

Effect of incorporating orange maize flour into beef sausage and its associated quality attributes

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Abstract: The aim of this study was to incorporate different percentages (0%, 10%, 20%, 30%, and 50%) of orange maize flour (OMF) into beef sausage, and to study the bioactive qualities of the OMF. Also, the physicochemical, microbiological and sensory characteristics of the OMF beef sausages were explored. Results from the bioactive analyses of the OMF indicated it had total carotenoid content of 14.31 µg/g, while the tannin content was 1.52 mg/g. The moisture content of the beef sausages decreased when OMF levels in the sausages were increased. The fat contents of the OMF beef sausages were significantly lower ($p < 0.05$) than that of the control, while the emulsion stability of the 30% OMF beef sausage was not statistically different to that of the control sausages. The iodine values of the OMF beef sausages tended to increase with an increased content of OMF. *Salmonella* spp. and *Staphylococcus aureus* were not detected in the sausages. *Escherichia coli* detected were within the range of 1.0 to 2.0 CFU/g. A consumer preference study showed the most acceptable sausage among the OMF beef sausages contained 20% OMF. This study showed that OMF has the potential to be used as a fat replacer; can maintain the sensory properties of beef sausages and prevent or limit microbial growth on beef sausages formulated with added OMF.

Keywords: orange maize flour, bioactive, total carotenoid, iodine value, fat replacer.

Introduction

Orange maize (OM) is a new crop that is bio-fortified with vitamin A. This crop is a staple food that provides substantial amounts of energy, vitamin A, antioxidants (carotene, xanthophylls, polyphenols), and vitamins C, E, and D (Alamu *et al.*, 2014). It is widely grown commercially in

Zambia, Nigeria, Zimbabwe, Tanzania and Ghana (Alamu *et al.*, 2015; Pixley *et al.*, 2013; Smale *et al.*, 2015; Hwang *et al.*, 2015). The polyphenols and the carotenoids present in OM flour (OMF) are known to be antioxidants, which play a vital functions as anti-carcinogenic, antioxidant, antiviral, antimicrobial, and anti-inflammatory molecules. The potency of this bio-fortified maize is to enhance vitamin A status in children (Gannon *et al.*, 2014; Palmer *et al.*, 2016). The presence of vitamins C and K, β-carotene and selenium in OMF could improve the functions of the human thyroid gland and immune system (Shah *et al.*, 2016).

Maize is used to produce products including corn flour, cornstarch, corn gluten, corn syrup, tortillas, tortilla chips, polenta cornmeal, corn oil,

popcorn, and cornflakes, but the usage of OM in food production is limited, despite its nutritional attributes. However, the intake of OM-based products could contribute to phenolic acid ingestion (Alamu, 2014) and other vital nutrients.

Thus, consumption of sausage with incorporated OMF could improve the vitamin A status in children, pregnant woman and lactating mothers and could provide the potential to scavenge generated free radicals, prevent protein energy malnutrition, reduce the risks of coronary heart disease, tumour incidence, and cancer, decrease blood cholesterol level and fat absorption and could have the potential to lower blood pressure (Jo *et al.*, 2015). Also, vitamin A is noted to play a very key role in good vision and cell differentiation, and therefore, OMF intake could prevent vitamin A deficiency that can result in blindness, increased child and maternal mortality, immune system function impairment and abnormal foetal development (WHO, 2009).

In this paper, some bioactive qualities of OMF, and the physicochemical properties, the sensory attributes and the microbiological quality of sausages with incorporated OMF were studied.

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Materials and methods

The solar dried OM was provided by the Department of Food Science and Technology, University for Development Studies, Tamale, Ghana. Adobo, white pepper, black pepper, chilli pepper, polyphosphate, iodised salt and purified water were purchased from a supermarket in Tamale, the northern region of Ghana. Lean meat (boneless beef) used for the sausage was obtained from the meat unit of the Animal Science Department, University for Development Studies. The boneless beef was acquired from a healthy adult cow slaughtered based on the approved code of ethics for slaughtering animals in Ghana. The lean meat was trimmed of subcutaneous fat and of connective tissues. The meat was stored in a refrigerator at $4\pm 1^\circ\text{C}$ for about 6–8 h and frozen at -18°C until it was processed.

Orange maize flour preparation

Five (5) kilograms of solar dried OM was milled into flour using a plate mill (Quaker City Grinding Co, Model 4-E, Phoenixville, PA, USA). It was then sieved through a 0.2 mm screen. The OMF obtained was kept in a container with fitted lid and was stored at room temperature until it was utilised.

Determination of bioactive compounds in solar dried OMF

Carotenoid extraction and its total content

To determine the total content of carotenoids, the method described by Lucia *et al.* (2012) was employed. In brief, 5 g of the OMF was weighed using a digital balance (Bel Engineering, model MA0434/05). The carotenoid was extracted by continuously adding 15 mL of acetone until a paste was obtained. This was transferred into a sintered-funnel (5 μm), which was fixed to a 150 mL Buchner flask. This was filtered under vacuum. This technique was repeated three times. The extract acquired was conveyed to a 200 mL sieve funnel containing 20 mL petroleum ether. The acetone removal was conducted by adding slowly ultrapure-water (Milli-Q-Millipore). The extract was then conveyed through a funnel to a 50 mL volumetric flask containing 10 g of anhydrous sodium sulphate. The volume was made up using petroleum ether, and the absorbance was read at 450 nm. The total carotenoid content was calculated using the following equation:

$$\text{Carotenoid content } \left(\mu \frac{\text{g}}{\text{g}} \right) = \frac{A \times V(\text{mL}) \times 10^4}{\lambda_{1\text{cm}}^{1\%} \times Z(\text{g})} \quad (1)$$

Where: A = absorbance; V = total extract volume; Z = sample weight; $\lambda_{1\text{cm}}^{1\%} = 2592$ (β -carotene extinction coefficient in petroleum ether).

