DIFFERENTIAL EFFECTS OF THE CURING AGENTS SODIUM ASCORBATE AND SODIUM NITRITE ON PROTEIN OXIDATION IN DRY FERMENTED SAUSAGES

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Abstract – The effects of sodium nitrite and sodium ascorbate on lipid and protein oxidation were studied in dry fermented sausages. Both compounds were added in a 2×2 factorial design based on the following four treatments: (1) a control without sodium ascorbate and sodium nitrite, (2) sodium ascorbate at 500 mg/kg, (3) sodium nitrite at 150 mg/kg, and (4) sodium ascorbate at 500 mg/kg and sodium nitrite at 150 mg/kg. Lipid oxidation was quantified by determining TBARS and protein oxidation was assessed by measurement of protein carbonyls (DNPH method) and the loss of thiol groups. Sodium ascorbate and sodium nitrite were separately able to reduce lipid oxidation. In contrast, the combined addition of sodium ascorbate and sodium nitrite resulted in higher amounts of carbonyl compounds compared to their separate addition or the treatment without any of both compounds. A loss of thiol groups was observed during ripening, which was not affected by the use of sodium ascorbate nor sodium nitrite.

Key Words – carbonyls, thiols, lipid oxidation.

I. INTRODUCTION

The stable character of dry fermented sausages is largely due to a combination of salting, bacterial acidification, drying and sometimes smoking. The salting process includes the addition of sodium chloride, nitrate and/or nitrite salts, and ascorbate salts. Nitrite and ascorbate salts are basic ingredients in fermented products since they are responsible for the development and stabilization of the desired red colour [1]. Nitrite also exerts antimicrobial activities [2]. Additionally, nitrite and ascorbate salts develop the cured flavour in fermented products [3]. Ascorbate salts prevent lipid oxidation [4], although pro-oxidant effects were reported as well [5].

Whereas the effects of nitrite and ascorbate salts on lipid oxidation have been extensively studied [6], their effects on protein oxidation have been overlooked. Nevertheless, protein oxidation is potentially important for meat fermentation since it implies modifications at the protein level which can alter the structure and functionality of proteins, compromising their technological and organoleptic properties [7].

The aim of the present study was to investigate the effects of sodium nitrite and sodium ascorbate on protein oxidation in dry fermented sausages.

II. MATERIALS AND METHODS

Dry fermented sausages were prepared with lean pork, pork backfat (27 %), sodium chloride (2.5 %) and a starter culture (*Lactobacillus sakei*, *Staphylococcus carnosus* and *S. xylosus*). Sodium ascorbate (SA) and sodium nitrite (SN) were used to obtain four treatments: (1) a control without SA and SN, (2) SA at 500 mg/kg, without SN, (3) SN at 150 mg/kg, without SA, (4) SA at 500 mg/kg and SN at 150 mg/kg. The batter was stuffed into casings with 50 mm diameter and ripened for 28 days. During the first two days, fermentation was performed at 24 °C and a relative humidity of 94 %. Temperature was dropped to 12 °C after the first two days of ripening and relative humidity to 82 % after the first two weeks. Samples were

taken at days: 0 (day of production), 2 (end of fermentation), 8, 14, 21, and 28 (end of ripening). This experimental set-up was repeated twice at two different days (two independent manufacturing processes). Residual ascorbic acid (AA) was analysed through HPLC [8]. Residual nitrite [9], TBARS (expressed as µg malonaldehyde equivalents (MDA eq.) / g sample) [8], loss of thiol groups [10] and carbonyl formation [11] were analysed spectrophotometrically. Data were further analysed using the general linear model ANOVA procedure with the fixed effects of addition or not of SA and SN and interaction term as well as day of ripening for carbonyl formation and loss of thiol groups. The data at days 0 and 2 were analysed separately for residual nitrite and residual AA with the fixed effects of addition or not of SA and SN and interaction term. The data at days 2 and 28 were analysed separately for TBARS with the fixed effects of addition or not of SA and SN and interaction term. A random effect of manufacturing process was included in all models.

