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IMPACT OF NITRATE AND NITRITE REDUCTION WITH HYDROXYTYROSOL ON ORGANIC DRY-CURED SAUSAGES: PHYSIOCHEMICAL TRAITS AND FOOD SAFETY

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I. INTRODUCTION

The utilization of nitrites and nitrates in the production of cured meats constitutes a long-standing practice, aimed at enhancing the sensory attributes of flavor and color, while concomitantly ensuring product preservation. However, growing health concerns and consumer demand for products with minimal or no additives have prompted researchers and the food industry to explore other alternatives to these preservatives, particularly in organic cured meats. The aim of this study was to evaluate the effect of the partial substitution of nitrate and nitrate salts by hydroxytirosol (HXT, by-product of olive tree) on the physicochemical characteristics of the dry-cured sausages and the critical challenge of controlling *Listeria monocytogenes*, a pathogen of significant concern in meat processing.

II. MATERIALS AND METHODS

The sausages were made by raw material (28.8% ham, 15.9% shoulder, 10.3% loin, 9.9% sirloin, 7.1% belly, 1.9% tender loin, 16.1% ribs and 10% pork trim) from an organic pig farm (three carcasses from cull sows, > 3 years of age, crossbreed Duroc x Gascon). Five treatments were compared, consisting of 35 dry-cured sausages for each formulation. The control formulations contained a concentration of 80 mg KNO_3 (TC1) or 80 mg $NaNO_2$ (TC2) per kg of meat. The experimental formulations contained 40 mg of KNO_3, and 100 mg and 300 mg of HXT powder per kg of meat for T100 and T300 respectively, and 40 mg $NaNO_2$ and 200 mg of HXT per kg of meat (T200). The HXT was commercial olive fruit dried powder extract with a 10% purity (Mediteaneox® H10, Euromed, Barcelona, Spain). The meat was kneaded until the ingredients (1.8% fine sea salt organic, 0.4% organic pepper powder, 0.1% organic grain black pepper and 1% cane sugar organic) were gently homogenized (15 minutes to get a paste with a kneader mixer). The paste was put in a chamber during 24 h at 3.1ºC and 52.2% relative humidity (RH). The five formulations were prepared (day 0) without a starter. The following day (day 1) the mixtures were stuffed into pork casings, using an automatic sausage filler/stuffing machine made of stainless steel. The natural pork casing (7-10 cm ø and 30-40 cm length) was previously desalted and washed. Each sample sausage weighed approximately 500 g, and they were hung on a trolley-rack and stored for three days at 3.1ºC and 52.2% RH. Afterwards the trolley-rack was put in an air-drying chamber set to 8ºC-10ºC (average 9.2ºC) and 76-83% RH for 70 days. Subsequently, all sausage samples were vacuum packed and refrigerated at 6°C until analysis in the laboratories. Five samples were taken randomly from each treatment and each day (day 1 and 70) for physiochemical measurements and microbiological tests. The color measurements (lightness (L*), redness (a*), yellowness (b*)) were determined using a portable colorimeter, Minolta CM-700d. Chroma (C*) (saturation index) was calculated from the a* and b* values as $(a^2 + b^2)^{0.5}$. Hue angle (h*) (discoloration) was calculated as h = arctangent (b*/a*). In addition, the reflectance spectra (400 - 700 nm wavelength) was recorded for calculating the metmyoglobin (MMb) formation, following the equation MMb= 100 × (1.395 – ((A572nm − A700nm) / (A525nm − A700nm))) [1]. The pH was measured within the inner surface after cutting the sausage in half, with a pH-meter equipped with a spear-tipped probe (Testo 205, Testo AG). Lipid oxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS index) analysis, following modifications [2] [3]. The residual nitrate content was determined by liquid chromatographic method (HPLC-UV) [4], while the nitrite residual content was carried out by spectrophotometric method (UV-vis) [5]. The presence of foodborne pathogens (*Clostridium perfringens* and *Listeria monocytogenes*) was tested by plate counting (UNE-EN ISO 14189:2017 and ISO 11290-2:2017) on day 70 of ripening. The data were analyzed using JMP Pro 16 software (USA), with a standard model including fixed effects of treatment, ripening day and their interactions. Values are presented as least square means and standard error of the mean. The level of significance was set at 0.05.

III. RESULTS AND DISCUSSION

There were effects of KNO_3 and $NaNO_2$ reduction and replacement by HXT on color parameters, pH, lipid oxidation (TBARS), residual NO_3 levels and *L. mono.* (Table 1). Treatment T200 showed larger C* values on day 70, indicating a higher saturation or color intensity of the samples compared to TC1, TC2 and T100. In groups with KNO_3 (TC1, T100, and T300) added on day 0, the %MMb (the oxidized form of myoglobin with ferric iron (Fe^{3+})) showed a two-fold increase from day 1 to day 70, consistent with previous findings [1]. However, this effect was not observed in $NaNO_2$ (TC2 and T200), which also had brine added on day 0, and the %MMb values were considerably higher in TC2 and T200 than in the rest on day 1. Consequently, by the end of ripening period (day 70), the %MMb levels