Determination of ascorbic acid

Ascorbic acid was determined using the dye-stuff titration method, which was expatiated according to AOAC (2006). OMF (5 g) was digested with oxalic acid (0.4 g/100 g). The aliquot was titrated against dyestuff that was formerly standardised using ascorbic acid solution. The ascorbic acid content was calculated using the following equation.

$$\text{Vitamin C (mg/100g)} = \text{titre value} \times 0.606 \times \frac{100}{\text{weight of sample}}$$

Determination of phytic acid content

The phytic acid content of the OMF was determined using the method of Alamu *et al.* (2014), wherein the atomic ratio of 4:6 Fe:P was used to calculate the phytic acid content.

Determination of tannin content

Tannin content determination was carried out using the AOAC (2006) method. OMF (5 g) was added to 50 mL of distilled water and agitated. The mixture was held for 30 min at room temperature and was filtered using Whatman no. 4 grade filter paper. The extract (2 mL) was put into a 50 mL volumetric flask. Similarly, 2 mL standard tannic solution (0.1 mg/mL tannic acid) and 2 mL distilled water were put in separate volumetric flasks and used as standards. A saturated sodium carbonate (Na_2CO_3 ; 2.5 mL) solution and Folin-C (1 mL) reagent were added to a separate flask and volume was made up to 50 mL and vortexed. Afterward 1.5 h, the OMF was filtered using Whatman no.4 grade filter paper. The absorbance was read at 760 nm against a reagent blank. Tannin was calculated according to equation (2) and is expressed as mg of tannic acid equivalence (TAE) per 100 g.

$$\text{Tannin} = \frac{(\text{standard concentration} \times \text{sample absorbance})}{(\text{standard absorbance} \times \text{weight of sample})} \quad (2)$$

Sausage preparation and processing

An adapted method of Afoakwa et al. (2015) with modification was employed for the preparation of the OMF beef sausages. Before the sausages were prepared, the lean meat and fat were stored at -18°C . A mixture of lean meat, fat, salt, ice, adobo, white pepper, black pepper, chilli pepper, polyphosphate, and water was considered as a control (OMF 0%). Different quantities (10%, 20%, 30% and 50%) of OMF, adobo, white pepper, black pepper, chilli pepper, polyphosphate, were added to the sausage, which constituted the experimental groups. The preparation of the ingredient mixtures using the correct ratio of fat and lean meat and different concentrations of OMF was carried out with caution. The mixture was minced at an emulsion temperature of 10°C for 10 min. The raw sausage emulsion was stuffed into cellulose casing using a sausage stuffer. Then, the sausages were tied manually into 10–13 cm lengths that were weighed and smoked for 45 min at 70°C in an electric sausage smoker. The sausages were kept in a refrigerator at 4°C .

Component analyses of the sausages

Moisture determination

The moisture content in the sausages was determined using AOAC (2006). In brief, 5 g of sausage sample was transferred into a previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105°C for 5 h until a constant weight was obtained. The sausage sample was again weighed after cooling in a desiccator. The percentage of moisture was then calculated using the equation:

$$\text{Moisture} = \frac{(\text{weight of wet sample} \times \text{weight of dry sample})}{\text{weight of wet sample}} \times 100[\%] \quad (3)$$

Protein determination

Protein content of the sausages was determined by employing the Kjeldahl method with KELPLUS nitrogen estimation system employing the method of AOAC (2006). Sausage (2 g) was placed into the digestion flask. A selenium tablet utilised as Kjeldahl catalyst was added to the sausage sample. Concentrated sulphuric acid (10 mL) was mixed with the sample and was fixed to the digester for 8 h until the solution obtained was clear. The cooled digest was placed into a 100 mL volumetric flask and made up to the mark with distilled water. The distillation apparatus was set

and rinsed for 10 min after boiling. Twenty-five millilitres (25 mL) of 2% boric acid was pipetted into a conical flask. Five (5) drops of methyl red were added to the flask as an indicator and the sample was diluted with 75 mL distilled water. The digest (10 mL) was made alkaline with 20 mL of 40% NaOH and distilled. The steam exit of the distillation apparatus was closed and the change of colour of the boric acid solution to green was timed. The mixture was distilled for 15 min. The filtrate was then titrated against 0.1 N HCl. The percentage total was calculated:

$$\text{Protein} = \% \text{ nitrogen} \times \text{conversion factor (6.25)} [\%] \quad (4)$$

Crude fat determination

Using the AOAC (2006) method, the fat content of the sausages was estimated using Soxhlet extraction apparatus. In brief, a previously dried (air oven at 100°C) 250 mL round bottom flask was weighed accurately. About 5.0 g of sausage sample was weighed on to a 22×80 mm paper thimble. About 150 mL of petroleum spirit B.P. $40\text{--}60^{\circ}\text{C}$ was added to the round bottom flask and the apparatus assembled. The Soxhlet extractor with a condenser connected was refluxed for 6 h on a heating mantle. After extraction, the thimble was removed and the solvent recovered by distillation. The flask and fat/oil were then heated in an oven at about 103°C to evaporate the solvent. The flask and contents were then transferred into a desiccator to cool. They were then weighed, the values recorded and the % fat was calculated according to equation (5).

$$\text{Fat} = \frac{W_4 - W_3}{W_2 - W_1} \times 100[\%] \quad (5)$$

Where: W_1 = weight of oven dried thimble, W_2 = weight of bottom flask with sample, W_3 = weight of round bottom flask, W_4 = weight of round bottom flask with fat.

