

## *Research Article*

# The Effect of Sodium Reduction and Replacement on the Chemical, Microbial, and Sensory Quality of a Traditional South **African Fresh Sausage**

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Sodium (Na) reduction and replacement were evaluated in traditional South African sausages (boerewors). The Na levels were adjusted to contain the Na inclusion limits of South African regulations for 2017 and 2019, on its own and in combination with different replacers (KCl and K-lactate). The effect of these treatments was evaluated in terms of chemical (ash, NaCl, Na, and chloride contents, pH, water activity  $(a_w)$ , lipid oxidative stability (TBARS), colour, and thaw and cooking losses), microbial (total viable count (TVC), coliform count, and yeast and mould count), and sensory (appearance, flavour, saltiness, and texture overall liking) characteristics. Lipid oxidative stability was comparable for all the treatments no longer than 6 days at 4°C. When the boerewors treatments were frozen for 180 days, the potassium-containing models with lowered levels of NaCl, indicated better secondary lipid oxidative stability than the models containing only Na. The red colour of the boerewors treatments was all negatively influenced by sodium. Higher concentrations of NaCl had a better effect on thaw, cooking, and total losses, which in turn will have a positive effect on texture and overall acceptability. The addition of NaCl better controlled the growth of the TVC until day 6 of storage at 4°C. However, KCl was a promising replacer regarding the control of coliforms. The Na levels and NaCl replacers assisted in inhibiting microbial growth. The sensory analysis indicated that consumers were only able to distinguish the negative control from the other treatments. A shelf life of between 3 and 6 days was recommended for boerewors with the treatments evaluated in this study.

## **1. Introduction**

Salt reduction and replacement in foods have been a major research topic in recent years due to the association of high salt levels with chronic diseases, e.g., high blood pressure and cardiovascular diseases [1, 2]. Salt reduction strategies should be investigated in all type of foods, because a single strategy does not necessarily fit every single food type and will have different effects on the microbial, chemical, and sensory characteristics of a food [3].

Boerewors is a traditional South African fresh sausage. It is so popular with South African consumers that Checkers, one of the big retailers in South Africa, calls out a competition for the best boerewors recipe on a yearly basis. Boerewors is,

however, one of the most regulated meat products in South Africa since it has a history of adulteration with plant proteins, mechanically deboned meat and organ meat [4].

Diets that include high amounts of salt (NaCl) were identified as one of the causes of chronic diseases [2]. South Africa was one of the first countries in the world to develop and implement wide-ranging mandatory legislation to reduce the sodium (Na) levels in processed food products [5]. The legislation was implemented in two phases. For fresh South African sausage, e.g., boerewors, the first limit of 800 mg Na/100 g sausage was made mandatory on 30 June 2016. The second limit comprised of 600 mg Na/100 g sausage and was mandatory from 30 June 2019 [6, 7]. Except for a study by Cluff et al. [8] on pork sausages, no other studies have yet been performed on the effect of these salt reduction legislations in boerewors.

Reduction of NaCl in large amounts is a major challenge in terms of upholding consumer acceptance [9]. Salt plays a significant role in meat processing, which include binding of water and fat [10]. Sodium chloride activates meat proteins, which lead to higher water binding. It also acts as a preservative, since it decreases the microbial growth by lowering the water activity ( $a_w$ ). It produces saltiness, which increases the perception of meat flavour. It is, therefore, evident that the overall quality of processed meat can be negatively impacted by reducing salt [8].

A number of alternative salt reduction and salt replacement strategies have been used in several studies, and one approach was to substitute NaCl with potassium chloride (KCl) or use KCl in combination with lowered levels of NaCl. The advantages of KCl are that it is similar to NaCl in molecular composition and the chloride anion helps with myofibrillar protein extraction which increases emulsion stability [11]. Inclusion levels of up to 50% (w/w) will assist in protein extraction; however, these levels will have a negative effect on sensory attributes since it imparts bitterness to products when included in quantities higher than 50% (w/w) [12].

Potassium lactate is widely used and known to act as antimicrobial; improve meat colour, juiciness, and tenderness; and decrease negative flavour attributes [13]. The US Department of Agriculture, Food Safety and Inspection Service permits inclusion levels up to 4.8% (w/w) in meat products. Studies on meat products indicated that an inclusion level of 3 to 4% was able to inhibit the growth of *Salmonella* [14].

This study is aimed at examining the chemical, microbial, and sensory effect of using lower salt concentrations according to the 2017 and 2019 regulations for Na limits in boerewors, on its own, and in combination with either KCl or potassium lactate.

