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EFFECT OF ROSEMARY LEAVES AND ESSENTIAL OIL ON TURKEY SAUSAGE QUALITY

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The purpose of this study was to evaluate the effect of rosemary essential oil (250–1000 ppm) or its leaves (0.5–2%) on the quality of turkey sausage. The addition of essential oil had no significant effect on the sausage texture and colour parameters. A high rosemary leaves level resulted in an increase in sausage hardness and chewiness and a decrease in lightness (L*) with respect to the control sausage. Sensory evaluation indicated that rosemary essential oil and its leaves increased the taste and the aroma scores of turkey sausage depending on the concentration. The obtained results also showed that rosemary leaves (0.5%) were more effective than essential oil in reducing total plate counts, TBARS, K_{232}, and K_{270} values during chill storage, in comparison with the control product. This will contribute to reducing the use of chemical additives, which are badly perceived by consumers, while increasing the sensory properties of such products.

Keywords: rosemary, essential oil, leaves, turkey sausage, quality

Turkey meat has been perceived and marketed as a healthy alternative to red meat due to its low fat and cholesterol contents. Large amounts of mechanically separated turkey meat can be produced and used for the manufacture of fresh sausages (Zouari et al., 2012). Sausages are present in the diet of different cultures. Generally, the shelf-life of sausage is determined by microbiological growth, colour and texture changes, and undesirable rancid flavours development (Oliveira et al., 2011). Lipid oxidation can be prevented by using various chemical additives. However, several questions about the safety of these chemicals used for food preservation were raised. In fact, they were suspected to have negative effects on consumer's health (Cassens, 1997). Thus, natural antioxidants have become highly demanded as alternatives to the chemical additives.

Plant secondary metabolites, such as essential oils and phenolic compounds of rosemary among various plants, have been widely studied for their antimicrobial and antioxidant activities, suggesting their use for food preservation. The antioxidant activities in rosemary extracts could be attributed to various phenolic compounds, such as carnosol, carnosic acid, rosmarinic acid, rosmanol, and rosemaridiphenol (Zaouali et al., 2013). Additionally, some reports have indicated that rosemary can retard lipid oxidation and prolong the shelf life of processed meat products (Liu et al., 2009). Unlike processed products made from beef and pork meat, only a few studies investigated the effect of functional ingredients on the properties of sausages made with turkey meat. Consequently, the objective of the present study is to

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investigate the effects of adding different levels of rosemary essential oil (250–1000 ppm) or its leaves (0.5–2%) on the chemical, texture, and sensory characteristics of nitrite-reduced turkey sausage, as well as determining their impact on quality during chill storage.

1. Materials and methods

1.1. Materials

Mechanically separated turkey (MST) meat, produced from turkey carcass, and turkey fat were obtained from local producer (Chahia, Tunisia). Analytical grade NaCl, $NaNO_2$ (E250), and sodium triphosphate (E451) were used. Modified starch (E1422) was from Sigma Chemical Co. (St. Louis, USA).

1.2. Essential oil extraction and analysis

Rosemary (*Rosmarinus officinalis* L.), collected from the south-eastern Tunisia, was dried in the shade until constancy of mass. After that, leaves were separated from stems and subjected to essential oil extraction by using a Clevenger-type apparatus. Essential oil composition was determined using A Hewlett-Packard 5890 series II gas chromatograph equipped with HP-5MS capillary column as previously described (Zouari et al., 2011).

1.3. Preparation of sausage

The control formulation (g/100 g sausage) consisted of: MST meat, 60; cold water, 23.6; turkey fat, 8; modified starch, 6; sodium chloride, 1.3; nitrite salt, 0.8 and sodium triphosphate 0.28 (Table 1). Nitrite salt is a mixture of sodium chloride and nitrite, and the final concentration of nitrite in the sausage is 150 ppm. In the sausage formulations (Table 1), containing different levels of rosemary essential oil (250–1000 ppm) or its ground leaves (0.5–2%), nitrite salt concentration was reduced to the half (0.4%). Sausages were prepared as described by Zouari and co-workers (2012).

Table 1. Added levels of REO and RL in turkey sausage base mix to obtain the studied formulations

Formulations (F)	REO (ppm)	RL (g/100 g)
1	0	0
2	250	0
3	500	0
4	750	0
5	1000	0
6	0	0.5
7	0	1.0
8	0	1.5
9	0	2.0

1.4. Turkey sausage analyses

1.4.1. Chemical composition and lipid oxidation. Moisture, crude protein, crude fat, and ash contents were determined according to AOAC (1995). Lipid oxidation in sausages was determined by the thiobarbituric acid reactive substances (TBARS) assay (mg malondialdehyde (MDA) equivalent/kg of sausage) and the conjugated diene as previously described (SOLDATOU et al., 2009).

