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Towards a healthier choice: Investigating no added nitrite beef sausage production using safe alternative approaches

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ABSTRACT

Replacing of sodium nitrite from meat processing is a new promising advance in clean label meat products. The current article explored the positive effects of total replacement of sodium nitrite with a combined antimicrobial and antioxidant mixture comprised of 400 ppm nisin (N), 25 ppm Nano sized zinc oxide (Z), 1% chitosan (C) and 1% roselle extract (R) on proximate and fatty acid composition, cooking loss, color analysis and sensory profile of beef sausage. Four types of beef sausage were prepared as following: NT sausage samples as control positive group containing 120 ppm sodium nitrite, NCR samples (400 ppm N, 1% C and 1% R), ZCR (25 ppm Z, 1% C and 1% R) and NZCR samples containing (400 ppm N, 25 ppm Z, 1% C 5and 1% R). The outcomes of the study displayed that there was no significant difference (P > 0.01) in proximate composition of control (NT) and reformulated (NCR, ZCR and NZCR) samples. Concerning fatty acid profiling, saturated (SFA) and monounsaturated fatty acids (MUFA) were the predominant portions in all sausage types and oleic acid was the prevalent one, moreover, reformulated sausages exhibited lower SFA and higher MUFA and PUFA improving fat quality of reformulated beef sausage. A significant difference in cooking loss percentage was observed in NZCR sausage samples compared to other sausages. Noteworthy, cooked reformulated sausages kept their red color after cooking and their a* values were close to those of NT. Sensory assessment (odor, taste and overall acceptance) of reformulated sausages were higher than NT especially for NZCR that recorded the highest scores. Overall, our findings established that combination of antimicrobials (nisin and Nano sized ZnO), antioxidant (chitosan) along with Roselle extract (for red color enhancement) could be potentially applied as a possible nitrite replacer to produce healthier product free from sodium nitrite.

Introduction

Synthetic meat preservatives are usually added to meat products to improve their quality and extend their shelf life, however, questions of safety and consumer partiality have increased attention and research into natural ones (Papadochristopoulos *et al*., 2021)

Sodium nitrite (NaNO2) is a multifunctional food preservative (E250) contributing to its outstanding control of sensory aspects (flavor development and pink color formation) , protection against fat and protein oxidations as well as microbial safety mainly against Clostridium botulinum (Parthasarathy & Bryan, 2012). Despite all its benefits, its toxicological implications are serious for its prospective involvement in highly carcinogenic nitrosamines formation (Sindelar & Milkowski, 2012). As a matter of fact, dissociation of secondary amines and nitrous acid can result in nitrosamines formation accelerated with reduced pH to 3.5, moreover, methemoglobinemia in newborn infants is related to nitrite (Morsy *et al*., 2022)

In response to consumer appeal for meat products that are safer, healthier and conserve their sensory characteristics, researchers and industry official bodies seriously attempt to decrease or eliminate nitrite from products, in this context, different constituents have been applied to substitute added nitrites ensuring that these additives can accomplish nitrite functions while maintaining quality in the end product (Alahakoon *et al*., 2015)

Nisin which is a polypeptide bacteriocin produced by fermentation of lactic acid bacteria, has antibacterial activity against Gram-positive bacteria as well as bacterial spores. Human proteases digests nisin, therefore, it is nontoxic and leaves no residues behind, consequently, it is applied as a prevalent food preservative (Todorov *et al*., 2022). Nisin can suppress pathogenic bacterial growth and its efficacy mainly depends on its diffusion throughout food products (Smaoui *et al*., 2023). The inhibitory activities of nisin is mostly through binding to lipid II, a substational cell wall precursor, therefore, disturb cell wall synthesis (Tang *et al*., 2020)

Many food grade nanoparticles as zinc oxide nanoparticles (ZnO/NPs) display potent antimicrobial activities and suitable to be applied as food preservatives (Zhang *et al*., 2023). Zinc oxide is one of the five GRAS zinc complexes by FDA (Mirhosseini & Arjmand, 2014). ZnO NPs are non-toxic, biocompatible and can be significant alternative for their strong antimicrobial and antifungal activities (Roy & Rhim, 2020)

Chitosan, a biodegradable natural polysaccharide (Hu & Gänzle, 2019), nontoxic , has film formation properties (Elsabagh *et al*., 2023) and has a noteworthy inhibitory activity against common food bacterial growth, yeasts and fungi. Consequently, chitosan is utilized as a common food preservative for vegetables, fruits and meat (Dai *et al*., 2022).

