

RESEARCH ARTICLE

POTENTIAL APPLICATION OF DIFFERENTLY PROCESSED EDIBLE AFRICAN PALM WEEVIL LARVAE (*Rhynchophorus phoenicis*) AS A FAT REPLACER IN SAUSAGE PRODUCTION

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ABSTRACT

Advocates for low-fat consumption have shifted focus to developing nutritious low-fat meat products from local, readily available raw materials. Edible African Palm Weevil Larvae (AFPWL) are a widely relished indigenous insect larvae whose properties are affected by processing methods. This study assessed the qualities of sausages produced with differently processed AFPWL. AFPWL, 65-80 days old (n=400), were asphyxiated at 4°C and processed in three forms: Raw (AFPWL-R), Moist Cooked (AFPWL-MC) and Smoked (AFPWL-S) before use in sausage production. Four types of Sausages (S): AFPWL-RS, AFPWL-MCS, AFPWL-SS and Lard Sausage (LS) were produced. Swelling (%) and Water Absorption Capacities (WAC) (%) were determined on the emulsion. Product yield (PY%), moisture (%), crude protein (%), fat (%), and organoleptic characteristics (9-point hedonic scale) were determined on freshly cooked sausage. Thiobarbituric Acid Reactive Substances (TBARS) (MDAmg/100 g) were assessed over a pooled storage of 21 days. Data were subjected to ANOVA and a significant test using DMRT at $P \leq 0.05$. Swelling capacity 9.64 (AFPWL-MCS) and 9.33 (AFPWL-SS) are similar ($P > 0.05$) but significantly higher ($P < 0.05$) than 7.77 (LS) and 6.93 (AFPWL-RS), while WAC 32.00 (AFPWL-SS) is similar to 37.00 (AFPWL-MCS) and 26.00 (AFPWL-RS) but higher ($P < 0.05$) than 23.00 (LS). All AFPWL sausages had higher PY (90.51-96.15) and Moisture contents (61.40-66.59). Crude protein (28.07) AFPWL-MCS and AFPWL-SS (27.91) were higher ($P < 0.05$) than LS (26.53) and AFPWL-RS (24.60). Fat (4.06) in AFPWL-RS was lower ($P < 0.05$) than 4.24 (LS), 4.35 (AFPWL-SS) and 4.46 (AFPWL-MCS). All AFPWL sausages were significantly ($P < 0.05$) tender, but AFPWL-SS sausage was the most acceptable. All AFPWL sausages had significantly higher TBAR values (0.53-0.58) when compared to 0.50 (LS). Processing of AFPWL before use in sausage production could lead to a novel insect-based meat product with unique, high nutritional and sensory properties. .

Keywords: Edible insect, functional properties, lard sausage, organoleptic properties, processing methods

INTRODUCTION

The high nutrient profile of meat and meat products makes them an important component in human diets (Brewer, 2012). They are rich in fat, mainly comprising saturated fatty acids (Desmond, 2006; Zhang *et al.*, 2010). However, there has been tremendous concern over the consumption of excess fat because of its significant contribution to various diseases such as high blood cholesterol, cardiovascular and cancerous diseases (Aggett *et al.*, 2005; Alexander *et al.*, 2010) that are life-threatening.

This growing concern over healthy food consumption has led to intense research into producing meat products with reduced fat content. However, this poses a significant challenge to meat product industry because of the technological importance of fat in meat products (Ansorena and Astiasaran, 2004; Jimenez-Colmenero, *et al.*, 2006; Carrapiso, 2007). Thus, the focused attention is on formulating and developing nutritious low-fat meat products from readily available local, low-fat raw materials such as edible insects.

Edible insects have been reported to play

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many important roles in different food systems (Tang *et al.*, 2019), such as nutritional (Zielińska *et al.*, 2015), accounting for about 5-10% of the total protein in the diet of the people who consume them (Ayieko and Oriaro, 2008). Their high protein, coupled with mono and polyunsaturated fats, accounts for their wide nutritional value (Gnana Moorthy Eswaran *et al.*, 2023), making them a potential food for human consumption. They are not only consumed as a snack but also as a side dish or in combination with other ingredients to produce different food products (Acosta-Estrada *et al.*, 2021).

African palm weevil (*Rhynchophorus phoenicis*) larva (AFPWL) is a popular edible insect larva globally consumed as food (Cheung, 2010). The larva is a highly nutritious mini livestock that is rich in protein, iron and other micro nutrients (Debrah *et al.*, 2019). It is highly relished due to its taste, nutritive value, texture, flavour and ease of preparation (Ijeomah and Alagoa, 2012) and can be consumed as part of a meal or as a complete food (Van Huis *et al.*, 2013). Furthermore, its mode of consumption, which can be in moist, cooked, dried, fried, or roasted form, differs among different geographical locations (Van Huis *et al.*, 2013; Ingweye *et al.*, 2020). Research has shown that application of these different processing methods/heat treatments to edible insects usually has an impact and influence on the end-products (Mishyna *et al.*, 2020).

Sausage is a popular, finely seasoned minced meat product made from either beef, pork, chicken or lamb. It is usually seasoned with spices, herbs, salt, preservatives, fillers, and other ingredients that contribute to its taste, flavour, and appealing appearance. The inclusion of AFPWL in sausage production has been reported to increase the nutritional qualities of sausage (Abo *et al.*, 2022). However, information on its inclusion in sausage when processed differently before subsequent use as a fat replacer is scanty. Therefore, evaluating the effect of differently processed AFPWL inclusion in sausage formulation on nutritional, functional and sensorial qualities as well as shelf stability is

imperative to promote its use in food formulations and food product development, especially in processed meat products such as sausage.

MATERIALS AND METHODS

Procurement of AFPWL

Edible AFPWL between 65-80 days of age were harvested from the mini livestock farm located at the Department of Crop Protection and Environmental Biology, University of Ibadan.

AFPWL preparation

Whole larvae were asphyxiated in the refrigerator at 4°C, allowed to thaw and then ground before use as raw. For the moist cooked processing method, the thawed larvae were placed in clean cellophane bags and boiled in water at 100°C for 20 minutes. After this, they were removed from the cellophane bags, cooled down to room temperature and then ground. The smoke processing method was achieved by smoking the thawed larvae in a smoke chamber at 50 °C for 20 minutes. After doneness, it was allowed to cool down to room temperature and then ground. Each set of grounded processed larvae was packed in an airtight container and used for further analysis and in sausage production.

