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Nutritional Composition and Histopathological Effects of Composite Biscuits Made From Finger Millet, Soybean, and Wheat Bran on Albino Wistar Rats

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Abstract

Biscuits are snacks consumed in between meals. Biscuit was produced from composite flour (finger millet flour, soybean flour, and wheat bran). Five samples; FSW1 (60% finger millet flour, 35% soybeans flour and 5% wheat bran) FSW2 (70% finger millet flour, 25% soybeans flour and 5% wheat bran) FSW3 (80% finger millet flour, 15% soybeans flour and 5% wheat bran) FSW4 (90% finger millet flour, 5% soybeans flour and 5% wheat bran) FSW5 (100% finger millet) of biscuit were produced from the composite flour and FSW6 (positive control). This study highlights the potential of finger millet, soybean flour, and wheat bran blends for the production of nutritious biscuits. Proximate composition biscuit and histopathology of Wistar rats were carried out. Proximate analysis showed increasing soybean flour content significantly increased protein (5.67-14.92%) and fat (19.65-26.59%) levels, moisture content reduced with values between (2.28-6.32%) and carbohydrate between (38.20-55.64%). Crude fiber ranged from 11.74-16.66%, with wheat bran contributing to higher values. Biscuits from the composite flours were fed to albino Wistar rats for 28 days before histopathological examination. Kidney histology showed no significant changes related to the feed composition, with some formulations displaying a nearly normal architecture. Liver histology revealed normal to nearly normal hepatic structure in most of the groups, with some sinusoidal dilation and diffusion. The heart histology indicated varying degrees of change, including connective tissue enlargement, muscle fiber changes, and lipid deposits in some formulations. The biscuit will provide a good source of protein and because of the high fiber content, it will aid digestion.

Keywords: *Composite Flour, Biscuit, Finger Millet, Soybean, Histopathology, Nutritional Quality, Wistar Rats*

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1. Introduction

Malnutrition remains a significant global health issue, affecting individuals of all ages (Ajala *et al*., 2024). Its impact extends beyond health, impeding efforts to reduce poverty and hampering economic growth (WHO 2020). According to the World Health Organization (WHO), a substantial portion of the world's population experiences malnutrition (WHO 2020). Therefore, it is essential to improve nutrition, address hunger, promote healthy living, and ensure overall wellness throughout the continent (Ajala *et al*., 2024). An emerging approach gaining momentum in tackling the malnutrition crisis in Africa involves developing diverse snack food items such as biscuit products (Adebowale *et al.* 2016; Adegbanke *et al*., 2024c; Jenfa *et al*. 2024a, 2024b).

Biscuit is a consumable product traditionally produced from wheat flour, fat, and sugar (Adegbanke *et al.*, 2019b). Recently, Biscuit manufacturing techniques have been developed to enhance nutritional quality. These treatments can serve as carriers for delivering essential nutrients and provide wholesome options for health-focused consumers and individuals with specific medical needs. Conventionally, cookies are known for their low protein content and minimal nutritional benefits (Klerks *et al.* 2023; Blasi *et al.*, 2024).

Biscuits can help address global protein deficiency, particularly among lower-income groups who are unable to afford costly Western foods (Darmon & Drewnowski, 2015). Cereal-like finger millet can be used to produce nutritious biscuits. Cookies can also be referred to as biscuit and can be produced from unripe plantain, wheat and watermelon seed Oludumila and Adetimehin, 2016 and Bambara groundnut Adegbanke *et al.,* 2019a.

