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Rice husk-fortified beef sausages: Cholesterol oxidation products, physicochemical properties, and sensory attributes

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ABSTRACT

The effect of rice husk flour (RHF) supplementation on the physicochemical properties, oxidative stability, and sensory attributes of beef sausages was examined. Beef sausages were formulated with either 0 % RHF + 15 % wheat flour (WF), RHF-0; 5 % RHF + 10 % WF, RHF-5; 10 % RHF + 5 % WF, RHF-10, or 15 % RHF + 0 % WF, RHF-15, and cooked at 200 °C for 20 min. RHF supplementation significantly increased dietary fiber while decreasing moisture content ($P < 0.05$). Beef sausages with RHF had lower L^*, a^*, TBARS, carbonyl content, hardness, chewiness, and gumminess, and a higher pH ($P < 0.05$). RHF-10 and RHF-15 sausages exhibited greater cook loss compared to other formulations ($P < 0.05$). The levels of 7-ketocholesterol decreased with increasing RHF levels, while 7α-hydroxycholesterol and 7-hydroperoxycholesterol were higher in RHF-0 and RHF-10 sausages compared to RHF-5 and RHF-15 sausages ($P < 0.05$). In addition, RHF improved the taste, juiciness, appearance, and overall acceptance of beef sausages. These findings suggest that RHF can be a valuable ingredient in beef sausages by increasing fiber content, reducing oxidative degradation, and maintaining sensory quality despite some moisture loss.

1. Introduction

Meat products lack dietary fiber, and are highly prone to oxidative deterioration (Das et al., 2020; Katsanidis & Zampouni, 2023). The absence of dietary fiber in meat products is often linked to an increased risk of chronic diseases, including cardiovascular diseases, colorectal cancer and type 2 diabetes (Geiker et al., 2021; Giromini & Givens, 2022; Guo, Ding, Liang, & Zhang, 2021). Furthermore, addressing oxidative spoilage in meat products is essential for reducing food waste, ensuring food safety, and extending shelf life (Das et al., 2020; Katsanidis & Zampouni, 2023; Nawaz et al., 2022). A wide range of plant-derived products serve as rich sources of dietary fiber and phytochemicals with antioxidant properties (Angulo-López et al., 2022; Das et al., 2020; Madane et al., 2020; Yadav, Pathera, Islam, Malik, & Sharma, 2018; Zhao, Guo, Liu, & Xiao, 2021). Such products offer the potential to act as functional ingredients that address dietary fiber deficiency and mitigate oxidative changes in meat products, while providing additional health benefits (Das et al., 2020; Haque, Ahmad, Azad, Adnan, & Ashraf, 2023; Mishra et al., 2023). Therefore, fortifying meat products with functional ingredients such as plant-derived products, which possess both antioxidant and fiber-enhancing properties, is of growing importance (Das et al., 2020; Ursachi, Perța-Crișan, & Munteanu, 2020).

Rice is one of the most widely cultivated and consumed grains globally, generating massive amounts of by-products, particularly rice husk (Mohidem, Hashim, Shamsudin, & Che Man, 2022). Traditionally, rice husk has been disposed of through burning, landfilling, or as a low-value agricultural input, which contributes to environmental pollution (Asfar, Asfar, Thaha, Kurnia, & Syaifullah, 2021; Geethakarthi, 2021). However, rice husk is a rich source of dietary fiber, particularly insoluble fiber (Fadaei & Salehifar, 2012; Kuan, Yuen, & Liong, 2012), which

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plays an essential role in maintaining digestive health (Baky et al., 2024; Tan et al., 2023), and can alter the technological quality of foods (Mishra et al., 2023; Moldakulova et al., 2021). In addition, rice husk contains a range of bioactive compounds, including phenolic acids and antioxidants (Frosi, Vallelonga, Colombo, Milanese, & Papetti, 2023; Kaur & Ubeyitogullari, 2023; Nnadiukwu, Onyeike, Ikewuchi, & Patrick-Iwuanyanwu, 2023), which contribute to various health benefits, and can extend the oxidative shelf-life of foods. Thus, with its rich composition of dietary fiber, antioxidants, and minerals, rice husk can be effectively incorporated into food products, transforming a waste material into a valuable resource.

