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Optimization of Weaning Food Developed From Sorghum Supplemented with Crayfish and Garden Egg Using Response Surface Methodology

Iwanegbe, I.[1], Obaroakpo, J. U.[2], Olukoya, F. O.[3]

[1,2,3] *Department of Food Technology, School of Applied Sciences and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.*

ABSTRACT: This study utilized an integrated approach for the formulation of sorghum-based weaning food fortified with crayfish and garden egg. Response surface methodology (RSM) was used to evaluate the best fit of optimized formulations, which were further analysed for their nutritional and functional qualities to ascertain the most suitable formulation as a weaning food for infants' growth and development. Generally, good reproducibility was obtained from weaning blends A to T. However, the result of P-value of sensory parameters from RSM design showed best fit of various factors in A (100:20:5 g/g sorghum-crayfish-garden egg weaning blend); L (60:30:20 g/g sorghum-crayfish-garden egg weaning blend); I (113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend); M (80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend); and O (80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend). Protein is an essential part of a weaning food, and the result showed that the highest protein content was observed in L-blend (26.35 ± 0.03%), while the lowest content was recorded in O blend at a mean value of 23.06 ± 0.03%. The acidity of a weaning food influences its organoleptic quality, and the result showed that at an acidic range of 6.20 ± 0.10 - 6.70 ± 0.10, the blends were considered suitable. Overall, all blends had relative acceptable level of protein digestibility; although compared with the control (134.35 ± 3.69 mg/g), the protein digestibility of M-blend had the highest mean value (185.23 ± 0.2 mg/g). Sorghum-based weaning food enriched with crayfish and garden egg could be a potential cheap source of alternative supplements to conventional weaning foods from cereals.

I. INTRODUCTION

Weaning foods are supplements to breast milk introduced to children between the ages of 6 months to 3 years. Thus, it is expected that in addition to the provision of adequate nutrition, weaning foods should possess proper functional and organoleptic qualities. According to the World Health Organisation, weaning foods should have a functional property and consistency suitable for easy consumption and digestibility. Traditionally, weaning foods are mostly made of cereals such as millet, sorghum and corn. Like most cereals, sorghum has been reportedly used as an alternative source of high quality protein in weaning foods production, due to the high cost and unavailability of animal products such as milk and legumes.

In developing nations such as African countries, cereal-based foods constitutes a major source of their staple foods (Grote, Fasse, Nguyen, & Erenstein, 2021). Specifically in Nigeria, where over 40% of the population live below poverty threshold as a result of prevailing economic conditions, the incidence of protein-energy malnutrition in infants has become prevalent and on the increase yearly (Agiriga & Iwe, 2009). Protein-energy malnutrition is a serious deficiency among children in developing nations. The low socio-economic class in Africa due to high costs of raw material and improper processing technologies, consequently affects the quality of weaning foods.

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Among cereals in Nigeria, sorghum is most commonly utilized among locals as an alternative source to commercially developed weaning foods. It is considered a staple food in Nigeria (Raheem, Moammar, Rhoda, & Alice, 2021). Studies have shown that although sorghum is bulky in nature, it is devoid of essential nutrients and minerals. Since traditional weaning foods are mostly made of these cereals, their nutritional qualities are particularly low in protein content, as well as low in vital nutrients required for the normal growth and development of children in rural parts of Nigeria. In the processing of most weaning foods, attention has been given to its physicochemical properties, rather than its nutritional quality.

It is a known fact that an integrated approach of weaning food of different food products (such as the inclusion of small amounts of animal-source foods), has reportedly improved the nutritional and bioavailable micronutrients in plant-based diets (Chadare *et al*., 2019). A few studies had reported the use of crayfish (*Paranephrops planifrons*); a fresh water crustacean in the development of a protein-rich weaning diets (Ajanaku *et al*., 2013). It is relatively cheap, affordable and readily available in all seasons of the year. Also, the fortification of sorghum blended with legumes has been utilized in the development of weaning foods. Although several studies have reported the fortification of cereal-based weaning foods with protein-rich sources, there is still paucity of information on the use of biomaterials such as garden egg for the fortification of weaning foods. In fact, there is a dearth of knowledge about studies on vegetable-fortified weaning foods. Garden eggs (*Solanum sp.*), although not popular, could be potential enhancer to nutritious diets of weaning foods, due to the fact that it is rich in vitamin A, folate, calcium, niacin, magnesium and gut-healing fibre (Asaolu & Asaolu, 2002); all of which could contribute to baby's growth and development.

In order to develop a weaning food with a superior biological and functionally acceptable product, this study utilized an integrated approach of sorghum-based weaning food fortified with crayfish and garden egg. The organoleptic acceptability of any weaning food is a necessary factor greatly considered in its development. Therefore, in this study, the sensory parameter of the weaning food formulation from sorghum, crayfish and garden eggs were first optimized using Response Surface Methodology (RSM) and Central Composite Design (CCD). RSM is a collection of mathematical and statistical techniques useful for the development and optimization of products as well as processes. The best fit of optimized formulations were further evaluated for their nutritional and functional qualities to ascertain the most suitable formulation as a weaning food for infants' growth and development.

II. 2.0 MATERIALS AND METHOD

2.1 Collection of materials

Dried guinea corn (*Sorghum bicolor*) (5 kg), Garden eggs (*Solanum* sp.) (4 kg), and crayfish (*Paranephrops planifrons*) (4 kg) were purchased from Uchi market of Etsako West Local Government Area, Auchi, Edo State Nigeria. All reagents used were analytical grade.

2.2 Preparation of sample blends

The flow charts for the preparation of the blends from sorghum, crayfish and garden eggs are shown in figure 1.

