



Effects of Rosemary Spice (*Rosmarinus Officinalis L.*) and Nitrite Pickling Salt Combination on Keeping and Organoleptic Quality of Beef Sausages

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ABSTRACT

The potential use in foods as antioxidants and antimicrobials coupled by increasing interest in the use of natural preservatives (for safety reasons) motivated the demonstration of the possibility of substituting rosemary spice for nitrite pickling salt in beef sausages. Five types of beef sausage with similar ingredients in type and quality except for the level of rosemary spice (*Rosmarinus Officinalis L.*) and nitrite pickling salt were prepared conventionally and stored at 5°C. The sausages had spice-nitrite pickling salt combinations from sample with no rosemary spice and 18gm nitrite pickling salt /Kg beef (standard sausage) to sample with 0.5% rosemary spice (based on sausage mass) and with no nitrite pickling salt. Microbial proliferation was monitored for 9 days and extent of rancidity development for 11 days as measured by absorbance of their light petroleum (4°C-6°C) extract at 269nm. Twenty panelists appraised the organoleptic quality using a hedonic scale of 7. It was found that rosemary spice can substitute nitrite pickling salt to produce organoleptically acceptable sausages of comparable microbiological quality - with 0.4% rosemary spice and 6mg/Kg nitrite pickling salt mixture as the optimum combination in microbial inhibition. However, it was demonstrated that rosemary spice/ nitrite pickling salt mixes are not effective (relative to rosemary spice and NPS when separate) in halting production of secondary products of lipid oxidation.

KEY WORDS: rosemary spice, nitrite pickling salt, beef sausage.

INTRODUCTION

Meat emulsion products including sausages contain mainly meat and fat as major ingredients. It then follows that the main mechanism of spoilage are microbial spoilage and fat deterioration which affect both the keeping and organoleptic quality of meat emulsions. In meat processing, reservoirs of microorganisms include meat components, seasoning and formulating ingredients, meat handlers with poor personal hygiene, animal skin and byproducts, slaughter house and the environment (air and water for processing), transport and storage facilities (Wilson et al, 1981). Poor hygiene practices in the slaughter house and processing plants e.g. sticking knives into meat, making deep cuts or failure to store equipment hygienically or sterilize them are another source of microbial contamination leading to spoilage. There is also mushrooming of micro and small scale meat processing in East Africa as clearly evident in the low-income dwellers in Nairobi particularly in the slum areas. These enterprises observe minimal precautions and practices to reduce the risk associated with microbial spoilage (Mwangi, 2002) and overuse of certain chemical ingredients such as nitrites.

The most widely used preservatives to prevent microbial spoilage in meat and meat products include nitrates or nitrites pickling salt, smoke and /or sodium metabisulphite. However, there has been increasing concern over their safety. Relatively old but comprehensive reviews on this matter was done by the American Council for Agricultural Science and Technology (Tompin, 1983) and by Hansen and Marsden (1987).

Nitrites and nitrates are responsible for nitrite-induced methaemoglobinaemia (Hansen and Marsden, 1987; Walley and Flanagan, 1987). In methaemoglobinaemia, haem iron is oxidised to iron (III) and therefore cannot function as an effective oxygen transporting protein (Sally et al. 2001). This may cause difficulty in breathing, pallor, dizziness or headache. This may consequently cause toxicity, which may result to death. Nitrites can also react with amines to produce nitrosamines, which are reported to be carcinogenic (Oiye and Muroki, 2002).