Cholesterol oxidation products (COPs) are a class of oxidized derivatives of cholesterol formed when cholesterol undergoes oxidative reactions in animal-derived foods (Deng et al., 2023; Kim, 2023). COPs negatively impact human health by contributing to the development of cardiovascular diseases, atherosclerosis, and other inflammatory conditions (Deng et al., 2023; Maldonado-Pereira, Schweiss, Barnaba, & Medina-Meza, 2018). Cholesterol oxidation in foods is influenced by factors such as exposure to heat, light, oxygen, storage conditions, and the presence of pro-oxidants like metals or unsaturated fats (Adeyemi et al., 2025; Barreira et al., 2023; Kim, 2023; Sulaimon & Adeyemi, 2024). Depending on their composition, some sausages are particularly prone to oxidative deteriorations owing to their high unsaturated fat content, exposure to oxygen during processing, and the presence of pro-oxidant factors like metal ions, salts, and heat treatments (Bolívar-Monsalve, Ramírez-Toro, Bolívar, & Ceballos-González, 2019; Manzoor, Haque, Ahmad, & Hopkins, 2023; Sam et al., 2021). Additives with antioxidant properties can mitigate cholesterol, lipid, and protein oxidation in foods by neutralizing free radicals, inhibiting oxidative reactions, and stabilizing reactive oxygen species, thus preserving food quality and extending shelf life (Barreira et al., 2023; Kim, 2023; Sulaimon & Adeyemi, 2024). Thus, because of the phytochemical constituents of rice husk, which exert antioxidant properties (Frosi et al., 2023; Kaur & Ubeyitogullari, 2023; Nnadiukwu et al., 2023), it is possible that incorporating rice husk would reduce the formation of COPs, and lipid- and protein-derived oxidation products in sausages. The quality-enhancing potential of some rice milling by-products, such as rice bran (Aviles et al., 2023; Choi et al., 2011; Malekian, Khachaturyan, Gebrelul, & Henson, 2014), has been studied in various meat products. However, the potential of rice husk to improve the quality attributes of meat products has not been explored. This study aimed at assessing the influence of rice husk supplementation on COPs, textural profile, physicochemical properties, oxidative status and sensory attributes of roasted beef sausages.

2. Materials and methods

2.1. Raw materials

Semimembranosus muscle (4.36 ± 0.55 kg per batch) from White Fulani cows within 48 h postmortem, and beef fat were procured from a meat shop. Rice husk was obtained from a local rice milling plant. Wheat flour, ginger, garlic, seasoning, salt, *Capsicum frutescens*, and *Piper nigrum* were sourced from grocery stores.

2.2. Preparation of rice husk flour

The rice husk was subjected to hot water washing at 50 °C for 15 min (Bandara, Gamage, & Gunarathne, 2020). Thereafter, the rice husk was oven-dried at 60 °C until a constant weight was achieved. The dried rice husk was pulverized in a blender and passed through a 0.20 mm sieve to obtain a fine flour. The rice husk flour (RHF) was packaged in Ziplock bags and stored at −20 °C until use.

2.3. Beef sausage preparation

The beef was trimmed of external fat and connective tissue, diced into small pieces, and minced using a food processor. The minced meat was manually mixed with other ingredients except wheat flour (WF) and RHF. The mix was split into four portions, and the corresponding levels of RHF were added as follows: RHF-0: Beef sausage containing 0 % RHF + 15 % WF; RHF-5: Beef sausage containing 5 % RHF + 10 % WF; RHF-10: Beef sausage containing 10 % RHF + 5 % WF; RHF-15: Beef sausage containing 15 % RHF + 0 % WF (Table 1). The sausage mix was homogenized and allowed to rest for 4 h at 5 ± 1 °C. The mix was divided into 50 g portions, placed in a stainless-steel sausage mold and cooked in an oven (WTC Binder FD53, Binder GmbH, Tuttlingen) at 200 °C for 20 min. Six separate batches of beef sausages were made, with each batch considered a replicate.

2.4. Chemical composition, and dietary fiber analyses

The proximate composition, and dietary fiber of RHF and beef sausages were determined using AOAC (2007) methods. Moisture was measured by drying the samples at 105 °C until a constant weight was achieved. Crude protein was analyzed using the Kjeldahl method, with a nitrogen-to-crude protein conversion factor of 6.25. Ether extract was calculated based on weight loss after an 8-h extraction with petroleum ether using a Soxhlet apparatus. Ash was determined by incinerating samples in a muffle furnace at 550 °C for 8 h. Crude fiber was determined gravimetrically as the residue remaining after the acid and alkaline digestions. Total dietary fiber (TDF) and insoluble dietary fiber (IDF) were measured using an enzymatic-gravimetric method, and soluble dietary fiber (SDF) was calculated by subtracting the IDF from the TDF.