Owing to their high phenolic profile, many spices, fruits and vegetables appear to be natural antioxidant sources for substitution of synthetic ones (Youssef *et al*., 2021). Roselle (*Hibiscus sabdariffa* L.), a tropical annual shrub, Senegal, China and Egypt are the key producing countries of it. Roselle calyces are utilized as a beverage in jellies, jam and fermented drinks or natural colorant sources. Above and beyond, hibiscus has a significant application in folk medicine as a diuretic, digestion improver and hypertension reducer (Shruthi *et al*., 2016). Related to its phenolic content, roselle antioxidant and antibacterial activities have been studied (Cid-Ortega & Guerrero-Beltrán, 2015). Anthocyanins are water soluble pigments responsible for red color of different vegetable and could be applied as alternates for synthetic colorants. The calyxes of Roselle are main sources of these pigments (Morales-Luna *et al*., 2019)

Insight of these factors, the current study aimed to evaluate the effects of a possible nitrite alternative comprised of (400 ppm nisin (N), 25 ppm Nano sized ZnO (Z), 1% chitosan (C) and 1% Roselle extract (R) on proximate composition, fatty acids, cooking loss, color analysis and sensory attributes and of fresh beef sausage as a combined strategy to replace the added nitrite salts in these products.

Materials and methods

Materials

Nisin (food grade with potency 106 IU/g), chitosan (92 % deacetylation and 4.0 × 105 M.W) were provided by Sigma-Aldrich (St. Louis, Mo., U.S.A.).

Preparation and characterization of nano sized zinc oxide

Nano sized ZO was prepared by employing the hydrothermal method as described by Ayoub *et al*. (2023) through the reaction of zinc acetate, $2H_2O$ and a base of NaOH. Morphological assessment of the nanoparticles was implemented using Transmission Electron Microscopy (TEM, JEM-1200 EXII, JEOL, Japan).

Preparation of roselle extract

For preparation of aqueous roselle extract, 100 grams of dried roselle calyces were weighed

and kept in a clean glass flask. About 900 mL of distilled water were added to the flask, heated until boiling for ten minutes and left to cool. After extraction time, filtration process was done by using a filter paper. The filtered extract was concentrated using a rotary evaporator. Water was removed from the concentrate through recirculating air incubator at 45˚C for 24 h (Gutiérrez-Alcántara *et al*., 2016).

Preparation of fresh beef sausage

Fresh beef sausage was formulated with beef meat (78.40%), beef fat (16.04), water (3%), ground garlic (0.40%), sodium chloride (1.36%), sodium tripolyphosphate (0.25%), white pepper (0.40%) and monosodium glutamate (0.15%). Firstly, beef meat and fat were carefully mixed with water and the other ingredients were gradually added. Sausage samples for different treatments were prepared as the following: NT— (120 ppm sodium nitrite), NCR — (400 ppm nisin, 1% chitosan and 1% roselle extract), ZCR — (25 ppm nano sized ZO, 1% chitosan and 1% roselle extract) and NZCR — (400 ppm nisin, 25 ppm nano sized ZO, 1% chitosan and 1% roselle extract)

The different combinations individually were manually stuffed into clean mutton casings. Sausage samples were kept in plastic bags, sealed, identified and kept refrigerated at 4°C till time of analyses (de Carvalho *et al*., 2021).

Proximate composition

Fat, protein and ash contents of fresh sausage samples were analyzed according to AOAC, (2005). Soxhlet extraction method was applied to determine fat content. This method elaborated using n-hexane in an extractor apparatus for 60 min at 90°C to extract fat content from sausage samples. Kjeldahl method was utilized to analyze samples protein contents. For ash content determination, sausage samples were dried in a muffle furnace at 500°C for 24 hours.