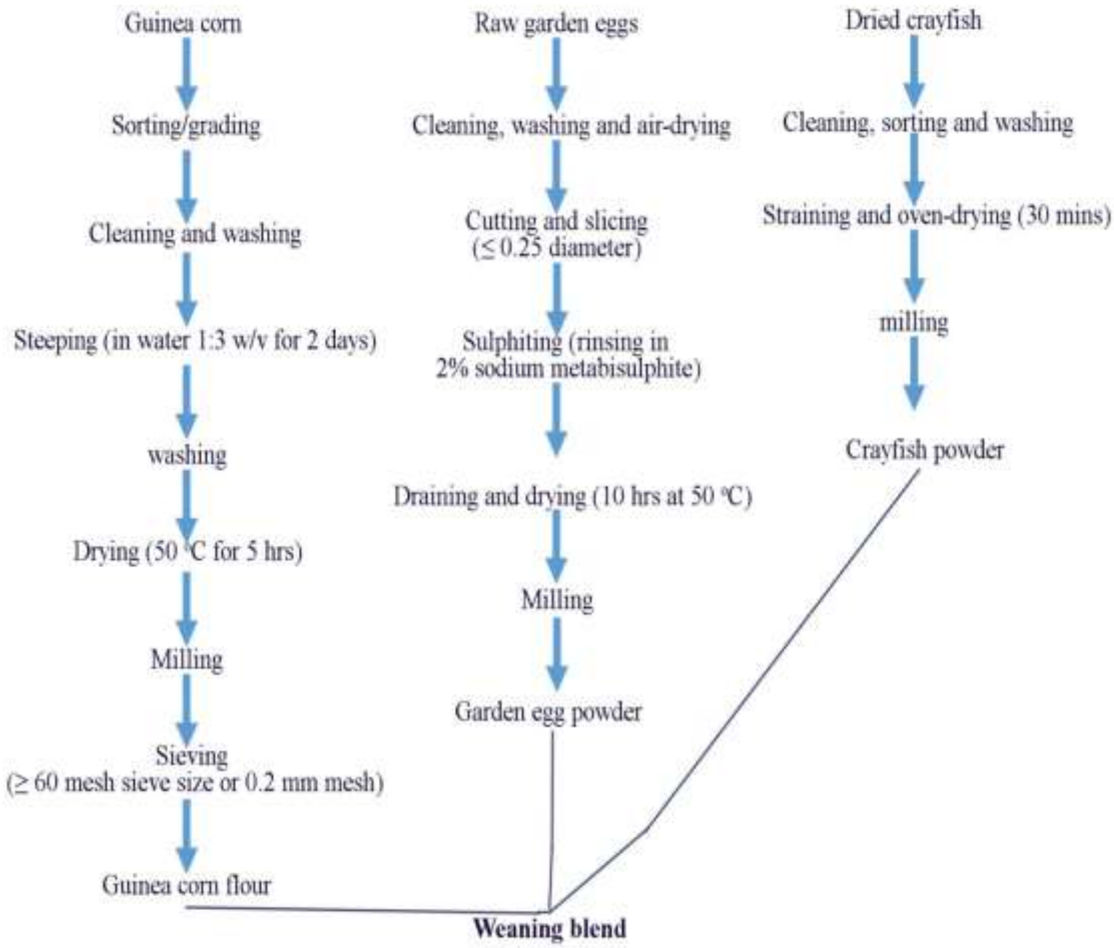

Figure 1: Flowchart for the preparation of sorghum (guinea corn flour), garden egg powder and crayfish powder blends.

2.3 Response surface methodology and generation of the optimal formulation

For the experimental plan, the following three parameters were selected: sorghum (at a range of 80 – 100 g/g), Crayfish (at a range of 20 – 30 g/g) and garden egg (at a range of 5 – 10 g/g). The complete model was developed using Design-Expert Software (version 6.0.8, Sate-Ease, Inc., Natick, MA, USA) was used for simultaneous optimization of the sensory parameters.

2.4 Proximate and physicochemical evaluation of optimized weaning formulation

Optimal formulations obtained from the responses in section 2.3 were further analysed for proximate and physicochemical evaluation. For proximate analyses, moisture, protein, fat, crude fibre and ash contents were determined according to the methods described by Montaño, Bonifacio and Rumbaoa (1999); and the carbohydrate content of each blend was calculated by difference.

The acidity of the optimized blends were determined using a pH meter (Orion Star T900). The potentiometric method was used for the determination of titratable acidity (TTA), and was expressed as the volume (in milliliters) of NaOH solution used in titration, to the nearest 0.05 mL.

2.5 Quantitative phytochemical screening of optimized weaning formulation

The total flavonoid content of the optimized weaning blends were determined by the aluminium chloride colorimetric method of Surana and Wagh (2017) with modifications. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight. The method described by Ejikeme, Ezeonu and Eboatu (2014) was used for the determination of tannins in the weaning formulations. The tannin content were quantified by a tannic acid standard curve and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g of dried sample and the soluble tannins calculated as:

$$\text{Soluble tannins} = \frac{\text{C (mg)} \times \text{extract volume (mL)}}{\text{DF } \times \text{aliquot (mL)} \times \text{sample weight (g)}}$$

Where: C = concentration extrapolated from standard calibration curve, DF = dilution factors.

The estimation of saponin content in the weaning formulations were determined according to the method described by Ejikeme *et al.* (2014) based on vanillin-sulphuric acid colorimetric reaction and expressed as saponin equivalents (mg SE/g sample) derived from a standard curve. The alkaloid content of the optimized weaning blends were determined according to the gravimetric method of Harborne (1984).

2.6 Functional analyses

Functional analyses of the optimized weaning formulations were done according to the slightly modified methods of Adejuyitan, Otunola, Akande, Bolarinwa and Oladokun (2009) to evaluate the water absorption capacity, swelling capacity, percentage solubility and foaming capacity.

2.7 Determination of the digestibility of optimized weaning formulation

2.7.1 *In vitro* starch digestibility (IVSD)

The IVSD was determined according to the method described by Singh, Neelam and Shruti (2012). Exactly 50 mg each of the samples was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.9). 0.5 mL of pancreatic alpha amylase (100 unit/mg) was added to the sample and incubated at 37 °C for 2 h. After incubation, 2 mL of 3, 5-DNS reagent was immediately added after incubation. After heating in a boiling water bath (5 – 15 min), 1.0 mL of 40% K-Na tartarate solution was added in the test tubes and allowed to cool at room temperature (25 °C). The solution was made up to 25 mL with distilled water and filtered prior to measurement of the absorbance at 550 nm, and the values were expressed as mg maltose per 100 mg of sample. A blank was run simultaneously. A standard curve was prepared using maltose.

2.7.2 *In vitro* protein digestibility (IVPD)

The IVPD of the optimized blends was determined by enzymatic method of Manjula and John (1991). 1 g each of the each blend was taken in triplicate and digested with 1 mg pepsin in 15 mL of 0.1 mL HCl at 37 °C for 2 h. The reaction was stopped by the addition of 15 mL 10% trichloro-acetic acid (TCA) and then filtered (Whatman No. 1). The TCA soluble fraction was assayed for nitrogen using the micro-kjeldahl method. Protein digestibility of the sample was calculated using the following formula:

$$\text{Protein digestibility (\%)} = \frac{N\ in\ supernatant - Blank\ N}{N\ in\ sample} \times 100$$

2.8 Statistics analysis

The data obtained from all analyses were statistically analyzed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Correlations between parameters were assessed by Pearson's correlation test, while Duncan multiple range tests applied to determine the differences between means.

III. RESULTS AND DISCUSSION

3.1 Organoleptic quality of sorghum-based weaning food weaning enriched with crayfish and garden egg.

The weaning formulation was obtained by the response surface analysis (RSM), based on the effect of the formulation on the colour, aroma, taste, texture and overall acceptability of the sorghum-based weaning food enriched with crayfish and garden egg. Twenty runs of experiment were evaluated for sensory attributes, and the three factors (sorghum, crayfish and garden egg blends) were taken into consideration.

Table 1: Experimental runs and actual values of factors used in central composite design.