1.4.2. Colour evaluation. Colour measurement parameters (Lightness (L*), redness (a*), and yellowness (b*)) of sausage samples were carried out using a Colour Flex spectrocolorimeter (Hunter Associates Laboratory Inc., USA).

1.4.3. Instrumental texture analysis. Texture analysis of sausages was done on samples, which were stored at least for 24 h at 4 °C, using a texturometer (Lloyd Instruments Ltd., UK) as described by ZOUARI et al. (2012).

1.4.4. Sensory evaluation. The sensory properties (firmness, taste, colour, aroma, and overall acceptability) were evaluated according to the method of MURRAY and co-workers (2001) by sixty panellists.

1.4.5. Microbiological analysis. Sausages were aseptically removed from the bags and 10 g of each sample were placed in a sterile bag containing 90 ml of sterile peptone (0.1%) water with 0.85% NaCl. After 1 min in a stomacher blender, appropriate decimal dilutions were prepared and pour-plated (1 ml) with Plate Count Agar (PCA) for the total count of mesophilic aerobic bacteria (30 °C for 48 h). All microbial counts were converted to logarithms of colony-forming units per gram of sample (log CFU g^{-1}).

1.5. Statistical analysis

All analytical determinations were performed in triplicate. One-way analysis of variance was conducted using SPSS software, 17.0. A difference was considered statistically significant when $P \leq 0.05$.

2. Results and discussion

2.1. Essential oil composition

The rosemary essential oil (REO) used in the present study was extracted by hydrodistillation from the dried leaves of *R. officinalis*. The average yield of oil was found to be 1.75% (v/w). The analysis of REO resulted in the identification of nineteen compounds accounting for 97.12% of the total oil. The REO was dominated by 1,8-cineole (27.42%), camphor (19.57%), camphene (14.58%), α-pinene (9.35%), borneol (6.71%), α-terpineol (5.36%), and bornyl acetate (3.70%) (data not shown). ZAOULI and co-workers (2013) showed that 1,8-cineole (35.8%), camphor (13.6%), and borneol (11.9%) were the main compounds of the essential oil of rosemary leaves from north-eastern Tunisia.

2.2. Chemical composition and instrumental analysis

The contents of moisture, crude protein, crude fat, and ash of the MST meat, which is the main component of sausages, were 72%, 13%, 8%, and 5%, respectively. Additionally, the contents of moisture, crude protein, crude fat, and ash of the formulated sausages were 67.9–69.6%, 8.26–8.68%, 9.70–10.87%, and 5.08–5.2%, respectively. The chemical composition showed no significant differences (P≥0.05) between formulations (data not shown). Table 2 shows that addition of REO had no significant effect (P≥0.05) either on the sausage texture or on the colour parameters measured in the formulations with respect to the control. Nevertheless, significant differences (P≤0.05) were found in textural parameters within the formulations containing rosemary leaves (RL) (Table 2). High level of RL resulted in lower elastic structure (F9) in comparison to the control sausage (F1). Moreover, F9 showed the highest hardness and chewiness values (Table 2). Addition of RL allowed an increase of sausage hardness with a dose dependant manner, which can be explained by the effect of fibres that strengthen the product structure. Previous studies reported an increase in hardness of meat sausage with added cereal or dietary fibre (García et al., 2002). The addition of RL also showed a significant effect (P≤0.05) on the lightness (L*) of the sausage (Table 2). The increased amount of RL added into turkey sausage reduced the L* value, which is in accordance with the results obtained by Liu and co-workers (2009). Sausage lightness seems to be the most informative parameter for colour changes in meat and meat products. The decrease of L* values can be explained by the dark green colour of RL. Moreover, a possible browning reaction occurring in the added RL may also contribute to this colour change. In fact, polyphenol oxidases presented in the plant tissues were able to oxidize phenolic compounds to corresponding quinones, which then condensed to dark compounds (Dogan & Dogan, 2004).

