Dog Rose as functional ingredient in ascorbic acid- and nitrite-free porcine frankfurters

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Abstract - **The effect of dog rose (*Rosa canina L.*, RC), which is rich in ascorbic acid and polyphenols, on lipid and protein oxidation, colour stability and texture of frankfurters is investigated. Four types of frankfurters were prepared: two containing 5 or 30g/kg RC extract without ascorbic acid and without nitrite (5RC and 30RC, respectively), a positive control (with ascorbic acid and nitrite; PC) and a negative control (without ascorbic acid, nitrite or RC extract; NC). During 60 days of chill storage, lipid and protein oxidation products were assessed and redness (a* value) as well as hardness were measured. Lipid and protein oxidation increased significantly during chill storage. Lipid oxidation was significantly higher in the NC samples compared to the PC, 5RC and 30RC samples. Significantly more protein oxidation occurred in the NC compared to the PC frankfurters and intermediate values were found for the RC samples, with no significant difference between the 5RC and 30RC samples. Regarding the a* values, significantly higher values were observed in the PC samples compared to the other treatments. Although the hardness of the frankfurters did not differ between treatments at the beginning of the storage experiment, 5RC and NC treated samples became significantly softer at the end of the storage period compared to 30RC and PC treated samples.**

Keywords – meat product, oxidative stability, dog rose extract

I. INTRODUCTION

Oxidation leading to degradation of lipids, proteins and pigments is one of the primary mechanisms of meat deterioration and can be prevented by including antioxidants in meat products. The increased public concern over the safety and toxicity of synthetic antioxidants presses the meat industry to find natural alternatives [1]. One of these natural alternatives is antioxidants extracted from fruits and preventing meat quality deterioration by the use of these fruit extracts is widely documented [2]. The main components contributing to the antioxidant effect in these extracts are phenolic compounds, due to their hydrogen-donating capacity and metal-chelating potential [3]. However, other antioxidants such as ascorbic acid and carotenoids may also contribute to the oxidative stability of meat products, not only because of their antioxidant capacity *in se*, but also because of their ability to act synergistically with phenolic compounds [4]. Dog rose (*Rosa canina L.*, RC) is rich in phenolic compounds and ascorbic acid [5] and is therefore believed to be a potential natural antioxidant. In fact, RC extracts have shown high antioxidant activities *in vitro* [6] and positive results on colour stability, texture properties as well as lipid and protein oxidation after addition to porcine burger patties [7]. However, one should be aware that the relative efficiency of phenolic-rich extracts when applied in different food matrices can not be predicted even for very well-characterised extracts [8]. Hence, the use of RC in different meat products needs to be further investigated. Furthermore, potential health risks related to the residual nitrite levels in meat and meat products demand for significant decrease in the use of sodium nitrite [9]. As RC contains considerable amounts of nitrates [10], replacing sodium nitrite by nitrate could result in lower residual nitrite concentrations, reducing the risk of N-nitrosamine formation during ingestion, without drastically affecting the oxidative stability of the meat product. Therefore, the objective of this research is to investigate the potential of RC as functional ingredient in ascorbic acid- and sodium nitrite-free porcine frankfurters in terms of texture, colour-, lipid- and protein oxidative stability.

II. MATERIAL AND METHODS

Frankfurters containing 700 g/kg porcine meat, 100 g/kg back fat, 180 g/kg water, 20 g/kg NaCl and 5 g/kg phosphate were produced in a pilot plant. Four types of frankfurters were prepared: 5RC and 30RC with 5 or 30 g/kg RC extract respectively and without ascorbic acid and nitrite, a positive control (with 0.5 g/kg sodium ascorbate and 0.1 g/kg sodium nitrite; PC) and a negative control (without sodium ascorbate, sodium nitrite or RC extract; NC). The frankfurters were stored in the dark (4 °C) for 60 days. The samples were analysed at days 1, 20, 40 and 60.

Colour measurements were performed in five fold on the internal section of the frankfurters using a Minolta Chromameter CR-300. The a* value (CIE L*a*b*colour system 1976) was assessed as a measure of the redness of the meat products.

Texture profile analysis was carried out at room temperature with a Texture Analyser TA-XT2i. Nine samples (height 2.0 cm) of each treatment were taken and subjected to a two-cycle compression test. The samples were compressed to 40% of their original height with a cylindrical probe of 5 cm diameter and a cross-head speed of 5 mm/s. The maximum force required to compress the sample (hardness, N/cm^2) was used for further analysis.