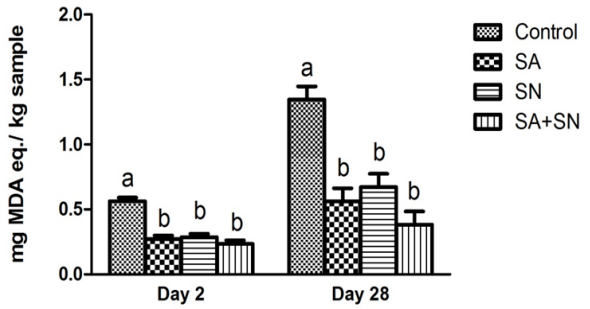
III. RESULTS AND DISCUSSION

Dry fermented sausages showed a final pH ranging from 5.4 to 5.6, following a mild fermentation and a weight loss in the final products of 30 %. No significant differences were recorded among treatments. The obtained weight loss and mild pH decrease were compatible with southern-type dry fermented sausages [12].

In this study, the initial residual nitrite was present in very low amounts compared to the ingoing dose (14.7 and 5.8 mg NO_2 / kg sample in the SN and SA+SN treatments respectively), confirming the high reactivity of this compound [13]. Nitrite reacts with AA [14] and this explains the tendency towards a lower amount in the SA+SN treatment compared to the SN treatment. At the end of fermentation and during the drying phase, residual nitrite showed a significant difference between the SN and SA+SN treatments (9.7 and 3.1 mg NO_2 / kg sample at the end of fermentation, respectively). In the treatments without sodium nitrite added, only traces of nitrite were detected.

Residual AA was not detected in sausages prepared without sodium ascorbate. The initial level of residual AA at day 0 was 156 mg AA / kg sample in the SA treatment. Residual AA tended to be lower in the SA+SN treatment compared to the SA treatment (75 mg AA / kg sample); indeed its reaction with nitrite in the SA+SN treatment might have speeded up its oxidation to dehydroascorbic acid. However, the contrary was observed at the end of fermentation, whereby the SA treatment tended to have lower amounts of AA than the SA+SN treatment (13 and 112 mg AA / kg sample, respectively). We speculate that the anticipated higher oxidative stability of the SA+SN treatment due to nitrite addition, especially against lipid oxidation, might have prevented further involvement of AA in oxidative reactions.

Figure 1. Nitrite and ascorbate effects on TBARS in dry fermented sausages at day 2 and 28 of ripening



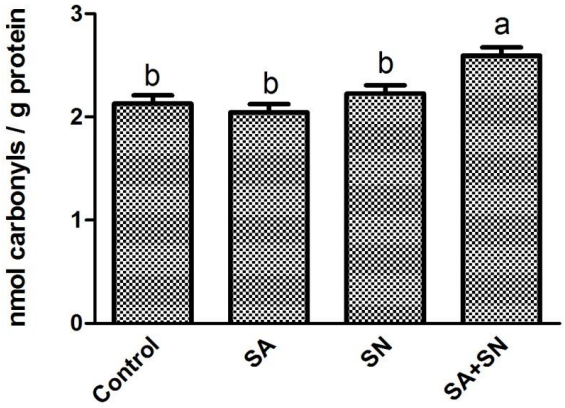
Error bars represent standard errors of the mean values. Superscripts denote significant differences between treatments within sampling days.

The SA, SN and SA+SN treatments had significantly lower MDA eq. compared to the control treatment, both after fermentation and at the end of ripening (significant interaction term; P=0.023 and P=0.049, respectively; Figure 1). The combined addition of nitrite and ascorbate resulted, although not significantly, in a further slight decrease of MDA eq. compared to the separate addition of nitrite or ascorbate. Ascorbic acid has the ability to scavenge reactive oxygen species and radicals [15]. Nitrite can limit the oxidation of lipids and the consequent formation of aldehydes in several ways [14]. Nevertheless, the most important antioxidant property seems to be its ability to react with lipid radicals blocking the oxidation chain reaction [16].