Lard preparation

The lard (fat) was obtained from pork, washed thoroughly with clean water, and then heat-rendered at 100°C for approximately 40 minutes until it melted into a liquid state. The rendered fat was cooled to room temperature, sieved (using a muslin bag) to remove all dirt, tissues, and sediments, ensuring a clean lard, and then refrigerated until use.

Casing preparation

Natural casing was used for the sausage production in this study. The small intestine of a ram (14-18 mm diameter), which was purchased from a reputable abattoir, was used as the casing. This was washed thoroughly, turned inside out and washed again. The intestine was placed in saline water for 24 hours as a means of storage and also to prevent contamination.

Smoke chamber

A smoke chamber was manually constructed at the Department of Animal Science, University of Ibadan, Nigeria and had layers made of iron rods where sausages were hung. First, it was heated up to a temperature of 180 °C before hanging the sausages for smoking (Figure 1).



Figure 1: Smoked sausage in the smoke chamber

Sausage production

The schematic representation of the production of sausages is presented in *Supplementary figure 2*. Sausage was produced according to the method of Wan Rosli *et al.* (2015). AFPWL and lard emulsions (Table 1) were formulated following the procedure of Jin *et al.* (2007) with slight modification, where AFPWL was replaced with lard.

Each emulsion formulation was stuffed in the casing with a sausage stuffer, and the lengths were linked together at approximately 2 inches continuously. The sausages were cooked for approximately 15 minutes to an internal temperature of 72 ± 1 °C. Sausages produced were further subjected to sensory panel assessment and laboratory analysis.

Parameters measured

Proximate composition analysis was carried out on the processed Larvae (L) (Raw (RL), Moist cooked (MCL), and Smoke (SL) and on the different types of sausage (AOAC, 2020).

Water holding capacity (WHC)

This was determined according to Suzuki *et al.* (1991) with slight modification.

$$WHC = \frac{100 - (A_r - A_m \times 9.47)}{(W_m - M_o)}$$

Where; A_m : Area of sausage samples (cm^2), A_r : Area of water released from the sausage (cm^2), W_m : Weight of sausage samples (g), M_o : Moisture content of sausage samples (%), and 9.47: a constant factor

Functional properties

Swelling capacity

This was determined following the procedures described by Okaka and Potter (1977) with slight modification. The emulsion was filled to the 10 ml mark of a graduated 100 ml flask, and then distilled water was added to reach a 50 ml volume mark. The flask was stoppered

Table 1: Sausage recipe with differently processed AFPWL

Ingredients (%)	Lard Sausage (LS)	AFPWL-RS	AFPWL-MCS	AFPWL-SS
Beef	70.00	70.00	70.00	70.00
Lard	12.50	-	-	-
Raw Larvae	-	12.50	-	-
Cooked Larvae	-	-	12.50	-
Smoked Larvae	-	-	-	12.50
Phosphate	0.50	0.50	0.50	0.50
Curing salt	1.30	1.30	1.30	1.30
Corn starch	2.00	2.00	2.00	2.00
Sausage seasoning	1.20	1.20	1.20	1.20
Ice flakes	12.50	12.50	12.50	12.50
Total	100.00	100.00	100.00	100.00

AFPWL-RS: African Palm Weevil Larvae Sausage Raw, AFPWL-MCS: African Palm Weevil Larvae Sausage, Moist Cooked, AFPWL-SS: African Palm Weevil Larvae Sausage smoked

tightly and the mixture was mixed thoroughly by inverting the graduated flask every 2 minutes for several times (approximately five times) to allow a homogenous mixture of the sample. After the desired mixture is achieved, the sample is allowed to stand still again for 8 minutes before reading the final volume occupied by the sample. This was carried out on all the emulsions.

$$\text{Swelling capacity (\%)} = \left(\frac{W_{e_{bm}}}{W_{e_{am}}} \right) \times 100$$

Where; $W_{e_{bm}}$: Weight of emulsion paste before mixing, and $W_{e_{am}}$: Weight of emulsion paste after mixing

Solubility

The procedure to determine the solubility of the emulsion involved weighing 1 g of the emulsion sample into a test tube containing 20 ml of distilled water. The mixture was heated in a water bath at a temperature of 60 °C for 30 minutes. At the end of heating, the sample was subjected to decanting and dried to a constant weight, and the solubility was expressed as,

$$\text{Solubility (\%)} = \left[\frac{\text{Initial}_{sw}}{\text{Dry}_{sw}} \right] \times 100$$

Where; Initial_{sw} : Initial sample weight, and Dry_{sw} : Dry weight of sample

Water absorption capacity (WAC)

An emulsion sample (2 g) was weighed and poured into a 200 ml beaker, to which 25 ml of distilled water was added. This was stirred with a glass rod continuously for 30 minutes to achieve uniformity. Thereafter, the dispersions were transferred into centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted, and the residue was used to determine the solids content, and water absorption capacity was expressed as a percentage.

$$\text{WAC(\%)} = \frac{(\text{Weight}_{es} - \text{Weight}_{se})}{\text{Weight}_{\text{sample}}} \times 100$$

Where; Weight_{es} : Weight of emulsion sample, Weight_{se} : Weight of sediment, and

$\text{Weight}_{\text{sample}}$: Weight of sample

Foaming capacity (FC)

One gram (1 g) of sample was weighed into a blender, and 50 ml of distilled water was added. The mixture was then whipped for 3 minutes at a medium speed. After this, the mixture was poured into a graduated measuring cylinder (100 ml) to measure the volume. Forming capacity (%) was expressed as follows:

$$\text{FC (\%)} = \left[\frac{\text{Volume}_{aw} - \text{Volume}_{bw}}{\text{Volume}_{bw}} \right] \times 100$$

Where; Volume_{aw} : Volume after whipping, and Volume_{bw} : Volume before whipping

Foaming/ Emulsion stability

This was determined by measuring the foam volume after standing and checking at intervals of 5 minutes until a stable volume was attained. Emulsion stability was then expressed as a percentage of the initial foam volume.

$$\text{Foaming stability (\%)} = \frac{\text{Initial}_{fv} \times 100}{\text{Final}_{fv}}$$

Where; Initial_{fv} : Initial foam volume, and Final_{fv} : Final foam volume

Cost of production

This is calculated by calculating the cost of producing 1kg of sausage. This cost includes the cost of procuring 1kg of meat, AFPWL, spices, energy used in the production and the human effort.

