PAPER

EFFECT OF ROSEMARY POWDER ON THE QUALITY OF DRY EWE SAUSAGES

I. ESSID*[1], S. SMETI[2] and N. ATTI[2]
[1]Department of Animal Resources, Fisheries and Food Technologies, Research unity UR-17AGR01, National Agronomic Institute of Tunisia, 43 Avenue Charles Nicole, Carthage University, Tunisia
[2]Animal and Forage Productions, National Institute of Agronomic Research of Tunisia, Carthage University, Tunisia
*E-mail address: essidiness@gmail.com

ABSTRACT

The aim of this study was to evaluate the effect of rosemary powder on the quality of dry ewe sausages. Color parameters (L, a* and b*), pH and microbiological profile were evaluated over the 6-day drying period. Sausages with 4 % rosemary powder added showed the lowest values of total viable counts and total coliforms. pH values of dry ewe sausages with rosemary powder decreased significantly during drying. Redness (a*) decreased significantly during the drying of control dry sausages against a stability in sausages added with rosemary powder. Finally, incorporating rosemary significantly affected dry sausages acceptability.

Keywords: dry ewe sausage, microbiological quality, physicochemical quality, rosemary powder, sensory quality

1. INTRODUCTION

Many countries have meat products whose tradition people wish to preserve as part of their history and culture. Dry ewe sausages are one of the oldest known traditional meat products in Tunisia. In the past, they were often homemade for religious celebrations. Nowadays, most dry ewe sausages are produced all year round both in butcher's shops and sausage manufacturing companies. The ewe meat and fat are finely ground and seasoned with salt and other spices. Chemical additives are not used. The mixture is then stuffed into natural casings. Sausages are dried naturally at ambient temperature until reaching the desired dehydration, and they are usually consumed without cooking. Despite, their short shelf life (not exceeding 2 weeks at 4°C), traditional dry cured ewe sausages are considered safe due to several factors such as reduced water activity and the addition of salt and spices. Sensorial attributes such as color and texture are linked to drying conditions (temperature and relative humidity).

Rosemary (*Rosmarinus officinalis L.*) is one of the most well-known Mediterranean *Lamiaceae*. Like other aromatic herbs and spices used in Mediterranean cuisine, it is used not only to improve or modify the flavor of foods, but also for its antioxidant and antimicrobial effects (LIU *et al*., 2009). The antioxidant activity of rosemary has been related with the presence of some phenolic diterpenes such as carnosic acid and carnosol (PIZZOCARO *et al*., 1994; GEORGANTELIS *et al*., 2007). In addition, several authors have reported that some compounds present in rosemary extract possess antimicrobial effects (DEL CAMPO *et al*., 2000; MCCARTHY *et al*., 2001; BALENTINE *et al*., 2006; LUND *et al*., 2007).

The widespread availability of the rosemary plant in Tunisia makes it easy to use as a preservative in meat products. Many studies have documented the effects of rosemary essential oils on the quality of meat products (STEPHANIE *et al*., 2002; MIELNIK *et al*., 2003; AHN *et al*., 2007; GARCÍA-DIEZ *et al*., 2016); however, few have focused on the effects of rosemary powder.

The aim of this work was to study the effect of rosemary powder on physicochemical, microbiological and sensorial characteristics of traditional dry ewe sausage.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of *Rosmarinus officinalis* L. were collected from Ouesslatia, (semi-arid bioclimatic zone of Tunisia) in June. Specimens of the plant were submitted to the herbarium division of the National Institute of Research on Rural Engineering, Water and Forests where identification was confirmed in the Laboratory of Forest Ecology. Rosemary leaves were dried in an oven (Ecocell Drying Oven, MMM Med center, Germany) at 60°C, ground with a grinder (Moulinex, France) and then stored in a moisture-proof container until use.

2.2. Meat sampling and sausage preparation

The meat used was taken from adult fat-tail Barbarine ewes. Ewes were fed oat hay and concentrate until slaughter at 48 kg. The carcasses were cut into leg, lumbar region, flank, thoracic region, neck, and shoulder following the procedure of COLOMER-ROCHER *et al*. (1972). The shoulders were dissected into muscle, fat and bone; the muscles were conserved at -20°C until sausage preparation.