Ash determination

Ash content in the sausages was estimated by employing the AOAC (2006) method. In brief, 5 g of dried sausage was placed in a weighed crucible and ignited until no charred particles remained in the crucible. The crucible was put in muffle furnace for 2 h at 600°C until a white ash was obtained. Thereafter, the crucible was cooled in a desiccator and reweighed. The percent ash was calculated using equation (6)

$$\text{Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100[\%] \quad (6)$$

Total carbohydrate determination

The total carbohydrate in the sausages was determined by the differential method, and this was achieved by subtracting percent amounts of crude protein, crude fat, moisture, fiber and ash from 100%.

$$\text{Carbohydrate} = 100 - [\%](\text{protein} + \text{fat} + \text{fiber} + \text{ash} + \frac{\text{moisture}}{\text{content}}) \quad (7)$$

Determination of pH

Using *ISO 2917* (2004) method, the pH of the sausages were measured using Crison pH meter. Prior to the pH reading, the pH meter was standardised using a buffer solution of pH = 7.02 and 4.00 to 20°C. In brief, 5 g sausage sample was homogenised with 20 mL distilled water for 10–15 s. The pH meter electrodes were inserted into the slurry, and the pH values recorded in triplicate.

Functional properties of the sausages

Water holding capacity

Employing the method of *Maltin et al.* (2003), empty containers used for this test were labelled and weighed. The sausage samples were pierced all around with a needle and were hung on a stick in the container. The new weight (weight of sample and container together) was measured and recorded, then the samples were kept in a desiccator for 24 h at room temperature. The samples were then placed in a bag and hung in an airtight container using a hook under the lid. After the required storage time, i.e. 24 h at room temperature, samples were weighed again. The water holding capacity (WHC) was calculated using equation (8):

$$\text{WHC \%} = \frac{T3 - T2}{T3 - T1} \times 100\% \quad (8);$$

Where: T1 is weight of empty container, T2 is weight of container and drained liquid, T3 is weight of container and whole sample.

Emulsion stability

The emulsion stability was measured according to the modified method as described by *Jitngarmkusol et al.* (2008). In brief, 1 g of each sample was dispersed in 16 mL of distilled water contained in falcon tube. The mixture was homogenised for 30 s. Approximately 2 mL of petroleum

ether was added, and the mixture was homogenised again for 30 s. Each emulsified sample was transferred into a centrifuge tube, heated in a water bath at 85°C for 15 min, cooled down to room temperature, and centrifuged at 1100 g for 5 min.

The emulsion stability (ES) was calculated using the equation:

$$\text{Emulsion Stability} = \frac{V_3}{V_1} \times 100[\%] \quad (9);$$

Where; V_1 is the volume of emulsion before centrifugation, V_3 is the volume of the remaining emulsified layer after centrifugation.

Determination of the iodine value of the sausages

The iodine value was determined by extracting fat from the sausage samples using the Soxhlet method. The fat obtained after extraction was kept in an oven to dry. After cooling in a desiccator, fats were transferred into centrifuge tubes. About 1g of each fat sample was weighed into conical flasks. Approximately 10 mL of chloroform was added and mixed. Hanus iodine solution (25 mL) was added with a pipette. The mixture in the flask was kept in the dark for 30 mins. About 10 mL potassium iodide solution and 100 mL distilled water was added and was the flask was shaken. The iodine liberated in the process was titrated with 0.1N sodium thiosulfate solution. The flask was stoppered and shaken vigorously while titrating until a pale-yellow colour was obtained. Exactly 2 mL of 0.5% starch solution was used and titration was continued until the blue color disappeared. Blank titration was also performed. The entire test was performed in triplicate. Iodine values were calculated according to equation (10).

$$\text{Iodine value} = \frac{(B-S) \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 0.127}{\text{Weight of sample}} \times 100[\%] \quad (10)$$

Where B = blank titre, S = sample titre and N = normality of $\text{Na}_2\text{S}_2\text{O}_3 - 0.1\text{N}$

Microbiological analyses

The microbial analysis of the sausages was performed using the method as described by *Philip et al.* (2002). Briefly, 25 g of sausage was placed in 225 mL buffered peptone water (Merck, Darmstadt, Germany) and homogenised in a stomacher for 2 min. The homogenate (1 mL) was pipetted into 9 mL of buffered peptone water to obtain a tenfold dilution. The solution was further step-wise diluted up to 10^{-5} dilution. The spread plate method was