#### 2. Materials and Methods

2.1. Sourcing of Lean Meat, Fat, Additives, and Spices. All the meat and fat used during this trial were sourced from an owner-operator meat processing plant in Bloemfontein, SA. Fresh pork, consisting of a minimum of 70% lean meat and 30% fat (70/30), fresh lean beef (80/20), on a dry weight basis, and pork back fat were used to manufacture the sausages. The meat was collected less than 24 h before required

and transported to the meat processing facility of the University of the Free State, where it was kept at 4°C until used. The spices, potassium chloride, and potassium lactate were obtained from B.T. Enterprises (Johannesburg, SA).

2.2. Formulation of Boerewors. The basis of the formulation was the two Na limits as set out in the South African Department of Health (SA DoH) Regulations [6, 7], and the formulations are given in Supplementary Table S1. The formulations of the treatments were as follows: treatment 1 was the negative control with 0.00% (w/w) added salt (NC); treatment 2 consisted of a combination of 0.55% (w/w) KCl and 1.25% (w/w) added NaCl as recommended by SA DoH [7] (K600); treatment 3 consisted of a combination of 0.55% (w/w) added NaCl as recommended by SA DoH [7] (L600); treatment 4 contained only the 1.25% (w/w) added NaCl as recommended by SA DoH [7] (N600) with no added replacer; and treatment 5, which was the positive control (PC), consisted of 1.80% (w/w) NaCl as recommended by SA DoH [6].

The spice formulation for all the treatments contained (%, w/w) coarse roasted coriander (0.12), coarse coriander (0.16), ground roasted coriander (0.16), ground coriander (0.18), ground clove stems (0.08), ground nutmeg (0.09), ground black pepper (0.03), ground white pepper (0.04), mustard powder (0.04), monosodium glutamate (0.25), dextrose (0.19), medium rusk (0.50), and sodium metabisulphite (0.06).

2.3. Manufacturing of Boerewors. Three separate replicates for each treatment were manufactured at least one month apart to compensate for variations in raw materials and processing, as well as environmental conditions. A single replicate consisted of a 6 kg boerewors batch, made for each of the five treatment groups. The sausage models were manufactured by using representative industrial procedures [15] and in compliance with the South African regulations for boerewors [5].

Fresh meat (beef (80/20) and pork (70/30)) was comminuted through a 13 mm mincing plate and fitted to a number 32 Okto mincer. The meat was thoroughly mixed with a butchery mixer to obtain a homogenous raw material mixture. The spice pack, together with the additives, was mixed with the ice water and left to stand for five minutes, to allow for hydration and the water-soluble additives to dissolve properly. This spice mixture was thoroughly mixed with the meat mixture, before being minced through a 4.5 mm mincing plate. Natural hog casings with a diameter of 28-32 mm were filled with the sausage mixture, using a manual sausage filler (Trespade, Crown National, Johannesburg, SA). This resulted in a single, continuous roll of sausage that had a weight of 6 kg per treatment. Each treatment's roll of sausage was cut into individual 150 g pieces for day 0 samples; 100 g pieces for day 3, 6, and 9 samples; and 50 g pieces for the 90- and 180-day samples.

Each individual sausage was placed in an expanded polystyrene (EPS) tray, containing an absorbent pad and was overwrapped with polyvinyl chloride (PVC) film. The sausages that were sampled on days 0, 3, 6, and 9 were stored at  $4^{\circ}$ C under retail refrigeration-type conditions, including fluorescent lighting, for fresh product shelf-life determination (4 sausages per treatment for each day of sampling). The sausages for the 90- and 180-day analysis, were, in addition to being overwrapped with PVC, vacuum packed and stored at  $-18^{\circ}$ C for frozen product lipid stability determination since fresh boerewors are sometimes frozen at  $-18^{\circ}$ C by consumers after purchasing. For sensory analysis, a 3 kg sausage roll, of all five treatment groups from each replicate, was stored at  $-18^{\circ}$ C for a week, until sensory analysis was carried out. The sausages from each replicate for the cooking loss analysis were weighed into 100 g pieces, placed in individual EPS trays containing absorbent pads, overwrapped with PVC film, and stored at 4°C for one day and then at  $-18^{\circ}$ C for 9 days, before cooking loss analysis was carried out.