The sausage mixture was prepared according to the following formulation: 80 % muscle, 20 % tail fat, 3 % salt and 1 % paprika (Kamy, Tunisia). Muscle and tail fat were minced and mixed in a rotating bowl meat cutter (Rowenta, Universo, Germany). The sausage mixture was divided into 3 batches. To the first and the second batches, 2 and 4 % (levels are results of sensorial analysis) of rosemary powder were added, respectively, corresponding to the samples labeled RP2 and RP4. The third batch, without rosemary powder, acted as a control (C).

Natural salted casings were purchased from a local market in Tunis and soaked in water prior to use. Subsequently, the sausage mixture was manually stuffed into casings (20 cm of length and about 4 cm of diameter) at approximately 25 g each and then dried at ambient temperature for 6 days. Relative humidity and temperature averaged 70 % and 18°C respectively. For sampling, five sausages were taken from each batch at days 0, 3 and 6 of drying for pH, color determination and microbiological analysis. Chemical composition and sensorial analysis were done after 6 days of drying.

2.3. Sausage 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 4.00).

A Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) was used to measure color directly on the surface. Color coordinates were calculated in the CIELAB space (CIE, 1986). The lightness (L*), redness (a*) and yellowness (b*) parameters were directly recorded (Hunt *et al.*, 1991). The 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 the microbiological analysis, 10 g of meat were collected aseptically from the center of each sausage, and diluted with 90 ml of sterile peptone water (Accumix, Belgium) using a Stomacher 80 Biomaster. Serial 10-fold dilutions were prepared in sterile peptone water. Appropriate dilution samples (1 or 0.1 ml) were poured or spread in duplicate on different growth media. Total viable counts were determined on Plat Count Agar (PCA) (Accumix, Belgium) after 48 h of incubation at 30°C; yeasts and molds on Sabouraud Agar (Accumix, Belgium) after 5 days of incubation at 25°C and coliforms on Desoxycholate Agar (Accumix, Belgium) after 24 h at 37°C for total coliforms and 44 °C for fecal coliforms (Guiraud, 1998).

2.5. Chemical composition

Sausage samples were dried by lyophilization (CHRIST, BETA 1-8 LD plus); samples of dry matter were ground (1 mm screen) and used for subsequent analyses. Total protein was determined using the Kjeldahl method (ID 942.01) and ash content was determined by ashing at 600°C for 8 h (ID 942.05) according to AOAC (1990). Water activity (a_w) of the sausage samples was determined at 25°C using a Thermoconstanter A_w Sprint Novasina TH500 (Switzerland).

2.6. Sensory analysis

After 6 days of drying, sausages were cooled at room temperature, then cut into 1x1 cm pieces. Each piece was then coded and served in random order to a sensory panel, which consisted of 13 panelists with experience in sensorial evaluation. Each parameter in the sensorial analysis was evaluated on a scale of 1 to 9 (1- low intensity; 9- high intensity). Color, odor, flavor, tenderness and overall acceptance were scored; then, mean values were calculated for each parameter.

2.7. Statistical analyses

Each parameter was measured three times and then averaged. All data were analyzed using a General Linear Model ANOVA with treatment (rosemary powder incorporation) and time as factors. Duncan's multiple range test was used to determine any significant difference between mean values, and evaluations were based on a significance level of $P < 0.05$. All statistical analyses were done using SAS (2002) Version 8.2.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

Moisture, protein and ash contents of the ewe sausages after drying are presented in Table 1. Results showed that incorporating rosemary powder significantly increased ($P < 0.05$) moisture in the traditional ewe sausages. Our results are in agreement with of those of JUNG *et al*. (2015). Indeed, rosemary powder appeared to improve water retention due to its antioxidant activity (ESTEVEZ and CAVA, 2006). The antioxidant activity of rosemary powder for protein degradation moderated the loss of sulfhydryl groups and the generation of carbonyl compounds and then maintained water holding ability (JUNG *et al*., 2015). Other studies have reported that moisture of dried sausages could be affected by both processing method and processing time (DALMIS and SOYER, 2008). The addition of rosemary powder did not affect protein and ash content. The protein content of dried ewe sausages was the same as that found by KOVAČEVIĆ *et al*. (2010) who showed that protein content of traditional dry sausages ranged between 26 and 53%. Water activity (Table 1), an important factor for microorganism growth, was not affected by the addition of rosemary powder, which contrasted with the results found by JUNG *et al*. (2015).