Product yield (PY)

Product yield of each sausage was determined following the procedure of Yang *et al.* (2007) using the following equation. This is expressed for the smoked sausage as a percentage as follows.

$$\text{PY (\%)} = \left[\frac{\text{Weight}_{ss}}{\text{Weight}_{rs}} \right] \times 100$$

Where; Weight_{ss} : Weight of smoked sausage, and Weight_{rs} : Weight of raw sausage

Colour

A colourimeter (Chroma Meter (Minolta Camera Co. LTD, CR-300, Osaka, Japan), which uses the CIE L * a * b * colour system, was used in determining the colour parameters such as lightness (L *), redness (a *), and yellowness intensities (b *). All measurements were carried out following the methodologies and procedures of Ansorena *et al.* (1997), Wroslstad *et al.* (2005) and Goncalves *et al.* (2007).

Sensory assessment of sausage

Sensory attributes such as aroma, flavour, tenderness and overall acceptability of freshly prepared sausage were evaluated using thirty (30) semi-trained panelists made up of students and staff of the Department of Animal Science, University of Ibadan. The panelists were briefed before the commencement of the evaluation process, and sausage samples were rated on a nine-point hedonic scale ranging from 9 (like extremely) to 1 (dislike immensely) (Chowdhury *et al.*, 2011). The carry-over effect of one sample flavour over the other was taken into consideration during the assessment. This was achieved by imploring the panelists first to chew unsalted crackers and then drink water at room temperature to rinse their mouths in between samples (Harry and Hildegard, 2010).

Microbiological analysis

Total Viable Count (TVC), Total Coliform Count (TCC) and Total Fungal Count (TFC) of the sausages were determined using the spread plate method. Tenfold of the homogenate was serially diluted and used for microorganism enumeration. One millilitre aliquot of each of the diluted samples was plated out on sterile MacConkey agar for the determination of TVC. In contrast, nutrient agar was used in determining the TCC and TFC. A colony counter was used for counting the colonies of microorganisms after incubation. The microbial data were transformed to log₁₀ as described by Kang *et al.* (2017) to meet the requirements of equal variance as well as standard distribution.

Lipid oxidation

The extent of oxidation in the sausages was assessed by measuring the levels of Thiobarbituric Acid Reactive Substance (TBARS) accumulated during storage under refrigeration conditions at 4 °C at intervals of 0, 7, 14, and 21 days. The procedure of Rosmini *et al.* (1996) was adopted with slight modifications. The amount of TBARS accumulated in each sample was reported as a pooled data over the storage period of 21 days and expressed as MDA mg/100 g.

Amino acid (AA) determination

This was determined using the modified method of Peace and Gilani (2005). The procedure consisted of two phases.

The hydrolysis phase (phase one): Sausage samples were prepared for hydrolysis by weighing 1 g of thoroughly ground sample into a 250 ml conical flask, 100 ml of 6M hydrochloric acid was then added, and the flask was covered with a stopper. The mixture was then heated in an oven and incubated for 16 hrs to hydrolyse the sample. After this, the mixture was filtered into another 250 ml conical flask using a double-layered Whatman No. 1 filter paper, and the conical flask was covered with a stopper.

Amino acid determination phase (phase two): The hydrolysate (2 ml) was pipetted into a 30 ml capacity test tube, 10 ml of buffered ninhydrin reagent was added, and the mixture was placed in a water bath containing boiling water and then heated for 15 minutes. After this process, the mixture was allowed to cool down to room temperature, and 3 ml of 50% ethanol was immediately added. A working standard amino acids total of 0-5 µg/ml was prepared from each of the standard solutions of amino acids to obtain the gradient factor from which the curve was calibrated for each of the amino acids. The buffered ninhydrin reagent was used to heat the working standards as done with the sample hydrolysate. The wavelength of colour developed by each of the amino acids was used to determine the specific amino acid.

$$AA \% = \frac{\text{Absorbance}_s \times \text{Gradient}_f \times \text{Dilution}_f}{10,000}$$

Where; Absorbance_s: Absorbance of sample, Gradient_f: Gradient factor, and Dilution_f: Dilution Factor

Fatty acid profiles

The HPLC method was used to determine the triglyceride profile of each cooked sausage. This was carried out purposely to characterise the fatty acid compositions of the sausages. The mole ratio was used in calculating the concentration of each fatty acid component of each triglyceride (Ortiz *et al.*, 2016).

Experimental design and statistical analysis

Four sausage emulsions were produced in a completely randomized design where the sausage with lard was used as the control and three other sausage types, namely AFPWL-R, AFPWL-MCS and AFPWL-S as the treatments. Each of the analysis carried out on the sausages were replicated three times. The differences between sample means were determined through one-way Analysis of

Variance (ANOVA) using the SAS 1999 package version 15.0. Where the analysis of variance indicates a significant difference in their means, Duncan Multiple Range Test (DMRT) was used to separate the means at $P \leq 0.05$.

RESULTS

Proximate composition of differently processed AFPWL

Crude protein content of MCL (26.80%) and SL (27.68%) was significantly higher ($P < 0.05$) compared to RL (19.63%) (Table 2). Ash content of MCL (4.45%) and SL (4.75%) is not different from each other ($P > 0.05$) but was significantly higher ($P < 0.05$) than RL (5.05%). Statistical variation exists ($P < 0.05$) in all the crude fat contents of the sausages, and they were 22.05% (RL), 24.30% (SL) and 25.15% (MCL). No statistical variation ($P > 0.05$) was observed between RL (61.69%) and MCL (62.1%) for moisture content, but these are significantly higher ($P < 0.05$) compared to 59.60% in SL.