2.4. Sample Preparation. The study was performed in triplicate for all analysis. The fresh product shelf-life evaluation sampling was done on days 0, 3, 6, and 9, and the frozen product shelf-life evaluation was done on days 0, 90, and 180. For each sampling interval, four sausages per treatment group per replicate were collected for quadruplicate chemical and microbial analyses. For thaw, cooking, and total losses, 12 sausages per treatment group per replicate were used.

After samples for microbial analyses were removed as eptically, the remaining sausage sample was used to fill three plastic cuvettes with tight fitting lids. Two cuvettes were frozen at  $-18^{\circ}$ C for chemical analyses. The third cuvette was used for same-day chemical analyses. The same process was repeated for each of the four samples per treatment group, per time interval, and per replicate.

2.5. Ash, NaCl, Na, and K Contents. Sodium chloride and Na contents were determined on day 0 samples. For NaCl, the Volhard method was used [16]. Sodium and K contents were determined by atomic absorption spectroscopy (AAS), using a Varian SpectrAA-300 spectrometer (SMM Instruments, Johannesburg, SA). For AAS analysis, the samples were reduced to a mineralized form free from any organic compounds by means of ashing [17].

2.6. pH. The pH was measured with a direct pH measurement probe (Model MA920, Milwaukee Instruments, Rock Mount, USA), coupled to a pH meter (Thermo Scientific, Orion 3-Star Plus Model, Labotec, Midrand, SA).

2.7. Water Activity  $(a_w)$ . The  $a_w$  was determined using a Novasina Thermoconstanter TH 200 (Labotec, Midrand, SA).

2.8. Lipid Oxidative Stability and Moisture Content. A 5 g sample was taken from each sausage, at each sampling interval, per treatment group, and per replicate and used for thiobarbituric acid reactive substance (TBARS) analysis [18]. Frozen samples were thawed overnight at 4°C. The TBARS results were quantified in terms of milliequivalents (mEq) malondialdehyde (MDA) per kilogram sample. The analysis was performed on the fresh sausages after 0, 3, 6, and 9 days of storage at 4°C. Sausage treatment samples were also frozen on day 0, and TBARS was then performed on overnight thawed samples after 90 and 180 days of storage at  $-18^{\circ}$ C. Moisture content (%) was determined by oven drying over-

night at 102°C and used as a second establishing parameter [16] as it is required in the calculation of TBARS.

2.9. Colour. Colour measurements were performed on days 0, 3, 6, and 9 of storage at 4°C, after the sample for microbial analysis was taken. Samples were left to bloom for 30 min. Measurements per sausage were done in sextuplicate. Although colour is usually measured in terms of redness  $(a^*$ -value), yellowness  $(b^*$ -value), and lightness  $(L^*$ -value), only the  $a^*$ -value colour measurements, which represents redness, were taken into account since several studies ([19] and references within reference [19]) indicated that  $a^*$ -value was a variable factor, while  $b^*$ - and  $L^*$ -values were giving opposite values from the  $a^*$ -value. The  $a^*$ -value is also regarded the most important colour characteristic in red meat products. Colour measurements were performed vertically using a Minolta CR 400 chromometer (8 mm measurement aperture) [20].

2.10. Thaw and Cooking Losses. Thaw and cooking losses were performed to indicate the water-holding capability of the treatments. Twelve sausages of each treatment group per replicate were kept frozen at  $-18^{\circ}$ C for 9 days, to simulate a short-term home-freezing scenario. After 9 days, the samples were placed at 4°C for 24 h for thawing. The samples were removed from the packaging and weighed. The sausages were then dry-cooked in a convection oven and preheated to 160°C, until an internal temperature of 72°C. During cooking, the baking tray was rotated 90° every 2 min, for even cooking conditions. Afterwards, the sausages were removed from the oven and air-cooled to room temperature before being weighed again. The thaw loss and cooking loss were calculated according to Cluff et al. [8].

2.11. Microbial Analyses. For the microbial analysis, a 10 g sample, from each product, was aseptically weighed into a sterile 207 mL Whirl-Pak<sup>TM</sup> bag (Lasec, Bloemfontein, SA), after which 90 mL of sterile 0.1 mol/L buffered peptone water (BPW) solution was added. The sample was homogenised (AME Stomacher Lab-Blender 400; Labotec, Johannesburg, SA) for 1 min. Further dilutions  $(10^{-2}-10^{-6})$  were made in 9 mL sterile 0.1 mol/L phosphate buffer. One millilitre volumes of each dilution were then pour plated on different selective media [21]. All media were sourced from Thermo Fisher (Pty) Ltd. (Randburg, SA).

