Effect of Natural Preservatives on the Microbial Quality, Lipid Stability and Sensory Acceptability of Boerewors

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Abstract– **Boerewors is a fresh sausage preserved with 450 mg/kg sulphur dioxide (SO_2). The preservative effects of rosemary extract (Ros) and chitosan (Chi; natural preservatives) were compared to SO_2. Eight boerewors models with different treatments were formulated. Microbial, colour, lipid and sensory characteristics were evaluated. Chi and Chi in combination with other preservative types had a significant effect on reducing the total bacterial and *Enterobacteriaceae* counts, comparable to SO_2. Chi, however, had a better effect on decreasing yeasts and moulds counts than SO_2. Chi also showed good colour properties comparable to SO_2. Ros showed comparable lipid stability to SO_2 and it showed significantly better lipid stability when compared to Chi. Ros had a better effect on the sensory taste when compared to Chi, but the SO_2 treatment was still preferred. Reduced levels of 100 mg/kg SO_2 showed good synergistic effects in combination with Chi as antimicrobial, colour stabilizer and in combination with Ros as antioxidant and improving the sensory properties.**

Keywords– **natural preservatives, microbial quality, chemical stability**

I. INTRODUCTION

In South African, boerewors is a traditional fresh sausage made from pork and beef meat. It is preserved with 450 mg/kg SO_2 which is used to lower bacterial counts [1] and preserve the bright red colour of the sausages [2]. In the US the use of sulphite agents in meat are not permitted [1,2] due to its relation to health problems (asthmatic attacks) and thiamine absorption deficiency [1,3].

Consumers are nowadays more aware of the use of chemical additives in food and food products. This has resulted in an increase in research on natural additives. Rosemary extracts have been shown to have anti-inflammatory, anitcarcinogenic, antidiuretic, hepatolonic protective as well as anti-microbial and anti-oxidant properties [4].

Chitosan is novel preservative with GRAS status. It is a deacetylated form of chitin derived from the shell of crabs and shrimps and the cell wall of fungi [1,5,6]. Chitosan is effective against the growth of bacteria, yeasts and fungi and possess anti-oxidant activities, water and lipid binding, chelating and emulsifying capacities [1,5,7].

Food quality in terms of microbial, physiochemical and sensory characteristics are very important for both the consumer and the food industry. Lipid oxidation is one of the main parameters that may affect these characteristics [4,7].

The objectives of this study was to study the effectiveness of rosemary extract, chitosan and SO_2, applied individually and in combination with each other, on the microbial quality, colour stability, lipid stability and sensory attributes of boerewors.

II. MATERIALS AND METHODS

A. Sausage preparation and sampling

Boerewors models were manufactured following typical industrial procedures [8,9] and in compliance with the South African regulations [10].

In the conventional boerewors, 0.0682% w/w of sodium metabisulphite was added which is equivalent to 450 mg/kg SO_2 (S). Rosemary extract (Ros) (Flavor'Plus^{TM} #050501, SharonBolel Chemical Marketing, South Africa) was added at a concentration of 0.026% w/w [7]. Chitosan models (Chi) contained 1.0% w/w chitosan (Sigma-Aldrich, USA) [5]. The combination models contained 100 mg/kg SO_2 [3].

The models were cut into 60–80 g pieces, placed in polysterene trays and wrapped with air-permeable polyethylene film. The models were stored at 4 °C under fluorescent light for 9 days.

B. Microbial analyses

The effect of the preservatives against a wide spectrum of micro-organisms, namely total bacteria, *Enterobacteriaceae* and yeasts and moulds, were evaluated. Microbial analyses were performed on days 1, 3, 6 and 9 on all eight treatments as previously described [9,11].

C. Colour stability determination

On days 1, 3, 6 and 9 each sausage was opened and redness colour (a* value) measured on 6 different positions on each sausage after 30 minutes bloom using a Minolta CR-400 chromometer to determine the effect of preservative type on colour stability.

D. Lipid stability determination

A 5 g sample was removed from each portion of sausage and used for thiobarbituric acid reactive substance (TBARS) analysis [12] to determine the effect of preservative type on lipid oxidation. TBARS were measured on day 1of production, after 6 days of storage at 4 ^{o}C and after 100 days at -18 ^{o}C.

E. Sensory Evaluation

For sensory analysis, preparation was as previously described [13]. A 75-member consumer panel was used to taste/evaluate and give their acceptability opinion on the cooked boerewors samples using a hedonic scale.

F. Statistical analysis

All the data were subjected to analysis of variance (ANOVA) while important interactions were further investigated by means of the Tukey-Kramer multiple comparison test [14].