Table 2: Proximate composition of differently processed AFPWL

Parameters (%)	Raw Larvae	Moist Cooked Larvae	Smoked Larvae	P-value
Crude protein	19.63 ^b ±0.95	26.80 ^a ±0.42	27.68 ^a ± 2.31	0.002
Ash	5.05 ^a ±0.21	4.45 ^b ±0.07	4.75 ^{ab} ±0.07	0.005
Ether extract	22.05 ^c ±0.21	25.15 ^a ±0.04	24.30 ^b ±0.04	0.000
Moisture	61.69 ^a ±0.06	62.15 ^a ±0.23	59.60 ^b ±0.58	0.013

^{abc} Means that on the same row with similar superscripts are not significantly different ($P > 0.05$)

Functional properties of sausage emulsion incorporated with differently processed AFPWL

The functional properties, as displayed in

Table 3, showed that no statistical variation ($P > 0.05$) exists in pH (5.82-5.87) of all the raw sausage emulsions.

Table 3: pH and functional properties of sausage emulsion prepared with differently processed AFPWL

Parameters	LS	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
pH (raw emulsion)	5.86	5.87	5.85	5.82	0.02
Swelling capacity (%)	7.73 ^b	6.93 ^c	9.64 ^a	9.33 ^a	0.04
Solubility (%)	87.50 ^a	87.00 ^a	77.50 ^b	81.00 ^b	0.001
WAC (%)	23.00 ^b	26.00 ^b	37.00 ^a	32.00 ^{ab}	0.02
Foaming capacity%	0.25 ^a	0.10 ^b	0.05 ^b	0.10 ^b	0.03
Emulsion stability % @ 15 mins	0.15 ^a	0.05 ^b	0.03 ^b	0.05 ^b	0.03

^{abc} Means with the same superscripts along each row are not significantly different ($P > 0.05$), LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Sausage Raw, AFPWL-MCS: African Palm Weevil Larvae Sausage, Moist Cooked, AFPWL-SS: African Palm Weevil Larvae Sausage smoked, WAC: Water Absorption Capacity

Swelling capacities of AFPWL-MCS (9.645%) and AFPWL-SS (9.33%) emulsions were not statistically different ($P > 0.05$) but higher ($P < 0.05$) than lard (7.73%) and AFPWL-RS (6.93%) emulsions. No variation exists ($P > 0.05$) in the solubility of lard (87.59%) and AFPWL-RS (87.00%) emulsions but these were higher ($P < 0.05$) than 77.50% and 81.00% recorded in AFPWL-MCS and AFPWL-SS emulsions, respectively. Water absorption capacity of lard emulsion (23.00%) was lower ($P < 0.05$) than AFPWL-RS (26.00%), AFPWL-S (32.00%) and AFPWL-MCS (37.00%). The forming capacity of lard emulsion was higher ($P < 0.05$) compared to the forming capacities recorded in AFPWL-RS, AFPWL-MCS and AFPWL-SS emulsions. Lard emulsion had higher ($P < 0.05$) form stability (0.15%) at 15 minutes

than AFPWL-RS (0.05%), AFPWL-MCS (0.03%) and AFPWL-SS (0.05%) emulsions.

Some physico-chemical characteristics and cost implications of sausages produced with differently processed AFPWL

As displayed in Table 4, there is no statistical difference in pH (5.91-5.96) of all sausages. WHC of AFPWL-SS (60.41%) was higher ($P < 0.05$) than LS (43.04%), AFPWL-R (43.13%) and AFPWL-MCS (44.22%). Product yield of AFPWL-SS (96.15%) was significantly higher ($P < 0.05$) than 88.71%, 90.51% and 91.62% obtained in LS, AFPWL-RS and AFPWL-MCS sausages, respectively. The cost of producing 1kg of sausages is ₦7,326.46, ₦7,125.51, ₦7,093.76 and ₦6,790.74 for LS, AFPWL-RS, AFPWL-MCS and AFPWL-SS, respectively.

Table 4: Physico-chemical characteristics, yields and cost of cooked sausage prepared with differently processed AFPWL

Parameters	LS	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
pH	5.96	5.93	5.96	5.91	0.020
WHC	43.04 ^b	43.13 ^b	44.22 ^b	60.41 ^a	0.026
Product Yield (%)	88.71 ^c	90.51 ^b	91.62 ^b	96.15 ^a	0.009
Cost/kg (₦)	7,326.46	7,125.51	7,093.76	6,790.74	
Cost/100g (₦)	732.65	712.55	709.38	679.07	

abcd Means with the same superscripts along each row are not significantly different ($P > 0.05$), L: Brightness; a*: redness; b*:-: yellowness, LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

Colour assessment of sausage produced with differently processed AFPWL

The colour parameters displayed in Table 5 revealed that lard and AFPWL-MCS sausages' brightness (L) (8.90 vs 7.73, respectively) were not different ($P > 0.05$). Still, these are higher ($P < 0.05$) than AFPWL-

RS (5.09) and AFPWL-SS (6.05) sausages. The redness and yellowness of lard sausage (54.56 and 63.38, respectively) were higher ($P < 0.05$) compared to AFPWL-RS (35.87 and 24.96), AFPWL-MCS (40.50 and 52.24) and AFPWL-SS (50.39 and 43.57).

Table 5: Colour differences of smoked sausage containing differently processed AFPWL

Colour	LS	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
L	8.90 ^a	5.09 ^b	7.73 ^a	6.05 ^b	0.006
a*	54.56 ^a	35.87 ^d	40.50 ^c	50.39 ^b	0.008
b*	63.38 ^a	24.96 ^d	52.24 ^b	43.57 ^c	0.033

abcd Means with the same superscripts along each row are not significantly different ($P > 0.05$), L: Brightness; a*: redness; b*:-: yellowness, LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

Proximate composition of sausage prepared with differently processed AFPWL

The proximate composition of the sausages as displayed in Table 6 revealed that significant variations exist ($P < 0.05$) among the moisture

(59.35- 66.59%), crude protein (24.60- 28.07 %) and ash (0.72- 0.97%) contents. The ether extracts contained in the AFPWL-RS sausage were lower ($P < 0.05$) than those in LS, AFPWL-SS and AFPWL-MCS.