Table 1. Effect of rosemary powder incorporation on chemical composition of dry ewe sausages.

Chemical composition	C	RP2	RP4	S.E.M	P-Value
Moisture (%)	15.7	18.5	20.7	1.22	0.001
Protein (% DM)	39.7	40.4	41.2	5.48	0.977
Ash (% DM)	7.8	7.7	8.3	0.47	0.590
Aw	0.75	0.72	0.78	0.01	0.450

C: control sausage; RP2: sausage added with 2 % of rosemary powder; RP4: sausage added with 4 % of rosemary powder; S.E.M: standard error of means.

3.2. Microbiological analysis and pH

Results of the microbiological analysis of the sausages during drying are presented in Table 2. Counts of total viable and coliform increased significantly during drying and were significantly affected (P <0.05) by the addition of rosemary powder. At the end of the drying period, sausages with 4 % rosemary powder added showed the lowest value of total viable counts (4.17 log cfu/g) and total coliforms (2.18 log cfu/g). Since there is no significant difference in water activity values between samples of dry ewe sausages, this result confirmed the antimicrobial effect of rosemary, widely seen when added directly to meat and meat products (AHN *et al*., 2007; ANGIONI *et al*., 2004; GEORGANTELIS *et al*., 2007; JUNG *et al*., 2015). The antimicrobial properties of rosemary, especially on Gram positive bacteria are related to some non-polar components such as phenolic diterpenes. Furthermore, phenolic diterpenes could inhibit Gram negative bacteria when in combination with factors which can disturb cell membrane permeability and/or integrity such as pH values and NaCl concentrations (Liu *et al*., 2009).
Yeast, mold and fecal coliform counts were not affected either by the drying period or by the addition of rosemary powder.

Table 2. Populations of microbial groups (cfu/g) during the drying of traditional ewe sausages.

	Batch	Days of drying		
		0	3	6
Total Viable Counts	C	4.39±0.352^{aX}	5.35±0.387^{aX}	6.07±0.889^{bX}
	RP2	3.98±0.18^{aY}	4.78±0.94^{bY}	5.38±0.98^{cY}
	RP4	3.77±0.27^{aY}	3.74±0.47^{aY}	4.17±0.95^{bY}
Molds & yeasts	C	3.38±0.74^{aX}	4.14±0.89^{aX}	5.27±0.72^{bX}
	RP2	3.58±0.83^{aX}	5.37±0.74^{bY}	5.36±0.98^{bX}
	RP4	3.26±0.46$^{a X}$	3.51±0.85^{aX}	5.22±0.65^{bX}
Total coliforms	C	3.81±0.32^{aX}	4.03±0.24^{abX}	4.31±0.85^{bX}
	RP2	4.29±0.46^{aY}	3.23±0.63^{bY}	3.11±0.51^{bY}
	RP4	4.16±0.59^{aY}	2.43±0.20^{aZ}	2.18±0.76^{bZ}
Fecal coliforms	C	2.61±0.68^{aX}	2.37±0.99^{aX}	2.44±0.51^{aX}
	RP2	2.81±0.72^{aX}	2.74±0.62^{aX}	2.95±0.30^{aY}
	RP4	2.38±0.23^{aX}	2.12±0.37^{aX}	2±0.56^{aX}

C: control sausage; RP2: sausage added with 2 % of rosemary powder; RP4: sausage added with 4 % of rosemary powder; values are the mean of three replicates; a, b et c: means in the same line for the same treatment with a different letter differ significantly (P<0.05); X, Y et Z: means in the same column for the same day with a different letter differ significantly (P<0.05).

The potential of hydrogen (pH) is usually ranked among the technological characteristics because it greatly influences meat processing and conservation. Table 3 shows that incorporating rosemary significantly affected pH values every day of drying except the first. For the control samples, the pH remained stable between Day 0 and Day 3 of drying, then significantly increased to a value of 5.83 on Day 6 (P <0.05). This rise can be attributed to the increase of total viable counts and total coliforms, which cause protein and amino acid degradation resulting in ammonia formation and consequently an increase in pH (GEORGANTELIS *et al*., 2007). For RP2 and RP4 sausages, pH values significantly decreased during the drying period (P < 0.05). These lower values are probably due to sausage acidification, although no sugar was added to the mixture. Rosemary is an herb rich in carbohydrates (Deef, 2007) and during the drying process, these sugars are used by bacteria to produce lactic acids responsible for the drop in pH. This was not the case in the control sausage because no rosemary powder was added.