Finger millet (*Eleusine coracana*), which originate from Ethiopia, is grown in over 25 countries across Africa and Asia. After sorghum, pearl millet, and foxtail millet are the fourth most important millet (Rakkammal *et al.*, 2024). This cereal is a crucial dietary staple that provides significant calories and proteins to large populations, especially economically disadvantaged groups (Chandra *et al.*, 2016). Finger millet is highly nutritious and renowned for its exceptional calcium levels that are essential for bone strength. This grain is abundant in lysine and methionine (Abioye *et al.,* 2022). Per 100 grams of finger millet, the mineral content includes 130–283 mg of phosphorus, 430–490 mg of potassium, 78–201 mg of magnesium, 162–398 mg of calcium, 49 mg of sodium, 2.3 mg of zinc, 3.3–14.39 mg of iron, 17.61–48.43 mg of manganese, and 0.47 mg of copper (Abioye *et al.,* 2022). Finger millet contains high levels of dietary fiber, which promotes digestive health, and polyphenols with antioxidant properties. However, millet has been reported to be toxic to animals when consumed (Antony, *et al*., 2003).

Soybean flour is notable for its high protein content and balanced amino acid profile, offering a solution to protein deficiencies in populations with limited access to animal-based proteins (Qin, *et al*., 2022). This characteristic makes them valuable for addressing nutritional challenges, particularly in biscuits. Soybeans contain beneficial plant compounds, such as isoflavones and phenolic substances, that promote human health (Wang & Komatsu, 2017). They contain high levels of isoflavones, which are known to have antioxidant and phytoestrogenic properties. Soybean crops are often exposed to substantial pesticide use and soil pollution can result in high levels of potentially toxic elements (PTEs) (Tóth *et al*., 2016). Soybeans can accumulate metals and contaminants, leading to health concerns among consumers. Soy-based drinks contain detrimental elements (Carmen *et al.*, 2020).

Wheat bran is another ingredient of biscuits that enhances texture, moisture retention, and nutritional content in products such as breakfast cereals and biscuits (Onipe *et al.,* 2015). Wheat bran, which is rich in insoluble fibers, promotes digestive health and supports the gut microbiome. It contains bioactive compounds, including phenolic acids and antioxidants, linked to lower risks of chronic health conditions such as obesity, type 2 diabetes, and colorectal cancer (Cui *et al*., 2013, Yao *et al*., 2022). However, wheat bran contains components such as phytic acid that can impede mineral absorption (Gupta *et al*., 2013). Incorporating soybean, finger millet, and wheat bran into biscuit formulations boosts the protein content and nutritional value. The addition of wheat bran increases the fiber content and meet the consumer demand for health-beneficial foods. Despite their nutritional advantages, these ingredients contain antinutritional factors (Costa *et al*., 2024). Anti-nutrients in soybeans can adversely affect food nutritional quality and potentially cause toxic effects (Nath *et al.*, 2022). Although health benefits are well documented, ensuring their safety for human consumption is crucial. This study aimed to examine the potential toxic effects of biscuits made from finger millet, soybean flour, and wheat bran and to seek a thorough assessment of their possible adverse health impacts.

2. Materials and Methods

Source of Materials and Animals

The main ingredients, consisting of finger millet (Eleusine coracana), soybean flour (Glycine max), and hard wheat bran (*Triticum aestivum*), as well as additional elements such as salt, sugar, and pepper, were procured from the Erekesans market located in Akure, Ondo State, Nigeria. Other materials and equipment were obtained from the Food Processing Laboratory of the Department of Food Science and Technology (FUTA). The experiment involved 30 healthy weanling Albino Wistar rats of both sexes, acquired from the Department of Biochemistry at FUTA in Nigeria. The study was conducted with the approval of the Federal University of Technology Akure ethical committee under the ethical number FUTA/SAAT/2016/015. All chemicals used in this study were of analytical grade.

Preparation of Finger Millet Flour, soy bean flour and wheat bran

Finger millet flour was prepared according to the method described by (Sengev *et al.,* 2010) as shown in figure 1. Soybean flour was prepared according to the process described by (Adelekan *et al.,* 2013), as illustrated in (figure 2). The production of wheat bran flour followed to the technique described by (Olaoye *et al*., 2014), as depicted in figure 3. The flours were stored in high-density polyethylene containers at room temperature until further analysis.

Determination of Proximate Composition of Biscuits

The proximate composition of the biscuit made from a blend of finger millet, soybean, and wheat bran flour was determined according to the procedure outlined by AOAC (2012).