The most common fat spoilage mechanism is autoxidation. Lipolysis is encouraged only after storage in conducive conditions, at which time, the microbial spoilage is evident and thus the product not suitable for

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consumption. Autoxidation is usually prevented by use of synthetic phenolic antioxidant such as dodecylgallate, octyl gallate, butylated hydroxyanisole (BHT) and butylated hydroxytoluene (BHT). Sodium metabisulphite also has antioxidant effect and can be used to prevent oxidation of fat. Vitamin E (α -tocopherol)- a natural antioxidant- is fairly widely used particularly in infant food. There is evidence that these chemical antioxidants have potential hazardous effects. Experimental data on gallate toxicity and studies on reproduction, teratogenicity and mutagenicity are available (van der Heijden *et al.* 1986). Both carcinogenic and anticarcinogenic properties have been reported for the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Botterweck, 2000). Cancer of the fore stomach in Syrian golden hamster has been reported after they had consumed large quantities of BHA (Hansen and Marsden, 1987). It is recommended that antioxidants include sodium metabisulphite (and with an exception of α -tocopherol) be not used in baby foods. Gunnison (1981) has reviewed data on sulphite chemistry and toxicity in *in vitro* systems with the perspective of potential mammalian toxicity and indicated that sulphites are most likely to be of toxicological significance in mammals.

In this era of increased concerns on safety of chemical food additives, promotion of readily and naturally available materials, and use of appropriate methods of preservation, advertent use of spices as preservatives has received increased attention. Despite their demonstrated potential use in food as antioxidants and antimicrobials, spices still remain primarily as food condiments (Okiye and Muroki, 2002). Billing and Sherman (1998) emphasize that the proximate reason spices are used obviously is to enhance food palatability. Spices are plants with intensive and distinctive flavor and aroma used in fresh or dry form (Belts and Grosch, 1983). Their benefits as preservatives are hitherto mostly inadvertent. But the ultimate reason is most likely that spices help cleanse foods of pathogens and thereby contribute to the health, longevity and reproductive success of people who find their flavors enjoyable (Billing and Sherman, 1998). Spices such as (*Salvia officinalis*), rosemary (*Rosemarinus officinalis*), oregano (*Oreganum heracleoticum*) have both antioxidant and antimicrobial effects (Davidson, 1983). The synergistic effect of combining the spices has also been suggested (Grohs and Kunz, 2000). The specificity of spices to specific micro-organisms have also been intensively been investigated in the earlier studies of spices (Webb and Tanner 1944; Zaika and Kisinger, 1979; Shelef *et al.* 1980) and in the recent past (Sema and Suleymena, 2007; Biljana *et al.* 2007).

In a comprehensive review by Shibamoto and Bjadanes (1993) on toxicological aspects of spices, none of these spices have been mentioned to be toxic at consumed levels. Further, these spices are locally grown and their increased industrial use could augment their economic value-consequently increasing the incomes of the farmers who grow them. These spices could thus substitute the advertent chemical preservatives and antioxidants discussed here to make these foods safe, stable and tastier.

With demonstrated potential use in food as antioxidants and antimicrobials spices can be substituted for some chemical additives. The antimicrobial potency of rosemary spice has been demonstrated in culture media (Shelef *et al.*, 1980) as well as in foods (Shelef *et al.*, 1984; Kisinger and Zaika, 1978). Rosemary spice is bacteriostatic at 0.3% and bactericidal at 0.5% (Davidson, 1993), just above 0.4% which is the organoleptic threshold, beyond which it is generally unacceptable (Okiye and Muroki, 2002). Antioxidant properties of rosemary spice in foods have also been investigated (Musa, 2003).

Numerous studies have been conducted to demonstrate the antioxidant and antimicrobial effects of rosemary spice in meat products and the results have been positive. This paper presents results in an investigation on the effect of combination of rosemary spice and nitrite pickling salt (99.95 parts of sodium chloride and 0.05 parts of sodium nitrite) on eating and keeping quality of beef sausages. In the attempt to demonstrate the possibility of substituting the spice for nitrite pickling salt (both as the sole antimicrobial and antioxidant), sensory assessment, microbial count and relative rate of development of lipid oxidation secondary byproducts in beef sausages were examined. The aim was to indicate the possibility of substituting rosemary spice for nitrite pickling salt while maintaining or improving the preservative and organoleptic properties.