Weaning blend	FACTORS			RESPONSES				
	Sorghum (g/g)	Crayfish (g/g)	Garden egg (g/g)	CL	AR	TS	TX	OAA
A	100	20	5	4.40	4.40	4.50	4.40	4.50
B	80	25	7.5	3.70	3.40	3.10	4.10	3.90
C	100	20	10	4.50	4.20	3.40	4.30	3.90
D	80	25	7.5	3.80	3.80	3.40	3.70	3.70
E	80	16.59	7.5	4.80	4.10	3.90	2.50	2.90
F	60	30	5	4.00	4.10	3.70	4.50	4.10
G	80	33.41	7.5	4.80	4.50	4.20	4.00	4.40
H	80	25	7.5	3.80	4.20	4.60	4.20	4.20
I	113.64	25	7.5	4.50	4.70	4.70	5.00	4.70
J	60	20	10	4.60	3.40	2.80	3.80	3.30
K	60	20	5	4.80	4.20	4.10	4.20	4.20
L	60	30	10	4.40	4.40	4.60	4.00	4.70
M	80	25	11.7	4.50	4.50	4.30	4.30	4.60
N	100	30	10	4.10	4.60	3.40	3.70	3.90
O	80	25	7.5	4.60	4.40	4.50	4.30	4.50
P	80	25	7.5	4.20	3.60	4.30	3.50	3.90
Q	80	25	7.5	4.80	4.30	4.40	4.20	4.50
R	100	30	5	3.90	4.10	3.10	3.40	4.10
S	80	25	3.3	4.50	4.20	4.10	4.00	4.10
T	46.36	25	7.5	4.60	4.60	4.20	4.30	3.90
X	100	-	-	5.00	4.80	4.80	4.90	4.90

KEY: CL = Colour, AR = Aroma, TS = Taste, TX = Texture and OAA = Overall acceptability. X - Control (100% sorghum blend).

The level of the response fixed for the optimization of the blends of sorghum-based weaning food enriched with crayfish and garden egg are described in table 2. The minimum and maximum colour score varied from 3.79 to 4.53 on a 5-point hedonic scale (Table 1). The quadratic model for colour was found to be not significant ($p>0.05$) in both crayfish and garden egg inclusion. The equation was obtained as:

Colour = 4.16 – 0.078 *A – 0.14 *B + 0.037 *C + 0.077 *A2 + 0.16 *B2 + 0.059 *C2 + 0.012 *A *B + 0.12 *A *C + 0.088 *B *C.

Where A = Sorghum blend, B = Crayfish blend and C = Garden egg blend.

Table 2: Level of response fixed for optimization of weaning blend.

Constraints	Goal	Lower limit	Upper limit	Intercept	F-value	P-value
Sorghum (g/100g)	Maximize	60	80	-	-	-
Crayfish (g/100g)	Is in range	20	25	-	-	-
Garden egg (g/100g)	Is in range	5	7.5	-	-	-
CL	Maximize	3.79	4.53	4.16	0.11	0.52
AR	Maximize	3.64	4.28	3.96	0.08	0.006
TS	Maximize	3.54	4.60	4.06	0.28	0.003
TX	Is in range	3.59	4.41	4.00	0.31	0.014
OAA	Maximize	3.90	4.30	4.10	0.20	0.283

Key: **CL** = Colour, **AR** = Aroma, **TS** = Taste, **TX** = Texture and **OAA** = Overall acceptability. [1]$p < 0.05$ indicates statistical significance. [2]$p > 0.05$ indicates that the lack of fit is not significant, which demonstrates that the specified model is adequate.

Figure 2 represents the 3-dimensional response surface view of colour. The coefficient of determination (R-square) was 0.35. The adjusted R-Square was of 0.23; while the adequate precision was 2.60.

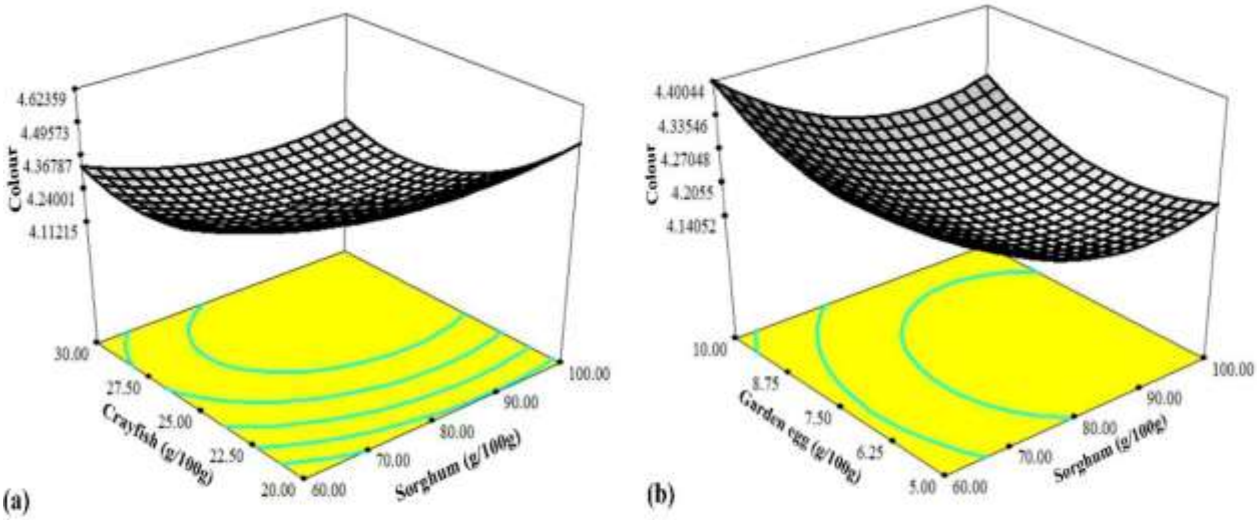

Figure 2: Effect of process variables on colour quality of weaning food. **(a)** Sorghum and crayfish as a function of colour **(b)** Sorghum blend and garden egg blend as a function of colour.

For aroma (figure 3), the coefficient of determination (R-square) was 0.55, with an adjusted R-square of 0.15 and an adequate precision value of 4.45 (< 4), which is the desirable value indicating an adequate signal. The inclusion of crayfish and garden egg resulted in a significant difference ($p<0.05$) in the weaning formulation.

Aroma = +3.96 + 0.10 *A + 0.12 *B + 0.022 *C + 0.19 *A2 + 0.063 *B2 + 0.081 *C2 – 0.10 *A *B + 0.100 *A *C + 0.23 *B *C.

Where A = Sorghum blend, B = Crayfish blend and C = Garden egg blend.