Ethical approval

The ethical committee of the Federal University of Technology Akure granted approval for this research, in accordance with the Animal Utilization Protocol Certification, under the ethical number FUTA/SAAT/2017/016.

Table 1: Formulation blends of biscuit

Samples	FSW1	FSW2	FSW3	FSW4	FS5
Finger millet flour	60	70	80	90	100
Soy beans flour	35	25	15	5	-
Wheat bran	5	5	5	5	-

FSW1: - Finger millet flour 60 %, soybean flour 35%, and wheat bran 5%; FSW2: - Finger millet flour 70%, soybeans flour 2%, and wheat bran 5%; FSW3: - Finger millet flour 80%, soybeans flour 15%, and wheat bran 5 %; FSW4: - Finger millet flour 90 %, soybean flour 5%, and wheat bran 5 %; FSW5: - 100% finger millet.

Formulation of Blends

A biscuit recipe was developed using the material balance equations (Table 1). The formulation was combined with 500 g flour, 200 g fat, 125 g sugar, 5.0 g salt, 115 ml eggs, 37.5 ml powdered milk, 1.5 g nutmeg, 12.5 ml vanilla flavoring, and 5.0 g baking powder. These ingredients are mixed to form a batter (Adeyeye & Akingbala, 2016).

Animal bioassay and feeding trial

Thirty healthy weanling albino Wistar rats (15 of each sex) were obtained from the Animal Laboratory of the Department of Biochemistry at the Federal University of Technology Akure. The rats were randomly placed in metabolic cages and fed standard rat pellets during a 7-day acclimatization period. After acclimatization, the rats were weighed and separated into six groups of five animals each. Table 2 outlines the feed composition for all diets, including nutribon, which was used as a control. The feeding experiment lasted for 28 days.

Digestion trial

A digestion trial was conducted at the end of the feeding trial. The rats' urine and faeces were collected in the last 7 days of experiments separately and preserved using 5% sulfuric acid for protein quality determination and nitrogen as outlined by Adegbanke and Jeremiah, 2024.

Histopathological analysis

Following the 28-day experimental period, the albino rats were deprived of food overnight but allowed free access to water. The rats were sacrificed under anesthesia following the guidelines of the Animal Handling Ethics Committee at the Federal University of Technology, Akure, Nigeria. Histological analyses were performed on the kidney, heart, and liver of the albino rats following the method proposed by Di-Fiore (1963). The kidneys and liver were immediately removed, samples were fixed in 10% neutral buffered formalin. Tissues were sectioned at 4-5 thickness, stained with haematoxylin and eosin (H and E), and examined microscopically at x 200 visual magnification.

Table 2: Animal Grouping Based on Diet

Rat Group	Diet Description	Water
WSF1	FSW1	Ad libitum
WSF2	FSW2	Ad libitum
WSF3	FSW3	Ad libitum
WSF4	FSW4	Ad libitum
WSF5	FSW5	Ad libitum
WSF6	FSW6	Ad libitum

WSF1: rat fed with FSW1; finger millet flour 60%, soybean 35%, and wheat bran flour 5%; WSF2: rat fed with FSW2; finger millet flour 70%, soybean 25%, and wheat bran flour 5%; WSF3: rat fed with FSW3; finger millet flour 80%, soybean 15%, and wheat bran flour 5%; WSF4: rat fed with FSW4; finger millet flour 90%, soybean 5%, and wheat bran flour 5%; WSF5: rat fed with FSW5; Negative Control: 100% finger millet; WSF6 rat fed with Nutribom: Control

Statistical Analysis

All analyses were performed in triplicate and the generated data were subjected to One-Way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 23.0. The means were separated using New Duncan Multiple Range Test (NDMRT) at 95% confidence level ($P \leq 0.05$).