2.5. Total phenol analysis

The total phenolic content (TPC) of RHF was determined using the Folin–Ciocalteu reagent, according to the method described by Singleton, Orthofer, and Lamuela-Raventós (1999). Briefly, 20 μL of the sample was diluted in 180 μL of deionized water, followed by the addition of 200 μL of the reagent. The mixture was vortexed, then 800 μL of Na_2CO_3 was added, and the solution was incubated for 30 min at 40 °C. The optical density was measured at 765 nm. TPC concentrations were calculated using a standard curve of gallic acid, ranging from 0 to 100 μg/mL.

2.6. Color, cook loss, and pH analyses

Color values, following the International Commission on Illumination's (CIE) L^* a^* b^* system, were assessed using a pre-calibrated colorimeter (WR-10, Shenzhen, China) with an 8 mm port size, a 10° standard observer, and a D65 illuminant. Cooked sausages were sliced

Table 1
Composition of beef sausage.

	Level of rice husk flour (%)			
Ingredient (%)	0	5	10	15
Beef	70.00	70.00	70.00	70.00
Wheat flour	15.00	10.00	5.00	0.00
Rice husk flour	0.00	5.00	10.00	15.00
Beef fat	10.00	10.00	10.00	10.00
Salt	1.50	1.50	1.50	1.50
Ice	1.50	1.50	1.50	1.50
Ginger	0.50	0.50	0.50	0.50
Garlic	0.50	0.50	0.50	0.50
Seasoning	0.50	0.50	0.50	0.50
Capsicum frutescens	0.25	0.25	0.25	0.25
Piper nigrum	0.25	0.25	0.25	0.25

perpendicular to their length, and three readings were taken from both the inner and outer sections. The results were then averaged. Cook loss was calculated based on the percentage difference in sample weight before and after cooking. The pH of cooked sausages was measured using a pH meter (MW102; Milwaukee® Instruments, Inc., Rocky Mount, NC, USA). Calibration of the pH meter was conducted prior to the measurements, following the manufacturer's instructions, using standard buffer solutions with pH values of 4.01 and 7.01 at 25 °C. The pH electrode was used in conjunction with the temperature probe to utilize the meter's automatic temperature compensation (ATC) capability. The measurement was performed on a homogenate prepared by mixing 5 g of the sample with 50 mL of deionized water.

2.7. Texture analysis

Texture profile analysis of the sausages was conducted at room temperature using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY), following the methodology outlined by Bourne (2002). A 25 kg load cell was used to calibrate the equipment, and the test involved two successive compression cycles with a 5-s interval between compressions. Samples measuring 2 cm in thickness and 1 cm in diameter were compressed to 30 % of their original height at a speed of 1 mm/s, using a P-35 probe (35 mm diameter, stainless steel). The parameters assessed were hardness, cohesiveness, gumminess, springiness, and chewiness. Gumminess was calculated by multiplying cohesiveness and hardness values. Chewiness was calculated by multiplying cohesiveness, springiness and hardness values.

2.8. Lipid oxidation and protein oxidation analyses

Lipid and protein oxidation analyses were performed on the sausages immediately after cooking. Lipid oxidation was assessed using the 2-thiobarbituric acid (TBA) method. One gram of the sample was mixed with 5 mL of a stock solution containing 0.375 % thiobarbituric acid, 15 % trichloroacetic acid, and 0.25 N HCl. After vortexing, the samples were heated in a boiling water bath for 10 min. Once cooled, the samples were centrifuged and filtered. The absorbance was then measured at 532 nm. TBARS values were calculated using a standard curve of malondialdehyde (MDA) and expressed as mg MDA/kg of sample.