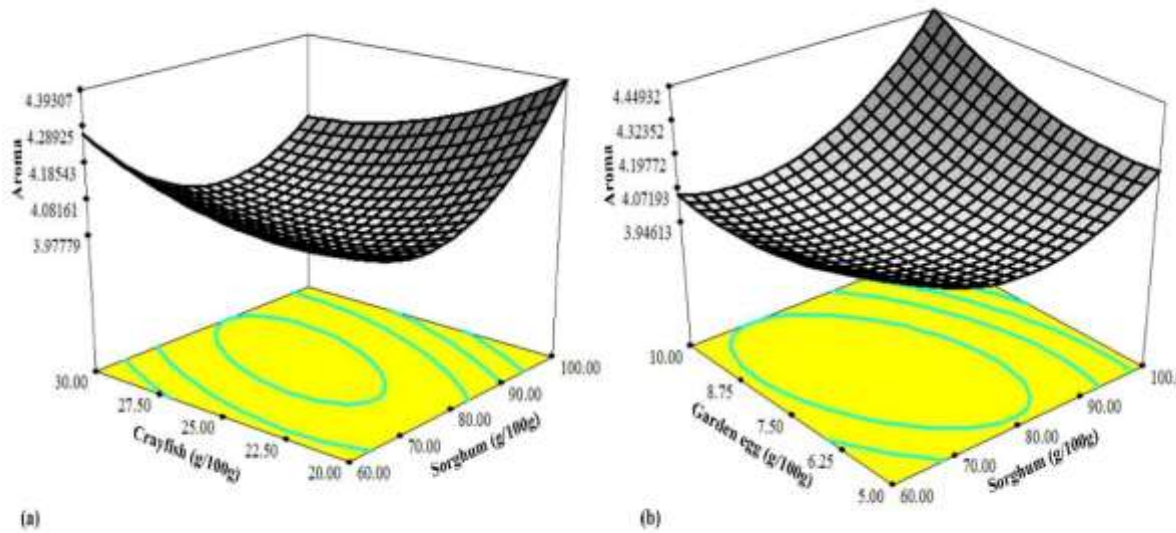

Figure 3: Effect of process variables on colour quality of weaning food. **(a)** Sorghum and crayfish as a function of aroma **(b)** Sorghum blend and garden egg blend as a function of aroma.

The fitted quadratic equation for taste response indicated (figure 4) an R-square value of 0.46, adjusted R-square of 0.02 and an adequate precision value of 4.19, indicating a significant difference in the weaning formulation at $p < 0.05$.

Taste = +4.07 + 3.002E - 0.03 *A + 0.037 *B - 0.063 *C + 0.027 *A2 – 0.11 *B2 - 0.062 *C2 - 0/.35 *A *B - 0.050 *A *C - 0.050 *A *C + 0.45 *B *C.

Where A = Sorghum blend, B = Crayfish blend and C = Garden egg blend.

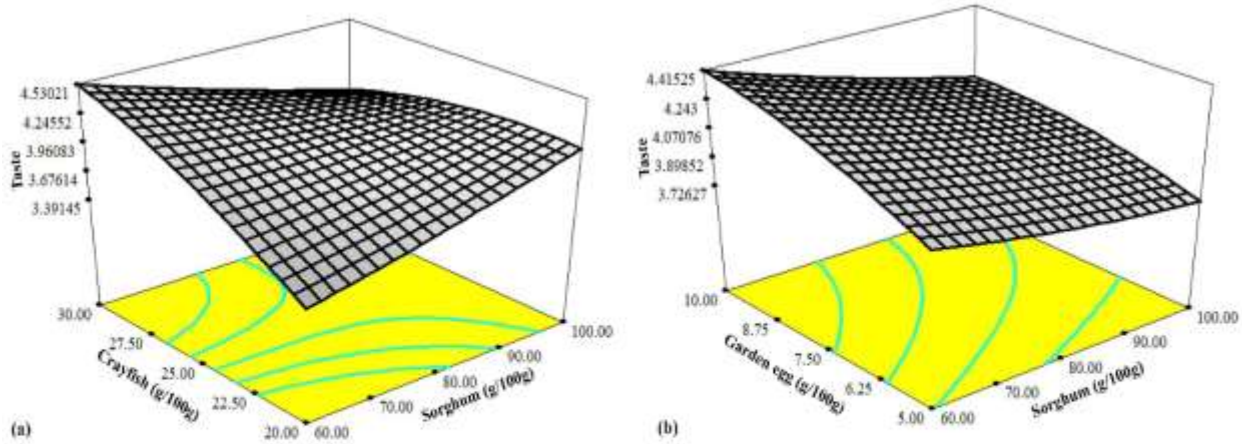

Figure 4: Effect of process variables on taste quality of weaning food. (a) Sorghum and crayfish as a function of taste (b) Sorghum blend and garden egg blend as a function taste.

For texture (figure 5), the equation of quadratic fit influenced by crayfish and garden egg inclusion was:

Texture = + 4.00 + 0.035 *A + 0.10 *B - 0.014 *C + 0.23 *A2 – 0.26 *B2 + 0.057 *C2 - 0.26 *A *B - 0.063 *A *C + 0.24 *B *C.

Where A = Sorghum blend, B = Crayfish blend and C = Garden egg blend.

The response of process variable as a function of texture resulted in a R-square value of 0.58, adjusted R-square of 0.21 and an adequate precision value of 5.01; thus, indicated a significant difference ($p<0.05$) in the weaning formulation.

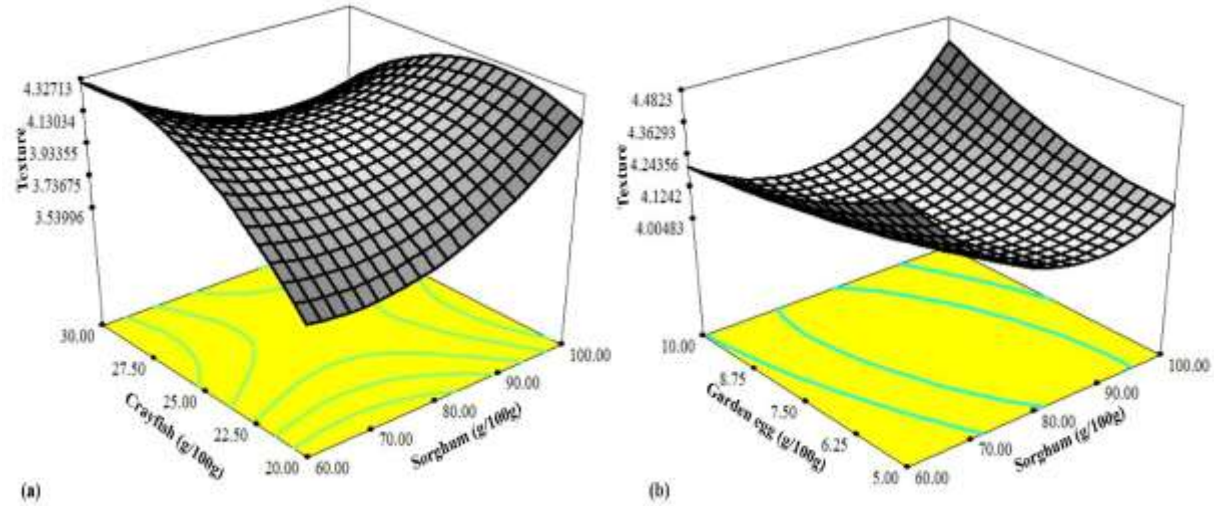

Figure 5: Effect of process variables on texture quality of weaning food. (a) Sorghum and crayfish as a function of texture (b) Sorghum blend and garden egg blend as a function texture.