MATERIALS AND METHODS

Materials

Fresh lean beef was purchased from Mihango butcher at Uthiru Township Nairobi twelve hours after slaughter. The butchery, which had no refrigeration facility, had brought the meat from Dagoretti Kikuyu County Council slaughterhouse. Chopped chilled pork back fat and nitrite pickling salt were obtained from Department of Food technology, University of Nairobi food processing pilot plant. Chilled water was purchased from the Nairobi City Council water system. Spices and starch were from Uchumi Supermarket, Nairobi while artificial collagen casings (22-32mm caliber) were purchased from Naturin-Werk, Wenheim Germany. All the chemicals were artificial grade reagents purchased from Kobian Chemicals Ltd, Nairobi.

Preparation of the spice extract

Water was added to the rosemary spice at a rate of 100ml/gm of the spice and heated to boiling on a TA (Czechoslovakia) model 099 hot on simerstat4. Boiling was obtained for two minutes after which the extract and the leaves were put in a 25 gauge polythene bag, sealed and then cooled in water bath at 22°C for 3 hours before being stored at 2°C for use later for processing of beef sausages

Processing of beef sausage.

Five types of sausages having similar levels of ingredients but different combination of levels of rosemary spice and nitrite pickling salts were made. The formulations for the sausages are presented in Table 1. These were:

- Sausages containing no rosemary spice and 1.8% nitrite pickling salts (based on weight of lean meat)-R00S18
- Sausages containing 0.2% rosemary spice (based on weight of sausage mass) and 1.6% nitrite pickling salts (based on weight of lean meat)-R02S16
- Sausages containing 0.3% rosemary spice (based on weight of sausage mass) and 1.2% nitrite pickling salts (based on weight of lean meat)-R03S12
- Sausages containing 0.4% rosemary spice (based on weight of sausage mass) and 0.6% nitrite pickling salts (based on weight of lean meat)-R04S06
- Sausages containing 0.5% rosemary spice (based on weight of sausage mass) and no nitrite pickling-R05S00

Table 1: Formulations for beef sausages[§]

Ingredients	Amounts ¹ /composition														
	R00S18			R02S16			R03S12			R04S06			R05S00		
	Gm	%BF	%SM	Gm	%BF	%SM	Gm	%BF	%SM	Gm	%BF	%SM	Gm	%BF	%SM
Beef	550	100	-	550	100	-	550	100	-	550	100	-	550	100	-
Pork	250	45.6	-	250	45.6	-	250	45.6	-	250	45.6	-	250	45.6	-
Ice water	200	36.4	-	200	36.4	-	200	36.4	-	200	36.4	-	200	36.4	-
STTP²	1.25	0.23	-	1.25	0.23	-	1.25	0.23	-	1.25	0.23	-	1.25	0.23	-
Starch	15	2.73	-	15	2.73	-	15	2.73	-	15	2.73	-	15	2.73	-
NPS³	9.9	1.8	-	8.8	1.6	-	6.6	1.2	-	3.3	0.33	-	0	0	-
Sodium chloride	0.0	0.0	-	1.1	0.2	-	3.3	0.6	6.6	1.47	-	9.9	-	1.8	-
Rosemary spice	0.0	-	0.0	2	-	0.2	3	-	0.3	4	-	0.4	5	-	0.5

¹Weight of major ingredients, namely meat, pork fat and ice water are referred to here as the sausage mass (SM). Their weight and that of starch, sodium tripolyphosphate, sodium chloride (common salt) and nitrite pickling salt, which can effect physical functionality of the sausages are given in reference to beef (BF), whereby beef is taken as 100 units. Spices whose effects on sensory characteristics of the sausage are given in reference to sausage mass (SM).

²STTP- Sodium tripolyphosphate

³NPS-Nitrite pickling salt

[§]Acronyms for R00S18, R02S16, R03S12, R04S06 and R05S00 as explained above.