Protein oxidation was assessed by quantifying carbonyl groups through their reaction with DNPH to form hydrazones following the method of Levine, Garland, and Oliver (1990) Briefly, 0.1 g of sausage sample was incubated with 1.0 mL of 20 mM DNPH solution for 1 h. Proteins were precipitated by adding 20 % (*v*/v) TCA and re-dissolved in the DNPH solution. The proteins were then precipitated again using 20 % (v/v) TCA. The resulting protein pellet was washed three times with 125 μL of an ethanol/ethyl acetate (1:1) mixture and re-suspended in 125 μL of 6 M/L guanidine hydrochloride. Absorbance was measured at 370 nm. The carbonyl content was calculated using the extinction coefficient for DNPH (ε = 22,000/M/cm) and expressed as mmol carbonyl/mg protein.

2.9. Extraction and determination of cholesterol oxidation products

Cholesterol oxidation products were assessed in the sausages immediately after cooking. Two grams of sausage was mixed with 6 mL of ethanol and 4 mL of 50 % KOH, and the mixture was incubated in the dark at 27 °C for 22 h. After incubation, 5 mL of distilled water and 10 mL of hexane were added, and the mixture was vortexed. The hexane fraction was separated, and the extraction with 10 mL of hexane was repeated three times. The combined hexane extracts were dried using a rotary evaporator. The residue was dissolved in 5 mL of hexane, transferred to a screw-top flask under nitrogen, diluted with 1 mL of mobile phase, and filtered through a 22 μm filter (Millipore, Maryland, MD, USA) for HPLC analysis. COPs were quantified with high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) using a UV (SPDFP-00 AVVP) detector. Separation was achieved using a Nova-Pak CN HP column (300 mm × 3.9 mm, × 4 mm, Waters, Milford, MA, USA) at 32 °C. The mobile phase consisted of hexane and 2-propanol (97:3, v/v) at a flow rate of 1 mL min^{-1}. COPs were identified by comparing the retention times and mass spectra of verified standards (Sigma-Aldrich, St. Louis, MO, USA) with those of the corresponding cholesterol oxidation products peaks in the sample.

2.10. Sensory analysis

The experimental procedure followed the guidelines of the Ethical Review Committee, University of Ilorin. Informed written consent was obtained from all assessors. Sensory acceptability was evaluated according to AMSA, American Meat Science Association (2015) guidelines, using a 9-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). The sensory parameters assessed included taste, flavor, texture, appearance, juiciness, and overall acceptability. Each sample consisted of 10 g of recently cooked sausage, served on coded plates. The sensory panel comprised 37 untrained females and 28 untrained males, aged 19–48 years, all familiar with beef sausage and drawn from the staff and students of the University of Ilorin. A sensory session was conducted for all batches, with the same group of assessors evaluating all the six batches. In each batch, each assessor evaluated four samples, corresponding to the four different treatments. Prior to the sensory analysis, assessors were briefed on the sensory attributes and evaluation procedures. Between samples, assessors were provided with water and unsalted crackers to cleanse their palates.

2.11. Statistical analysis

Data were analyzed using analysis of variance for a completely randomized design in SAS software. Treatments were treated as fixed effects, while batches were considered random effects. For sensory data, assessors were also treated as random effects. A significance level of $P < 0.05$ was applied, and mean differences were separated using Tukey's HSD test.

3. Results and discussion

3.1. Chemical composition, dietary fiber, and total phenolic content in rice husk flour

The chemical composition, dietary fiber, and total phenolic content of RHF are outlined in Table 2. The proximate composition values are consistent with those reported in previous studies (Fadaei & Salehifar, 2012; Kuan et al., 2012; Nnadiukwu et al., 2023). The total dietary fiber in RHF was higher than the 59 % reported by Kuan et al. (2012) but was comparable to the 67 % reported by Fadaei and Salehifar (2012). The

Table 2
Chemical and dietary fiber composition and total phenolic content in rice husk flour.

Item	(%)
Moisture	5.22
Crude protein	3.03
Crude fat	1.55
Ash	18.40
Crude fiber	37.30
Total dietary fiber	70.45
Insoluble dietary fiber	67.95
Soluble dietary fiber	2.50
Total phenolic content (mg GAE[1]/g)	13.77
pH	6.75

[1] gallic acid equivalent.

total phenolic content of RHF (13.77 mg GAE/g) was similar to the 14.9 mg GAE/g reported by Gao et al. (2018) and the 9.4 mg GAE/g reported by Elkatry et al. (2023), but lower than the 20.7–29.9 mg GAE/g reported by Wisetkomolmat et al. (2023). The chemical and dietary fiber composition, and phytochemical content of rice husk could be influenced by factors such as rice variety, agronomic conditions, processing methods, and storage conditions. The high dietary fiber content in RHF suggests potential health benefits and may improve the technological properties of meat products. In addition, the elevated phenolic content indicates that RHF could enhance the oxidative stability of meat products, making it a promising functional ingredient for meat applications (Elkatry et al., 2023; Wisetkomolmat et al., 2023). The pH of RHF was 6.75, likely due to its high mineral (ash) content. This value is comparable to the 6.91 reported by Kolar and Jin (2019).