Lipid oxidation products (hexanal, heptanal, octanal and nonanal) were analysed from the headspace of minced frankfurters using solid-phase micro extraction (SPME) coupled to GC/MS device [11]. Four samples of each treatment were analysed and results were provided in arbitrary area units (AAU). The sum of oxidation products was calculated and used for statistical analysis.

Protein oxidation products (α-amino adipic semialdehyde (AAS) and γ-glutamic semialdehyde (GSS)) were derivatized with p-aminobenzoic acid (ABA) and analysed using fluorescence HPLC [12]. Samples were analysed in quadruplicate and the sum of AAS and GGS was calculated and used for statistical analysis. Results are expressed as nmol carbonyls/mg protein as quantified using an ABA standard curve.

The data were analysed using the general linear model ANOVA procedure considering time and treatment as independent variables. Mean differences between groups were tested using Tukey's post hoc test operating at a 5% level of significance (SPSS for Windows 15.0).

III. RESULTS

The results concerning lipid and protein oxidation in frankfurters during chill storage are summarised in Table 1. During chill storage, lipid oxidation products significantly increased for all treatments and a clear effect of RC was observed. Compared to NC samples, a three to five fold lower level of lipid oxidation products was found in 5RC and 30RC treatments at the end of the storage period. No dose effect on lipid oxidation was found based on the absence of a significant difference between the 5RC and 30RC treatments.

Also protein oxidation increased during the chill storage period (Table 1). Similar to lipid oxidation, there were significant treatment effects with the highest protein oxidation products in NC samples and the lowest amounts in PC samples. Intermediate values were found for the RC treatments, significantly different from PC and NC treatments. Like for lipid oxidation, no significant difference between the two RC doses was observed.

The effect of RC on colour stability and hardness is presented in Table 2. In general, the RC-treated samples resulted in significantly two fold lower a* values compared to nitrite treated samples (PC). Nevertheless, in the 30R- treated samples significantly higher a* values were observed compared to the 5RC and NC treatments. However, after 40 days of storage, the a* values of the NC and 5RC treated samples increased significantly and the a* values of the 30RC samples decreased slightly, resulting in no significant differences between 30RC and NP or 5RC. During the whole period of storage no change in a* value was observed for the PC samples.

Considering the results of texture, no differences in hardness were found between the treatments at the start of the experiment, while at the end of the experiment the hardness of 5RC- and NC-treated samples were significantly lower compared to 30RC- and PC-treated samples. Throughout the storage period, significant differences between treatments and storage days were observed, but no clear patterns could be found.

IV. DISCUSSION

The amount of oxidation products increased during chill storage, indicating that oxidation occurred. Importantly, incorporation of RC inhibited both lipid and protein oxidation. These results confirm that RC can act as an effective antioxidant compound in frankfurters and they compare well with the effects found in cooked burger patties [6;7]. Present results

also agree with Estevez et al. [11] where the lowest hexanal and carbonyl compounds were found in frankfurters with the highest phenolic content. Note that the RC extracts are as efficient as the nitrite and sodium ascorbate addition in PC samples for lipid oxidation, while the nitrite and sodium ascorbate addition was more effective compared to both 5RC and 30RC for protein oxidation. Noteworthy are the higher a* values in the 30RC-treated frankfurters compared to the NC and 5RC samples right after manufacturing. Perhaps some nitrosopigments were formed, as RC contains high amounts of nitrates [10]. However, nitrate is only effective after being reduced to nitrite, which can be accomplished by microorganisms found in the natural flora of meat [13] and potentially reducing compounds present in the RC extracts. Although this reduction is only possible in raw batters and not in cooked meat products, no more than 4-6 ppm nitrite is necessary for cured colour development in frankfurters (Fox, 1987, as cited in [14]). It would thus be of interest to study the potential of RC in colour formation in more detail.

Table 1 Protein and lipid oxidation during chill storage of frankfurters with added dog rose extract

		5RC	30RC	PC	NC	SEM[3]
Lipid oxidation[1]	**Day 1**	$1.59^{c,y}$	$3.77^{b,x}$	$1.65^{b,y}$	$3.44^{c,x}$	0.30
(AAU $\times 10^6$)	**20**	$3.30^{bc,y}$	$3.61^{b,y}$	$2.50^{ab,y}$	$20.44^{b,x}$	1.98
	40	$4.74^{b,y}$	$6.04^{ab,y}$	$4.08^{a,y}$	$27.55^{ab,x}$	2.59
	60	$10.78^{a,y}$	$7.40^{a,y}$	$4.22^{a,y}$	$34.50^{a,x}$	3.16
	SEM[4]	0.93	0.53	0.36	3.11	-
Protein oxidation[2]	**Day1**	$0.35^{c,y}$	$0.36^{c,y}$	$0.14^{b,z}$	$0.89^{c,x}$	0.08
(nmol carbonyls/mg protein)	**20**	$0.76^{bc,y}$	$0.55^{c,y}$	$0.18^{b,z}$	$1.43^{bc,x}$	0.13
	40	$1.26^{b,y}$	$1.14^{b,y}$	$0.49^{a,z}$	$1.86^{b,x}$	0.13
	60	$1.95^{a,y}$	$1.49^{a,y}$	$0.56^{a,z}$	$2.92^{a,x}$	0.23
	SEM[4]	0.17	0.12	0.05	0.20	-