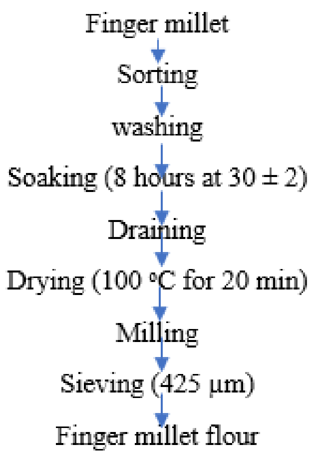


Figure 1: Production of finger millet flour (Sengev *et al.*, 2010)

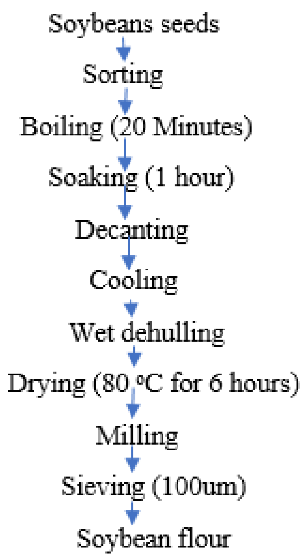


Figure 2: Production of soybean flour, (Adelekan *et al.*, 2013)

Figure 3: Flowchart for the Production of Wheat Bran Flour (Olaoye *et al.*, 2014)

3. Results and Discussion

Proximate analysis for Biscuit Samples

The proximate analysis of the biscuit samples made from finger millet, soybean, and wheat bran is shown in Table 3. Protein content ranged from 14.92% (FSW1) to 5.67% (FSW4), with the control sample FSW5 at 11.85%. All formulations showed significant differences ($P \leq 0.05$) in protein content. A decreasing protein trend was observed as the proportion of soybean flour decreased. Banureka and Mahendran (2011) found biscuits with 10% soy flour had protein levels between 5% and 9.9%, surpassing those made solely from wheat flour. The biscuit sample's fat content varied from 26.59% in the control to 19.65% in FSW4. A correlation was observed between increased soybean flour percentage and increased fat content. These findings align with Farzana and Mohajan (2015), who reported higher soy flour proportions in mushroom-fortified biscuits increased fat content from 17.36% to 20.89%. Roger *et al*., (2022) examined nutritional composition of biscuits from wheat, sweet potato, and soybean composite flour, revealing significant variations ($P < 0.05$) in fat content, ranging from 0.55 in sweet potato flour to 27.3 g/100 g in soybean flour. The fiber content ranged from 16.66% (FSW1) to 11.74% (FSW4), of the control sample was 15.28%. A decrease in the proportion of soybean reduced the fiber content. This was likely due to wheat bran, as the soybean was de-hulled. Okpalanma *et al*. (2020) found biscuits made from cassava, wheat, and soybean flours had fiber contents between 0.09% and 0.31%. The fiber content of the wheat biscuit control's sample differed significantly from that of the other samples. These fiber-rich biscuits may help address constipation and health issues related to fast food consumption (Khalid *et al*., 2022). The Moisture content ranged from 2.28% in FSW1 to 6.32% in the control sample. The control sample had the highest moisture content, whereas FSW1 had the lowest moisture content. A significant difference was observed between the control and other samples, but no substantial variation was found between FSW2 and FSW3. As the proportion of finger millet flour increased, the moisture content also increased. Finger millet flour absorbs and retains more moisture than soybean and wheat bran flours. High moisture content in biscuits can reduce shelf life and increase vulnerability to microbial growth (Ajala, *et al*., 2024). Conversely, the lower moisture content of FSW1 suggested better shelf stability. Low moisture content is crucial for food preservation, extending shelf life, and inhibiting microbial spoilage. According to (Ajala, *et al.*, 2024), products with high moisture content (below 12%) have shorter shelf stability than those with lower moisture (below 12%). Current moisture levels are suitable for biscuit production, ash content varied from 1.79% (control) to 2.96% (FSW3), with FSW3 and FSW4 showing significantly higher levels, indicative of their mineral-rich composition. The ash content exceeded the range reported by Jayaweera *et al.*, (2018) for biscuits made from sprouted sorghum-soybean-finger millet (1.41% to 2.16%). Jayaweera *et al.*, (2018) noted that increasing soybean flour content increased ash levels, while this study found a decrease with reduced soybean content and an increase with higher finger millet content. A higher ash content in supplemented biscuits is significant for bowel movement and constipation reduction. Carbohydrate content ranged from 38.20% (control) to 55.64% (FSW4). FSW4 with 90% Finger Millet Flour had the highest carbohydrate content, making it suitable for high-calorie diets, whereas formulations with higher soybean flour (FSW1, FSW2) had significantly lower values (40.29 and 45.70%).