Table 6: Proximate composition of sausages prepared with differently processed AFPWL

Parameters (%)	LS	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
Moisture	59.35 ^d	66.59 ^a	61.40 ^c	63.59 ^b	0.03
Crude protein	24.60 ^c	26.53 ^b	28.07 ^a	27.91 ^a	0.04
Ash	0.97 ^a	0.72 ^d	0.87 ^b	0.79 ^c	0.01
Ether extract	4.24 ^b	4.06 ^c	4.46 ^a	4.35 ^{ab}	0.03

^{abcd} Means that in the same row with similar superscripts are not significantly different ($P > 0.05$), LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

Sensory characteristics of sausage prepared with differently processed AFPWL

The sensorial attributes (Table 7) revealed no significant differences ($P > 0.05$) in aroma

(4.25- 5.21), tenderness (5.75- 6.07), juiciness (5.19- 5.38) and flavour (4.19- 5.25) of the sausages. The overall acceptability score of AFPWL-SS (6.65) was higher ($P < 0.05$) than LS (5.86), AFPWL-RS (5.79) and AFPWL-MCS (6.22) sausages.

Table 7: Sensory characteristics of smoked sausage with differently processed AFPWL

Sensory properties	Lard sausage	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
Aroma	5.21±0.58	4.63±0.13	4.38±0.75	4.25±0.25	0.49
Flavour	5.00±0.25	4.19±0.44	5.13±0.88	5.25±0.00	0.51
Tenderness	5.75±0.13	6.07±0.57	6.07±0.30	6.13±0.88	0.55
Juiciness	5.32±0.69	5.38±0.25	5.19±0.44	5.32±0.32	0.45
Overall acceptability	5.86±0.72 ^c	5.79±0.32 ^c	6.22±0.65 ^b	6.65±0.65 ^a	0.02

^{abc} Means that on the same row with similar superscripts are not significantly different ($P > 0.05$), LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

Selected fatty acid profile of sausage incorporated with differently processed AFPWL

The selected fatty acid amounts as displayed in Table 8 showed that AFPWL-MC sausage had higher ($P < 0.05$) amounts of each of the selected fatty acids, followed by AFPWL-SS,

LS and AFPWL-RS. The ranges of the fats were 4.01- 5.00 (lauric), 5.67- 7.12 (stearic), 5.12- 6.41 (palmitic), 6.08 - 7.60 (arachidonic), 5.63- 6.49 (oleic), 5.59- 7.01 (linoleic), 5.06- 6.37 (palmitoleic), 2.33- 2.89 (propionic), and 4.55- 5.69 (myristic), respectively.

Table 8: Composition of fatty acids of sausage with differently processed AFPWL

Fatty acid (%)	Lard sausage	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
Lauric	4.39 ^c	4.01 ^d	5.00 ^a	4.59 ^b	0.07
Stearic	6.24 ^c	5.67 ^d	7.12 ^a	6.53 ^b	0.03
Palmitic	5.65 ^c	5.12 ^d	6.41 ^a	6.14 ^b	0.02
Arachidonic	6.69 ^c	6.08 ^d	7.60 ^a	6.96 ^b	0.02
Oleic	6.19 ^c	5.63 ^d	7.04 ^a	6.49 ^b	0.02
Linoleic	6.15 ^c	5.59 ^d	7.01 ^a	6.46 ^b	0.01
Palmitoleic	5.58 ^c	5.06 ^d	6.37 ^a	5.83 ^b	0.06
Propionic	2.55 ^c	2.33 ^d	2.89 ^a	2.68 ^b	0.08
Myristic	5.05 ^c	4.55 ^d	5.69 ^a	5.23 ^b	0.01

^{abcd} Means that in the same row with different superscripts are significantly different ($P < 0.05$), AFPWL-RS= African Palm Weevil Larvae Raw Sausage, AFPWL-MCS African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS African Palm Weevil Larvae Smoked Sausage

Amino acid composition of sausage incorporated with differently processed AFPWL

The selected amino acids depicted that isoleucine (9.46), leucine (14.14), lysine (5.60) and threonine (7.36) contents in lard sausage were statistically different ($P < 0.05$) from the isoleucine, leucine, lysine and threonine found in AFPWL-RS, AFPWL-MCS and AFPWL-SS sausages (Table 9).

Histidine (7.08) and tryptophan (9.35) in AFPWL-MCS were significantly higher ($P < 0.05$) than those found in lard, AFPWL-R and AFPWL-S sausages. The amount of methionine (6.45) in AFPWL-RS sausage was higher than in LS (3.94) and AFPWL-MCS (4.44). The valine in LS (4.05) and AFPWL-SS (4.05) were higher ($P < 0.05$) than in AFPWL-RS and AFPWL-MCS.

Table 9: Percentage compositions of selected amino acids of smoked sausage prepared with differently processed AFPWL

Amino acids	Lard sausage	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
Histidine	4.93 ^d	5.77 ^b	7.08 ^a	5.24 ^c	0.03
Isoleucine	9.46 ^a	6.01 ^b	4.17 ^d	5.43 ^c	0.01
Leucine	14.14 ^a	10.79 ^c	10.63 ^d	11.19 ^b	0.01
Lysine	5.60 ^a	4.99 ^c	4.92 ^d	5.17 ^b	0.01
Methionine	3.94 ^d	6.45 ^a	4.44 ^c	4.68 ^b	0.03
Threonine	7.36 ^a	4.93 ^c	6.94 ^b	4.62 ^d	0.02
Tryptophan	8.31 ^d	9.15 ^b	9.35 ^a	8.75 ^c	0.03
Valine	4.05 ^a	2.25 ^c	3.97 ^b	4.05 ^a	0.02

^{abcd} Means that on the same row with different superscripts are significantly different ($P < 0.05$), LS: Lard sausage, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

Microbiological status and levels of Thiobarbituric acid reactive substances (TBARS) in AFPWL fortified sausage

The Total Heterophilic Counts (THC) (0.09-0.11) in each of the sausages were similar ($P > 0.05$) (Table 10). At the same time, Total Coliform Counts (TCC) found in AFPWL-RS

were significantly higher than 0.05, 0.02 and 0.03 recorded in LS, AFPWL-MCS and AFPWL-SS, respectively. The amount (0.58) of TBARS generated in AFPWL-SS during storage was significantly more ($P < 0.05$) than 0.55 (AFPWL-MCS), 0.53 (AFPWL-RS) and 0.50 (lard sausage).