used to plate 0.5 mL of the 5 dilution levels. Total Plate Count Agar (Oxoid) was used for total aerobic plate count determination. Some microorganisms were isolated and identified from this agar. *Salmonella-Shigella* Agar (Oxoid) was used for *Salmonella* enumeration, Baird Parker Agar (Oxoid) was used for *Staphylococcus aureus* and MacConkey Agar (Oxoid) was used for *Escherichia coli* detection. Agar plates for *Salmonella* and total aerobic plate count was incubated at 37°C. Baird Parker Agar plates for *S. aureus* were incubated at 35°C, while MacConkey Agar plates for *E. coli* were incubated at 44.5°C. In all, the incubation period was 24 h, after which, suspect colonies of *Salmonella*, *Shigella*, and *E. coli* were identified prior to counting the colonies. Suspected *Salmonella* colonies on *Salmonella-Shigella* agar plates were recognised as light pink with dark/black centres and *Shigella* were colourless, black colonies were suspect *S. aureus* on Baird Parker agar plates, while pink colonies of *E. coli* were observed on the MacConkey agar plates. The citrate test was used as a confirmatory test for the suspect *E. coli* even though it is a common test for *Salmonella* and *Shigella* as well. In this test, Simmons citrate (Oxoid) agar plates was prepared according to the manufacturer's instructions and all suspected *E. coli* colonies transferred aseptically with further incubation at 37°C for 24 h, after which, results were observed and recorded. Simmons citrate agar after preparation looked green; *Salmonella*, if positive in the citrate test, can change the colour to blue, whereas *E. coli* does not change the colour of the medium. For *S. aureus*, the use of Baird parker together with its supplement (rabbit plasma fibrinogen) was enough and, hence, no further test was needed.

Sensory analysis of the sausages

Sensory analysis was conducted using 50 untrained panellists. Panellists were asked to indicate how much they liked or disliked each of the sausages. Quality attributes (colour, texture, flavour, taste) and overall acceptability were scored based on five-point hedonic scale (1=dislike extremely, 2=dislike moderately, 3=neither like nor dislike 4=like moderately 5=like extremely). All sausages were prepared by grilling for about 10 mins. Sausage pieces (1.5 cm long) were wrapped in aluminium foil and different samples were kept in separate plastic bags in an ice chest. They were then served to the panellists with three digit codes on disposable plates in a randomised order for evaluation.

Panellists were asked to rinse their mouth with water between tasting samples.

Statistical analysis

One-way ANOVA was performed to determine differences in characterisation parameters of the sausages with different levels of OMF incorporated. Minitab was used for the analysis of proximate data, XLSTAT-2016 for sensory data and SPSS version 17.0 for microbial data analysis. The significance was determined using the Tukey's pairwise comparison at $p < 0.05$.

Results and discussion

Bioactive qualities of the bio-fortified orange maize flour

The total carotenoid in the bio-fortified OMF was 14.31 ± 0.3 µg/g. From a comparative point of view, the values found in this study were higher than those reported for the conventionally bred bio-fortified orange maize varieties (6–9 µg/g) of vac and for white maize (2 µg/g) (Pixley et al., 2013). In this study, ascorbic acid was not detected in the OMF. This could be attributed to the drying method employed, which probably degraded it. Also, it could be attributed to the fact that ascorbic acid is sensitive to heat treatment, and so processing the OMF could have contributed to the ascorbic acid loss (Odriozola-Serrano et al., 2007).

The OMF contained tannin (1.52 ± 0.5 mg/g). The concentration of tannins greatly varies among different cultivars, as well as among plants belonging to the same cultivar, and values of 0.8–70 mg/g, expressed as catechin equivalent, have been reported by McMillan et al. (2007), so the amount of tannin in the OMF used in the current study is in this range (McMillan et al., 2007). However, the total content of tannin observed in this study is difficult to compare with the total tannin contents of other plants found in the literature, because methods of analysis, maturity stage of the plants, the type of plant cultivars and the plant part used for analysis can be different (Mamta and Shashi, 2015). Besides, tannins are a diverse group of compounds present in foods at variable levels. Hence, their consumption is virtually universal. Also, differences at the chemical level among the different tannins have resulted in diverse methods for measuring their concentrations. This could be why it is problematic to quantify the consumption levels of these compounds among dissimilar populations. The effect of food tannins on health is a public health issue (Mamta and Shashi,

2015), although tannins have prophylactic effects, noted to be cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, amongst others. These attributes may be due to their free radical scavenging abilities and the potential to activate antioxidant enzymes (Mamta and Shashi, 2015).

A relatively low content of phytic acid was determined in the OMF (6.34±0.6 mg/100g). Phytic acid is the primary phosphorus storage compound in cereals, legumes, nuts and oil seeds, and it accounts for up to 90% of the total phosphorous content, contributing as much as 1.5% of seed dry weight (Lan and Lott *et al.*, 2005). This suggests that the phytate in the flour has the potential to increase the bio-accessibility and bioavailability of minerals and proteins. However, phytates should be 25 mg or <100 g (Onomi *et al.*, 2004). The phytate content in the current research was in close agreement with Khan *et al.* (1991) and Nawab Khan and Manzoor (2006), who indicated a decreased concentration of phytic acid during heat treatment. Shahidi and Nacz (1995) showed that polyphenols are not distributed evenly in plant tissues; hence, fractional food processing can lead to loss or enrichment of some phenolic compounds.

Proximate Composition of the Sausage

As depicted in Table 1, the moisture contents of the sausages were significantly different ($p < 0.05$), and ranged between 61 to 67%. As the percentage of OMF incorporated increased, the moisture content decreased ($p < 0.05$). This might be as a result of OMF's ability, due to its fibre content, to hold most of the water as bound water (Choi *et al.*, 2009). A similar result for moisture was reported by Afoakwah *et al.* (2015). Additionally, the fat

contents of the OMF beef sausages (Table 1) were lower ($P < 0.05$) than the control (18.04±2.13) beef sausages. This was expected since the control sausages were formulated with a higher percentage of fat. Tobin *et al.* (2012) obtained similar results in their study where sausages prepared with high-fat levels had a higher fat content.