Table 3: Proximate composition of biscuit

Sample	Protein (%)	Fat (%)	Fibre (%)	Moisture (%)	Ash (%)	Carbohydrate (%)
FSW1	14.92 ± 0.16a	25.63 ± 1.36a	16.66 ± 0.10a	2.28 ± 0.08d	1.81 ± 0.01d	40.29 ± 0.81d
FSW2	12.36 ± 0.11b	23.99 ±0.79ab	11.76 ± 0.07d	2.72 ± 0.06c	1.88 ± 0.01c	45.70 ± 1.27c
FSW3	7.96 ± 0.11d	22.03 ±1.10bc	12.11 ± 0.02c	2.76 ± 0.28c	2.63 ± 0.03b	52.20 ± 0.66b
FSW4	5.67 ± 0.11e	19.65 ± 1.03c	11.74 ± 0.16d	4.67 ± 0.06b	2.96 ± 0.03a	55.64 ± 1.02a
FSW5	11. 85 ± 0.16c	26.59 ± 0.97a	15.28 ± 0.09b	6.32 ± 0.04a	1.79 ± 0.04d	38.20 ± 1.29d

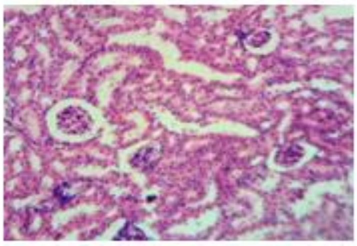
Mean ± standard deviation (SD). Values with different superscripts in the same row are significantly different (p<0.05.
FSW1: finger millet flour 60%, soybean 35% and wheat bran flour 5%; FSW2: finger millet flour 70%, soybean 25% and wheat bran flour 5%; FSW3: finger millet flour 80%, soybean 15% and wheat bran flour 5%; FSW4: finger millet flour 90%, soybean 5% and wheat bran flour 5%; FSW5: Negative Control: 100% finger millet(control); FSW6: Positive Control: Nutribom.

Histological examination of the of Kidneys

Histological evaluation of the kidneys from Albino Wistar rats fed composite biscuits and basal diets is shown in Figure 4. WFS5 exhibited acute tubular necrosis (TN) in the proximal convoluted tubule, whereas the glomeruli (GR) remained intact. WSF1 displayed nearly normal kidney histology. WSF6 shows intact glomeruli (GR) and potential immunological material (IM) deposition in the glomerular basement membrane. WSF2 demonstrates acute tubular necrosis (TN) and glomeruli room destruction (DGR). WSF3 indicates an intact glomeruli room with possible renal tubule destruction (DRT). WFS4 revealed an intact glomerular room (GR), potential immunological material (IM) deposition, and widespread acute tubular necrosis (TN) in the proximal convoluted tubule. No histopathological or gross changes were linked to feed composition. A study by (Grunz-Borgmann *et al.*, 2020) found, through blinded histological assessment, that glomerulosclerosis and tubulointerstitial fibrosis were reduced in rats on a soy diet. The kidney shape and colour observed in this study are consistent with those reported by (Al-Samawy, 2012).

