Reformulation of dry sausages with natural plant extracts prevents oxidation even in the absence of nitrates

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I. INTRODUCTION

Dry sausages are part of French gastronomy and represent 8% of the total food consumed weekly in France. Appreciated for their sensorial appeal and nutritionally relevant with highly digestible proteins, to name just a few qualities, dry-cured sausages suffer from the presence of food additives like nitrite and nitrate. These additives may lead to harmful nitroso-compounds [1, 2]. Therefore, the replacement of such additives while preserving health and sensorial properties has been the subject of numerous studies, including formulation with polyphenolic compounds. Known for their antioxidant (through scavenging free radicals or chelating metals) and anti-nitrosating properties, polyphenols can be provided by plant byproducts like grapeseed or olive pomace from olive oil extraction [3]. Reformulation provides technological leverage to reduce or even exclude nitrite/nitrate inputs in processed meats. Nitrite/nitrate in dry-fermented pork sausages/salamis can be replaced through formulation with a grapeseed/olive pomace mixture, while controlling microbiological safety [4, 5], albeit with possible color or taste alteration. Preliminary studies have defined an acceptable concentration from a sensory point of view, set at 6 mM eq gallic acid. The aim of the present study was to investigate different sources of polyphenols, alone or in combination with nitrates, to highlight possible synergistic effects on oxidation and prevention of nitrosation.

II. MATERIALS AND METHODS

The sausages were made at the Technical Institute ADIV (Clermont-Ferrand, France), with 87% of porcine shoulder meat and 13% of porcine back fat. Meats were ground at 6 mm diameter and then stuffed in porcine casings of 55 mm diameter. Additions were 160 mg/kg $NaNO_3$ and/or 6 mM polyphenol extracts: green tea GT, olive grape OG, fruit cocktail FC. Products were flavored with 1.50 g/kg of ground grey pepper. Starters (namely *Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus* (Lallemand, France)), dextrose and lactose (0.15 g/kg; 5.50 g/kg and 6.00 g/kg, respectively) were added to acidify the sausages during the fermentation step. Seven formulations of dry sausage were produced: 0 $NaNO_3$ / 0 $NaNO_2$; 0 $NaNO_3$ / 0 $NaNO_2$ + GT; 0 $NaNO_3$ / 0 $NaNO_2$ + OG; 160 $NaNO_3$ / 0 $NaNO_2$; 160 $NaNO_3$ / 0 $NaNO_2$ + FC; 160 $NaNO_3$ / 0 $NaNO_2$ + GT; 160 $NaNO_3$ / 0 $NaNONO_2$ + OG. The process was similar to that described by [6]. After the fermentation step, the pH increased slowly to final values between 5.1 and 5.2. No significant pH difference was noted for 5 of the 7 trials or for weight loss (44%), except for trials including OG with or without preservatives. For these conditions, pH values after fermentation and drying were higher (+0.1 point) than in other tests.

Determination of thiobarbituric acid reactive substances (TBARS) and nitroso-compounds: The dry sausages were ground in liquid nitrogen to avoid any oxidation and 1 g aliquots were stored at -80°C before use. TBARS were determined as described by [2] and expressed in mg/kg MDA. Nitroso-compounds (nitrosothiols & non-volatile nitrosamines), and residual NO_2 and NO_3, were assessed using the Griess method and expressed in ppm [7]. Nitrosylated heme and total heme were measured as described in [6] and expressed in % and ppm, respectively. Data were analyzed with Jamovi software (version 2.3.26). Variance analysis (ANOVA) and a Tukey *post-hoc* test, as well as PCA were carried out. The significances were given by p values. Every biochemical analysis was performed in triplicate and the results are systematically given as mean ± standard deviation.

III. RESULTS AND DISCUSSION

The results are presented in table 1. In the absence of nitrates, practically no nitrites were detected and the residual nitrates corresponded to the nitrates intrinsically present in muscle. Adding nitrates to the formulation increased both residual nitrites and nitrates. Residual nitrate levels were 50% higher with green tea extracts. Nitroso-compounds, *ie*, RSNO and NNO, were hardly detected, whatever the formulation. The percentage of nitrosyl iron was lower in the absence of nitrates in the formulation,

but with nitrates we observed a decrease of nitrosyl iron when polyphenols were provided in the fruit cocktail. This difference could be due to the nature of polyphenols and their ability to chelate metal. Lastly, the oxidation level of dry sausages was the highest in the absence of nitrates or polyphenols, but was drastically reduced when adding polyphenols. The oxidation level was tantamount to that of the formulation with nitrates alone (160/0). Moreover, in combination with plant extracts, the oxidation level was reduced by almost $^1/_3$, highlighting a synergistic effect of nitrates and polyphenols.

Formulation NO3/ NO2								
	PP extract	residual NO2 ppm	residual NO3 ppm	RSNO ppm	NNO ppm	Total heme ppm	% Fe-NO	MDA mg/kg
0/0	/	0 +/- 0 a	6.77 +/- 0.18 a	0 +/- 0	0.037 +/- 0. 032	197 +/- 21 a	29.8 +/- 1.4 a	0.133 +/- 0.007 a
0/0 - GT	Green Tea	0.18 +/- 0.03 b	6.18 +/- 0.09 b	0.017 +/- 0.001	0 +/- 0	158 +/- 2 b	31.1 +/- 1 a	0.075 +/- 0.009 b
0/0 - OG	Olive Grape	0 +/- 0 a	6.63 +/- 0.19 a	0 +/- 0	0.057 +/- 0. 050	199 +/- 1 a	27.3 +/- 0.5a	0.078 +/- 0.004 b
160/0	/	2.91 +/- 0.05 e	9.63 +/- 0.06 c	0.065 +/- 0.001	0.096 +/- 0.017	184 +/- 3 a	50.7 +/- 0.5 b	0.076 +/- 0.004 b
160/0 - FC	Fruit Cocktail	2.69 +/- 0.02 d	9.92 +/- 0.15 d	0.047 +/- 0.001	0.025 +/- 0.04	285 +/- 3 c	39.6 +/- 0.5 c	0.056 +/- 0.004 c
160/0 - GT	Green Tea	5.38 +/- 0.08 f	14.2 +/- 0.2 f	0.098 +/- 0.001	0.108 +/- 0.082	313 +/- 25 d	46.6 +/- 0.4 b	0.063 +/- 0.006 c
160/0 - OG	Olive Grape	2.12 +/- 0.02 c	11.3 +/- 0.1 e	0 +/- 0	0.006 +/- 0.01	233 +/- 2 c	56.3 +/- 2.4 b	0.052 +/- 0.001 c
P		p<0.001	p<0.001	p>0.05	p>0.05	p<0.001	p<0.001	p<0.001

Table 1: Mean+/- SD of residual nitrites, residual nitrates, nitroso-compounds (RSNO, NNO, Fe-NO), total heme and lipid oxidation (MDA) in dry sausages formulated with or without nitrates and with or without 6 mM plant extract (green tea GT, olive Grape OG, fruit cocktail FC)

IV. CONCLUSION

Dry sausages did not contain meaningful amount of harmful nonvolatile nitrosamines, which is a public health result worth mentioning. Replacing nitrates in dry sausages is possible with 6 mM plant extract (green tea and olive grape), with limited lipid oxidation. Moreover, the combination with nitrates highlighted a synergistic action on lipid oxidation, therefore ensuring better preservation of the food product. Further investigations are planned to evaluate how the polyphenol extracts protect against oxidation and nitration in digestive conditions, which are known to increase oxidation and the release of NO.

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