III. RESULTS AND DISCUSSION

A. Microbial analyses

The acceptable total microbial quality standard is 6.00 log cfu/g [15]. The Ros and Ros+S treatments could not conform to this standard over the 9 day storage period. The Chi, Chi+S and Ros+Chi+S produced comparable preservative action to the S treatment (Fig. 1). Similar effects of the different preservatives were observed in another study [7].

Enterobacteriaceae is an indicator for hygienic quality of food products. The eight treatments met the standard of <5.00 log cfu/g for *Enterobacteriaceae* counts for all the storage days [15].

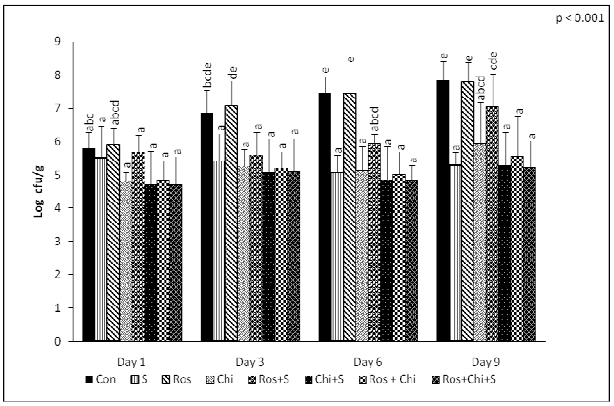

Fig. 1 Effect of preservative types and storage time on the total bacterial counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations

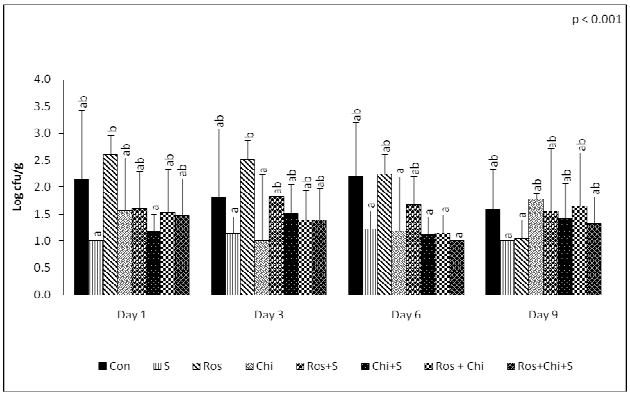

Fig. 2 Effect of preservative types and storage time on the *Enterobacteriaceae* counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations

The S and Chi+S treatments gave significantly better preservative action against *Enterobacteriaceae* than the Ros treatment on days 1 and 6 (Fig. 2). The S, Chi and chitosan in combination with other preservatives (Chi+S, Ros+Chi and Ros+Chi+S), maintained the counts of *Enterobacteriaceae* at 1–1.5 log cfu/g during the storage time of 1–6 days which is in agreement with other studies [5,7].

The S, Ros and Ros+S treatments were not able to maintain the yeasts and moulds counts in this study (Fig. 3). Yeasts and moulds are known to be resistant to SO_2 [1]. The Chi and Chi containing models were the most effective in controlling yeasts and moulds over 9 days storage. This was in contradiction with another study [6] due to the lower concentration of 50–500 mg/kg chitosan that was used.

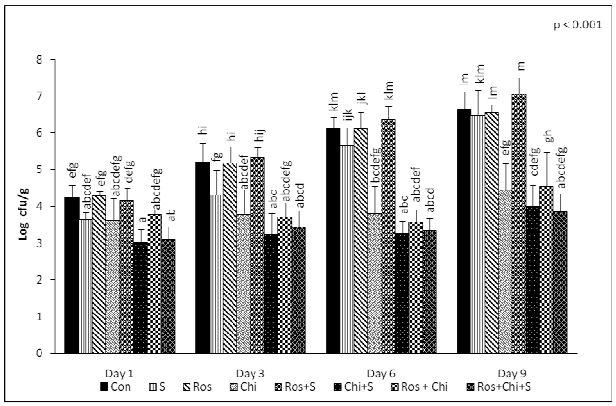

Fig. 3 Effect of preservative types and storage time on the yeasts and moulds counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations

B. Colour stability

There was a significant decrease in the redness (a*) values of all the treatments during the storage time of 1–9 days (Fig. 4).