The ash content in the sausages showed a gradual increase as the OMF concentration increased. A significant difference ($p < 0.05$) between the control beef sausage and the 50% OMF and 30% OMF beef sausages was observed. The increasing value for the ash content detected in the OMF incorporated sausages indicates the increasing mineral content in the sausages, which confirms that OMF is a rich source of mineral elements that are vital for biological functions in humans (Ullah *et al.*, 2010; Nascimento *et al.*, 2014).

The protein content in the OMF sausages increased as the content of OMF increased (Table 1). The protein results obtained were higher than those recorded by Méndez-Zamora *et al.* (2015) who used inulin and pectin in low-fat frankfurter sausages. OMF, which contains a higher quantity of carbohydrate, resulted in increasing carbohydrate content as the OMF content increased. Quinoa flour led to an increase in carbohydrate content and a decrease in moisture in sausages that were prepared with tilapia fillet waste (Zapata and de la Pava, 2018).

pH of the Sausages

As the amount of OMF in the sausages increased, the sausage pHs also increased (Figure 1). Sausage containing 50% OMF had a significantly higher pH of 6.03 than did 10% OMF sausage or control sausage, the pH of which was 5.93 ($p < 0.05$).

Table 1. Proximate composition of beef sausages with added orange maize flour (g/100g) and statistical differences

Type of Sausage	Moisture Content	Fat Content	Ash Content	Protein Content	Carbohydrate Content
Control	66.77 ±1.81 ^a	18.04±2.13 ^a	1.49 ±0.26 ^b	12.34±1.26	1.36 ±0.63 ^b
OMF 10%	66.52 ±0.88 ^a	16.94±1.25 ^{ab}	1.57±0.10 ^b	12.28±0.10	2.69±1.53 ^{ab}
OMF 20%	63.72 ±0.08 ^{ab}	14.97±2.13 ^{ab}	1.95±0.17 ^{ab}	13.46 ±2.03	5.90±2.68 ^{ab}
OMF 30%	63.69 ±1.42 ^{ab}	12.41±1.57 ^{ab}	2.23±0.00 ^a	15.50±2.88	6.17±0.10 ^{ab}
OMF 50%	61.03 ±1.17 ^b	11.21±2.30 ^b	2.25±0.10 ^a	16.68±0.23	8.83±2.00 ^a
p value	0.024	0.037	0.010	0.165	0.042

Legend: Means with the same superscript letters in the same column are not significantly different at $P < 0.05$; OMF: orange maize flour.

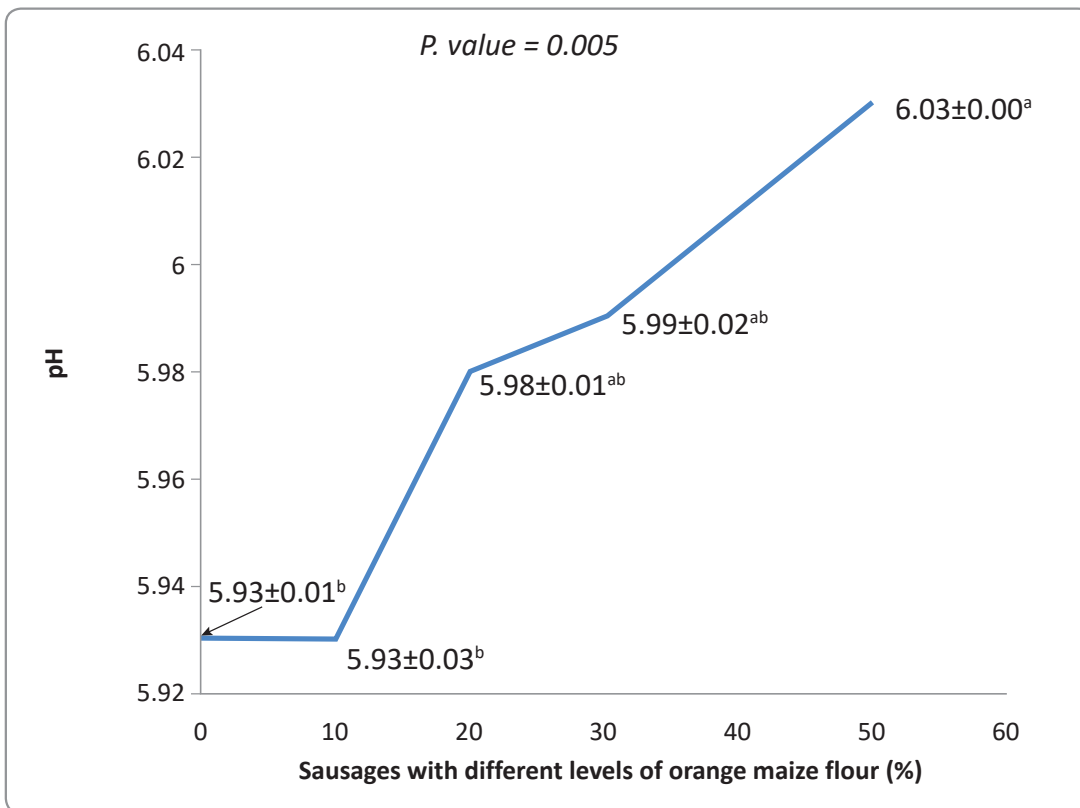


Figure 1. pH of sausages with different levels of incorporated orange maize flour.