5RC: 5 g/kg *Rosa canina;* 30RC: 30 g/kg *Rosa canina*; PC: Positive control, NC: Negative control;[a–c] Effect of storage: values with a different letter within a column of the same treatment are significantly different ($P < 0.05$); [x-z] Effect of treatment: values with a different letter within a row of the same storage day are significantly different ($P < 0.05$);[1] Sum of hexanal, heptanal, octanal and nonanal [2] Sum of α-amino adipic semialdehyde and γ-glutamic semialdehyde;[3] Standard error of the mean within the same storage day (n=16).[4] Standard error of the mean within the same treatment (n=16).

Table 2 Colour a* value and hardness of frankfurters with added dog rose extract during chill storage

		5RC	30RC	PC	NC	SEM[3]
a* value	**Day 1**	$6.11^{b,z}$	$6.81^{a,y}$	11.85^{x}	$5.93^{bc,z}$	0.56
	20	$6.05^{b,z}$	$6.61^{b,y}$	11.80^{x}	$5.89^{c,z}$	0.56
	40	$6.64^{a,y}$	$6.79^{a,y}$	11.97^{x}	$6.69^{a,y}$	0.48
	60	$6.29^{ab,y}$	$6.57^{b,y}$	11.91^{x}	$6.68^{ab,y}$	0.48
	SEM[2]	0.07	0.03	0.04	0.12	-
Hardness	**Day 1**	$3.16^{b,y}$	3.44^{xy}	$3.26^{ab,xy}$	$3.63^{a,x}$	0.06
($10^3 \times N/cm^2$)	**20**	$3.06^{b,y}$	3.50^{x}	$2.87^{b,y}$	$2.85^{b,y}$	0.06
	40	$3.86^{a,x}$	3.88^{x}	$3.17^{ab,y}$	$2.89^{b,y}$	0.10
	60	$2.95^{b,y}$	3.71^{x}	$3.38^{a,xy}$	$2.82^{b,y}$	0.11
	SEM[3]	0.09	0.06	0.07	0.09	-

5RC: 5 g/kg *Rosa canina;* 30RC: 30 g/kg *Rosa canina*; PC: Positive control, NC: Negative control;[a–c] Effect of storage: values with a different letter within a column of the same treatment are significantly different ($P < 0.05$);[x-z] Effect of treatment: values with a different letter within a row of the same storage day are significantly different ($P < 0.05$);[1] Standard error of the mean within the same storage day, n=20 (a* value) or n=36 (hardness);[2] Standard error of the mean within the same treatment, n=20 (a* value) or n=36 (hardness).

What is unusual about the results, especially for the NC treatments, is that the a* values increased during storage, while a loss in redness was expected. It is difficult to explain this increase as various endogenous factors can change the conditions of the meat, such as pH, reducing conditions, degree of denaturation, and reactivity of endogenous meat compounds, which can affect the chemical state, structure, and reactivity of the pigments. Some of these factors may result in pinking of cooked meat [15].

Texture is a major parameter of cooked sausages and consumer acceptance of food products strongly depends on textural characteristics. Therefore, when changing the composition of a well known meat product such as frankfurters, one should verify that the desired textural characteristics are maintained. In work of Dong et al. [16], altering nitrite concentrations resulted in changed texture attributes and the nitrite concentration was negatively correlated with hardness. At the onset of the storage experiment no differences in hardness were found, indicating that the RC treatment had no adverse effect on the hardness during the processing of the frankfurters. At the end of the experiment, however, only the 30RC treatment resulted in the same hardness as the ascorbate and nitrite-treated frankfurters (PC).

V. CONCLUSION

Addition of dog rose revealed clear protection against lipid and protein oxidation in frankfurters during 60 days of chill storage. Dog rose extract has the potential to contribute to colour formation and did not substantially affect the hardness of the sausages. Further studies will include an assessment of microbiological risk and sensory research on the acceptability of these frankfurters to verify if RC can be used to extend the shelf life of nitrite-free frankfurters.

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