The sausages were processed as follows in the food processing pilot plant of Department of Food Technology and Nutrition, University of Nairobi. The chilled beef and pork fat were minced separately through a 5mm plate using an electric mincer. The meat was then cut with the spices, nitrites pickling salt and one third of the ice in a Killia (Switzerland) silent cutter until the mass was uniform but ensuring that the temperature did not exceed 10°C. Starch, fat and the rest of the ice were then added and cutting continued until the mass was judged to have cut fine but also ensuring that the temperature did not exceed 14°C. The mass was immediately filled into castings using a manual filler with due care not to incorporate air. The sausages were then linked at about 15cm intervals ensuring that they were firm, other than soft.

The sausages from each batch were then packed in 250 gauge polythene bags in lots containing four sausages each and used in studies of keeping quality (microbiological and rancidity tests) and organoleptic characteristics as described below.

Microbiological analysis

The total counts of sausages stored at 5°C were determined on day 1 (immediately after processing), 2, 4 and 7 as follows. Twenty grams from each treatment (batch) were weighed out and transferred into a sterile blender (Corydon, England). Four hundred and fifty milliliters of 0.85% sterile saline solution was added and the sausages macerated for two minutes. Serial dilutions of up to 10⁷ were made. The dilution 10⁶ and 10⁷ were plated in plate count agar (PCA) an duplicates and then incubated at 35°C for 48 hours and number of colonies with viable growth

counted, and recorded in colony forming units per gram sample apart from the microbial load recorded after averaging the two readings, the change ratio was calculated by dividing the microbial load on day 4 and 9 of storage with the load on day 1 (load – immediately after processing).

Assessment of extent of lipid oxidation secondary products

The extent of development of lipid oxidation secondary products was determined on day 4, 7 and 11 of storage as follows. One sausage was drawn from each batch and cut into pieces of about 0.2 cm. Forty grams were then weighed into tarred porcelain and then dried in air circulating oven at about 80°C for about 6 hours. The sample was ground in a mortar and pestle and then transferred into 250 ml flat bottom flask. Two hundred milliliters of light petroleum (40 – 60°C) was added and the content then agitated in Gerber (Switzerland) shaker at 200rpm for one hour. The extract was filtered with Whatman No. 41 filter paper. The filtrate was then diluted with the light petroleum (1 part to 5 parts of petroleum) and absorbance read at 269nm in a Beckmann (Zurich Switzerland) spectrophotometer. In addition to absorbance measured on days 4, 7 and 11, the percent increases were calculated, taking absorbance on day 4 as the base and absorbance on day 11 as absorbance at end of storage. That is,

$$\text{Relative absorbance} = \frac{\text{Absorbance reading on day 11} - \text{Absorbance reading on day 4}}{\text{Absorbance on day 4}}$$

Sensory evaluation of the sausages

Immediately after preparation, the sausages were taken from each batch and scaled in hot water until a core temperature of 70°C was reached. It was then held in water for a further three minutes. The sausages were put in 250 gauge polythene bags, cooled in cold water at 22°C and then presented to taste panel of 20. The panelists were asked to rate its sensory quality (i.e. colour, taste and general acceptability) on a hedonic scale of 7 where 7 represented like extremely, 6 like very much, 5 like moderately, 4 neither like nor dislike, 3 dislike moderately, 2 dislike very much and 1 dislike extremely. Dry wheat bread slices to be chewed after each sample was tasted and water to rinse the mouth were provided. The data was entered in the statistical Package for Social Scientists (SPSS) version 10 and subjected to analysis of variance (ANOVA).

RESULTS

Microbial load of sausages

Immediately after processing (day 1), R00S18 (sample containing 18gm NPS and no rosemary spice) had highest count of 4.60×10^7 cfu/gm (Table 2). The microbial count increased with increase in the amount of rosemary spice but decreased to 5.00×10^6 cfu/gm when 0.5% of rosemary spice was added (with no NPS as an ingredient).