3.2. Chemical composition, and dietary fiber content of beef sausages

Moisture levels decreased ($P < 0.05$), while ash, crude fiber, TDF, IDF, and SDF levels increased ($P < 0.05$) as the amount of RHF increased (Table 3). The ether extract and crude protein content of the beef sausages were not significantly affected by RHF supplementation. The reduction in moisture content is consistent with the higher cook loss (Table 4) observed in the RHF-supplemented sausages. Rice husk is primarily composed of insoluble fibers such as cellulose, hemicellulose, and lignin, which do not dissolve in water and have limited moisture absorption and retention capacity. This limited water-binding ability explains why adding rice husk to sausages results in lower moisture retention. Similar to our findings, the addition of rice bran reduced moisture content in chevon sausages (Malekian et al., 2014) and chicken patties (Aviles et al., 2023). In addition, dried carrot pomace, and wheat bran addition lowered the moisture content in chicken sausages (Yadav et al., 2018).

The increase in ash and fiber contents reflects the naturally high ash and fiber levels in RHF (Fadaei & Salehifar, 2012; Kuan et al., 2012), suggesting that rice husk flour can enhance the nutritional value of sausages by boosting dietary fiber, making it a beneficial ingredient for improving the healthfulness of meat products. Likewise, dried carrot pomace, and wheat bran addition enhanced the TDF, IDF, and SDF of chicken sausages (Yadav et al., 2018). The recommended daily intake of dietary fiber is 25–30 g with an insoluble-to-soluble fiber ratio of 3:1 (USDA, 2015). Thus, consuming 100 g of RHF-5, RHF-10, and RHF-15 would provide approximately 18–22 %, 32–39 %, and 45–54 % of the recommended daily fiber intake, respectively.

3.3. Color, pH and cook loss

Meat color is a key indicator of freshness and quality, significantly influencing consumer acceptance as it imparts their perception of taste, safety, and overall appeal. RHF supplementation significantly reduced the lightness and redness of cooked beef sausages (Table 4). The color of the RHF used in this study was light brown. Thus, incorporating RHF into the sausage matrix resulted in a darker and less red product. Further, fiber can interact with water and fat, which affects surface reflection and reduces the overall lightness of meat products (Aviles et al., 2023). While yellowness was significantly affected by RHF supplementation, the trend was non-linear. The RHF-5 sausages had lower yellowness compared to other formulations, except for RHF-10. The initial decrease in yellowness could be attributed to the interaction between rice husk components and the sausage matrix, which may have masked yellow hues. At higher concentrations, RHF may contribute yellow pigments or alter fat distribution, thereby increasing yellowness. Similarly, the addition of rice bran fiber reduced the color coordinates of pork sausages (Choi et al., 2011), while rice bran supplementation lowered the color coordinates of chicken patties (Aviles et al., 2023).

Cook loss was lower ($P < 0.05$) in the RHF-0 and RHF-5 sausages compared to the RHF-10 and RHF-15 sausages (Table 4). This was likely due to the poor water-binding capacity of insoluble fibers, which are abundant in RHF. Moreover, the addition of RHF may weaken the water-fat-protein emulsion, allowing more water to escape during cooking. Contrary to our findings, the addition of rice bran reduced cook loss in pork sausages (Choi et al., 2011) and chicken patties (Aviles et al., 2023). These discrepancies could be attributed to the differences in product formulations and the water-binding capacities of the supplements. While RHF was used as a substitute for wheat flour in this study, rice bran replaced pork (Choi et al., 2011) and breast meat (Aviles et al., 2023) in other studies. Compared to RHF, wheat flour contains starch, which can absorb water and form a gel during cooking, helping to retain moisture in the sausage. Further, rice bran has a relatively high fat content, whereas RHF is very low in fat. When rice bran is used as a meat substitute, it may absorb and retain more water during cooking. The supplementation with RHF caused an increase in pH, as the pH of RHF in this study was 6.75, and its addition led to a gradual rise in the pH of the beef sausages. A similar pH increase was observed with the supplementation of rice bran fiber in pork sausages (Choi et al., 2011).