Fatty acids determination by Gas Chromatography (GC-FID)

Fatty acid composition analysis was conducted using a modified approach depending on a method showed by Zahran and Tawfeuk (2019). The technique involved fatty chains conversion into fatty acids methyl esters (FAMEs) (trans-methylation). Later, FAMEs were separated by gas chromatography system (an HP 6890 plus and Supelco™ SP-2380 capillary column). Detection was performed by a flame ionization detector (FID). Injector and detector temperature was 250°C while the temperature of column started at 130°C and raised gradually (5°C/ min) until reached 230°C and maintained for 10 min. Helium served as gas carrier at a rate of flow (1.2 mL/min). 1 μL of sample was dissolved in n-hexane and introduced into split injector with a splitting ratio (50:1). For FAMEs identification, their retention times were in comparison to other established FAME standards and composition of fatty acids was expressed as total peak are relative percentage (Zahran & Tawfeuk, 2019).

Cooking loss of sausage

Sausage samples from each treatment were weighed (w1), cooking was performed using a water bath (80°C for 15 min) to attain an internal temperature about 72°C. Whole of cooked sausage samples were cooled and then kept refrigerated till determination time. After peeling of sausage casings, the weight was calculated as w2. Cooking loss for samples was set through utilizing the attached equation: cooking loss (%) = (w1 − w2) / w1 × 100 (Yang *et al*., 2015).

Instrumental color analysis of cooked sausage

Cooked sausage samples were evaluated at Cairo University Research Park (CURP) at Faculty of Agriculture using (Chroma meter, model CR 410, Japan. Color was conveyed applying CIE L*, b* and a* color system (CIE, 1976). Three spectral readings were utilized for each sample (International Commission on Illumination, 1978). Standardization using white standard was performed before assessment. Mean values were set relying on six readings for a tray with two measurements taken per slice.

Sensory evaluation

The sensory properties of sausage were assessed by 20 tasting panel from Food Hygiene and Control department. Samples of 2.5 cm length from each variant were heated in a microwave for 30 s then presented to panelists randomly. Panelists were asked to evaluate the odor, taste and overall acceptance. Scores ranged from 1 till 10 (1: the lowest and 10 for the highest score). The sensory analysis was carried out three times for each sample (Ruiz-Capillas *et al*., 2015)

Statistical analysis

Statistical assessment was applied using Graph Pad Prism 8.0.2. Statistical data used Two-way analysis of variance (ANOVA) and $p < 0.01$ (Greenhouse & Geisser, 1959). The statistical investigations were directed to explore the impact of different treatments on product quality. Post-hoc analysis was performed using Tukey's HSD test to explore which treatments were significantly dissimilar from others when two-way ANOVA gave significant results. Data was conveyed as mean ± SD of 3 triplicates.

Results

Characterization of the prepared nano sized zinc oxide

Fig. 1 shows the TEM image of nano sized ZO prepared by hydrothermal method. The image shows that formation of ZO nanoparticles with an evident hexagonal structure. The average diameter of the particles is 20 nm.

Proximate composition

The impacts of nisin and nano- ZO along with chitosan and roselle extract as a possible nitrite replacer on composition of fresh sausages are described in Fig. 2. Fat, protein, and ash contents ranged from 15.33 to 15.61, 21.62 to 23.3 and 2.48 to 2.95, respectively, in all sausage variants. Notably, the compositional parameters comprising fat, protein, and ash did not exhibit significant differences among different treatments (P > 0.01).

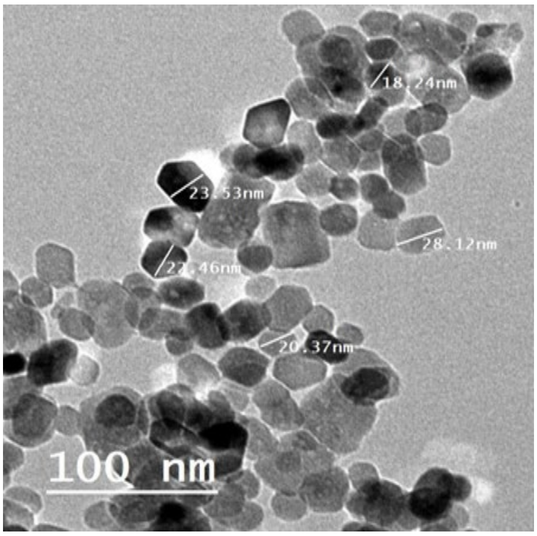

Fig. 1. TEM image of the prepared nano sized ZO.