Table 2: Microbial loads of beef sausages stored at 5°C over a period of 9 days¹

Day	Microbial load ²				
	R00S18	R02S16	R03S12	R04S06	R05S00
1	4.60×10^7	1.02×10^7	1.94×10^7	3.00×10^7	5.00×10^6
2	1.40×10^6	4.00×10^6	5.60×10^6	1.60×10^6	4.00×10^6
4	1.20×10^6	6.50×10^6	6.50×10^6	5.50×10^7	1.00×10^6
7	2.10×10^7	2.00×10^7	9.60×10^7	1.24×10^7	1.20×10^7
9	3.30×10^7	4.00×10^7	3.40×10^7	2.42×10^7	6.00×10^7
Day 4/day 1 ³	0.03	0.64	0.34	1.83	0.20
Day 9/Day 1 ⁴	0.72	3.92	1.75	0.81	12.00

¹The values are average of two readings

²Acronyms as in Table 1

³Count on day 4 divided by count on day 1

⁴Count on day 9 divided by count on day 1

The microbial count fell until day 4 in all samples (except for R04S06) then increased for the rest of the storage period (to day 9). The greatest fall in the count until day 4 was in sample R00S18 (0.03 times the count on day 1). The highest count increase on day 4 was observed in sample R04S06 that was 1.83 times the original count. In the sample R04S06, the sample with 0.4% rosemary spice and 3.3 gm NPS/Kg, the microbial load increased to day 4 (1.83 times the day 1 count). Whilst the sample with no spice (R00S18) had the highest microbial count on day 1, sample with highest spice dosing (R05S00) had the highest load on day 9. For the intermediate samples, highest microbial load observed in each sample was spice dose-dependent- the higher the spice level, the earlier the highest load was reached: day 9 for R02S16, day 7 for R03S12 and day 4 for R04S06. For both extreme samples, lowest counts were observed on day 4. At this point, the counts for these two samples were comparable

(1.2×10^6 cfu/gm for R00S18 and 1.00×10^6 cfu/gm for R05S00). Samples R05S00 recorded the lowest count on day 4. All intermediate samples recorded the lowest count on day 4 and was comparable to sample with no rosemary spice.

At the end of storage, day 9, sample R04S06 – sample with 0.4% rosemary spice and 3.3 gm NPS/Kg had the lowest microbial load with R05S00 having the highest. This sample (R05S00), also recorded the highest increase; the count was 12 times the count in day 1. Sample R00S18 recorded the highest decrease (0.72 times the count on day 1) and was comparable to sample R04S06 (0.81 times the count on day 1).

Production of secondary products of lipid oxidation

The extent of formation of secondary lipid oxidation products, as shown by absorbance at 269nm, which is indicative of degree of development of rancidity, is shown in Table 3. The higher the absorbance, the more the relative production of secondary products of fat oxidation.

Absorbance observed on the days 1-3 could not be detected because secondary products of oxidation were not yet substantially formed. Absorbance on day 4 of storage shown by the extract from the sample without rosemary spices (R00S18) was the highest of all samples. It was more than that of: R02S16 (2.3 times), R03S12 (1.5 times), R04S06 (2.7 times) and R05S00 (1.9 times).

On the day 7, the highest absorption was still in sample R00S18 while there were practically minimal changes for the samples R02S16 and R04S06. On the same day, it was also observed that the absorbance of R00S18 was 1.6 times higher than R03S12, more than twice as much as that of R02S16 (2.5 times), and 3 times in R04S06 and R05S00.

