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Dietary incorporation of distillated rosemary (*Rosmarinus officinalis* L.) leaves in ewe diets: effects on dry sausages quality

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Abstract – The aim of this work was to study the effect of dietary incorporation of distillated rosemary (*Rosmarinus officinalis* L.) leaves (DRL) on physical, microbiological and sensorial characteristics of a traditional dry sausage. Fourteen Barbarine ewes were randomly assigned into two homogeneous groups. Ewes of the Control group (C) received daily 850 g oat hay and 350 g commercial concentrate. The diet of the experimental group (DRL) was modified via substitution of the hay, at the level of 17%, with DRL. After slaughter, the sausage batter (80 % of shoulders muscles, 20 % fat tail and 4 % salt) was prepared and dried. During a natural drying (days 0, 2, 4 and 6), one sausage from each batch was taken for analysis. Lightens (L*) values decreased significantly during the drying period ($p < 0.05$); the highest value of L* were recorded for DRL samples without significant difference with C samples. Similarly, redness (a*) values decreased significantly with the drying time; it ranged from 12.3 to 8.6 and from 10.4 to 9.7 for C and DRL samples, respectively. However, DRL administration did not present significant effects on this parameter. The yellowness (b*) decreased during drying and was affected significantly ($P < 0.05$) by DRL incorporation at day 0 (14.9 vs. 12.6 for C and DRL samples, respectively). The results shows that the chroma (C*) indices were similar between groups except at day 0 (19.5 v 16.5 for C and DRL groups respectively; $P < 0.05$). The Total Viable Counts were affected ($P < 0.05$) by the rosemary incorporation in the ewe diet, they were lower for DRL sausage. Sausages' eating quality was not affected by DRL administration. DRL use as animal feed and meat quality preservative should be encouraged.

Keywords: distillated rosemary leaves; dry ewe sausage; microbiological quality; physicochemical quality; eating quality.

1. Introduction

Facing climate changes and nutrient shortages, small ruminants production systems based on pasture, became dependent on expensive feed supplements and high concentrate diet (Atti et al., 2003). This is why farmers mostly undernourished low productive animals such as older ewes (Bhatt et al., 2012). Ewes reaching the end of their productive life cycle are usually sold at low prices due to their poor live weight and low carcass yield. In this context, the valuation of this type of meat can have a promising nutritional tool (Bhatt et al., 2012). This type of meat can be sold fresh or processed into charcuterie. Dry ewe sausages are one of the oldest known traditional meat products in Tunisia; they are dried at ambient temperature until reaching the desired dehydration, which can alter their quality by reducing their shelf life.

The demand for natural foods has been increasing actually; furthermore, meat industry products with lower nitrites and salt content are needed in order to provide healthy natural products (Garcia -Lopez at al., 1998). Moreover, species such as rosemary, thyme, oregano can be used as natural preservatives given their bacteriostatic and antioxidant properties (Smeti et al., 2013; Yagoubi et al., 2018).

In this context, distillated rosemary (*Rosmarinus officinalis* L.) leaves (DRL), a source of bioactive components including polyphenols, were incorporated in sheep diet resulting in higher growth performances and extended meat shelf life (Nieto et al., 2010; Yagoubi et al., 2018). Therefore, the aim of the present study was to determine the effect of DRL incorporation in cull Barbarine ewe's diet on the, microbial alteration, color stability and sensorial quality of their dry sausage.

2. Materials and methods

This experiment was conducted at the National Institute of Agricultural Research of Tunisia (INRAT) and the National Institute of Agricultural of Tunisia (INAT). All the research methods employed in this study meet ethical guidelines and also adhere to Tunisian legal requirements and the Halal slaughtering procedure.

2.1. Animals and diets

Fourteen Barbarine fat-tail ewes were randomly assigned on the basis of their age, weight and body condition into two homogeneous groups. Sheep in the Control group (C) received daily a basal diet consisting of 850 g oat hay and 350 g commercial concentrate. The diet of the experimental group (DRL) was modified via substitution of the hay, at the level of 17%, with DRL which were collected from Ouesslatia, (semi-arid bioclimatic zone of Tunisia) in September after the distillation season. Animals were fed diets for 60 days and then slaughtered at body weight of 43 ± 3 kg.

2.2. Meat sampling and sausages preparation

Cold carcasses (24 h after slaughter) were cut into leg, lumbar region, flank, thoracic region, neck, and shoulder, following the procedures of Colomer-Rocher et al. (1972). Shoulders muscles were separated and conserved at –20°C until sausages preparation and analysis.

The sausage batter was prepared according to the following formulation: 80 % muscle, 20 % fat tail and 4 % salt (Kamy, Tunisia). Muscle and fat tail were minced and mixed in a rotating bowl meat cutter (Rowenta, Universo, Germany).