Table 3
Chemical composition, and dietary fiber in beef sausages containing varying levels of rice husk flour.

Item (%)	Level of rice husk (%)				SEM	*P* value
	0	5	10	15		
Moisture	56.05^{a}	53.65^{b}	53.30^{bc}	52.70^{c}	0.45	0.007
Crude protein	17.48	16.44	16.28	16.35	0.80	0.119
Ether extract	11.72	12.09	11.95	11.63	0.08	0.071
Ash	3.31^{d}	4.59^{c}	5.65^{b}	6.41^{a}	0.04	0.001
Crude fiber	2.07^{d}	4.12^{c}	6.29^{b}	8.26^{a}	0.06	0.000
Total dietary fiber	1.55^{d}	5.65^{c}	9.65^{b}	13.56^{a}	0.15	0.027
Insoluble dietary fiber	1.43^{d}	4.57^{c}	9.02^{b}	12.67^{a}	0.05	<0.001
Soluble dietary fiber	0.13^{d}	0.38^{c}	0.63^{b}	0.89^{a}	0.01	<0.001

^{a,b,c,d}Means with different superscripts in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

Table 4
Color, cook loss and pH of beef sausages containing varying levels of rice husk flour.

Item	Level of rice husk flour (%)				SEM	*P* value
	0	5	10	15		
*L**	43.2^{a}	41.2^{ab}	39.2^{b}	36.5^{b}	0.98	0.001
*a**	12.0^{a}	10.4^{b}	10.5^{b}	10.1^{b}	0.27	0.004
*b**	15.7^{a}	12.6^{c}	13.7^{bc}	14.7^{a}	0.42	0.000
Cook loss (%)	3.41^{b}	3.75^{b}	5.91^{a}	6.70^{a}	0.87	<0.001
pH	5.78^{c}	5.88^{b}	5.86^{b}	5.98^{a}	0.03	<0.001

^{a,b,c}Means with different superscripts in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

3.4. Textural profile

There exists a relationship between the overall tenderness of meat products and the textural characteristics of hardness, gumminess, chewiness, cohesiveness, and springiness (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003). Supplementing RHF affected the hardness, chewiness, springiness and gumminess of beef sausages ($P < 0.05$) but did not affect the cohesiveness (Table 5). The RHF-15 and RHF-10 sausages had lower hardness and gumminess compared to the RHF-0 sausages. The hardness and gumminess of the RHF-5 sausages did not differ from those of other sausages. Rice husk fiber can interfere with the formation of the meat protein gel matrix that is responsible for holding the sausage together. This disruption weakens the overall structure, making the sausage softer and less cohesive. As a result, both gumminess and chewiness are reduced since the sausage becomes easier to break down during chewing. The effect of fiber addition on the textural properties of meat

Table 5
Textural profile of beef sausages containing varying levels of rice husk flour.

Item	Level of rice husk flour (%)				SEM	P value
	0	5	10	15		
Hardness (N)	28.2[a]	24.6[b]	22.2[bc]	19.2[c]	0.95	<0.001
Cohesiveness	0.81	0.80	0.80	0.78	0.01	<0.001
Springiness	0.81[a]	0.75[b]	0.75[b]	0.77[ab]	0.01	0.009
Gumminess	22.8[a]	19.7[ab]	17.8[bc]	15.1[c]	0.89	<0.001
Chewiness (N/cm)	18.5[a]	14.9[b]	13.2[bc]	11.6[c]	0.73	<0.001

[a,b,c]Means with different superscripts in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

products varies across the literature. Zhao et al. (2021) observed a reduction in hardness, chewiness, and gumminess in pork meat balls supplemented with Kiwi fruit pomace fiber. Conversely, Aviles et al. (2023) reported that the addition of rice bran fiber increased hardness and gumminess in chicken patties. Similarly, Yadav et al. (2018) found that supplementing wheat bran and dried carrot pomace enhanced the hardness, chewiness, and gumminess of chicken sausages. It is important to note that in studies reporting increased hardness, chewiness, and gumminess, the dietary fiber source was used as a substitute for meat or fat, whereas in our study, the meat and fat contents remained constant, while RHF was used to replace wheat flour. Differences in product formulations, meat products, dietary fiber sources, types, and their concentrations may explain the discrepancies in the textural properties of meat products in response to fiber addition.