Overall, the process variables resulted in a R-square value of 0.45, adjusted R-square of 0.22 and an adequate precision value of 5.84 (figure 6). Among all weaning blends, no significant difference ($p<0.05$) was observed. The fitted quadratic equation for general acceptability of the weaning formulation was:

Overall acceptability = + 4.10 + 0.11 *A + 0.25 *B - 0.019 *C - 0.21 *A *B - 0.063 *A *C + 0.24 *B *C.

Where A = Sorghum blend, B = Crayfish blend and C = Garden egg blend.

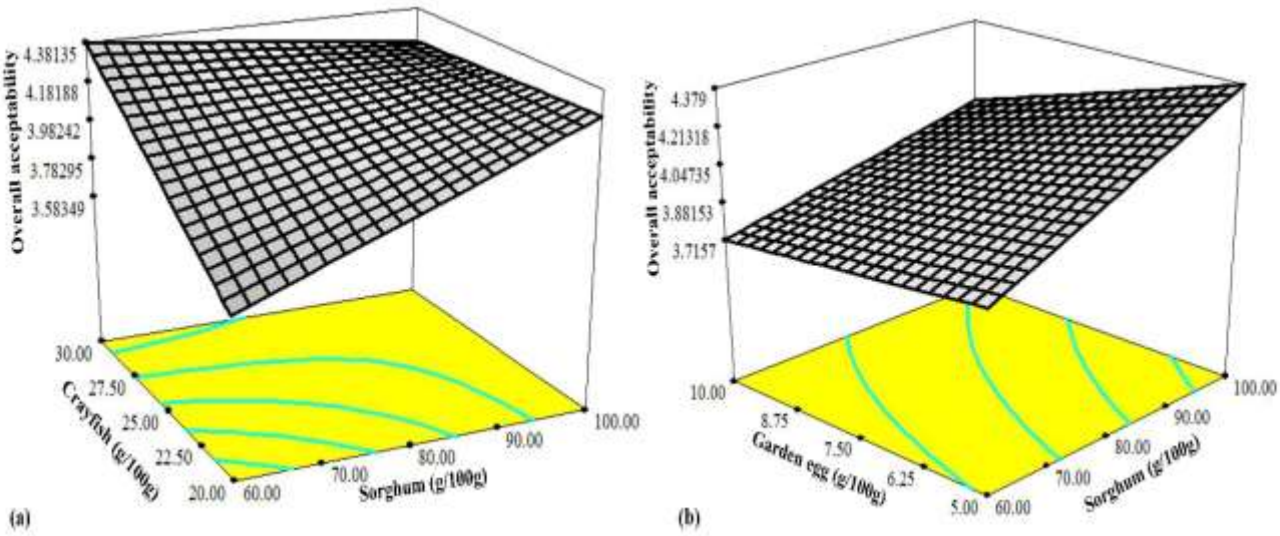

Figure 6: Effect of process variables on overall acceptability of weaning food. (a) Sorghum and crayfish as a function of texture (b) Sorghum blend and garden egg blend as a function texture.

According to the p-value of parameters on sensory properties, the best fit of various factors on the responses from RSM were sorted and observed in A, I, L, M and O; indicating that these factors or their interaction had the best fit with regards to the organoleptic quality of the blends.

3.2 Proximate composition of the optimized weaning blend

Generally, good reproducibility was obtained from weaning blends A - T. However, the factors A, I, L, M and O were observed as best fit; thus, were further investigated for nutritional and functional qualities. The results of the proximate composition (table 3) showed that among the best fit of optimized blends, moisture content was significantly low. However, the least moisture content was observed in weaning blends L and O at mean values of 10.42 ± 0.02 % and 10.42 ± 0.02 % respectively. Moisture content in flour samples are usually expected to be low enough for proper storage or the enhancement of shelf stability (Destrosier, 2004; Ihekeronye & Ngoddy, 1985).

The result of the protein content showed significant differences ($p<0.05$) among all weaning blends. While the highest protein content was observed in L-blend (26.35 ± 0.03%), lowest content was recorded in O blend at a mean value of 23.06 ± 0.03%; and was not significantly different ($p>0.05$) from that of M-blend (23.09 ± 0.03%). The expectedly high protein content observed in L-blend could be attributed to the increased inclusion of crayfish. A high-balanced protein content is an essential for a good-quality weaning food (Wu & Xu, 2019).

The result of the ash content showed significant differences ($p<0.05$) among all samples. The highest ash content was observed in L-blend (5.36 ± 0.04%). An enhanced ash content could concomitantly influence the availability of mineral in the weaning blend (Folorunso, Akintelu, & Oyebamiji, 2019). Mineral is an essential component in weaning foods. Interestingly, the L-blend also recorded the highest content of fibre at a mean value of 2.33 ± 0.02%, and could be attributed to the addition of garden eggs; a vegetable predominantly rich in fibre (Igwe, Akunyili, & Ogbogu, 2003). Dietary fibre is a known factor significant in the prevention of diet-dependent diseases (Barber, Kabisch, Pfeiffer, & Weickert, 2020). The result of the fat content in the weaning blends showed that L-blend had the highest fat content at a mean value of 8.32 ± 0.03%; while the lowest fat content was observed in I-blend (5.68 ± 0.05%).

Table 3: Proximate composition of sorghum-based weaning food weaning enriched with crayfish and garden egg

Parameter	Weaning formulation				
	A	L	I	M	O
Moisture (%)	10.71 ± 0.01^{b}	10.42 ± 0.02^{c}	11.73 ± 0.03^{a}	10.72 ± 0.00^{b}	10.42 ± 0.04^{c}
Ash (%)	3.30 ± 0.01^{d}	5.36 ± 0.04^{a}	3.01 ± 0.01^{e}	4.28 ± 0.08^{b}	3.60 ± 0.02^{c}
Protein (%)	18.11 ± 0.02^{d}	26.35 ± 0.03^{a}	19.35 ± 0.01^{c}	23.09 ± 0.03^{b}	23.06 ± 0.03^{b}
Fat (%)	7.89 ± 0.00^{c}	8.32 ± 0.03^{a}	5.68 ± 0.05^{e}	7.05 ± 0.03^{d}	8.10 ± 0.02^{b}
Fibre (%)	1.40 ± 0.01^{c}	2.33 ± 0.02^{a}	1.34 ± 0.01^{d}	1.65 ± 0.02^{b}	1.30 ± 0.02^{e}
Carbohydrate (%)	58.61 ± 0.03^{b}	46.77 ± 0.03^{e}	58.92 ± 0.01^{a}	53.27 ± 0.05^{d}	53.57 ± 0.05^{c}

KEY: **A** = 100:20:5 g/g sorghum-crayfish-garden egg weaning blend; **L** = 60:30:20 g/g sorghum-crayfish-garden egg weaning blend; **I** = 113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend; **M** = 80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend; and **O** = 80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend. Values are means ± standard deviation of triplicate determination. Means with the different superscript in the same column are significantly different ($P<0.05$).