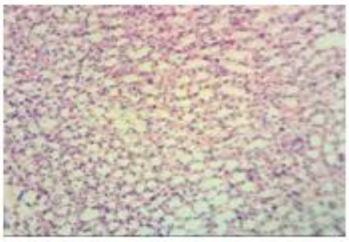
Histology of Liver of Albino Wistar Rats Fed with Composite Biscuit

As shown in figure 5, WSF5 shows sinusoid dilation, WSF1, WSF6, and WSF3 have normal hepatic architecture with organized cells and sinusoids lined by Kupffer cells, WSF3 displays extensively diffused sinusoids, and WSF4 has a nearly normal structure. Biscuit-induced liver toxicity affects transaminases, AST and ALT, which indicate liver function (Hilaly *et al.*, 2004) and serve as potential toxicity biomarkers (Rahman *et al.*, 2001). Damage to liver parenchymal cells typically elevates blood levels of both transaminases (Anderson and Borlak, 2008). Rat liver is a dark brown organ beneath the diaphragm, primarily on the right side. It has four lobes: the largest median, right lateral, left lateral, and small caudal lobes. The liver is crucial for cholesterol synthesis and breakdown and is the main site for metabolism, including drug processing. It also regulates glucose production and releases free glucose from stored hepatic glycogen (Anderson and Borlak, 2008).



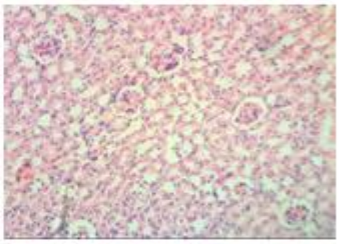
Photomicrograph (Mag x400) of kidney of WSF1 fed with FSW1.

FSW1: Finger millet 60 %, soybeans 35 % and wheat bran flour 5 %



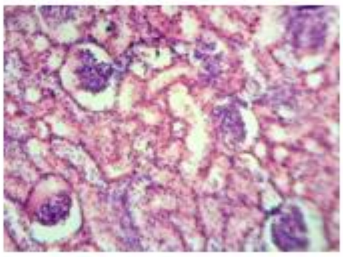
Photomicrograph (Mag x400) of kidney of WSF2 fed with FSW2

FSW2: Finger millet 70 %, soybeans 25 % and wheat bran flour 5 %.



Photomicrograph (Mag x400) of kidney of WSF3 fed with FSW3

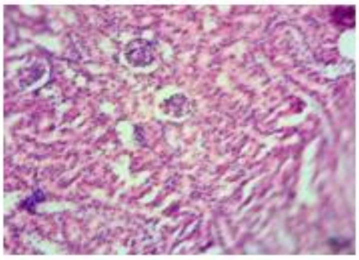
FSW3: Finger millet 80 %, soybeans 15 % and wheat bran flour 5 %.



Photomicrograph (Mag x400)

of kidney of WSF4 fed with FSW4

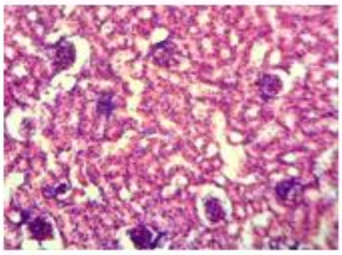
FSW4: Finger millet 90 %, soybeans 5 % and wheat bran flour 5 %.



Photomicrograph (Mag x400)

of kidney of WSF5 fed with FSW5 (Mag x400)

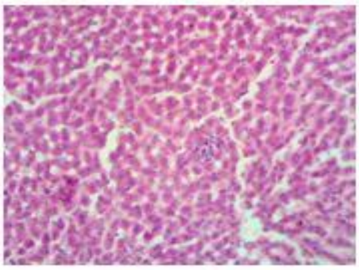
FSW5: Finger millet 100 %



Photomicrograph (Mag x400)

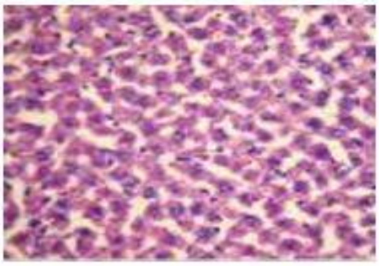
of kidney of WSF6 fed with FSW6 (Mag x400)