Fatty acid composition

Effects of substitution of sodium nitrite with a sustainable replacer compromising nisin and nano- ZO along with chitosan and roselle extract on fatty acid composition of fresh beef sausage are presented in Fig. 3. Saturated and monounsaturated fatty acids were the main fractions in all sausage samples followed by PUFA. Concerning the individual fatty acids, oleic acid C18:1n9 was the prevalent one in nitrite added (NT) and reformulated (NCR, ZCR and NZCR) treatments followed by stearic and palmitic acids.

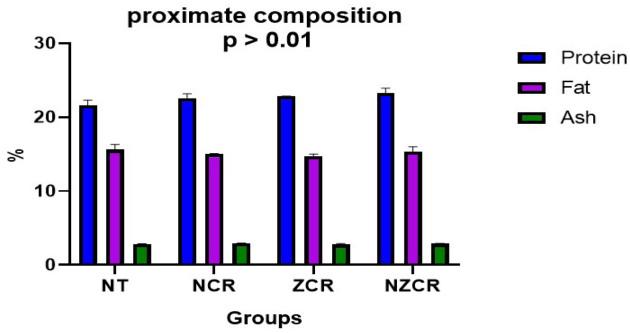

Fig. 2. Effects of nisin, nano- ZO, chitosan and roselle extract incorporation as a nitrite replacer on proximate composition of fresh sausage.

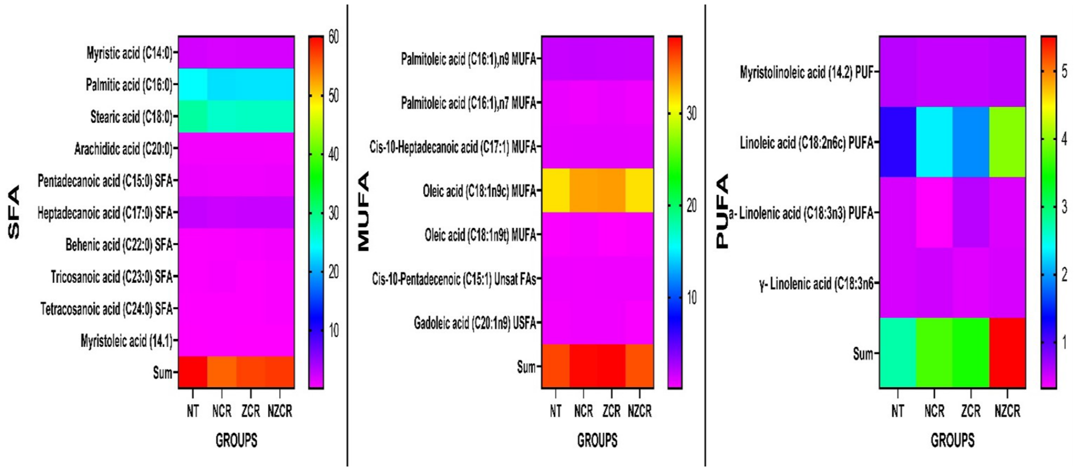

Fig. 3. Effects of nisin, nano- ZO, chitosan and roselle extract incorporation as a nitrite replacer on fatty acid composition of fresh sausage.

Cooking loss

Sausage batters incorporated with nitrite replacer constituents (nisin, nano- ZO, chitosan and roselle extract) showed lower cooking loss percentages Fig. 4. The best impact was obtained by NZCR that recorded 18.58 %.

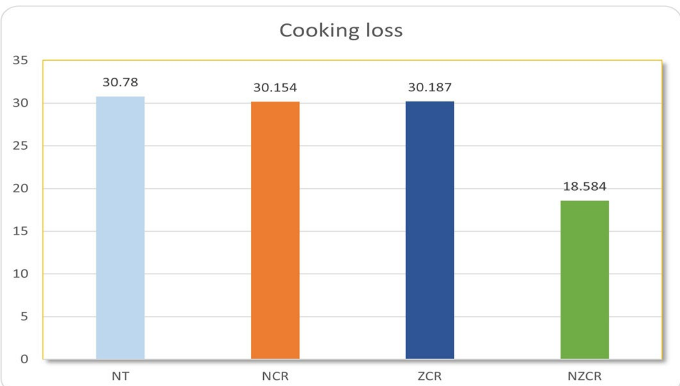

Fig. 4. Cooking loss percentages of nitrite added and reformulated sausage integrating a nitrite replacer compromising (nisin, nano sized- ZO, chitosan and roselle extract).