Table 3: Absorbance at (269nm) reading of beef sausages lipid oxidation secondary products of sausages stored at 5°C for 11 days¹

Day	Absorbance reading ²				
	R00S18	R02S16	R03S12	R04S04	R05S00
4	0.56	0.24	0.29	0.21	0.30
7	0.63	0.27	0.38	0.21	0.21
11	1.10	0.54	0.73	0.58	0.57
% Change between days 4-11	96.43	125.00	151.72	176.19	90.00

¹The values are average of two readings

²Acronyms as in Table 1

Sample R00S18 still had the highest absorbance on the day eleven followed by R03S12. On the same day, extracts of samples R02S16, R04S06 and R05S00 had lower absorbance reading and were comparable- 0.54, 0.58 and 0.57 respectively. At this point of storage, the increase in absorbance ranged from 90-170% in all. The lowest increase in absorbance (90%) was observed in sample with 0.5% rosemary spice and no NPS (R05S00) but was comparable to increase (96.43%) in sample with no rosemary spice and with 18gm NPS/Kg. The highest absorbance was observed in R04S06. The spice/NPS combinations did not have significant effect on the absorbance.

Sensory quality of sausages

There was no significant difference in sensory qualities in all the sausage samples (Table 4). There were also neither treatments nor panelist effect. However, R00S18 (no-spicy sample) and R02S16 (lowest spice concentration) had higher scores for all the characteristics as compared to other samples, which had practically similar scores. This subtle difference can be attributed to the effects of the spice. Even though the spice taste is generally familiar, spicy foods are rarely consumed by majority of Kenyans. The effect is most marked in the case of color assessment where spicy samples score comparatively low (albeit not significantly different within the samples). All samples were far from being rejected since scores were between 4.7 and 5.9 on the scale of 7.

Table 4: Sensory score of different types of sausages

Sample ¹	Sensory parameters ²		
	Appearance (colour)	Taste	General Acceptability
R00S18	5.7a	5.8a	5.9a
R02S16	5.4a	5.9a	5.5a
R03S12	4.7a	5.3a	5.0a
R04S06	4.9a	5.3a	5.0a
R05S00	4.7a	5.2a	5.3a

¹Acronyms as in Table 1

²Figures in the same column followed by the same letters are not significantly different from each other at p=0.05. Each score is an average of 20 observations

DISCUSSIONS

Microbial stability

The lowest microbial count of sample with 0.5% rosemary spice (and no NPS) on day 1 compared with other samples was attributed to the bactericidal effects of rosemary spice which is reported to take place when the spice is used at this level (Shelef *et al.*, 1980; Davidson, 1983). Shelef *et al.* (1980) also found out that at 0.3% level, the spice is known to be bacteriostatic. Bactericidal effects of the rosmarinic acid, which is a phenolic compound and the terpene fractions found in rosemary spice is dose dependent due to the high levels of these compounds. The antimicrobial effect of nitrites is slow due to the mechanism of action against microbes as explained by Jay (1987). This explains the highest microbial count in sample with highest level of NPS on the day 1.

The increase of microbial load in sample with 0.4% spice and 6mg/kg NPS combination after fourth day-to-day 9 was rapid compared to that of other samples. This could be explained by the fact rosemary spice is not in levels sufficient to exhibit full bactericidal effects (but rather only bacteriostatic effect) and the NPS concentration is the lowest. Bacteriostatic effect of 0.3%-0.4% rosemary spice could possibly be gradual. Rosemary inhibitory effect is attributed to terpene fractions, which is composed of borneol, aneole, pinene and camphor (Jay, 1987). Phenolic compounds have also been implicated. The highest decrease in microbial load (by the 9th day) was observed in sample with no spice and standard NPS composition and this is attributed to then potent NPS in the active form (Jay, 1987). However, this is comparable to the sample with 0.4% spice and 6mg/kg NPS combination.