In comparison to the results obtained by Choi et al. (2009), higher pH (6.47) was observed in sausages formulated with rice bran fibre, which was attributed to the effect of the minerals, such as iron, phosphorus,

and calcium, present in the fibre. Higher pH values according to Kristinsson and Hultin (2003) are desirable for better water holding capacity, juiciness and other sensory parameters of sausages.

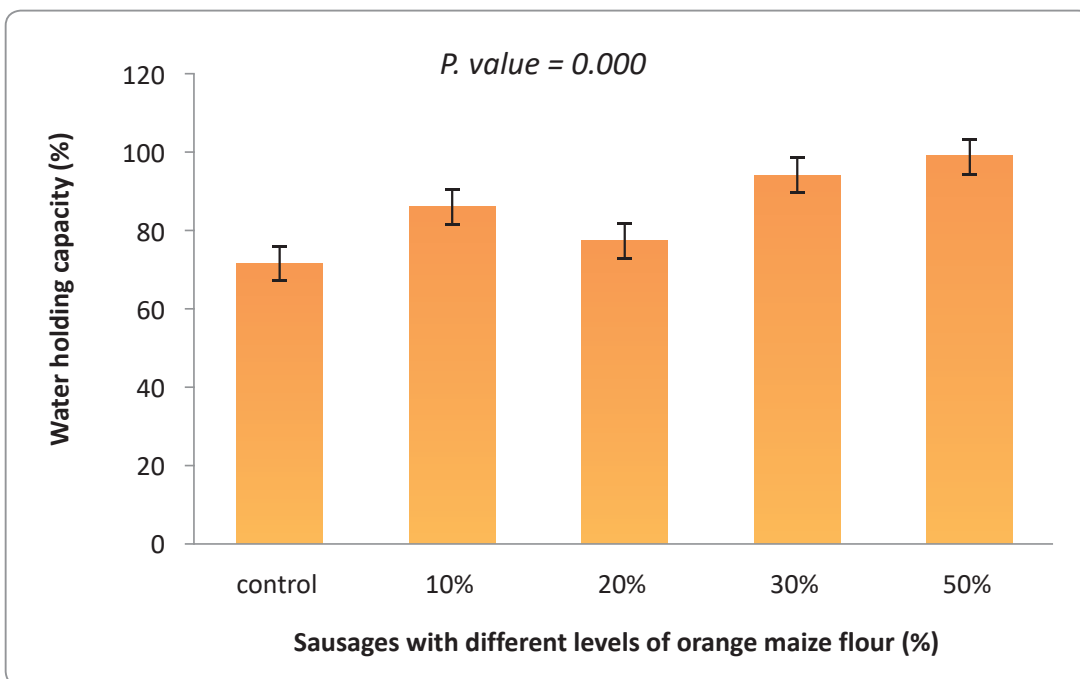


Figure 2. Water holding capacity of sausages with different levels of incorporated orange maize flour.

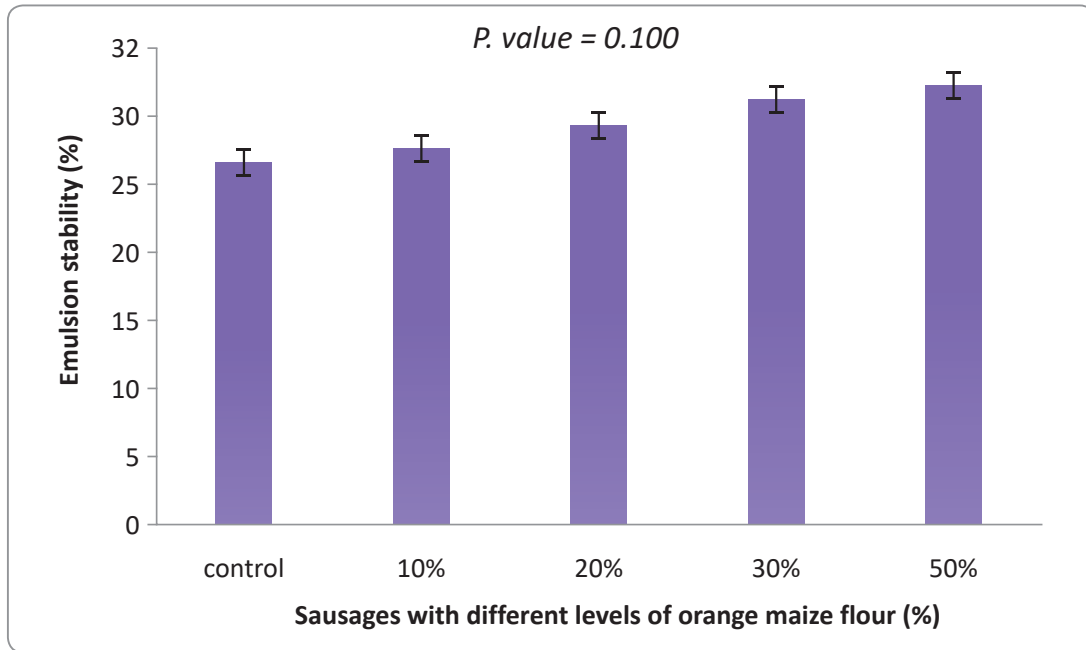


Figure 3. Emulsion stability of sausages with different levels of incorporated orange maize flour.