Color analysis of cooked beef sausage

CIE L*, a*, and b*of beef sausage variants are represented in Fig. 5. Redness a* values of nitrite added was 6.32 compared to 6.34, 7.54 and 6.80 for NCR, ZCR and NZCR, respectively, Lightness L* values decreased in reformulated sausages, while b* values increased compared to nitrite added samples. It was noteworthy that redness a values of nitrite replacer added treatments were close to those of nitrite added treatments.

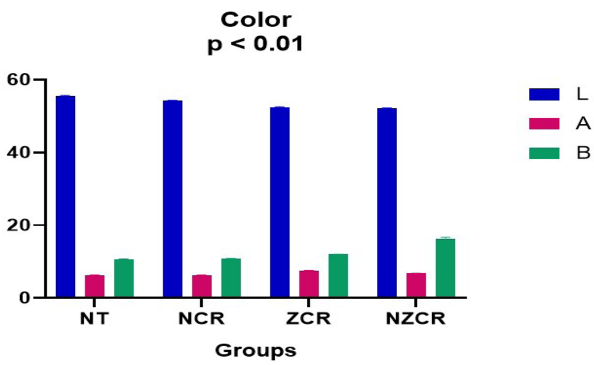

Fig. 5. Impacts of sodium nitrite replacement with (nisin, nano sized- ZO, chitosan and roselle extract) mixture on color parameters of cooked beef sausage.

Sensory Assessment of cooked beef sausage:

Nitrite replacer mixture (nisin, nano sized- ZO, chitosan and roselle extract) had significantly improved the sensory characteristics of cooked beef sausage in terms of odor, taste and overall acceptability Fig (6). The best sensory scores were recorded by NZCR followed by ZCR and NCR sausage samples and finally NT treated samples.

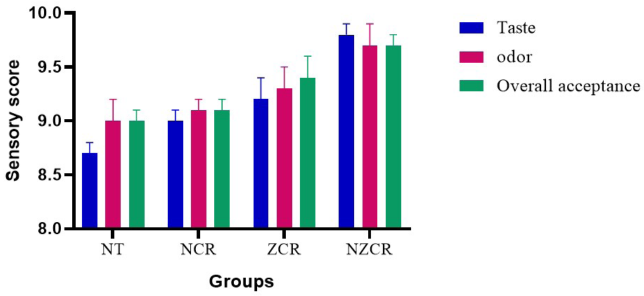

Fig. 6. Sensory Characteristics in terms of odor, taste and overall acceptability of nitrite added and reformulated sausage samples incorporating nitrite replacer mixture (nisin, nano sized- ZO, chitosan and roselle extract).

Discussion

Owing to its high carcinogenicity, several research have attempted to replace sodium nitrite with different and variable replacers. The current study evaluated the extent of the effects resulted from total replacement of sodium nitrite with a sustainable nitrite replacer containing (400 ppm nisin (N), 25 ppm nano sized- ZO (Z), 1% chitosan (C) and 1% roselle extract (R) from fresh beef sausage batter. N and Z were chosen for their potent antibacterial actions, C for its antioxidant activity and R for red color capability. Proximate and fatty acid compositions of nitrite added (NT) and reformulated (NCR, ZCR and NZCR) samples were investigated, moreover, cooking loss percentage, color parameters and sensory assessment of cooked sausage were inspected. The findings demonstrated that substitution of sodium nitrite with these substances did not alter proximate composition (fat, protein and ash) and fatty acid composition compared to nitrite treated samples, furthermore, they could decrease cooking loss %, retain red color after cooking and enhance sensory attributes of beef sausage in terms of (odor, taste and overall acceptability)

The outcomes of the present study showed that addition of the nitrite replacer constituents into sausage recipe did not alter the compositional parameters of sausage (fat, protein and ash) likely as the raw materials used were the same. Similarly, Hampikyan and Ugur (2007) found that different concentration of nisin had no effect on proximate composition of turkish fermented sausage compared to control sample and data obtained by Ozaki *et al*. (2020) reported that proximate composition of fermented cooked sausage was not affected by chitosan integration/treatment