Table 2. Experimental results of textural and colour parameters for turkey sausages formulated with different levels of REO or RL

Formulations (F)	Hardness (N)	Elasticity (mm)	Chewiness (Nmm)	L*	a*	b*
1	5.61±0.43[b]	8.10±0.59[a]	22.65±0.75[c]	57.27±1.21[a]	14.14±0.60 [d]	11.82±0.60[a]
2	5.16±0.55[b]	8.91±0.43[a]	22.08±0.68[c]	60.01±1.40[a]	13.35±0.40[de]	10.87±0.41[a]
3	5.57±0.62[b]	8.86±0.66[a]	22.09±0.74[c]	59.78±1.50[a]	13.53±0.14[d]	11.01±0.73[a]
4	5.94±0.85[b]	8.52±1.62[a]	23.04±2.63[c]	62.14±3.10[a]	13.71±0.60[d]	10.94±0.36[a]
5	5.94±0.91[b]	8.09±0.31[a]	22.16±1.56[c]	60.74±1.20[a]	13.36±0.20[d]	10.75±0.29[a]
6	6.13±0.62[b]	8.15±0.66[a]	23.51±1.44[c]	57.12±0.47[a]	13.35±0.40[d]	10.59±0.23[a]
7	7.54±0.40[ab]	8.0±1.01[a]	27.73±1.69[b]	55.13±1.02[b]	12.99±0.20[b]	10.54±0.20[a]
8	7.57±0.32[a]	7.51±0.45[a]	28.86±1.10[b]	52.53±0.61[c]	11.95±0.10[c]	10.78±0.34[a]
9	7.96±0.20[a]	7.21±0.25[ab]	31.46±1.51[a]	28.02±0.70[d]	10.67±0.10[a]	10.94±0.63[a]

Values followed by the same letter in the same column showed no statistically significant differences (P≤0.05).

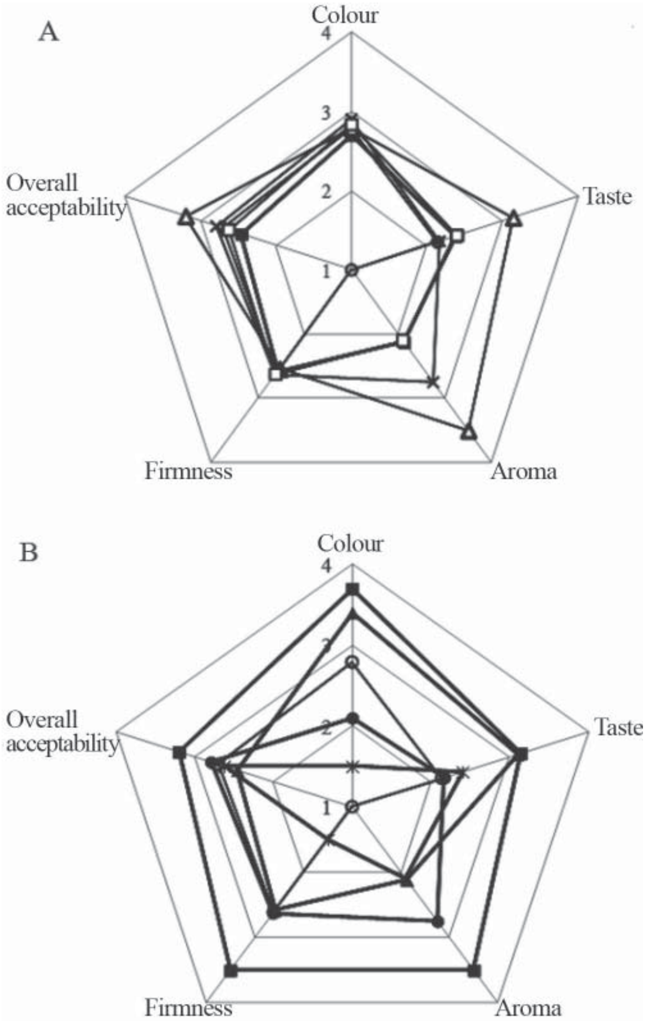

Fig. 1. Sensory scores of sausages formulated with different levels of A) REO: F1: —○—; F2: —■—; F3: —△—; F4: —×—; and F5: —□—; B) RL: F1: —○—; F6: —■—; F7: —▲—; F8: —●—; and F9 —✳—