Standard plate count agar (SPCA; Oxoid 0463) was used to enumerate the total viable counts and incubated at 32°C for 48 h. Violet red bile agar +4-methylumbelliferyl- $\beta$ -Dglucuronide (VRBM; Oxoid CM0978) was used for total coliform counts, as well as for detection of *E. coli*. Incubation was at 37°C for 48 h, and fluorescence under ultraviolet light (366 nm, CAMAG Universal UV Lamp) was used as an indication of the presence of *E. coli*. Yeast and mould enumerations were done using Rose-Bengal chloramphenicol agar (RBCA; Oxoid CM0549), with chloramphenicol supplement (Oxoid SR0078), incubated at 25°C for four days. After incubation, all the colonies on the media were enumerated using a colony counter [21].

2.12. Consumer Sensory Evaluation. Samples of each treatment group per replicate were defrosted overnight at 4°C. The same cooking method was employed as for the *thaw* and cooking losses described previously. The cooked sausages were cut into pieces each with a length of ~2 cm and placed individually in small glass bowls that were covered with squares of aluminium foil. The bowls were kept warm at  $55^{\circ}$ C until just before serving. Each container was marked with a randomised, three-digit code unique to each sample. Five glass bowls representing the five treatment groups were arranged from left to right on a serving tray, in ascending order of the three-digit codes, ensuring that the samples were evaluated in a random order from one consumer to the next. The five samples were evaluated by each respondent in one session.

A 75-member consumer panel of staff and students from the agriculture building of the University of the Free State was used. The panel consisted of 63% females and 37% males, ranging from 19 to 61 years of age, with an average age of between 20 and 29 years, respectively. The sensory evaluation was performed in five individual booths. Multiple panels of five individuals per panel were performed. The booths were fitted with three overhead light fittings with three red coloured bulbs emitting only red light to mask any possible colour variations between different samples.

Although a consumer panel was used in this study, a screening method, using a wood ice-cream stick dipped in a 2% (w/v) NaCl solution, was used to exclude nonsalt tasters from the sensory panel. Each respondent received a printed, 5-page questionnaire consisting of five nine-point hedonic rankings per page, ranging from 1 = dislike extremely to 9 = like extremely [22]. The respondents were then expected to rank each sample individually for the following attributes: appearance (condition of the sausages like possible shrinkage or bursting of the sausage, colour was not considered), flavour, saltiness, texture (loose or firm texture), and overall liking. Bottled water was presented at 20°C as a palate cleanser between samples.

Written informed consent was obtained from all the participants, which was approved by the University of the Free State Ethics Committee (UFS-HSD2017/1198).

2.13. Statistical Analyses. All results were captured in Microsoft Excel 2018. The experimental design consisted of five treatments and three replicates, for all methods of analysis. An ANOVA (NCSS Statistical Software package, version 11.0.20) was used to determine the effect that added NaCl levels and/or replacer treatments had on various quality parameters of the boerewors. The Tukey-Kramer multiple comparison test ( $\alpha = 0.05$ ) was carried out to identify significant differences between the treatment means [23].

#### 3. Results and Discussion

3.1. Ash, NaCl, Na, and K Contents. The significantly different (p < 0.001) percentages of ash content were a result of the different amounts of NaCl added in each of the different treatment formulations (Table 1). The treatments with higher percentages of ash correlated directly with treatments that had higher amounts of added NaCl and added replacers, because the inorganic matter that was left mostly consisted of metal oxides, which were enhanced by the amount of NaCl added [24].

Sodium chloride was present at a base level of  $0.21 \pm 0.04\%$  in the NC (Table 1), with all contributions made by formulation components, other than added NaCl. The NaCl content of each treatment group significantly (p < 0.001) increased with increasing amounts of added NaCl. The K600 treatment did show a higher NaCl percentage (Table 1) which could be attributed to the Volhard method of analysis that has a limiting factor in terms of accuracy, because the amount of NaCl was calculated from the amount of Cl present in the sample. This entirely omitted the Na contributed to the final product. Potassium in the K600 treatment was added as KCl, which then gave a higher value.

With regard to the K values, the K600 and L600 had significantly (p < 0.001) higher values than the rest of the treatments (Table 1). This could be ascribed to the potassium in the KCl and the lactate being added as K-lactate.