Concerning fatty acid composition, results demonstrated that application of nitrite replacer mixture resulted in no impact on fatty acid composition. Saturated and monounsaturated fatty acids were the predominant type of fatty acids in all sausage treatments, the sum of SFA and MUFA was higher than 85 % of total fatty acids in all sausage samples came in consistence with Pintado *et al*. (2016). Oleic acid was the dominant individual fatty acid in different sausage variants agreed with data recoded by da Silva *et al.* (2019). It can be observed that the nitrite replacer mixture could improve the nutritional quality of beef sausage with higher MUFA, PUFA and lower SFA compared to nitrite added treatments. These effects could be attributed to the endogenous lipase enzyme playing a role in these observed lipid changes during storage and ripening sausages, similar results were obtained by Zhao *et al*. (2011)

Cooking loss represented water holding capacity of sausage samples, results exhibited that NZCR group samples showed the best water loss prevention 18.58% compared to 30.78% for nitrite treated samples. The results designated that the fresh sausage treated with nisin, zinc oxide nanoparticles and chitosan combination could be conducive for keeping better WHC. This positive result may be due to inhibition of proteolytic enzymes activities contributing to muscle tissue spoilage and water loss by the combination of chitosan, ZnO-NPs and nisin. Similar results were recorded by Zhao *et al*. (2019), their results showed that chitosan, nisin, and tea polyphenols combination could improve water-holding capacity of fresh pork and Suo *et al*. (2017), their results exhibited that zinc oxide nanoparticles could maintain water-holding capacity of pork meat during refrigerated storage.

Color is the most important sensory attribute in consumer's valuation of any type of food (Teixeira *et al*., 2022). Results displayed that reformulated sausage samples (NCR, ZCR and NZCR) could retain a* redness values similar to those obtained by nitrite treated sausages. The red color is probably due to anthocyanin pigment in roselle extract in reformulated sausage. Results were consistent with Ismail *et al*. (2022), their data revealed that roselle extract could retain redness of buffalo meat patties. The stability of red color after cooking in reformulated sausages is contributed to the antioxidant potential of chitosan (Mojaddar Langroodi *et al*., 2021) integrated in the replacer formula, moreover, the antioxidant properties of the roselle extract itself (Lyu *et al*., 2020) gave an assistance to obtain better color stability after ripening. lower L* values resulted may for dark color formation caused by browning reaction (Malelak *et al*., 2017) or due to low concentration of roselle extract as L* values increased with increased extract concentration (Sipahelut *et al*., 2023). From the color profile results, roselle extract could be applied for red color development in meat products for its anthocyanin content, similar results were recorded by Santos *et al*. (2022), while, the red color in nitrite-added treatment is owing to nitrosohemochrome formation that give pink color in meat products after cooking (Honikel, 2008).

The current investigation displayed that nitrite substitute mixture could significantly improve the sensorial profile (odor, taste and overall acceptability) of beef sausage. The best results were demonstrated by NZCR group samples. The acidic taste of roselle was avoided through applying a low concentration of the extract in meat batter. Sensory profile was generally enhanced for the combined antibacterial and antioxidant effects of the alternate constituents. Nisin and nano sized- ZO play as potent antibacterial compounds (Engelke *et al*., 1992; Ahmadi *et al*., 2020) and chitosan acts as a prevailing antioxidant compound (Duran & Kahve, 2020)

Results agreed with Huang *et al*. (2020), their data showed that nisin combined with monascus color and beet red could enhance sensory parameters of cured meat, Ahmadi *et al*. (2020), their data displayed that zinc oxide nanoparticles could improve sensory evaluation of refrigerated chicken meat., and Gaba *et al*. (2022), their data showed that chitosan could enhance the sensory acceptability of fresh meat and Ismail *et al*. (2022) for sensory properties amelioration of buffalo meat

Conclusion

The current study provided a suitable nitrite replacer to avoid health risks associated with nitrite additive. The replacer involves 400 ppm nisin, 25 ppm nano sized ZO, 1% chitosan and 1% roselle extract. The findings represented that mixture applied cause no significant modification in proximate composition of beef sausage compared to nitrite added. Fatty acid composition did not alter after nitrite substitute had been utilized, moreover, mixture constituents could successfully enhance fat quality by lowering SFA and increasing MUFA and PUF. Cooking loss percentage was significantly improved, red color of the product in reformulated sausages was reserved even after processing and sensory parameters were enhanced.

Conflict of interest

There are no conflicts of interest declared by the authors.

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