2.3. Sensory evaluation

Sensory analysis of formulated sausages was carried out by checking colour, aroma, taste, firmness, and overall acceptability (Fig. 1). Sensory evaluation showed that REO had no effect on the firmness and the colour, which is in concordance with the instrumental analyses. However, the sausages containing REO displayed an increase of sensory attribute (aroma and taste) scores compared to the control sausage (Fig. 1A). Aroma is an important factor influencing the eating quality of meat and consumer decisions. Therefore, the lowest aroma score observed for the control sausage can be explained by the presence of turkey fat, which is known by its high susceptibility to oxidation and it causes changes in these attributes. F3 showed the highest score for overall acceptability, which can be explained by the specific volatiles identified in REO. Results of this study also showed that incorporation of RL improved all sensory attributes for turkey sausage (Fig. 1B). F6 presented the highest scores for all attributes, while high level addition of RL (more than 0.5%) decreased sensory preference due to a too condensed aroma. Liu and co-workers (2009) demonstrated that some volatiles, such as alcohols (1,8-cineole, α-terpineol, myrtenol, and *p*-cymen-8-ol), ketones (acetone and camphor), one of the ether (anethole), alkenes (α-pinene, camphene, β-pinene, myrcene, and verbenene), acids (formic acid and acetic acid), esters (ethyl acetate and methyl eugenol), and phenols (estragol, thymol, and carvacrol), were identified from the fresh chicken sausage containing 1500 ppm of RL. Addition of REO or RL modified the organoleptic properties of turkey sausage depending on the concentration. Therefore, F3 (500 ppm essential oil) and F6 (0.5% leaves), which displayed the highest scores for the overall acceptability, were selected to study their stability during chill storage.

2.4. Stability of sausages

In the new formulated sausages, it was chosen to reduce the nitrite salt concentration to half compared to the control sausage. In fact, nitrite was the main ingredient responsible for the production of the characteristic pink colour of cured meats. Additionally, nitrite inhibited lipid oxidation, exerted antibacterial activity, and contributed to desirable flavour of meat products (Martin, 2001). However, nitrite was suspected to have negative effects on consumer's health (Cassens, 1997). Therefore, the objective of this part is to determine the effects of REO or RL on lipid and microbiological stability during storage at 4 °C for 30 days.

2.4.1. Prevention of lipid oxidation in meat sausage system. Lipid oxidation represents a major cause of meat quality deterioration during chill storage. In the present study, fat oxidation was evaluated on F1, F3, and F6. Thus, TBARS, K_{232}, and K_{270} were chosen as markers for lipid oxidation (Fig. 2). Results showed that TBARS and specific extinctions coefficients of F1 increased during storage period. Reduction of TBARS-value in the control sausage after 15 days of storage can be due to the decomposition of malondialdehyde. Interestingly, addition of REO (F3) or RL (F6) brought about a reduction of TBARS and specific extinctions values, which remained lower than those corresponding to the control ($P \leq 0.05$). One can notice that RL were more effective than REO in reducing lipid oxidation during storage period. Indeed, contrary to REO, which is essentially composed of monoterpenes, it was reported that RL were characterized by high amounts of phenolic compounds and condensed tannins. Thus, the high antioxidant activity previously mentioned for RL extracts was attributed to their high phenolic content (37.4 mg gallic acid equivalent/g dry mass), such as rosmarinic acid, carnosic acid, and carnosol (Zaouali et al., 2013). These results suggested that addition of 0.5% RL, characterized by their high antioxidant efficiency, improved the oxidative stability of turkey sausage during chill storage.

2.4.2. Microbial analysis. Table 3 shows that the total plate counts of all sausage samples increased during chill storage. Control sausage (F1) and that with REO (F3) had similar total plate counts. However, sausage with RL (F6) had comparatively lower microbial counts. In fact, at day 30, the total plate counts of sausage containing 0.5% of RL was 3.32±0.22 log CFU g^{-1}, which is significantly ($P \leq 0.05$) lower than that of the control (4.41±0.32 log CFU g^{-1}). It is worthy to note that although nitrite concentration was reduced to the half, RL were more effective than REO in reducing total flora, which can be explained by their high total phenolic content. Such antimicrobial effect on the chicken and pork sausages due to the addition of rosemary was also previously reported (Liu et al., 2009). Some non-polar components such as phenolic diterpenes, which were isolated from rosemary, were indicated to be responsible for its antimicrobial properties (Fernández-Lopez et al., 2005).