Figure 4: Histology of Kidneys of Albino Wistar Rats Fed with Composite Biscuit



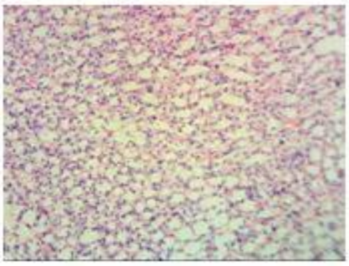
Photomicrograph of liver fed with FSW1 (Mag x400)

FSW1: Finger millet 60 %, soybeans 35 % and wheat bran flour 5 %.



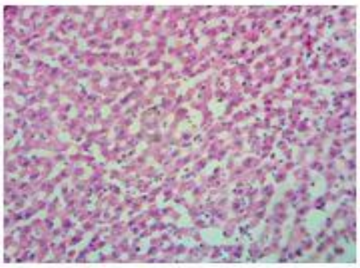
Photomicrograph of liver fed with FSW2 (Mag x400)

FSW2: Finger millet 70 %, soybeans 25 % and wheat bran flour 5 %.



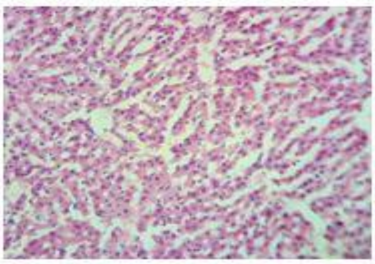
Photomicrograph of liver fed with FSW3 (Mag x400)

FSW3: Finger millet 80 %, soybeans 15 % and wheat bran flour 5 %.



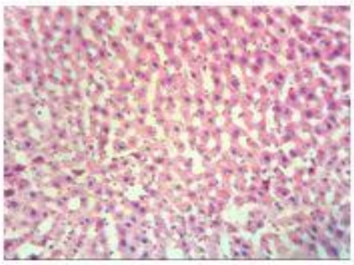
Photomicrograph of liver fed with FSW4 (Mag x400)

FSW4: Finger millet 90 %, soybeans 5 % and wheat bran flour 5 %.



Photomicrograph of liver fed with FSW5 (Mag x400)

FSW5: Finger millet 100 %



Photomicrograph of liver fed with FSW6 (Mag x400)

Figure 5: Histology of Liver of Albino Wistar Rats Fed with Composite Biscuit.

Histology (heart) of Albino Wistar Rats Fed with Composite Biscuit

The evaluation of the effects of Epinephrine on the left ventricular muscle kidney focused on myocyte morphology and physiology. As shown in figure 6, histopathological findings of Epinephrine after 28 days included inflammatory cell infiltration, myocyte degeneration, collagen accumulation, capillary endothelial changes, and perivascular fibrosis. Similar studies have reported significant inflammatory cell infiltration in the aortic wall (Miyajima *et al*., 2014) and atrium (Yu *et al*., 2010). Miyajima *et al*. (2014) showed that periodontitis-activated monocytes and macrophages adhere to aortic endothelial cells. WSF5 indicates normal a heart architecture with a regular cardiomycete arrangement, normal connective tissue (NCT), and muscle fiber (MF). WSF1 shows severe connective tissue enlargement (SEC), vacuolation, and depletion of cardiomycetes. WSF6 signifies irregular vacuolation and muscle fiber enlargement. WSF2 shows depleted connective tissue (DCT) and diffuse muscle fibers (DMF). WSF3 shows severe irregular connective tissue drainage (SIC) or vacuolation. WSF4 signifies an enlarged muscle fiber (EMF), depleted connective tissue (DCT), and lipid deposits (LD).