Subsequently, the sausage mixtures were manually stuffed into natural sheep casings (20 cm of length and about 4 cm of diameter) and then dried in ambient temperature for 6 days. At each sampling period (Days 0, 2, 4 and 6) two sausages from each batch were taken for pH and color determination and microbiological analysis. Sausages from each batch were taken at day 6 for chemical composition and sensory analysis.

2.3. Sausages color and pH analysis

The pH was measured on sausages with a penetrating electrode connected to a portable pH-meter (HANNA instruments, Romania) after calibration with two buffers (7.00 and 4.01). A Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) was used to measure color directly on the sausage' surface. Each sample was measured twice and then averaged. Color coordinates were calculated in the CIELAB space (CIE, 1986). The lightness (L*), redness (a*) and yellowness (b*) parameters were recorded (Hunt et al., 1991); hue angle (H*) and chroma (C*) indices were calculated as $H^* = \tan^{-1}(b^*/a^*) \times 57.29$, expressed in degrees and $C^* = (a^{*2}{}_+ b^{*2})^{1/2}$. H* is the attribute of a color perception denoted by blue, green, yellow, red, purple, etc; C* is related to the quantity of pigments and high values represent a more vivid color and denote lack of greyness (Miltenburg et al, 1992).

2.4. Microbiological analysis

For microbiological analysis, 10 g of each sample of sausage batch were collected aseptically, transferred into a sterile plastic bag and were homogenized with 90 ml of peptone water (Pronadisa, Spain) using a Stomacher 80 Biomaster. Serial dilutions were then made. Total viable counts (TVC) were determined on Plat Count Agar incubating at 30°C for 24 h; lactic acid bacteria (LAB**)** were counted on MRS agar (Pronadisa, Spain) after 48 h of incubation at 30°C; staphylococci on Mannitol Salt Agar (Pronadisa, Spain) after 37 h of incubation at 37°C; yeasts and molds on Sabouraud Agar (Pronadisa, Spain) after 5 days of incubation at 25°C and *Enterobacteriacea* on Violet Red Bile Glucose Agar (Pronadisa, Spain) after 24 h at 37°C (Guiraud, 1998).

2.5. Chemical composition

Samples of sausage were dried by lyophilisation; samples of dry matter (DM) were ground (1 mm screen) and used for subsequent analyses. Nitrogen was determined by Kjeldahl method (ID 942.01) and mineral content was determined by aching at 600°C for 8h (ID 942.05) according to AOAC (1990).

2.6. Sensory analysis

For sensory analysis, samples of sausage were roasted in aluminum paper in a pre-heated oven at 180ºC. Each sample was cut into pieces of 1x1 cm and each piece was coded and then served in random order for testing by 18 panelists to evaluate each sample for color intensity odor, tenderness and flavor using a ten-point scale (1, minimum; 10, maximum). Bread and water were provided for panelists to freshen the mouths between each two samples.

2.7. Statistical analysis

Statistical analyses were performed with SAS (2002). For microbiological analysis and instrumental meat color and pH variables, a repeated-measures ANOVA was carried out using the MIXED procedure of SAS. The model included rosemary incorporation, drying period and interactions among them as fixed effects. Then, the test Duncan was used to compare rosemary incorporation and drying period effects. Another ANOVA with the rosemary incorporation as the fixed effect was performed for sausage chemical composition and sensory evaluation, using the GLM procedure of SAS. The level of significance was set at 0.05 and trends were discussed for p-values comprised between 0.05 and 0.10.

3. Results and discussion

3.1. Dry ewe sausage chemical composition

the rosemary incorporation in ewe's diet did not affect the chemical composition of dry sausages (Table 1) made from Barbarine ewe's meat ($P < 0.05$). The dry matter of sausages was in the range of 85 %. The amount of protein found in this product (40 % DM) is in agreement with those of Kovačević et al. (2010) who showed that the protein content of traditional dry sausages ranged between 26 and 53%. The absence of difference in the chemical composition of sausage is related to the iso-energetic and iso-proteic composition of ewes' diet.

3.2. Sensory analysis

The effects of DRL administration on the sensory attributes of dry sausages are shown in table 2. Sausages' color intensity was significantly higher ($P = 0.02$) for the control group (6.1 vs. 5.4 for C and DRL groups, respectively). However, sausages' odor, flavor and tenderness were similar between groups ($P > 0.05$) and DRL administration did not affect these parameters.

Similarly, the exogenous addition of rosemary extract did not affect the odor or flavor of cooked beef slices (O'Grady et al., 2006). Results suggest that rosemary byproducts may have benefic effects on the sensory quality of lambs' meat through its protective role on proteins against oxidation (Balentine, et al., 2006; Djenane et al., 2003). However, the small quantity of DRL used in the present study can explain the lack of effects on sensory attributes of dry sausages.