The acidity of a weaning food could influence its organoleptic quality (Koraqi, Durmishi, Azemi, & Selimi, 2020). Figure 7 represents the acidity (pH) and titratable acidity (TTA) of the optimized weaning blends. In comparison with the control, the highest pH value was observed in L-blend (6.70 ± 0.10). However, the result of the TTA showed the highest mean value in L-blend (18.00 ± 1.00), and was not significant different ($p>0.05$) from that of M-blend (18.00 ± 0.98). Relatively, as weaning foods, the blends were considered suitable in acidic levels.

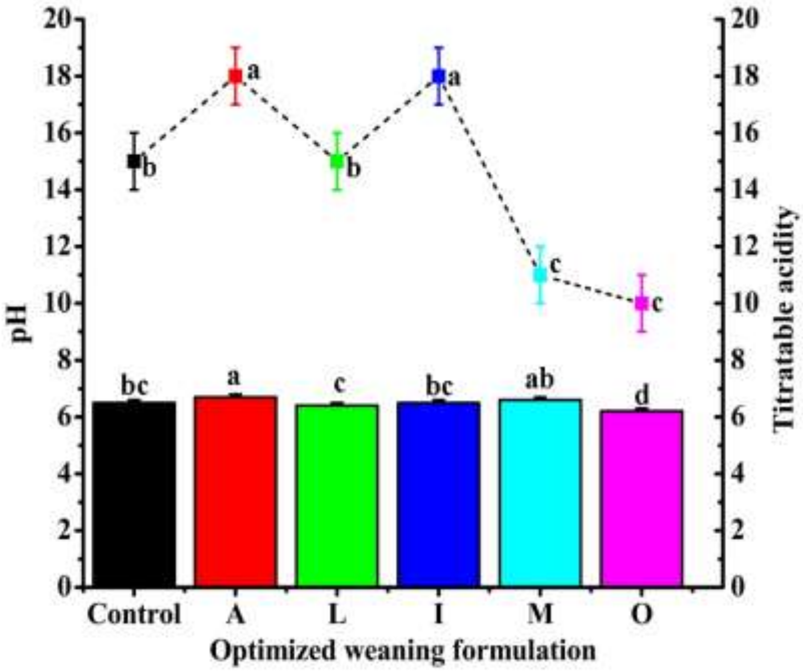

Figure 7: pH and titratable acidity of optimized weaning blends of sorghum-based weaning food weaning enriched with crayfish and garden egg.

KEY: Line graph indicates titratable acidity of the blends. Bar graph indicates pH of the blends. **A** = 100:20:5 g/g sorghum-crayfish-garden egg weaning blend; **L** = 60:30:20 g/g sorghum-crayfish-garden egg weaning blend; **I** = 113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend; **M** = 80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend; and **O** = 80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend. Values are means ± standard deviation of triplicate determination. Means with the different superscript on the same line are significantly different ($p<0.05$).

The functional properties of the blends are shown in figure 8. The water absorption capacity (WAC) of the blends was observed to be highest in the L-blends (200 ± 1.00%), and was not significantly different from that of the O-blend (figure 8a). The lowest WAC was observed in I-blend at a mean value of 200 ± 1.00%. In solubility (figure 8b), the results showed that L-blend and M-blend had the highest level at mean values of 8.50 ± 0.01% and 8.50 ± 0.00% respectively; while the lowest level of solubility was observed in A-blend (2.00 ± 0.01%). The result of the swelling capacity (figure 8c) of the weaning blends showed the highest and lowest mean values in A-blend (480.00 ± 1.00%) and O-blend (409.00 ± 1.00%). The foaming capacity (figure 8d) of the blends showed the highest mean value in I-blend (14.03 ± 0.06%); while the lowest capacity was observed in A-blend (4.00 ± 0.01%).

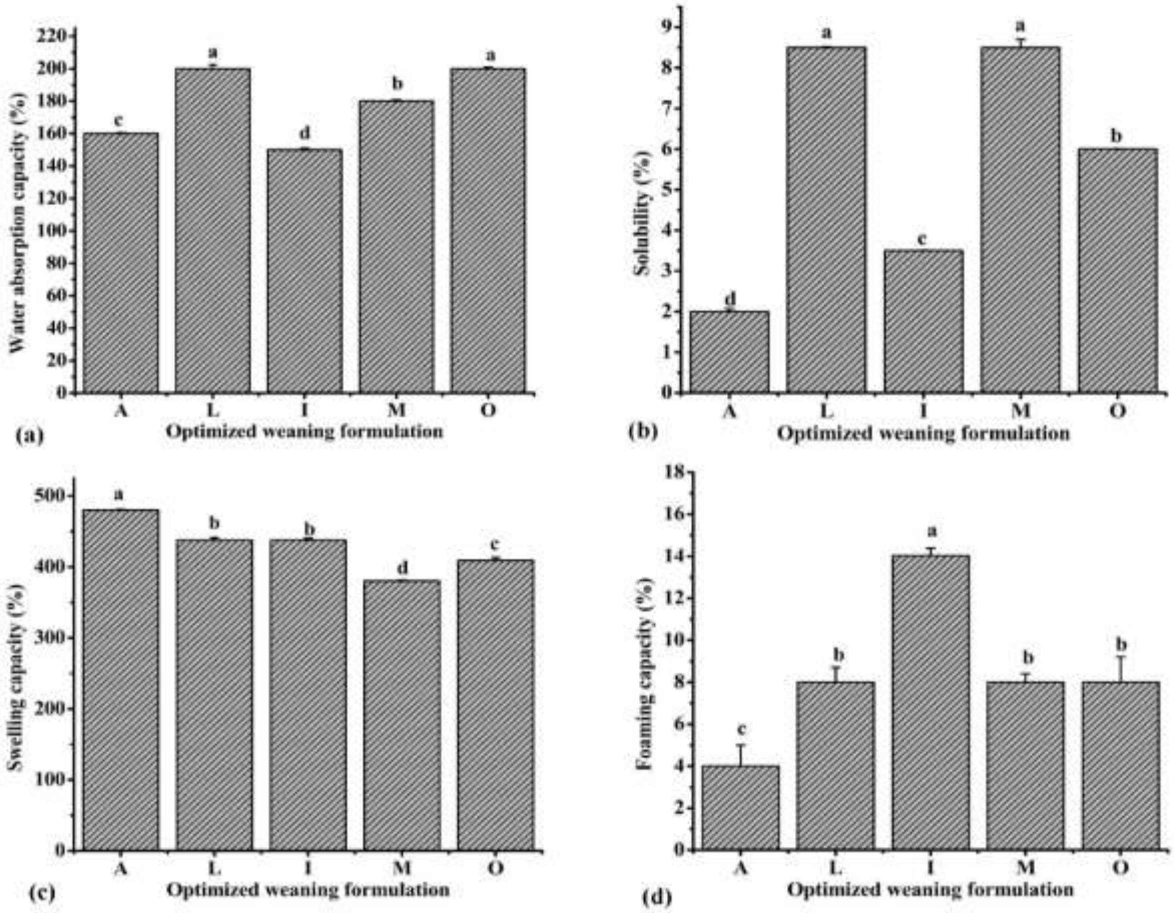

Figure 8: Functional properties **(a)** Water absorption capacity **(b)** Solubility **(c)** Swelling capacity **(d)** Foaming capacity of optimized weaning blends of sorghum-based weaning food weaning enriched with crayfish and garden egg.