3.2. *pH*, *Water Activity, and Moisture Content.* The addition of NaCl lowered the pH of the models, and the NC, therefore, had a significantly (p < 0.001) higher pH value than the rest of the treatments (Table 1). The dissociated Na<sup>+</sup> and Cl<sup>-</sup> ions of NaCl determine the functions thereof in meat mixtures. When added to minced meat, the negative charge on the proteins is increased by the Cl<sup>-</sup> ions. The isoelectric point then shifts to a lower pH when Cl<sup>-</sup> ions are adsorbed onto positively charged groups of myosin [25]. The findings in this study correlated with results obtained in a study done on the effect that NaCl and storage time had on the physicochemical and sensorial properties of beef meatballs [26].

The PC, which also had the highest NaCl content, had the lowest  $a_w$  value (Table 1). This can be attributed to the fact that NaCl lowers  $a_w$  by binding available free water [24]. Moisture content was not influenced by any of the treatments (Table 1) which was in accordance with Stanley et al. [11].

3.3. Lipid Oxidative Stability. Lipid oxidation in meat is accelerated through catalytic reactions by the synergistic working of NaCl with heme and nonheme iron in meat, because of its prooxidant reactions [27]. A first threshold of 0.50 mEq MDA/kg [28] and a secondary threshold of 1 mEq MDA/kg [29] exist for the detection of rancidity.

There was no significant difference between treatments at 4°C on days 0, 3, and 9 (Figure 1). All the TBARS values were below that of an organoleptic threshold of 0.5 mEq MDA/kg [28]. The salt content of all the treatments was not high enough to initiate lipid oxidation at 4°C during the nine days of storage.

Sausages that were kept frozen at -18°C for 90 days showed a significant (p < 0.001) difference in TBARS values between treatments (Figure 2). The N600 and PC treatments were significantly (p < 0.001) higher than the rest of the treatments, and their values also exceeded the 0.50 mEq MDA/kg threshold. The NC had the lowest secondary lipid oxidation. After 180 days of storage at -18°C, the secondary lipid oxidation had progressed to such an extent that only the NC and K600 treatments were below the 1.00 mEq

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Treatment	NC	K600	L600	N600	РС	Sign. level
Formulated Na (mg/100 g)	110.03	600.12	599.78	600.38	816.69	
% ash	$1.03^{a} \pm 0.04$	$3.07^{c} \pm 0.11$	$2.73^b\pm0.27$	$2.63^b\pm0.22$	$3.69^d \pm 0.15$	p < 0.001
% NaCl	$0.21^a\pm0.04$	$1.78^d\pm0.04$	$1.44^{c} \pm 0.04$	$1.40^b\pm0.04$	$1.92^e\pm0.06$	p < 0.001
Na (mg/100 g)	$131.89^{a} \pm 16.63$	$607.68^{b} \pm 46.95$	$610.49^{b} \pm 122.22$	$628.02^{b} \pm 111.06$	$855.21^{\circ} \pm 45.25$	p < 0.001
K (mg/100 g)	$227.03^a\pm9.60$	$580.87^{d} \pm 42.89$	$401.23^{c} \pm 70.12$	$302.11^{b} \pm 56.66$	$340.73^{bc} \pm 14.84$	p < 0.001
pН	$5.67^{b}\pm0.05$	$5.52^a \pm 0.05$	$5.57^{a} \pm 0.14$	$5.50^a \pm 0.11$	$5.51^a\pm0.11$	p < 0.001
Water activity	$0.9606^{ab} \pm 0.0135$	$0.9609^{ab} \pm 0.0084$	$0.9576^{ab} \pm 0.0165$	$0.9632^{\rm b}\pm 0.0102$	$0.9491^{a}\pm 0.0053$	<i>p</i> = 0.036
Moisture content (%)	$63.09 \pm 2.93$	$62.61 \pm 2.67$	$62.47 \pm 3.01$	$63.18 \pm 3.49$	$61.75 \pm 2.63$	p = 0.470

TABLE 1: Effect of NaCl replacer treatment on chemical properties of fresh boerewors directly after manufacturing (n = 12).

Means with different superscripts in the same row differed significantly. Means  $\pm$  standard deviation. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl).



FIGURE 1: The effect of added NaCl level on the TBARS of boerewors stored at 4°C for up to 9 days (n = 12). NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.



FIGURE 2: The effect of added NaCl and/or replacer levels on the TBARS values of boerewors stored at  $-18^{\circ}$ C for up to 180 days (n = 12). NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.