3.3. Sausage color

Table 3 shows that L* values, expressing brilliance in color, decreased significantly during the drying period (P<0.05). KOVAČEVIĆ *et al.* (2010) reported that this loss of clarity could be attributed to drying time. This decrease in L* values was in fact due to water loss (SANABRIA *et al.*, 2004). The results indicated that adding rosemary did not have a significant effect on the lightness of dry sausages.

Table 3. Effect of rosemary powder incorporation on the pH and color parameters of dry ewe sausages.

Parameters	Batch	Days of drying		
		0	3	6
pH	C	5.59±0.13^{aX}	5.62±0.06^{abX}	5.83±0.07^{bX}
	RP2	5.58±0.01^{aX}	5.47±0.12^{bXY}	5.41±0.1^{abY}
	RP4	5.55±0.06^{aX}	5.48±0.16^{abY}	5.44±0.07^{bY}
L	C	47.93±4.82^{aX}	38.36±5.04^{bX}	37.68±5.02^{bX}
	RP2	46.29±3.65^{aX}	38.37±5.6^{bX}	38.11±4072^{bX}
	RP4	43.51±3.66^{aX}	36.36±3.83^{bX}	36.12±4041^{bX}
a*	C	17.98±3.22^{aX}	15.21±2.84^{abX}	12.08±1.94^{bX}
	RP2	7.19±1.74^{aY}	7.37±1.4^{aY}	7.03±1.37^{aY}
	RP4	5.83±1.49^{aY}	3.51±0.22^{bY}	3.82±0.31^{bZ}
b*	C	27.66±3.53^{aX}	25.76±2.71^{abX}	21.15±2.78^{bX}
	RP2	28.63±3.19^{aX}	23.51±2.68^{bX}	22.82±3.39^{bX}
	RP4	23.43±3.95^{aY}	22.11±3.18^{bY}	19.12±1.78^{aX}
C	C	33.06±4.04^{aX}	30.14±4.04^{abX}	24.71±3.58^{bX}
	RP2	29.59±2.63^{aX}	24.71±3.06^{bY}	23.92±3.13^{bX}
	RP4	24.27±2.6^{aY}	19.46±3.49^{bZ}	22.44±1.75^{aX}
H	C	56.96±4.15^{aY}	60.21±4.5^{aZ}	59.81±5.34^{aZ}
	RP2	75.80±3.71^{aX}	72.79±4.54^{aY}	72.57±3.71 ^{aY}
	RP4	75.87±3.48^{aX}	79.71±2.65^{aX}	80.12±1.18 ^{aX}

C: control sausage; RP2: sausage added with 2 % of rosemary powder ; RP4:sausage added with 4 % of rosemary powder; values are the mean of three replicates; a, b, c: means in the same line for the same treatment with a different letter differ significantly (P<0.05); X, Y, Z: means in the same column for the same day with a different letter differ significantly (P<0.05).

Redness (a*) decreased significantly during the drying of control sausages, probably due to the oxidation of myoglobin to metmyoglobin (BALENTINE *et al.*, 2006). Sausages with 2 % rosemary powder (RP2) showed no change in redness over time (P > 0.05), versus a decrease in sausages RP4 between Day 0 and Day 3 followed by stability until Day 6 of the drying process. This stability may be attributed to the phenolic components of rosemary, which exert an antioxidant effect inhibiting the oxidation of myoglobin and thus maintaining the red color of samples (BALENTINE *et al.*, 2006; SMETI *et al.*, 2013).