3.3. Sausages colour and pH analysis

Table 3 shows the effects of DRL feeding and drying period on CIELab color and pH in sheep meat sausages. The interactions between DRL incorporation and drying period did not show any significant effect on colour and pH, thus they are not reported in Table 3. The incorporation of DRL did not affect the pH values for all days of drying. For Control and experimental samples, the pH increased between day 0 and day 2 then decreased to reach a value of 5.34 and 5.23, respectively on day 6 of drying ($P < 0.05$). t The L* values decreased significantly during the drying period ($p < 0.05$). However, throughout the drying period the highest value of L* were recorded for DRL samples without significant difference with C samples. It was reported that the brightness is correlated with the surface state of the meat and the loss of clarity could be attributed to the drying time (Kovacevic et al., 2010). Moreover, it has been reported that an increase in L* value may be related to an increase in metmyoglobin formation (Anton, et al., 1993).

Redness (a*) values decreased significantly with the drying time; it ranged from 12.33 to 8.61 and from 10.40 to 9.69 for C and DRL samples, respectively. However, DRL administration did not present significant effects on this parameter. These results agree with those of Nieto et al., (2010), working on DRL effects on ewes meat, who found that a* values decreased during chilled storage without significant effects of DRL addition throughout the storage.

Coordinate b* (yellowness) decreased during drying (14.88 to 11.73 and 12.55 to 11.21 for C and DRL samples, respectively) and was significantly affected by DRL ($P < 0.05$) with differences between C and

DRL samples appearing at day 0 (14.88 vs. 12.55 for C and DRL samples, respectively). So DRL incorporated in the ewes' diet delayed the appearance of these yellowish tones, usually associated to oxidation and deterioration.

The results show that at day 0, the degree of saturation differed significantly between groups ($P < 0.05$), the Control samples exhibited the highest C* value (19.53) against 16.46 for DRL samples. Subsequently, the degree of saturation drop throughout the drying period for all samples; but this drop was less important for DRL groups (from 16.46 at day 0 to 15.51 at day 6) compared with control samples (from 19.53 at day 0 to 14.65 at day 6) which confirms the effectiveness of rosemary by-products against myoglobin oxidation (Camo et al., 2008).

3.4. Microbiological analysis

Table 4 shows the effects of DRL feeding and drying time on TVC, total and fecal coliforms, LAB and mould and yeasts. The interaction between DRL incorporation and drying period did not show any significant effect on Microbiological profile, thus they are not reported in Table 4. TVC were affected ($P < 0.05$) by the rosemary incorporation in the ewe diet. Since the initial day until the end of drying period, TVC was higher for C than RDL sausages confirming the antibacterial power of rosemary by-products Many authors showed that administration of rosemary in ewe diet affect significantly the microbiological quality of the lamb meat (Nieto et al., 2010; Ortuno., et al., 2015). By contrast, Ismail et al., (2001) observed that addition of rosemary herbs decoctions had no effect on the microorganisms found in raw chiken. On the other hand, many studies confirmed the antimicrobial effect of rosemary, widely demonstrated when added directly to meat and meat products (Ahn et al., 2007; Georgantelis et al., 2007, Jung et al., 2015; Essid et al., *in press*).

The initial TVC were less than 4 log CFU/g and they augment to reached 5.5 log CFU/g at the 4^{th} day of drying of the two types of sausages. These values were lower than 5.69 log CFU/g, the threshold of European legislation (Regulation EC 2073/2005). At the end of drying period (day 6) the TVC were 6.45 and 6.08 log CFU/g for C and RDL, respectively. Our results are still lower than that of Jung et al. (2015) who found that TVC in dry sausages were about 7 log CFU/g. The LAB increased from 2 to 3log CFU/g during drying of sausages ($P<0.05$), this result confirms the good adaptation of LAB to the meat environment and their faster growth rates during fermentation and ripening of sausages (Zdolec et al., 2008; Zhao et al., 2011). Initial counts of total and fecal coliforms were similar for both types of sausages (3 log CFU/g). Counts of coliforms increased during drying but did not exceed the standard value of 3.69 log CFU/g for both types of sausages showing the hygienic conditions of sausage Treatment. The count of coliforms depends on the hygienic quality of the raw materials and the handling conditions during processing (Casquete et al., 2012). They are considered useful indicators of post-processing contamination. Yeast and molds appear from the second day of drying and their numbers keep low when compared to other groups of microorganisms. This evolution compare well with most studies on fermented sausages (Drosinos et al., 2005; Fernandez-Lopez et al., 2008). Capita et al. (2006) reported that molds and yeasts in dry fermented sausages are frequently found in low numbers when compared with other microbial groups.

4. Conclusion

The effect of dietary incorporation of distillated rosemary (*Rosmarinus officinalis* L.) leaves on the microbiological, physico-chemical and sensory qualities of dry ewe sausages was investigated. The results showed that rosemary incorporation did not affect sensorial parameters and chemical composition. However, it delays the appearance of the yellowish tones, usually associated to oxidation and improved the microbial statue of sausage by reducing the TVC. Thus, the use of this available by-product (DRL) should be encouraged to reduce animal feed cost and improve meat product quality.

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