FIGURE 3: Redness  $(a^*)$  value of five boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period (n = 12). NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

MDA/kg threshold, with  $0.80 \pm 0.08$  and  $0.92 \pm 0.08$  mEq MDA/kg values, respectively. The K600 and L600 treatments that contained K and lactate as respective replacers therefore helped to reduce secondary lipid oxidation. This was in agreement with Tan and Shelef [30], who found that combinations of K-lactate with NaCl in treated fresh ground pork improved the fat stability during storage at -20°C. This is ascribed to the ability of potassium lactate to scavenge super-oxide and hydroxyl (OH) radicals [14]. Sodium chloride is a prooxidant [30], which was the reason why the NaCl-containing treatments showed higher lipid oxidation values than the KCl treatment.

3.4. Colour. The red ( $a^*$ -value) colour of meat plays a vital role for consumers when purchasing a meat product [31]. The red colour of meat comes from when the myoglobin is exposed to oxygen, which then results in the formation of red oxymyoglobin [32]. The NC showed the highest value throughout the four sampling days, and significant differences (p < 0.001) were found between the treatments (Figure 3). As NaCl levels increased, redness decreased which could be ascribed to NaCl having a denaturing effect on myoglobin proteins, which decreases their ability to bind oxygen and give a red colour. In a study by Stanley et al. [11] on pork sausage patties, salt replacement with KCl did not affect the  $a^*$ -value of the patties. However, in fresh ground beef,  $a^*$ -value rapidly decreased in NaCl and K-lactate treatments at 2°C after 4 days [30].

3.5. Thaw, Cooking, and Total Losses. Thaw, cooking, and total losses have a significant influence on consumer preference as it affects the moisture content and tenderness of food [33]. The thaw losses indicated that the NC had a significantly (p < 0.001) higher value ( $4.81 \pm 0.047\%$ ) than the rest of the treatments and in contrast with the PC that had the lowest value of  $2.03 \pm 0.29\%$  (Figure 4). This indicated that the solubilization capacity of myofibrillar proteins is directly related to the amount of salt added in the formulation. Both of the replacer formulations had higher (p < 0.001) thaw losses compared to the two NaCl-only treatment groups.

The PC treatment had a significantly (p = 0.002) lower cooking loss value (13.30 ± 4.68%) than the NC and N600. The L600 had significantly (p = 0.002) higher cooking loss than the K600 and PC treatments (Figure 4). The PC, which contained the highest amount of NaCl, had the lowest cooking loss of 13.30 ± 4.68%, compared to the L600 treatment that had cooking losses of 20.36 ± 4.94%. The reason for the lower cooking loss of the PC could be ascribed to the enhanced WHC in the presence of salt, which led to reduced cooking losses [34].

The PC had the lowest total loss value of  $15.33 \pm 4.74\%$ . The PC and K600 treatments had significantly (p < 0.001) lower total losses compared to the NC and L600 treatments. The L600 treatment had an especially high total loss of  $23.13 \pm 4.16\%$ , which differed significantly (p < 0.001) from the K600 and PC. In terms of thaw, cooking, and total losses, higher concentrations of NaCl did prove to have a positive effect in reducing these losses, which in turn would influence texture and overall acceptability.

3.6. Microbiological Analyses. There were no significant differences between treatments for TVC on days 0 and 3 (Table 2), and the values complied to the <6 log CFU/g [33]. On days 6 and 9, however, the significantly (p = 0.049and p = 0.020, respectively) higher values of the NC showed that the addition of NaCl had an inhibitory effect on microbial growth. There were, however, no significant differences between the rest of the treatments on days 6 and 9 which indicated that the different replacer combinations had similar inhibitory effects on microbial growth. These results are in agreement with those of Cluff et al. [8] who reported no significant differences in TVC for 1, 1.5%, and 2% (w/v) NaCl inclusion levels in fresh pork sausages.

The TVC values of the NC, K600, and L600 treatments on day 6 and all the treatments on day 9 were higher than 6 log CFU/g. Only the PC and N600 sodium reduction treatments were <6 log CFU/g on day 6. This implies that higher concentrations of NaCl better controlled the growth of the TVC until day 6 of storage at 4°C than the K600 and L600 treatments.



FIGURE 4: Thaw, cooking, and total losses of five boerewors formulations based on different added NaCl and/or replacer levels (n = 12). NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl). Means with different superscripts for the same parameter differed significantly. Error bars represent standard deviations of means.

TABLE 2: Effect of NaCl replacer treatment on microbiological parameters of boerewors stored at 4°C for 9 days (n = 12).