3.5. Cholesterol oxidation products, lipid oxidation and protein oxidation

COPs are formed during the processing and storage of animal products, posing potential health risks (Deng et al., 2023). Therefore, it is crucial to reduce their levels to enhance both the safety and quality of the final product. In this study, seven COPs were detected, but only three were significantly affected by RHF supplementation (Table 6). The level of 7-ketocholesterol, the major COP, decreased as the RHF level increased in beef sausages ($P < 0.05$). Rice husk likely reduced selected COPs in beef sausages due to its high fiber content (Fadaei & Salehifar, 2012; Kuan et al., 2012), which can bind cholesterol (Okrathok & Khempaka, 2020; Shen, Weihao, & Cao, 2020; Zhao et al., 2021) and reduce its availability for oxidation. In addition, rice husk contains antioxidant compounds (Frosi et al., 2023; Kaur & Ubeyitogullari, 2023; Nnadiukwu et al., 2023) that may inhibit oxidative processes, further preventing the formation of COPs. Consistently, the addition of rice fiber reduced 7-ketocholesterol concentration in restructured beef roasts (Kim, Godber, & Prinaywiwatkul, 2000).

The effect of RHF on 7α-hydroxycholesterol and 7-hydroperoxycholesterol followed a non-linear dose-response pattern. Specifically, the concentrations of 7-hydroperoxycholesterol and 7α-hydroxycholesterol were higher in RHF-0 and RHF-10 sausages compared to RHF-5 and RHF-15 sausages. This inconsistency may be attributed to the differences in fiber-water interactions, antioxidant activity, and the ability of the fiber to bind cholesterol at various concentrations, leading to a non-linear effect on cholesterol oxidation. The trend in 7-hydroperoxycholesterol and 7α-hydroxycholesterol concentrations could also be linked to the specific properties and formation pathways of these COPs. 7-Hydroperoxycholesterol is an intermediate compound in the cholesterol oxidation pathway, being the initial product formed when cholesterol is oxidized by triplet oxygen (Nogueira, Costa, Crotti, & Bragagnolo, 2010; Smith, 1996). 7α-Hydroxycholesterol is a secondary oxidation product, often derived from the decomposition of 7-hydroperoxycholesterol (Nogueira et al., 2010; Smith, 1996). The conversion of 7-hydroperoxycholesterol to 7α-hydroxycholesterol is influenced by factors such as the presence of metal ions, heat, or continued exposure to free radicals (Nogueira et al., 2010; Smith, 1996). The unstable, reactive nature of 7-hydroperoxycholesterol makes it more sensitive to early-stage oxidation processes, which appears to be less controlled in the RHF-0 and RHF-10 sausages. The formation of 7α-hydroxycholesterol, as a secondary oxidation product, reflects the progression of oxidation, which might be mitigated in RHF-5 and RHF-15 sausages likely due to the antioxidant action and better inhibition of free radical formation.

The RHF-supplemented sausages showed lower carbonyl and TBARS values than the RHF-0 sausages (Table 6), suggesting that rice husk reduced lipid and protein oxidation by providing insoluble fiber (Fadaei & Salehifar, 2012; Kuan et al., 2012) and antioxidant compounds (Frosi et al., 2023; Kaur & Ubeyitogullari, 2023; Nnadiukwu et al., 2023), which limit oxygen exposure and neutralize free radicals that initiate oxidative reactions. Likewise, *Psyllium* husk addition lowered the TBARS in chicken patties (Mehta, Ahlawat, Sharma, Yadav, & Arora, 2013), while rice bran supplementation improved the antioxidant capacity of chevon sausages (Malekian et al., 2014). In addition, the supplementation of Kiwi fruit pomace fiber reduced TBARS in pork meat balls (Zhao et al., 2021).

3.6. Sensory properties

Despite the high cook loss and low moisture content, RHF

Table 7
Sensory properties of beef sausages containing varying levels of rice husk flour.