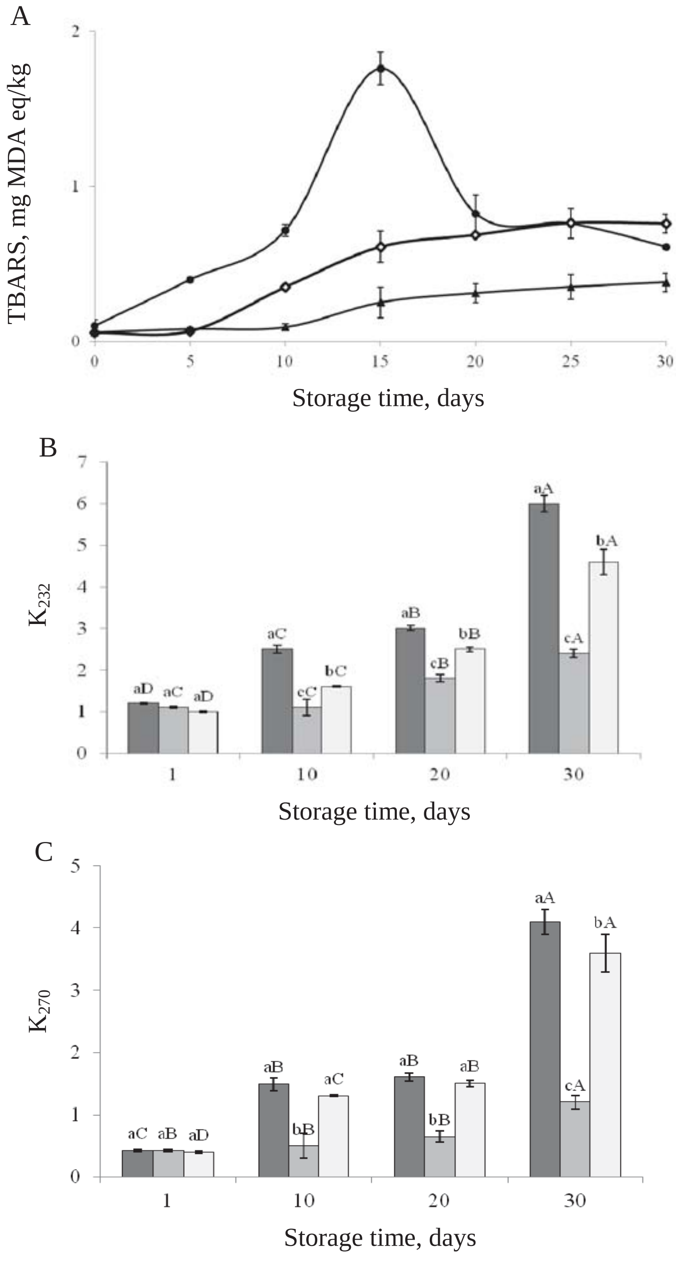

Fig. 2. Lipid oxidation during sausage storage at 4 °C: Changes in TBARS (A), K_{232} (B), and K_{270} (C). Different lower case letters were used at the same time of storage and indicated significant differences between samples. Different capital letters used within different time of storage indicated significant differences between samples ($P \leq 0.05$) A): F1: —●—; F3: —◇—; and F6: —▲—; B, C): F1: ■; F3: □; and F6: ■

Table 3. Total plate counts of microorganism in turkey sausages formulated with REO (500 ppm) or RL (0.5%) during storage at 4 °C.

	Total microorganism, log (CFU g^{-1})			
Storage, days	1	10	20	30
Formulation 1	$3.44{\pm}0.15^{cA}$	$3.54{\pm}0.21^{bA}$	$3.76{\pm}0.41^{bA}$	$4.41{\pm}0.32^{aA}$
Formulation 3	$3.01{\pm}0.10^{dB}$	$3.32{\pm}0.05^{cA}$	$4.25{\pm}0.21^{bA}$	$4.43{\pm}0.10^{aA}$
Formulation 6	$2.04{\pm}0.20^{cC}$	$2.54{\pm}0.24^{baB}$	$3.00{\pm}0.10^{aB}$	$3.32{\pm}0.22^{aB}$

Different lower case letters in the same line within different time of storage indicate significant differences. Different capital letters in the same column within different formulations indicate significant differences ($P \leq 0.05$).

3. Conclusions

The obtained results showed that the use of rosemary leaves as functional and natural ingredient of the mechanically separated turkey meat sausage did not alter the colour and texture properties. More importantly, these data suggested the potential use of savoury rosemary leaves, while reducing the chemical additive nitrite, in order to improve the oxidative and microbiological stability of turkey sausage. This fact pertains to current market trends and consumers' requests for natural products.

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