Day	Treatment	Total viable count (log cfu/g)	Sign. level	Coliform count (log cfu/g)	Sign. level	Yeast count (log cfu/g)	Sign. level	Mould count (log cfu/g)	Sign. level
0	NC	$6.12\pm0.38$		$3.53^b\pm0.14$		$3.47\pm0.20$		$1.17 \pm 0.22$	
	K600	$5.87 \pm 0.31$		$2.84^a\pm0.15$		$3.41\pm0.09$		$1.26\pm0.21$	
	L600	$5.90\pm0.22$	p = 0.232	$3.11^{a}\pm0.39$	p < 0.001	$3.38\pm0.15$	p = 0.324	$1.28\pm0.34$	p = 0.641
	N600	$5.83 \pm 0.36$		$3.04^a\pm0.24$		$3.34\pm0.18$		$1.32\pm0.25$	
	PC	$5.88 \pm 0.35$		$2.99^{a} \pm 0.21$		$3.45\pm0.20$		$1.24\pm0.20$	
3	NC	$5.51 \pm 0.57$		$2.93^{b}\pm0.11$		$4.12\pm0.53$		$1.79\pm0.45$	
	K600	$5.45\pm0.62$		$2.52^a\pm0.31$		$4.16\pm0.37$		$1.59\pm0.44$	
	L600	$5.44\pm0.55$	p = 0.967	$2.71^{ab}\pm0.16$	p < 0.001	$4.07\pm0.47$	p = 0.959	$1.89\pm0.22$	p = 0.371
	N600	$5.34 \pm 0.67$		$2.52^a\pm0.29$		$4.05\pm0.48$		$1.68\pm0.43$	
	PC	$5.48 \pm 0.53$		$2.61^a\pm0.29$		$4.16\pm0.31$		$1.72\pm0.32$	
6	NC	$6.17^{\rm b}\pm0.31$		$2.94^{d} \pm 0.15$		$5.59^{b}\pm0.19$		$1.76\pm0.44$	
	K600	$6.00^{a} \pm 0.37$		$2.33^{ab}\pm0.19$		$5.42^{ab}\pm0.17$		$1.49\pm0.37$	
	L600	$6.11^{a} \pm 0.27$	p = 0.049	$2.67^{\rm c}\pm 0.15$	p < 0.001	$5.40^a\pm0.20$	p = 0.025	$1.65\pm0.31$	p = 0.322
	N600	$5.76^{a} \pm 0.50$		$2.51^{bc} \pm 0.14$		$5.55^{ab}\pm0.10$		$1.58\pm0.37$	
	PC	$5.92^{a} \pm 0.35$		$2.25^a\pm0.26$		$5.46^{ab}\pm0.13$		$1.42\pm0.58$	
9	NC	$6.71^{b} \pm 0.29$		$2.91^{b} \pm 0.13$		$6.19^{c} \pm 0.11$		$1.81^b\pm0.40$	
	K600	$6.32^{a} \pm 0.39$		$2.40^a\pm0.28$		$5.95^{bc}\pm0.35$		$1.56^{ab}\pm0.48$	
	L600	$6.69^{a} \pm 0.29$	<i>p</i> = 0.020	$2.43^{a} \pm 0.23$	p < 0.001	$5.96^{bc} \pm 0.37$	<i>p</i> < 0.001	$1.44^{ab}\pm0.44$	<i>p</i> = 0.037
	N600	$6.36^{a} \pm 0.41$		$2.43^{a} \pm 0.20$		$5.64^{ab} \pm 0.32$		$1.32^{a} \pm 0.22$	
	PC	$6.50^{a} \pm 0.35$		$2.32^{a} \pm 0.28$		$5.49^a \pm 0.53$		$1.45^{ab}\pm0.35$	

Means with different superscripts in the same column within the same day differed significantly. Means  $\pm$  standard deviation. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl).

The addition of NaCl and replacers in treatments had significant effects on the coliform counts on days 0, 3, 6, and 9 (Table 2). No *E. coli* was present in any of the samples.

On days 0, 3, and 9, the NC had a significantly (p < 0.001) higher value than the rest of the treatments. Even though the four treatments with added NaCl did not differ



FIGURE 5: Consumer sensory rankings of five boerewors formulations based on different added NaCl and/or replacer levels (n = 75). NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl); PC = positive control (1.80% added NaCl). Means with different superscripts for the same parameter differed significantly.

significantly from each other, the K600 and PC treatments had lower values than the rest. This indicated that the treatment with KCl as replacer was comparable to the PC treatment which implies that KCl was a promising replacer regarding the control of coliforms. The values obtained were also lower than the maximum limit of 4 log CFU/g [35]. These findings were in agreement with Bidlas and Lambert [36] who indicated that KCl has an equivalent antimicrobial effect on pathogenic organisms, compared to NaCl. They also stated that partial or complete replacement of NaCl by KCl is possible after using a modification of the R.J. Lambert and R. Lambert [37] model.