Water holding capacity of the sausages

Control sausages had the lowest WHC, while the 50% OMF sausages had a significantly higher WHC (Figure 2). This indicates that OMF has good WHC. However, the 20% OMF sausage had lower WHC than that of 10% OMF sausage. *Serdaroglu and Degirmencioglu* (2004) showed that corn flour (2% and 4% level) increased moisture retention in treated meat balls. Also, the increase in WHC caused

by the OMF proves OMF has a good ability to form gel (*Rodriguez-Furlan et al., 2014; Petersson et al., 2014*). This could probably make OMF a suitable candidate for incorporation into sausages. WHC is the ability of meat to retain its water or added water during the use of external forces like cutting, heating, grinding or pressing (*Judge et al., 1990*). WHC is a significant indicator of dietary fibre, suggesting the inclusion of fibre in food product formulation (*Petersson et al., 2014*).

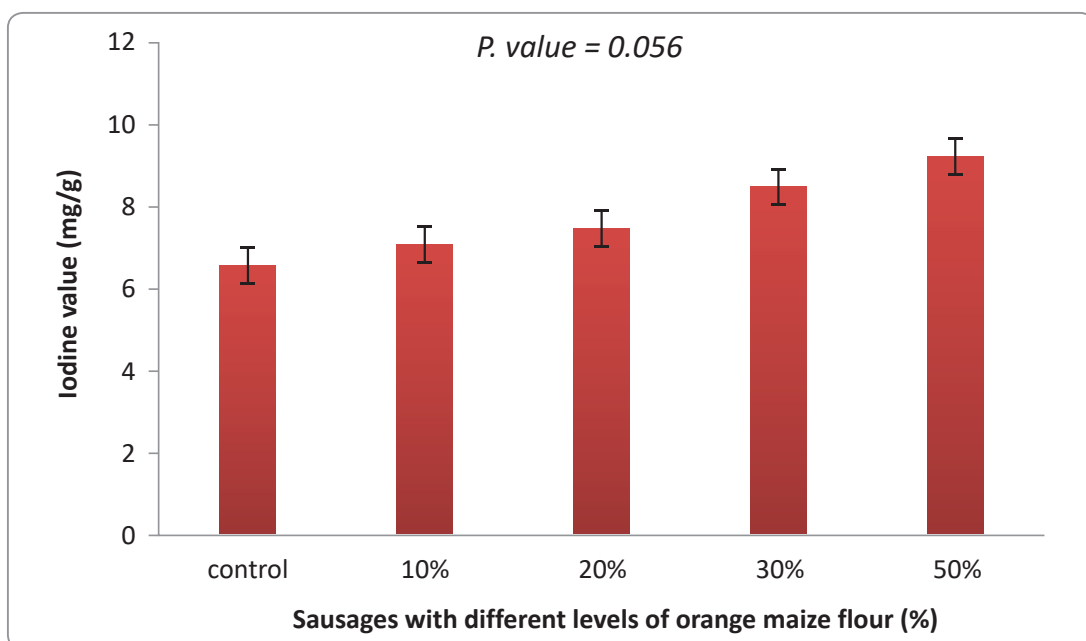


Figure 4. Iodine values of sausages with different levels of incorporated orange maize flour.

Emulsion stability of the sausages

As shown in Figure 3, the emulsion stability, an indicator of unseparated fat and water retained by meat proteins upon heat processing, increased with increasing OMF content in the sausages. However as reported by Alvarez and Barbut (2013), a reduction in fat content of meat products leads to a drop in emulsion stability, because fat is needed to stabilise batters. However, in our study, the emulsion stabilities of the OMF beef sausages were comparable with the high fat control sausages, since no significant difference was observed ($p < 0.05$). This suggests that OMF exhibited a fat-like property and also acted as an emulsifier.

Iodine value of the sausages

Iodine value was determined (Figure 4) to estimate the unsaturation level of the fat present in the formulated sausage products. The results indicated the unsaturation level improved with the increased

addition of OMF, although there was no significant difference between the sausages ($p < 0.05$). This result confirms the fact that maize contains oil, which is rich in poly- and monounsaturated fatty acids (CRA, 2006). This rise in the unsaturated fat could make the product more desirable for consumption since unsaturated fat is considered as a healthy dietary fat.

Microbiological quality of the formulated sausages

As shown in Table 2, the effects of OMF in the sausages stored at 4°C were studied on days 0, 4, 8 and 12 to determine its microbiological qualities. Furthermore, the presence of *E. coli*, *Salmonella* spp. and *S. aureus* was determined. On day 0 and on day 4 of storage, there were no detectable bacteria in any of the sausages. However, on day 8, a total plate count of 11.50 CFU/g and 5.00 CFU/g were observed for the control and 10% OMF sausages

Table 2. Microbiological quality (CFU/g) of the sausages.