were similar among groups. The pH decreases from day 1 to day 70, which favored the myoglobin oxidation (and reduced browning), below 5.5. The formulation without HXT (control TC1 and TC2) resulted in increased lipid oxidation (0.1-0.3 mg/kg more malondialdehyde) compared with HXT treatments (T100-200-300 mg HXT/kg). The residual NO_3 concentration was significantly higher in the TC2 formulation (80 mg $NaNO_2$/kg added), compared to T200 and T300, which would confirm previous findings observing that NO_2 can be transformed to NO_3 [6]. Finally, there were differences observed in *L. mono.* counts between the treatments; final counts were found to be up to 2.5 log cfu/g higher in the treatments with 50% nitrate reduction (T100 and T300) than in the maximum permitted dose treatment (TC1) and the maximum permitted nitrite (TC2) and 50% nitrite reduction (T200).

Table 1 – Color, physicochemical and microbial parameters in raw (day 1) and dry-cured loins (day 70)

	Days of storage	TC1	T100	T300	TC2	T200
Color parameters at day 1 and at the end of cured process (day 70)						
C*	1	46.0 ±1.24^{a,x}	45.9 ±1.24^{a,x}	45.6 ±1.24^{a,x}	43.9 ±1.24^{a,x}	46.7 ±1.24^{a,x}
	70	42.3 ±1.24^{b,yz}	40.1 ±1.24^{b,z}	45.3 ±1.24^{a,xy}	39 ±1.24^{b,z}	46.5 ±1.24^{a,x}
h*	1	44.5 ±2.82^{a,x}	43.5 ±2.82^{a,x}	42.2 ±2.82^{a,x}	42.1 ±2.82^{a,x}	49 ±2.82^{a,x}
	70	35.1 ±2.82^{b,x}	37.4 ±2.82^{a,x}	39.5 ±2.82^{a,x}	33.7 ±2.82^{a,x}	39.7 ±2.82^{b,x}
%MMb AMSA	1	15.7 ±1.37^{a,y}	17.2 ±1.37^{a,y}	15.1 ±1.37^{a,y}	32.4 ±1.37^{a,x}	33.5 ±1.37^{a,x}
	70	28.5 ±1.37^{b,x}	29.7 ±1.37^{b,x}	28.9 ±1.37^{b,x}	28.8 ±1.37^{a,x}	27.8 ±1.37^{b,x}
Physicochemical quality and microbiological evolution of cured sausages samples during ripening process						
pH	1	5.8 ± 0.03^{a,x}	5.8 ± 0.03^{a,xy}	5.7 ± 0.03^{a,y}	5.7 ± 0.03^{a,y}	5.9 ± 0.03^{a,x}
	70	5.4 ± 0.03^{b,xy}	5.5 ± 0.03^{b,x}	5.4 ± 0.03^{b,y}	5.4 ± 0.03^{b,xy}	5.3 ± 0.03^{b,y}
TBARs (mg MDA /kg carn)	1	0.2 ± 0.03^{a,x}	0.2 ± 0.03^{a,x}	0.2 ± 0.03^{a,x}	0.2 ± 0.03^{a,x}	0.2 ± 0.03^{a,x}
	70	0.4 ± 0.03^{b,x}	0.2 ± 0.03^{a,x}	0.2 ± 0.03^{a,x}	0.3 ± 0.03^{ab,x}	0.2 ± 0.03^{a,x}
NO_3 (mg/kg)	70	18.8 ± 1.4^{xy}	18.8 ± 1.4^{xy}	16 ± 1.4^{y}	24.8 ± 1.4^{x}	15 ± 1.4^{y}
NO_2 (mg/kg)	70	6 ± 0.46^{x}	5.7 ± 0.46^{x}	5.3 ± 0.46^{x}	7.2 ± 0.46^{x}	5.9 ± 0.46^{x}
L. mono. (log cfu/g)	70	2.8 ± 0.24^{yz}	3.6 ± 0.24^{xy}	4.4 ± 0.24^{x}	2.0 ± 0.24^{z}	1.9 ± 0.24^{z}

$^{x-z}$ Means within trait and day with no common letter differ significantly (P<0.05); $^{a-b}$ Means within treatment and trait with no common letter differ significantly (P<0.05); C*= chroma, h*= hue; %MMb= Metmyoglobin; TBARS = Thiobarbituric acid reactive substances; NO_2= residual nitrite level; NO_3= residual nitrate level; *L. mono.* = *Listeria monocytogenes*.

IV. CONCLUSION

Reducing the amount of added nitrates and nitrites to 50% of the maximum allowed in raw-cured sausages with HXT has little impact on the cured sausage's pH and colour attributes. The reduction of nitrite appeared to have no significant impact on microbiological safety. Conversely, the reduction of nitrates may potentially engender a more conducive environment for the proliferation of *L. mono.* However, HXT could reduce the lipid oxidation in cured sausages. This highlights the need for technological adjustments to ensure product safety.

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