Yeasts and moulds are indicators of the spoilage potential of a food. The yeast counts varied between 3.34 and 6.19 log CFU/g, and the mould counts varied between 1.17 and 1.89 log CFU/g over the 9-day storage period at 4°C. There were no significant differences in yeast counts between treatments on days 0 and 3 and on days 0, 3, and 6 for mould counts. On days 6 and 9, the yeast count of the NC differed significantly (p = 0.025 and p < 0.001, respectively), and for the mould count, it was significantly (p = 0.037) higher on day 9, than the other treatments. The reason for the NC to have significantly higher numbers is ascribed to no NaCl being present in these models.

For the rest of the treatments, for both yeast and mould counts, there were significant differences (p = 0.025 (day 6 yeasts) and p < 0.001 (day 9 yeasts), respectively, and p = 0.037 for mould count on day 9). This is in agreement with the study by Teixeira et al. [19] on Bizaro pork sausages, which indicated that yeast and mould counts significantly increased with storage time. However, on days 6 and 9 for yeasts and day 9 for moulds, there were no significant differences between any of the treatments that contained NaCl. This again indicated that all the treatments with NaCl and replacers had comparable results in controlling the growth of yeasts and moulds.

3.7. Sensory Analysis. The consumer panel continuously ranked the NC treatment significantly (p < 0.001) lower than

the other four treatments for all five of the chosen sensory attributes (Figure 5). For appearance, flavour, saltiness, texture, and overall liking, no significant differences in rankings were found between the K600, L600, N600, and PC treatments (Figure 5). Panel members were only able to distinguish between the NC and the rest of the treatments. This meant that the taste of the boerewors will not be negatively affected by either using lower levels of salt or by partially replacing salt with 0.55% (w/w) potassium lactate or 0.55% (w/w) potassium chloride. This was in agreement with a study by Stanley et al. [11] which found that there were no significant differences in pork sausage patties between treatments that contained NaCl and KCl as a salt replacer.

## 4. Conclusions

This study, therefore, indicated that, although both KCl and K-lactate will be regarded suitable replacers of NaCl in boerewors, especially in combination with lowered levels (1.25% w/v) of NaCl, the lowered NaCl inclusion level (1.25% w/v) was able to ensure the chemical, microbial, and sensory stability of boerewors. However, if the Na in NaCl in boerewors needs to be replaced with a healthier alternative, this study indicated that KCl will be a suitable candidate. In this study, a KCl concentration of 0.55% (w/v) in combination with 1.25% (w/v) NaCl did not have a negative taste result in the boerewors. It is, however, important to remember that the addition of KCl in concentrations above 0.5% tends to leave a bitter taste in the meat product.

#### **Data Availability**

Data is available on request from the corresponding author.

#### **Ethical Approval**

This study was approved by the Natural and Agricultural Sciences Research Ethics Committee of the University of the Free State with ethical clearance number UFS-HSD2017/1198.

### Disclosure

We acknowledge that an earlier version of this manuscript has been presented in the Book of Abstracts of the 65<sup>th</sup> International Committee of Meat Science and Technology Congress in 2019 with the link https://digicomst.ie/wp-content/uploads/2020/05/2019\_11\_32.pdf.

#### **Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

## **Authors' Contributions**

A.H. and C.H. were responsible for the conceptualization. A.H., C.H., M.C., C.B., E.R., and B.v.W. were responsible for the methodology. A.H. was responsible for the software. A.H., C.H., M.C., C.B., E.R., and B.v.W. were responsible for the validation. A.R. was responsible for the formal analysis. A.R. was responsible for the investigation. A.H., C.H., and C.B. were responsible for the resources. A.R. and A.H. were responsible for the data curation. A.R. was responsible for the writing—original draft preparation. A.H., C.H., M.C., E.R., and B.v.W. were responsible for the writing—review and editing. A.R. was responsible for the visualization. A.H. and C.H. were responsible for the supervision. A.H. was responsible for the project administration. A.H. was responsible for the funding acquisition. All authors have read and agreed to the published version of the manuscript.

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#### Supplementary Materials

Supplementary Table S1: sausage treatment formulations used in this study. (*Supplementary Materials*)

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