E., HERZON, A., GONZÁLES-LAREDO, R.F., IBARRA-PÉREZ, F.J., ZAMBRANO-GALVÁN, G. and GALLEGOS-INFANTE, J.J. 2007. Antioxidant and antimutagenic activity of phenolic compounds in three different color groups of common bean cultivars (*Phaseolus vulgaric*). Food Chem. *103*, 521–527.

RUIZ-MOYANO, S., MARTIN, A., BENITO, M.J., HERNANDEZ, A., CASQUETE, R. and CORDOBA, M.G. 2011. Application of *Lactobacillus fermentum* HL 57 and *Pediococcus acidilactici* SP979 as potential probiotics in the manufacture of traditional Iberian dry-fermented sausages. Food Microbiol. *28*, 839–845.

SANABRIA, C., MARTIN-ALVAREZ, P.J. and CARRASCOSA, A.V. 2004. Colour and moisture changes during the manufacture of Iberian dry-cured ham caused by some biotic and abiotic factors. Food Sci. Technol. Int. *10*, 269–275.

SEKIKAWA, M., KAWAMURA, T., FUJII, H., SHIMADA, K., FUKUSHIMA, M. and MIKAMI, M. 2003. Effect of arginine on growth of lactic acid bacteria for fermented sausage under high concentration of salt. Hokkaido Anim. Sci. Agric. Soc. *45*, 135–141.

SIMITZIS, P.E., DELIGEORGIS, S.G., BIZELIS, J.A., DARDAMANI, A., THEODOSIOU, I. and FEGEROS, K. 2008. Effect of dietary oregano oil supplementation on lamb meat characteristics. Meat Sci. *79*, 217–223.

SOLIGNAT, G. 1999. Drying-ripening. In *Technology of Meat and Cured Products* (P. Durand, ed.) pp. 278–391, Editions Tec & Doc, Paris.

ULTEE, A. and SMID, E.J. 2001. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. Int. J. Food Microbiol. *64*, 373–378.

VELIOGLU, Y.S., NAZZA, G., GAO, L. and OOMAH, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. *46*, 4113–4117.

WANG, B., PACE, R.D., DESSAI, A.P., BOVELL-BENJAMIN, A. and PHILLIPS, B. 2002. Modified extraction method for determining 2-thiobarbituric acid values in meat with increased specificity and simplicity. J. Food Sci. *67*, 2833–2836.

WHO. 2014. Food safety and foodborne illness. World Health Organization Fact Sheet 399, revised November 2014, Geneva.

ZAHO, L., JIN, Y., MA, C., SONG, H., LI, H., WANG, Z. and XIAO, S. 2011. Physio-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices. Meat Sci. *88*, 761–766.

ZDOLEC, N., HADZIOSMANOVIC, M., KOZACINSKI, L., CVRTILA, Z., FILIPOVIC, I., SKRIVANKO, M. and LESKOVAR, K. 2008. Microbial and physiochemical succession in fermented sausages with bacteriocinogenic culture of *Lactobacillus sakei* and semi-purified mesenterocin Y. Meat Sci. *80*, 480–487.