KEY: **A** = 100:20:5 g/g sorghum-crayfish-garden egg weaning blend; **L** = 60:30:20 g/g sorghum-crayfish-garden egg weaning blend; **I** = 113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend; **M** = 80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend; and **O** = 80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend. Values are means ± standard deviation of triplicate determination. Means with the different superscript are significantly different (p<0.05).

The quantitative phytochemical screening of the weaning blends are shown in figure 9. Several phytochemicals in plant products have previously been regarded as antinutrients due to their characteristic astringent or unpalatable influence in organolepic properties of food. In addition, they have been implicated in deleterious effects related to the absorption of nutrients and micronutrients. However, recent studies have demonstrated that most of these phytochemicals could act as bioactive compounds effective in the reduction of blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides (Shahidi & Ambigaipalan, 2015). In weaning foods, the presence of phytochemicals in small amounts could act as protective agents against infant dietary disorders or malnutrition.

The result of the alkaloid content (figure 9a) in the weaning blends showed the highest mean value in O-blend (17.43 ± 0.04%); while the lowest content was observed in A-blend at a mean value of 12.50 ± 0.24%. From the results, it was observed that as alkaloid content was higher in blends with increased garden egg addition. According to the reports of Eze and Kanu (2014), alkaloids are responsible for the astringent bitter taste in garden eggs.

In traceable amount, flavonoids could be beneficial in weaning formula. The result of the flavonoid content are shown in figure 9b. Although, sorghum is naturally rich in phytochemicals, the inclusion of vegetables in the form of garden eggs further enhanced its phytochemical content. The finding of this study also coroborated with the report of Maria, Sara, Amalia and Matteo (2018) in such a way that the flavonoid content was observed to be highest in L-blend (0.85 ± 0.04 mg/g) and O-blend (0.95 ± 0.04 mg/g) in which garden eggs were most included.

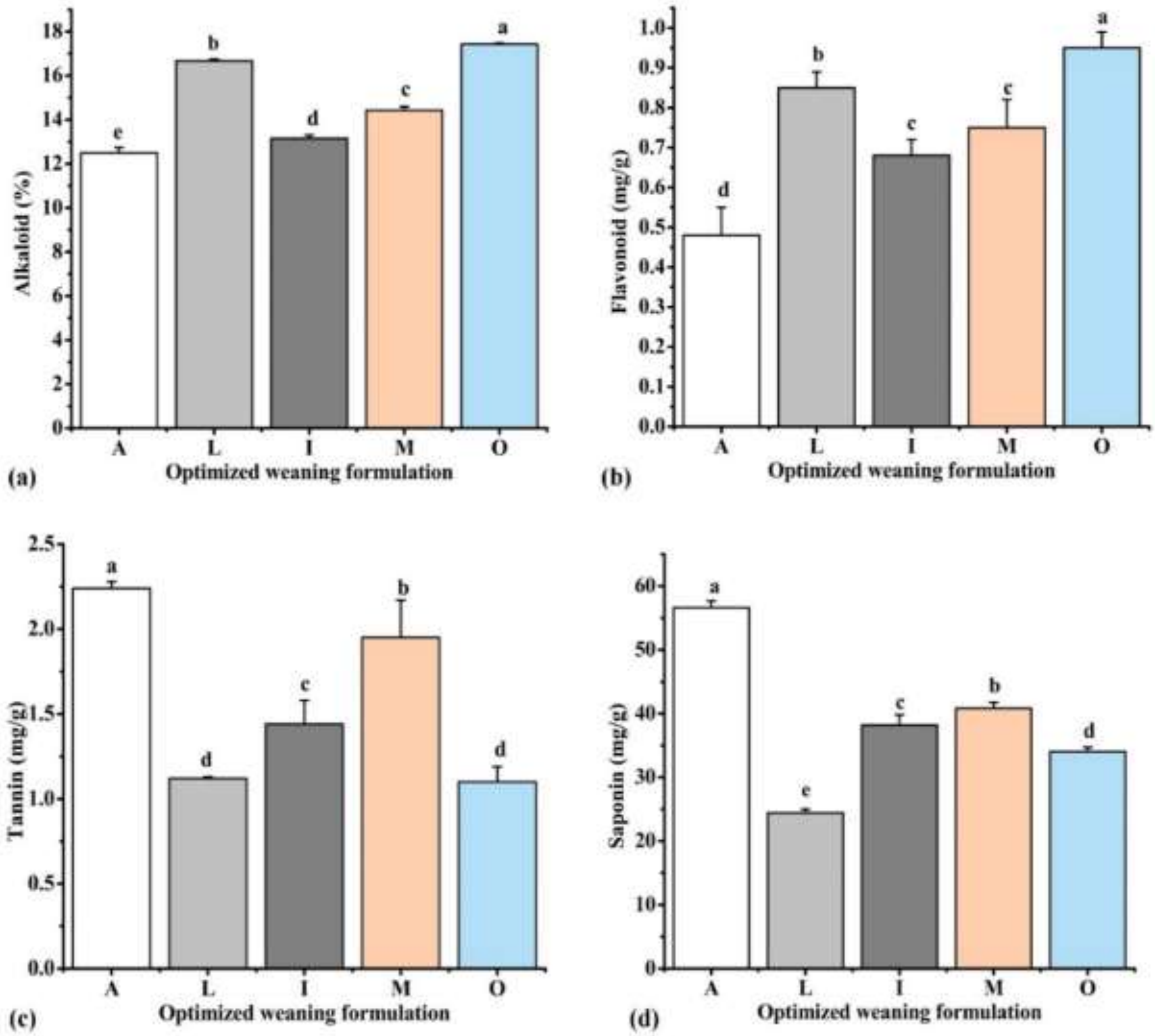

Figure 9: Quantitative screening of phytochemicals in optimized weaning blends of sorghum-based weaning food weaning enriched with crayfish and garden egg **(a)** Alkaloid content; **(b)** Flavonoid content; **(c)** Tannin content; and **(d)** Saponin content.