Table 10: Levels of Thiobarbituric Reactive Substances (TBARS) and microbial status of sausage over a storage period of 21 days as influenced by incorporation of differently processed AFPWL

Parameters (cfu/g log ₁₀)	Lard sausage	AFPWL-RS	AFPWL-MCS	AFPWL-SS	P-Value
TBARS (mg MDA/100 g)	0.50 ^d	0.53 ^c	0.55 ^b	0.58 ^a	0.001
THC	0.11	0.10	0.10	0.09	0.152
TCC	0.05 ^b	0.11 ^a	0.02 ^b	0.03 ^b	0.001
TFC	ND	ND	ND	ND	

^{abcd} Means that on the same row with similar superscripts are not significantly different ($P > 0.05$), THC: Total Heterophilic Counts, TCC: Total Coliform Counts, TFC: Total Fungi Counts, TBARS: Thiobarbituric Reactive Substances, AFPWL-RS: African Palm Weevil Larvae Raw Sausage, AFPWL-MCS: African Palm Weevil Larvae Moist Cooked Sausage, AFPWL-SS: African Palm Weevil Larvae Smoked Sausage

DISCUSSION

Information and knowledge of the nutritional composition of food supplemented with edible insects can influence attitudes towards insects as food. Although the nutritional composition of insects varies according to the species, the processing methods adopted in

preparing them also affect their composition. This is evident from the proximate composition of differently processed AFPWL reported in this study. For instance, the ash content of the processed larvae, irrespective of the processing methods, was higher than 4.90% (dried) and 1.24% (roasted) reported

by Agbemebia *et al.* (2020) but lower than 7.70% (roasted) reported by Edijala *et al.* (2009). The contents of protein in roasted larvae (30.69% and 29.57%) as reported by Agbemebia *et al.* (2020) were higher than what was obtained in all the processed larvae in this study. The nutrient compositions of AFPWL, as obtained in this study, showed that its inclusion in sausage production will make the product more nutritious (high protein) and healthy (low fat) for consumption. These nutritional benefits were also reported by Agbango *et al.* (2021) and Abo *et al.* (2022) when lard was partially replaced with palm weevil larvae in frankfurter production. The nutrient composition of AFPWL used in this study further confirmed that insects are rich in relevant dietary nutrients and, when added to processed foods, will increase the nutritional contents (Acosta-Estrada *et al.*, 2021).

The functional properties of food provide important information about its various uses in the food industry (Oladipupo *et al.*, 2020). These properties offer details on how ingredients, when added to food, will behave during preparation and cooking, and how they will also impact the finished food products, especially in terms of appearance, texture, structure, and taste (Awuchi *et al.*, 2019). The functional characteristics of a particular ingredient /food is crucial for predicting and evaluating more precisely how such an ingredient will behave when added into different food systems (Kaur and Singh, 2006; Siddiq *et al.*, 2009; Suresh and Samsher, 2013). Knowledge of these functional properties will further indicate whether such a newly added component can be used as a stimulant or replace the conventional food components (Kaur and Singh, 2006; Siddiq *et al.*, 2009; Suresh and Samsher, 2013). Therefore, assessing the functional properties of AFPWL fortified sausage emulsion, which has undergone different processing methods, is crucial. Reports have shown that heat treatments of food typically alter the three-dimensional structure of proteins, ultimately reducing the water retention capacity (Osundahunsi *et al.*, 2003) of such food. Therefore, there is a need

to assess the functional properties of the AFPWL fortified sausage emulsion.

The food solubility, which is the ability of a food to dissolve in a solvent, either water or oil, is a reflection of both the chemical and functional properties of such food (Awuchi *et al.*, 2019). Swelling tends to change the hydrodynamic characteristics of food (Oladipupo *et al.*, 2020), and the volume (mL) taken up by the swelling of one gram (1 g) of food material under specific conditions is usually referred to as swelling capacity or swelling index (Awuchi *et al.*, 2019).

The amount of water (moisture) that is taken up during the processing of food to achieve the desirable consistency and also produce a quality food product is usually referred to as Water absorption capacity (WAC) (Awuchi *et al.*, 2019). It is a valuable tool in food formulation as it gives information on the degree or extent of protein hydration, which depends on the nature of amino acids present and protein conformation of the food (Omotoso and Adedire, 2008).

Results of this study revealed that all AFPWL emulsions had improved WAC, which could be a result of the high WAC (127.33%) of the larvae (Ekpo *et al.*, 2010), implying that the larvae can easily be incorporated into aqueous food formulations as reported by Ekpo *et al.* (2010). The result further elucidated that processing (moist cooking and smoking) the larvae before their use in sausage production positively affected the swelling as well as water absorption capacities of the emulsions. This implies that incorporating processed larvae into sausage production is beneficial, as a higher WAC in food formulation improves handling characteristics, such as product bulking (Iwe *et al.*, 2016), as evidenced by the high product yield of AFPWL-fortified sausages.

The increased volume (swelling capacity/index) of sausage with processed AFPWL could be attributed to the high WAC because a direct relationship exists between swelling and water absorption capacities; thus, a higher swelling index implies a higher WAC

(Ikpeme-Emmanuel *et al.*, 2012). This further implies that processing methods such as moist cooking and smoking of AFPWL before its use in sausage production will confer a high water binding capacity to the emulsion/sausage, thus improving the reconstitution ability (Kulkarni *et al.*, 1991; Ajanaku *et al.*, 2012). Again, the high WAC recorded in the emulsion incorporated with smoked larvae may be a result of rehydration of the smoked larvae when added to the emulsion. This is because dehydration of the larvae occurs during smoking, and upon incorporation into sausage, they will absorb water; thus, the high water absorption capacity observed in AFPWL-SS.

The pH, type of protein, processing methods, viscosity and surface tension are factors that determine the foam formation and foam stability of a food (Yasumatsu *et al.*, 1972). The low foaming ability observed in the AFPWL emulsion, irrespective of the processing methods of the AFPWL, could be due to the high fat content of the AFPWL because high oil content in food hinders foam formation (Omotoso and Adedire, 2008). Such food will lack the ability to resist the changes and alterations in their physicochemical properties over time (McClements, 2004), thus the low emulsion stability recorded in AFPWL sausages. The low foaming ability of AFPWL emulsions might also be due to the fact that the globular proteins in AFPWL are highly ordered, thereby resisting surface denaturation. The overall assessment of the functional properties of the AFPWL emulsion in this current study further confirmed that AFPWL will be highly desirable for chopped meat or powdered food production, as reported by Ash *et al.* (2017). This is evident in the high product yields from all the AFPWL sausages. Furthermore, the high product yield of AFPWL sausages could probably be due to their low cooking losses and high water-holding capacities when compared with other AFPWL emulsions. This observation also accounts for the low cost of production of the AFPWL sausages, as the higher yield from these sausages will increase the marketability of the product. This is because WHC and yield are directly

proportional.