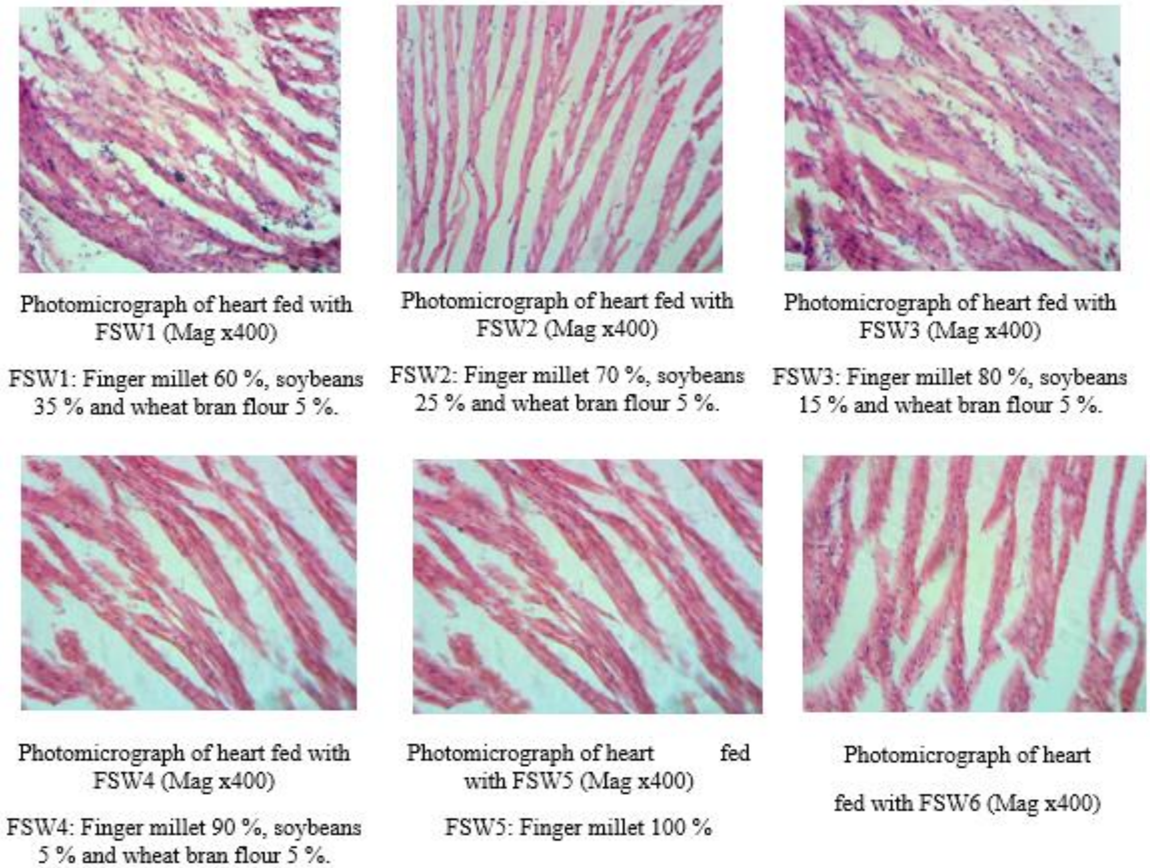


Figure 6: Histology of hearts of Albino Wistar Rat Fed with Composite Biscuit

4. Conclusion

The nutritional composition and potential health effects of composite biscuits made from finger millet, soybean, and wheat bran can be understood through proximate analysis and histological evaluations. Substantial variations in protein, fat, fiber, moisture, ash, and carbohydrate levels were found among the biscuit samples. Although the composite biscuits did not cause major pathological changes in the kidneys and liver, some differences in the tissue structure were noted during histological examination. These biscuits, fortified with finger millet, soybean, and wheat bran, are viable options for improving dietary intake, particularly for combating nutritional deficiencies. Nevertheless, it is crucial to strike a balance between nutritional advantages and possible health risks when developing optimal formulations to ensure consumer well-being.

Conflicts of interest

The authors declare no competing interest

Author contributions

The authors contributed equal to the research process, Literature writing, review and editing of the article.

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