KEY: **A** = 100:20:5 g/g sorghum-crayfish-garden egg weaning blend; **L** = 60:30:20 g/g sorghum-crayfish-garden egg weaning blend; **I** = 113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend; **M** = 80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend; and **O** = 80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend. Values are means ± standard deviation of triplicate determination. Means with the different superscript are significantly different (p<0.05).

Tannin is a phytochemical that predominantly binds or precipitates proteins and various other organic compounds such as amino acids and alkaloids. Figure 9c showed the content of tannin in the optimized weaning formulation. The highest content of tannin was observed in A-blend at a mean value of 2.24 ± 0.04 mg/g; while the lowest content was observed in O-blend (1.10 ± 0.09 mg/g). Interestingly, a negative correlation (data not shown) was observed between tannin and alkaloid contents, which could be attributed to their ability to form bound compounds in synergy (Adamczyk, Simon, Kitunen, Adamczyk, & Smolander, 2017). The increase in the saponin content could be attributed to the sorghum and garden egg inclusion. According to the report of WHO (2003), without exception to weaning foods, the permissible level of tannin content in foods is given as 1.5 mg /100 g. Thus, the report of this study indicated that the I-blend, L-blend and O-blend were considered most permissible in terms of tannin content.

The result of the saponin content of the optimized weaning blend are shown in figure 9d. Although, saponins has previously been misconstrued as an antinutrients, recent study has demonstrated that their potentials as bioactive compounds could best be exploited at safe levels (Gómez de Cedrón *et al.*, 2020), which were mostly measured by the removal of their astringent bitter tastes. The result of this study showed that the highest and lowest saponin content were recorded in A-blend and L-blend at mean values of 56.64 ± 1.02 mg/g and 24.42 ± 0.58 mg/g respectively. Invariably, among the weaning blends evaluated, L-blend could be considered safer in terms of saponin content.

Most of the phytochemicals present in the blends could have been influenced by inclusion of garden eggs. The consumption of vegetables early in life is currently reeported as an important strategy towards decreasing adverse health events that might occur in adulthood. According to Ogden, Carroll, Kit and Flegal (2014), more than a third of children and adolescents considered overweight or obese; thus, it is critically important to reduce chronic disease factors such as inflammation and oxidative stress.

The digestibility of the optimized weaning blends are shown in figure 10. Compared with the control (134.35 ± 3.69 mg/g), the protein digestibility of M-blend had the highest level (185.23 ± 0.2 mg/g). Overall, all blends had relative levels of protein digestibility which could be attributed to the modifications that occurred in the inherent protein during natural fermentation. Laminu, Modu and Numan (2011) reported that natural fermentation contains proteolytic bacteria which degrade proteins into simple proteins; thus improve digestibility.

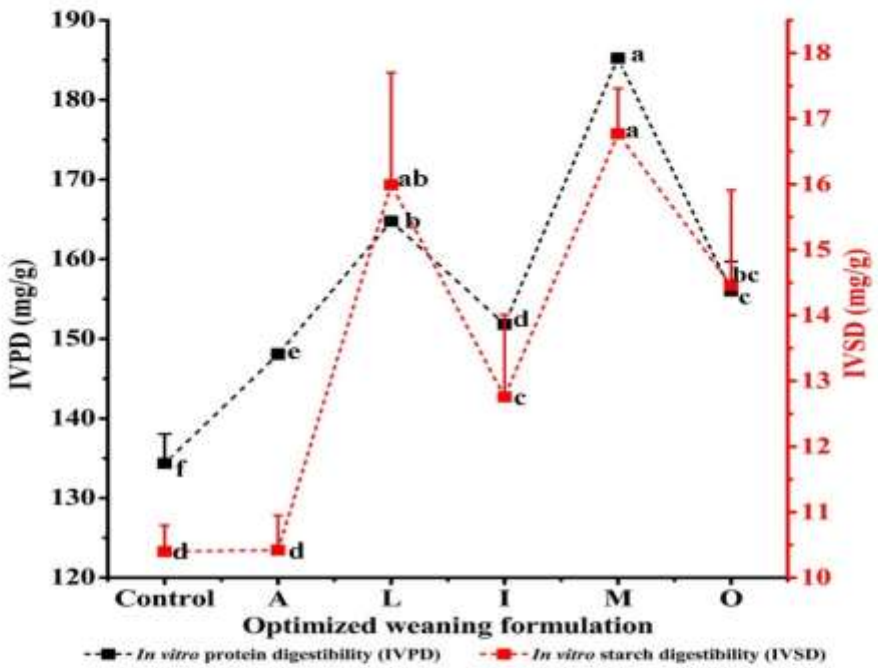

Figure 10: *In vitro* protein and starch digestibility of optimized weaning blends of sorghum-based weaning food weaning enriched with crayfish and garden egg.

KEY: A = 100:20:5 g/g sorghum-crayfish-garden egg weaning blend; **L** = 60:30:20 g/g sorghum-crayfish-garden egg weaning blend; **I** = 113.64:25:7.5 g/g sorghum-crayfish-garden egg weaning blend; **M** = 80:25:11.7 g/g sorghum-crayfish-garden egg weaning blend; and **O** = 80:25:7.5 g/g sorghum-crayfish-garden egg weaning blend. Values are means ± standard deviation of triplicate determination. Means with the different superscript on the same line are significantly different ($p<0.05$).

The *in vitro* starch digestibility (figure 10) showed that there were significant differences ($p < 0.05$) among all weaning blends. In comparison with the control (10.40 ± 0.40 mg/g), M-blend had the highest level of starch digestibility at a mean value of 16.77 ± 0.69 mg/g. A number of factors such as gelatinization, granule particle size and starch-protein interaction, have been shown to affect the digestion of starch in foods (Gahlawat & Sehgal, 2008). Thus, it was presumed that the formulation of sorghum, crayfish and garden egg in M- blend permitted better digestibility of the starch, as partial starch breakdown to dextrins could have occurred, attributed to its higher acidity.

IV. CONCLUSION

The influence of some selected parameters on sorghum-based weaning food enriched with crayfish and garden egg were evaluated using response surface methodology (RSM), and the optimal formulations were obtained. The developed optimized weaning formulation has the potential to meet certain nutritional needs of infants in terms of protein, fibre, digestibility and organoleptic acceptability. Thus, it could enhance the utilisation of crayfish, garden eggs and other biomaterials. In conclusion, sorghum-based weaning food enriched with crayfish and garden egg could be a potential cheap source of alternative supplements to conventional weaning foods from cereals. Consequently, their formulation could alternatively replace most commercial complementary food products, and play an effective nutritional role in the diets of infants in the world at large.

Credit authorship contribution statement

Iwanegbe Izuwa: Conceptualization, Data curation, Writing - review & editing and funding acquisition. Obaroakpo Joy Ujiroghene: Investigation, Writing - original draft, data curation and software. Olukoya, F. O.: Validation and visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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