With respect to protein oxidation, little is known about the effects of AA and nitrite. Formation of carbonyl compounds is the most studied

modification due to oxidation in meat products, usually based on the DNPH method [17]. In the present study, the amount of protein carbonyls analysed across the six sampling days during ripening was significantly higher (approximately 20 % more) in the SA+SN treatment (significant interaction term; P=0.009; Figure 2). Protein carbonyls can be formed by metal-catalysed oxidation, non-enzymatic glycation, adduct formation with carbonyl compound and peptide backbone cleavage [17]. Metal-catalysed oxidation forms carbonyls in the side chains of arginine, lysine, proline and threonine [18]. Ascorbic acid, normally considered a reducing compound, can be pro-oxidant by reducing oxidized metal ions forms which can generate oxygen radicals through the Fenton reaction. However, in this study, the only addition of ascorbate did not provoke an increase of carbonyls, therefore it seems unlikely that the added SA acted as pro-oxidant.

Figure 2. Nitrite and ascorbate effects on protein carbonyls (DNPH) in dry fermented sausages across days of ripening

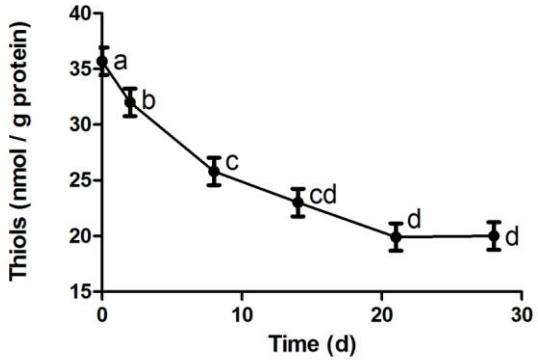


Error bars represent standard errors of the mean values. Superscripts denote significant differences between treatments.

Reducing sugars can also form carbonyls via glycation of lysine residues [17] and once AA is oxidized to dehydroascorbic acid, it is capable of glycating proteins [19]. Consistently, the reaction between nitrite and AA oxidizes the latter to dehydroascrobic acid [20] and this fact might have triggered the formation of carbonyls via glycation in the SA+SN treatment. Malondialdehyde and 4-hydroxynonenal are also carbonyl compounds derived from lipid oxidation which can bind and add carbonyl groups to proteins. However, in this study, the control treatment did not show higher carbonyl compounds although it displayed higher MDA eq. than the other treatments. In dry fermented sausages, lean pork and backfat are coarsely minced and meat and fat particles are clearly defined. As a consequence, interactions between lipid oxidation products and proteins might be more limited in this type of products compared to more finely comminuted products.

In addition to carbonyl compounds, the loss of thiol groups has been used as a marker of protein oxidation [7]. In this study, a significant loss of thiol groups occurred in the four treatments during ripening (Figure 3). However, the loss of thiol groups was not affected by the use of sodium ascorbate nor sodium nitrite, which, evidently, were not able to either prevent and promote it. The oxidation of thiols leads to formation of, among others, disulphide bonds causing aggregation between proteins [21].

Figure 3. Loss of thiol groups in dry fermented sausages during ripening



Error bars represent standard errors of the mean values. Superscripts denote significant differences among time points.

These protein-protein interactions might be involved in the formation of a matrix which is necessary for the development of the desired sliceable sausage texture [22].

IV. CONCLUSION

The results of the present study suggest that nitrite and ascorbate act differently against lipid and protein oxidation. While both ascorbate and nitrite

were able to reduce MDA formation, their simultaneous addition might increase the formation of carbonyl compounds in proteins. However, further studies are needed to better understand the reactions involved and to assess their actual impact on technological, nutritional and organoleptic qualities.

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