LIU *et al*. (2009) found that adding rosemary powder to fresh chicken sausage increased a* and decreased L values. Similarly, GEORGANTELIS *et al*. (2007) explained that many factors such as differences in the oxidation pattern of myoglobin under conditions of reduced enzymatic activity, storage temperatures, packaging methods, muscle type and light intensity and differences in the meat spices studied might contribute to the variations in rosemary color retention efficiency between different studies.
Results showed that values of b* decreased significantly for all sausage samples during drying (P <0.05), but values stayed relatively high (>19). KOVAČEVIĆ *et al*. (2010) reported that the higher b* values in the sausages are probably related to the presence of yellow carotenoids (ß-carotene and cryptoxanthin) from paprika. With their combined polyene chain, which acts as a chromophore, carotenoids are often responsible for the bright colors of certain fruits and vegetables. This chain is also responsible for the instability of the carotenoids against oxidation, light and heat, which may in turn. This instability of the carotenoids may be the cause of a decrease yellowness index during drying (CHANFORAN, 2010). On the other hand, rosemary powder did not affect the b* values of sausages.
On Day 0, not all types of sausages exhibited the same degree of saturation; the control samples had the highest Chroma when compared to samples RP2 and RP4. Thereafter, the control samples saturation level dropped throughout the drying period. This continuous decline was not observed in sausages with rosemary added, thus demonstrating the effectiveness of rosemary by-products against myoglobin oxidation (CAMO *et al*., 2008).

3.4. Sensory analysis

Color is one of the most important points in sensory evaluation, helping the consumer accept or reject particular foods. Figure 1 showed that rosemary powder significantly affected sausage color (P <0.001). Sausages with rosemary powder were darker, which could increase consumer sensory preferences. Our results differed from those of LIU *et al*. (2009) who found that the addition of rosemary decreased color preferences of fresh chicken sausages during refrigerated storage.

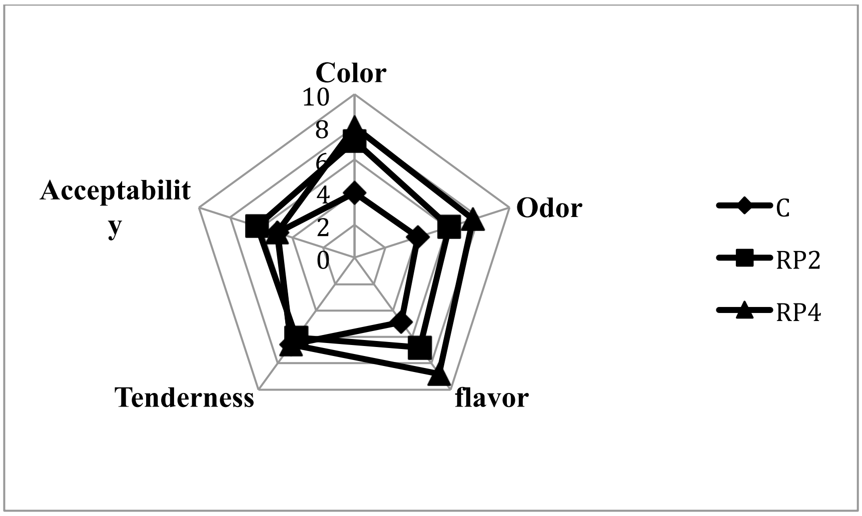

Figure 1. Sensory evaluation of dry ewe sausages.
C: control sausage ; RP2: sausage added with 2 % of rosemary powder ; RP4: sausage added with 4 % of rosemary powder.

Figure 1 shows that incorporating rosemary significantly affected the odor of the sausages ($P < 0.05$). In fact, aromatic substances allowed tasters to distinguish between samples. Similarly, it has been documented that certain compounds found in rosemary (verbenone, borneol, camphor) may give food a specific odor, even at low concentrations (CARRILLO *et al*., 2006). Likewise, incorporating rosemary significantly affected sausage flavor ($P < 0.05$). However, sausage tenderness was similar between groups ($P > 0.05$). In addition, rosemary significantly affected the acceptability of dry sausages ($P < 0.05$) in favor to RP2 samples, which were the most appreciated by panelists. These results agreed with previous investigations showing a beneficial effect of rosemary by-products on the sensory quality of ewe meat products (DJENANE *et al*., 2002; DJENANE *et al*., 2003; BALENTINE *et al*., 2006).

4. CONCLUSIONS

The effect of rosemary powder on the microbiological, chemical and sensory qualities of dry ewe sausages was investigated. The results obtained showed that rosemary powder added at 2 and 4 % improved microbiological quality by decreasing total viable and total coliform counts. Similarly, moisture and pH were affected by adding rosemary powder. Therefore, sausages added with 2 % of rosemary powder were the most appreciated by the panelists. This level of inclusion could be of interest for ewes' sausage preparation.

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