Selection of food by consumers is usually influenced by some intrinsic factors such as visual appearance and colour. The colour characterisation and colour stability of a product are measured through colour parameters such as L, a* and b* (García-Esteban *et al.*, 2003). As presented in this study, the summary of the 3-dimensional colour parameters of AFPWL sausages showed that L, a* and b* values were positive. This positive L, a* and b* imply that the incidence of brightness, redness and yellowness are detected on the surface (Pathare *et al.*, 2013). Although the lard sausage was comparatively brighter, as indicated by the higher L value when compared with AFPWL sausages. The low L value recorded in AFPWL-SS might be attributed to the golden brown colour that might have occurred (a characteristic of smoked product) during smoking of the AFPWL. Higher a* values were also recorded in all sausages, irrespective of what was added, indicating a greater redness in these products. The reduction in a* values of sausages prepared with raw and moist cooked palm weevil larvae could be that these larvae in this form caused a partial denaturation of nitrosomyoglobin pigment, which might be due to lipid oxidation (Gilani 2002; Fernández-Lopez *et al.*, 2004) or lactic acid production (Pérez-Alvarez *et al.*, 1999) in these products. The positive values recorded in the colour measurements of all the AFPWL sausages also implied that the sausages did not undergo any noticeable colour changes. Additionally, the colour quality will be acceptable to the consumers.

In this study, all AFPWL sausages exhibited increased protein content, a pattern observed by Agbango *et al.* (2021) in frankfurter-type sausages substituted with palm weevil larvae. Notably, the protein content in the AFPWL sausages of this study was higher than the 14.72-18.18% reported by these researchers. The high fat contents of AFPWL sausages in this study also affirmed the report of Acosta-Estrada *et al.* (2021), who opined that insect-enriched food had high fat content. The

results from this study further confirmed that processing of AFPWL (moist cooking or smoking) before its inclusion in sausage production will significantly increase the nutritional composition of the sausage. For instance, sausages prepared with processed AFPWL had higher protein contents, which could be attributed to the heat treatment, such as the cooking methods the palm weevil larvae have undergone. This is because cooking leads to the coagulation of proteins in food, which in turn increases the protein content of the food. The differences in the nutritional profile of sausages with processed AFPWL could be due to the different processing methods the AFPWL were subjected to before usage. This is because the type of cooking method a food is made to undergo has a significant impact on the nutrient content of that food. The high moisture contents of AFPWL sausages (when compared with lard sausages) implied that higher nutrient availability for the consumer after consumption. This further suggests that inclusion of this larvae in food (as animal source protein) can help improve dietary quality, as opined by Van-Huis *et al.* (2013). The attraction of a product to the consumer is a function of the sensory quality of such products (Cavalheiro *et al.*, 2013). However, these sensory qualities are dependent on the combination of several factors, which include materials, storage and the facilities used, as well as the processing conditions (Curt *et al.*, 2004). Therefore, evaluating the sensory characteristics of all the AFPWL sausages produced in this study is important because a slight modification in food formulation usually influences sensory attributes. Again, little information is available on consumers' reactions to consumption and acceptance of insect-based foods (Payne *et al.*, 2016), of which sausages produced with AFPWL are a part. Generally, the processing methods carried out on edible insects will not only affect the nutritional quality (Akullo *et al.*, 2018) but also the sensory properties of end-products (Mishyna *et al.*, 2020). However, this is contrary to the observations in this study, as most of the sensorial characteristics assessed on the sausages were not different from each other, except in overall

acceptability. This suggests that the inclusion of AFPWL in sausage production had little or no negative impact on the sensorial qualities of the sausage, which may have contributed to its high acceptance. These little or non-differences in the sensorial attributes of AFPWL and lard sausages could be a result of the high fat content of AFPWL sausages, which is assumed to have positively influenced the flavour and texture (Akullo *et al.*, 2018), thus the high sensory liking scores by the sensory panel for all AFPWL sausages. This implied that the high fat content could have contributed to the preference and high acceptability of AFPWL sausages (Cerdeira *et al.*, 2001; Olowu *et al.*, 2012) because the fat content of food contributes to the palatability of food. Aroma is an important criterion used in determining the acceptability of formulated foods (Ibironke *et al.*, 2012). This is because it usually determines the general acceptance of food before it is put inside the mouth, thus forming an integral part of taste. The aroma of all the sausages did not differ, implying no repulsiveness to any of the sausages when perceived. The panelists' indifference to the flavour and juiciness of AFPWL sausages compared to lard sausages can be attributed to the high fat content of AFPWL (Ekpo and Onigbinde, 2005), which is assumed to have been deposited into the sausage, thereby contributing to its flavour and juiciness. This is because the fat from animal protein contributes to the palatability and taste of food (Miller, 2004), which is one of the functions performed by lard in sausage production.

The overall acceptability of a product provides information on the extent to which consumers like it (Araújo *et al.*, 2017), which in turn determines whether they will become repeat buyers of the product. The overall acceptability score of all AFPWL sausages, regardless of their preparation method, indicated that they are slightly within the acceptable market range. This is because when sensory characteristics are assessed on a 9-point hedonic scale, the overall acceptability ratings should fall between 5.5 and 7.5 for the product to be considered within the marketable range (Potts *et al.*,

2017). This high acceptability of AFPWL sausages was also reported by Agbango *et al.* (2021). Again, it is evident from the results of this study that the methods of preparation of AFPWL before inclusion significantly affected the overall acceptability of the AFPWL sausages, as indicated by the panelists who preferred sausages with processed AFPWL. The high acceptability of sausage with moist cooked and smoked AFPWL sausage could be attributed to the processing methods the AFPWL had undergone before usage. This is because sensorial characteristics such as flavour and tenderness of food are developed during cooking. The high acceptance of sausage incorporated with smoked AFPWL showed that the panelists relished the product, which might be due to the characteristic flavour / taste usually associated with smoked products.