Sensory properties	Level of rice husk flour (%)				SEM	P value
	0	5	10	15		
Taste	5.7[b]	7.2[a]	6.7[a]	7.3[a]	0.35	0.005
Flavor	5.7	6.0	6.2	6.7	0.39	0.321
Texture	5.6[b]	6.2[ab]	7.1[a]	7.3[a]	0.40	0.006
Appearance	5.0[b]	6.6[a]	6.3[a]	6.4[a]	0.56	0.012
Juiciness	5.6[b]	6.4[a]	6.3[a]	6.5[a]	0.50	0.018
Overall acceptability	5.4[b]	7.0[a]	6.7[a]	7.4[a]	0.63	0.002

[a,b,c]Means with different superscripts in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

Table 6
Cholesterol oxidation products, lipid oxidation, and protein oxidation in beef sausages containing varying levels of rice husk flour.

Parameter	Levels of rice husk flour (%)				SEM	P value
	0	5	10	15		
Cholesterol oxidation product (μg/100 g)						
20α-hydroxycholesterol	4.40	3.87	3.99	3.73	0.39	0.663
25-hydroxycholesterol	2.20	1.37	2.20	2.07	0.33	0.103
7α-hydroxycholesterol	2.09[a]	0.14[b]	1.98[a]	0.15[b]	0.08	0.001
7-ketocholesterol	77.46[a]	70.75[b]	66.79[bc]	62.95[c]	3.34	0.004
22R-hydroxycholesterol	3.02	3.11	3.07	3.13	0.11	0.891
7β-hydroxycholesterol	8.39	8.41	8.33	8.22	0.62	0.996
7-hydroperoxycholesterol	2.30[a]	0.35[b]	2.00[a]	0.42[b]	0.06	<0.001
TBARS (mg MDA/kg)	0.32[a]	0.24[b]	0.21[b]	0.17[c]	0.02	0.013
Carbonyl (mmol/mg protein)	0.30[a]	0.21[b]	0.20[b]	0.20[b]	0.01	0.001

[a,b,c]Means with different superscripts in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

supplementation enhanced ($P < 0.05$) the taste, juiciness, texture, appearance, and overall acceptance of beef sausages (Table 7). This is likely due to the potential of rice husk to improve the structural integrity and mouthfeel through fiber addition. The RHF may have contributed to a desirable texture, and an appealing appearance by interacting with other sausage ingredients, compensating for the moisture loss while still delivering a satisfying sensory experience. Similarly, the addition of rice bran fiber enhanced the sensory attributes of frankfurters (Choi et al., 2015).

4. Conclusion

The supplementation of RHF in beef sausages significantly imparted their physicochemical properties, oxidative stability, and sensory attributes. Increasing the RHF levels led to an increase in dietary fiber while reducing the moisture content, color coordinates, and textural attributes, such as hardness, gumminess, and chewiness. Although the 10 and 15 % RHF resulted in a greater cook loss, RHF supplementation improved the sensory attributes of beef sausages. In addition, RHF effectively reduced lipid oxidation, and protein oxidation, as well as selected COPs, particularly 7-ketocholesterol. Overall, RHF supplementation enhanced the nutritional profile, and oxidative stability of beef sausages while maintaining desirable sensory attributes. Further studies could explore the long-term stability and shelf life of beef sausages supplemented with RHF, particularly under different storage conditions.

Consent statement

The experimental protocol was carried out according to the guidelines of the Ethical Review Committee of the University of Ilorin. I**nformed written consent** was obtained from all individuals who participated in the sensory assessment.

CRediT authorship contribution statement

Kazeem D. Adeyemi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Isiaka O. Kolade:** Resources, Methodology, Investigation. **Amidat O. Siyanbola:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Faidhat O. Bhadmus:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Rafiat M. Shittu:** Resources, Methodology, Investigation. **Hakeem Ishola:** Resources, Methodology, Investigation. **Chanporn Chaosap:** Resources, Methodology, Investigation. **Panneepa Sivapirunthep:** Resources, Methodology, Investigation. **Kehinde M. Okukpe:** Resources, Methodology, Investigation. **Victoria O. Chimezie:** Resources, Methodology, Investigation. **Oluwasayope I. Alli:** Resources, Methodology, Investigation. **Rasheed O. Sulaimon:** Investigation, Methodology, Resources. **Babatunde H. Ajao:** Resources, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

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