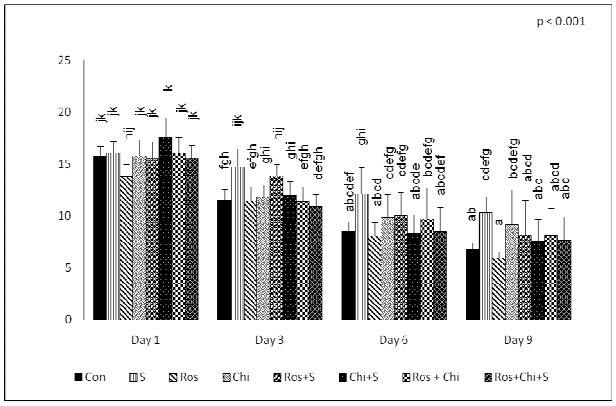

Fig. 4 Effect of preservative types and storage time on the a* value (redness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations

The redness of meat is an important aspect for consumers purchasing meat and meat products. The S treatment showed a significantly higher a* value to that of the Ros, Con and Ros+Chi+S treatments from days 3–9. The Ros treatment showed a significantly higher reduction of a* value when compared to the S treatment at 3–9 days storage time.

In this study the effect of chitosan in maintaining the redness colour was comparable to the S treatment at day 9. This was in agreement with other studies and linked to Chitosan's capacity to bind water and lipid in meat [1,5].

C. Lipid stability

The results of the lipid stability of the boerewors treated with different preservatives are presented in Fig. 5. The higher the TBARS value, the higher the rancidity of the product. At TBARS value of between 1 and 2, rancid off-flavours become detectable by taste panels [16].

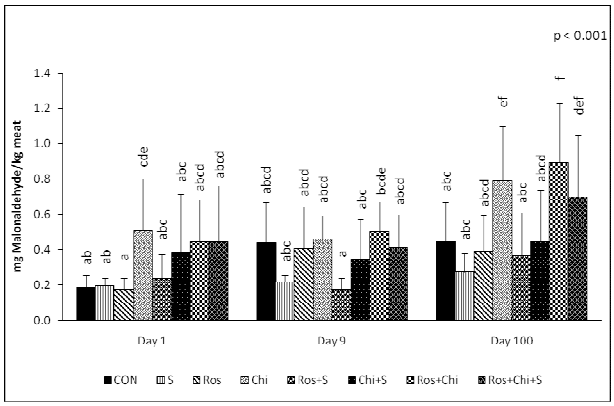

Fig. 5 Lipid stability measured in mg malonaldehyde/kg meat of boerewors treated with different preservatives and stored for 9 days at 4 ^{o}C and 100 days at -18 ^{o}C. Results with different superscripts are significantly different. Error bars represent standard deviations

The Chi and Chi containing models were not able to maintain the lipid stability in this study. The rosemary extract had good antioxidant properties when compared to the chitosan, and this has been speculated to be due to the phenolic compounds in rosemary that have high antioxidant properties [4].

However, the S treatment maintained the TBARS values best from day 1–100. A synergistic effect was observed in the Ros+S treatment, with values comparable to that of the S treatment during a storage time of 100 days. The low TBARS values of the treatments at day 100 may be due to further oxidation of MDA to other organic products which are not determined by the reaction with TBA [5].

D. Sensory evaluation

The results of the taste preference of the boerewors treatments are shown in Table 1. The S treatment was preferred by most consumers. The Con, Ros and

Ros+S treatments were more preferred by consumers compared to the Chi treatments which are in agreement with other studies [4].

The high score for the S treatment was probably due to the fact that consumers are used to a sausage with this kind of preservative. Rosemary also showed a positive sensory score due to improving the flavour of the sausages which was in accordance to findings in other studies [4].

Table 1 Mean values for the taste preference of boerewors samples manufactured with different preservatives. Samples that share the same superscript letter (a, b, c) are not significantly preferred to one another (equally liked), although there is a small difference in means

Treatment	Sensory Score
No preservative (control)	6.7^{bc}
S (450 mg/kg SO_2)	6.8^{c}
Ros	6.3^{bc}
Chi	5.2^{a}
Ros + S	6.2^{bc}
Chi + S	5.2^{a}
Ros + Chi	5.8^{ab}
Ros + Chi + S	6.0^{ab}
p	< 0.001

IV. CONCLUSIONS

In the current study, chitosan and its combinations was the most effective as anti-microbial. Rosemary extract showed good antioxidant and sensory attributes while SO_2 was effective in maintaining the colour of the sausages and had good sensory attributes. Chi, Chi+S and Ros+Chi gave comparable antimicrobial and antioxidant properties to the S treatment. More research is needed to find the perfect combinations.

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