The nutritional value of food largely depends on the quality of the protein it contains, which in turn is determined by the amino acid composition (Ash *et al.*, 2017). Qualitative analysis of essential amino acid contents of AFPWL sausages when compared with lard sausages showed that AFPWL sausages contain essential amino acids, suggesting that AFPWL would confer these essential amino acids on the sausage. This further confirmed that African Pam weevil larvae contain some indispensable amino acids, as reported by Fogang-Mba *et al.* (2021). However, irrespective of the amino acids, the concentrations/amount contained in sausage with smoked AFPWL were low, probably due to loss of some nutrients from the AFPWL during its processing as a result of the high temperature (smoke temperature) the AFPWL had undergone before usage in sausage production.

The obtained result of the free fatty acid compositions revealed that the fatty acid compositions of the sausage were enhanced when AFPWL were used in sausage formulations. For instance, arachidonic, linoleic and oleic acids were abundant while stearic and palmitic acids dominated the saturated fats in sausage enriched with palm

weevil larvae. Propionic fatty acids were in small amounts in all AFPWL sausages, irrespective of the processing methods, and also in 100% lard sausage. Additionally, the different processing methods administered on the AFPWL were observed to have altered the ratio of individual fatty acids present in the AFPWL sausages, as revealed in this study. It would have been expected that the fatty acid compositions of sausages formulated with raw AFPWL would be high because it has not passed through any form of heat processing, which might have caused some nutrient losses. However, the reverse was observed with raw AFPWL sausages having lower concentrations irrespective of the fatty acid.

The high TBARS recorded in all AFPWL sausages may be linked to the high fat contents of AFPWL (Ekpo and Onigbinde, 2005) coupled with the high percentage of unsaturation of this fat (Koffi *et al.*, 2017; Gbogouri *et al.*, 2013). This is because the fat characteristics of the AFPWL are expected to be reflected in the AFPWL sausages. Since food with high unsaturated fat is prone to oxidation, the AFPWL sausages are susceptible to lipid oxidation.

Delicacy foods can be poisonous if they contain disease-causing pathogens; therefore, assessing the microbiological qualities of food is necessary to ensure that the nutritive quality is not compromised by microbial content (Ebenebe and Okpoko, 2015). This will also increase consumer confidence in the safety of the food. The microbiological quality results showed that no fungi were detected in all the sausages, and other microbes were present in low counts. The reduced microbial load of these sausages could be attributed to the acidic nature (low pH) of the products because microbiological safety of sausages is usually ascertained by ensuring a low pH, usually around 4.5-5.0 (Cavalheiro *et al.*, 2013). Although no significant differences exist among the microbial loads of all AFPWL sausages, the microbial loads of the sausage with smoked AFPWL were numerically low. This could be attributed to the low moisture content of the smoked larvae, as high moisture content in raw larvae catalyzes spoilage

reactions. In contrast, smoking leads to dehydration of the larvae, thereby improving conservation (Debrah *et al.*, 2019).

The values of THC obtained in all the sausages were below the microbial acceptable level of 10^4 for class A satisfactory level and $10^4 < 10^5$ for class B acceptable level (Microbial guidelines, 2007) for sausages. The hygienic preparation and careful handling during the processing of the sausage could be responsible for this. There was no fungal growth throughout the storage period. This indicates a lack of mycotoxins in the product, as the presence of fungus in food substances should be considered a major food safety concern, given that some fungi produce mycotoxins that are injurious and deleterious to health. This further emphasises the microbial safety of the sausages made in this study for human consumption.

CONCLUSION

The unique functional properties, the appreciable increase in nutritional quality, coupled with the low cost of production of the AFPWL fortified breakfast sausages, have shown that inclusion of AFPWL in sausage production could lead to novel insect-based sausage products. The panelist's preference for sausage with processed AFPWL showed that processing the larvae, especially through smoking, before inclusion in sausage preparation increased its palatability and at the same time its marketability. This form of processing also improved the shelf stability of the sausage as reflected in the low microbial load of the sausage with smoked AFPWL, thereby improving food safety and security.

AUTHOR CONTRIBUTION

OAB provided the concept and the experimental design of the study. FBT preparation of samples, execute the experiment and collate the results. AOR is involved in the preparation of samples, writing, reading, editing the manuscript, and providing final approval of the manuscript.

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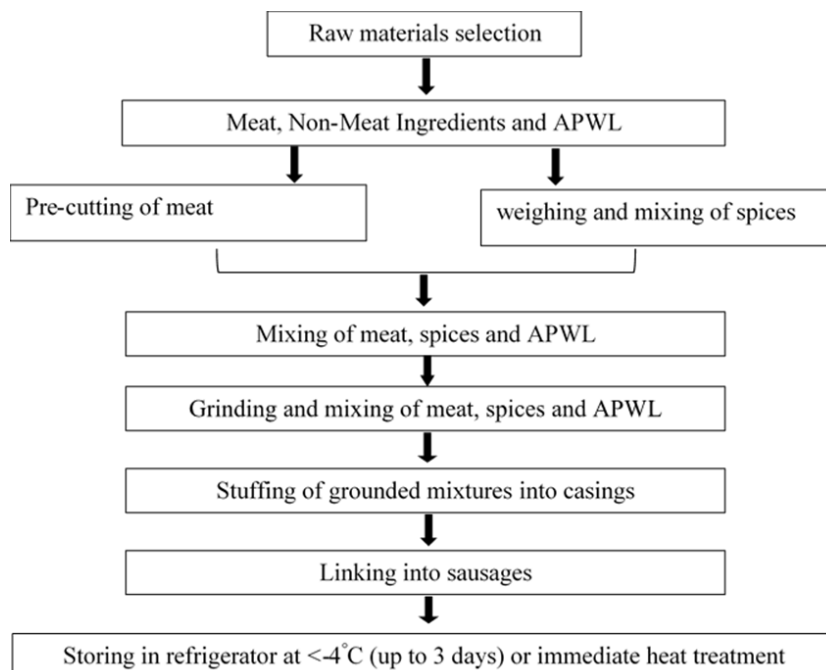
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Supplementary Figure 1: Flow chart of sausage production (Wan Rosli *et al.*, 2015)