Sausage	Day of Storage			
	Day 0	Day 4	Day 8	Day 12
Total Plate Count				
Control	nd	nd	11.50±2.12 ^a	21.00±2.83 ^b
OMF 10%	nd	nd	5.00±2.83 ^c	18.50±2.12 ^d
OMF 20%	nd	nd	nd	14.50±7.78 ^e
OMF 30%	nd	nd	nd	7.50±2.12 ^f
OMF 50%	nd	nd	nd	7.50±0.71 ^f
Salmonella spp.				
Control	nd	nd	nd	nd
OMF 10%	nd	nd	nd	nd
OMF 20%	nd	nd	nd	nd
OMF 30%	nd	nd	nd	nd
OMF 50%	nd	nd	nd	nd
E. coli				
Control	nd	nd	1.00±0.00 ^a	3.50±2.12 ^b
OMF 10%	nd	nd	1.00±0.00 ^a	2.00±1.01 ^a
OMF 20%	nd	nd	nd	1.00±1.41 ^b
OMF 30%	nd	nd	nd	1.50±0.71 ^c
OMF 50%	nd	nd	nd	2.00±0.00 ^a
Staphylococcus aureus				
Control	nd	nd	nd	nd
OMF 10%	nd	nd	nd	nd
OMF 20%	nd	nd	nd	nd
OMF 30%	nd	nd	nd	nd
OMF 50%	nd	nd	nd	nd

Legend: Means with the same superscript letters in the same column are not significantly different at $P < 0.05$; OMF: orange maize flour; nd: not detected; OMF: orange maize flour

Table 3. Mean ranks of sensory properties of sausages and statistical differences.

Treatment	Colour	Texture	Flavour	Taste	Overall Acceptability
Control	114.00	133.11ab	126.32abc	144.47b	129.16ab
OMF 10%	133.58	130.56ab	152.82c	142.71b	101.17a
OMF 20%	139.41	118.70ab	108.76ab	101.41a	153.71b
OMF 30%	112.05	145.66b	140.76bc	139.5 ⁶ b	144.94b
OMF 50%	128.46	99.47 a	98.84 a	99.35 ^a	98.52a
p value	0.206	0.012	0.000	0.000	< 0.0001

Legend: Means with the same superscript letters in the same column are not significantly different at $p < 0.05$; OMF: orange maize flour

respectively, and on day 8, an *E. coli* count of 1.00 CFU/g was observed in both the control and 10% OMF sausages. On day 12, the total plate count increased significantly in almost all sausage formulations, but the highest *E. coli* counts were measured in control sausage. However, the results were all within acceptable level of microbiological quality for processed meat, since according to Nel *et al.* (2004), the maximum limit of *E. coli* in meat and meat products is 10 CFU/g. The presence of *E. coli* in high numbers indicates the presence of organisms originating from faecal sources (Nel *et al.*, 2004). The presence of *S. aureus* can be used as indicator of personal hygiene since the source is usually the skin/hand of food handlers (Mulder, 1996). Also, the lower microbial counts in the OMF sausages could be attributed to the microbial inhibition potential of the OMF due to its total carotene concentration (Arief *et al.*, 2014).

Sensory evaluation of the sausages

Table 3 shows the mean ratings of sensory attributes for each sausage. There were significant differences ($p < 0.05$) in consumer preference among the sensory parameters assessed except for colour,

which showed no significant difference. Consumer preference scores based on texture were the lowest for 50% OMF sausages as compared to the other sausages, suggesting inclusion of 50% OMF negatively affected the texture. The consumer preference taste score was higher for control sausage, but this was similar to the taste scores for 10% and 30% OMF sausages. The sausage with the most preferred flavour was 10% OMF sausage. Table 3 shows the mean consumer preference score for overall acceptability was given to sausages with moderate addition of OMF (20% and 30% OMF sausages achieved the highest scores).

Conclusions

In conclusion, the tannin and the phytic acid contents of the OMF were low, while the physico-chemical properties of the OMF beef sausages were comparable to those of the control beef sausages. The microbiological results were all satisfactory, showing levels of specific bacteria below the maximum allowable levels for meat and meat products. Thus, OMF can be used to substitute fat in sausages without sausage sensory characteristics being affected.

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Uticaj uključivanja brašna narandžastog kukuruza u proizvodnju kobasica od goveđeg mesa i osobine kvaliteta proizvoda

Arthur Vera, Newlove A. Afoakwah

Apstrakt: Cilj ove studije bio je uključivanje, u različitim procentu (0%, 10%, 20%, 30% i 50%), narandžastog kukuruznog brašna (OMF) u kobasice proizvedene od goveđeg mesa i proučavanje bioaktivnih osobina OMF-a. Takođe, ispitivane su fizičko-hemijske, mikrobiološke i senzorne karakteristike OMF kobasica. Rezultati bioaktivnih analiza OMF-a su pokazali da je ukupan sadržaj karotenoida u OMF bio od 14,31 µg/g, dok je zabeležen sadržaj tanina 1,52 mg/g. Sadržaj vlage u kobasicama smanjio se sa povećanjem udela OMF-a. Sadržaj masti kobasica od goveđeg mesa – OMF bio je značajno niži ($p < 0,05$) u poređenju sa kontrolom, a stabilnost emulzije 30% OMF kobasice nije se statistički razlikovala od kontrolnih kobasica. Vrednosti joda OMF goveđih kobasica pokazuju trend porasta sa povećanjem koncentracije OMF-a. *Salmonella* spp. i *Staphylococcus aureus* nisu otkriveni u kobasicama. Otkrivena *Escherichia coli* bila je u opsegu od 1,0 do 2,0 CFU/g. Najprihvatljivija OMF goveđa kobasica je bila ona sa 20% narandžastog kukuruznog brašna. Ova studija je pokazala da narandžasto kukuruzno brašno ima potencijal da se koristi kao zamena za masti, uz očuvanje senzornih svojstava i sprečavanje mikrobnog rasta u goveđim kobasicama.

Cljučne reči: narandžasto kukuruzno brašno, bioaktivnost, ukupni karotenoid, jodna vrednost, goveđa kobasica

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