The increase of microbial load in sample with maximum spice and no NPS after the fourth day was very rapid compared to that of other samples, which were relatively gradual. These observations could be attributed to growth of lactic acid bacteria, some of which are less susceptible to bacteriostatic and bactericidal effects rosemary spice (Jay, 1987). NPS is known to inhibit lactic acid bacteria (Macdougall *et al.*, 1975). This explains the highest microbial load at the end of the storage period in sample with no NPS (6.00×10^7 cfu/gm) and the highest increase (12 times after storage). The observation that viable count in sample with 0.2% spice and 16mg/kg NPS can be explained by the fact that some lactic acid bacteria are able to grow better when sausage mass has 0.2% rosemary spice as compared to when the level is 0.3% and 0.4%. Lowest count on day 9 was observed in sample 0.4% spice and 6mg/kg NPS (2.42×10^7 cfu/gm) and this was 0.81 times the count on the first day. This could be due to combined effort of the spice and NPS. This sample had highest rosemary spice among samples with both rosemary spice and nitrite pickling salts. It also had modest concentration of NPS and thus a possible optimum of spice/NPS combination. Sample with highest NPS (3.30×10^7 cfu/gm) however had the highest decrease (0.72 times the day 1 count) on the day 9 but the count was comparable to sample with sample of 0.2% rosemary spice (4.00×10^7 cfu/gm) and 0.3% (3.40×10^7 cfu/gm).

Halting of lipid oxidation

The highest absorbance of standard sausage formulation with no spice on day 4, 7 and 11 is expected since it is the sample with no added extra antioxidants (from rosemary spice). Rosemary spice has rosmarinic acid carnosol and carnosic acid, which are reported to be potent in its antioxidant activity (Allen and Hamilton, 1989). There is however indications that antioxidant activity of rosemary extracts mainly depend on carnosic content (Hudson, 1990). The higher absorbance observed in sample containing 0.3% rosemary spice *vis a vis* other samples with the spice on days 7 and 11 may be explained by higher microbial load observed over whole storage period in this sample (Table 2). Some lactic acid bacteria species produce hydrogen peroxide (Allen and Hamilton, 1989), which can promote autoxidation of fat and consequently lipid oxidation by products (Hudson, 1990) hence high absorbance reading. There was no demonstration that samples with spice/NPS combinations had relatively high propensity to halt increases in secondary products of lipid oxidation. This is a compared to the sample with only NPS and only with the spice. There seem to be no comparative advantage in combining rosemary spice and NPS in halting the production of secondary products of lipid oxidation.

Sensory evaluation

Rosemary spice is organoleptically acceptable levels 0.1 - 0.4% as reported by Oiyee and Muroki (2002). However there is no indication that the out of the acceptable range (0.5% spice level), the acceptability of the sausages are reduced significantly in terms of appearance, taste and general acceptability. It is evident that substitution of rosemary spice for NPS does not influence in a significant way the taste organoleptic quality of beef sausages. This is because both tastes are generally familiar and the spice and NPS are within the acceptable organoleptic levels.

CONCLUSIONS

In short term, NPS without rosemary spice (*Rosmarinum Officinale* L.) is more potent in stabilizing microbial proliferation than in the long term storage where its effect is closely compared to 0.4% spice and 6mg/kg NPS combination. There is no indication that inhibition of microbial proliferation increases with spice substitution for NPS. For microbial inhibition, 0.4% rosemary spice and 6mg/kg NPS mix is potentially the optimum rosemary spice/NPS combination. It was also demonstrated that no level of spice/NPS substitution has appreciable effect in limiting the production of fat oxidation in sausages - and that standard NPS dosing and 0.5% rosemary spice concentration (with no NPS) are most potent in limiting production of secondary products of lipid oxidation. No level of altering of spice/NPS combination amounts to any significant change in the acceptability of sausages in terms of colour, taste and generally acceptability. Rosemary spice can therefore substitute nitrite pickling salt to produce organoleptically acceptable sausages of comparable microbiological quality. However, rosemary spice/NPS mixes are not relatively effective in halting production of secondary products of lipid oxidation.

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