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Optimization of the Response Surface Method in the Elaboration of a Complementary Food

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Abstract

Access to food is a humanitarian right where every person has the right to eat a qualitative quantity of food, enough to meet their basic needs. Hunger and malnutrition affecting infants and young children in most developing countries are two critical situations and constitute a public health problem. The objective of this work is to realize a formulation of infantile flour by using local products. The methodology applied is that of surface responses using the Box-Behnken matrix with three factors such as yam flour (X_1: 40 - 50 g), herring flour (X_2: 10 - 20 g) and pigeon pea flour (X_3: 20 - 30 g). The optimal experimental conditions of the mixture were established on the composite flour to be cooked, and the effects of the factors on the responses MC, AC, LC, PC, CC, E, FC and WAC meeting the quality criteria based on the standard norms for infant porridges were evaluated. A total of 15 sets of experiments where three levels were assigned to each factor at the top, middle and bottom, and with three additional replicated center points were performed. Statistical results (ANOVA) showed that the experimental data were correctly fitted into a second-order polynomial model with multiple regression coefficients ≥0.6. Using the objective parameters for each response, based on the standard infant flour quality norm by applying the desirability function, yielded optimal conditions for the infant flour formulation to be cooked. These conditions gave: 46.263 g of yam flour; 15.859 g of herring flour and 30 g of pigeon pea flour.

Subject Areas

Food Science & Technology

Keywords

Box-Behnken, Infantile Flour, Formulation, Optimization, RSM

1. Introduction

Nutrition in the first 1000 days of a child's life is critical for long-term physical and mental development [1]. According to WHO, malnutrition is a medical condition resulting from the relative or absolute deficiency or excess of one or more essential nutrients [2]. It is therefore a public health problem in the world, particularly in developing countries [3]. Malnutrition is responsible for at least half of all child deaths worldwide, which shows that it is more dangerous than any infectious disease [4]. From birth to 6 months of age, all of the child's nutritional needs are met by breast milk [5] [6] which has multiple benefits [7]. Beyond this age, breast milk is no longer sufficient to fully cover energy and protein needs [6]. It is therefore necessary to introduce into the young child's diet food supplements in liquid or semi-solid form to add to the breast milk supply [8]. These intakes must contribute to the dietary balance of children [9] and must be of satisfactory sanitary and organoleptic quality [10]. During this same period of supplemental feeding, physical changes can be noticed including thinness as well as stunted growth [11] [12]. The quality of the supplementary food used during this period is therefore of great importance. However, good quality infant flours that have been the subject of numerous works and WHO recommendations existing on the market are imported industrial products and are of high cost. Therefore, they are not accessible to all. In developing countries, particularly in the Congo, mothers often use traditional porridges of cereal flours or cassava roots as supplementary food, which are rich in starch but poor in protein [13] [14]. When these infant meals are cooked, they are mostly heavy, viscous, indigestible and difficult for children to swallow, due to the swelling of the starch; this forces mothers to add water in the preparation in order to make these porridges more fluid, thus reducing the dry matter content and consequently the energy density [5] [15] [16] [17]. The cooking process used by mothers cannot cover the nutritional needs of young children, especially when mealtimes are not regular. This is why, for several years, the FAO/WHO has advocated the production of supplementary foods of sufficient nutritional quality, based on locally available and accessible products, in order to meet the nutritional needs of African children [10] [18]. In order to meet this recommendation, we have been interested in improving supplemental food of good nutritional quality using local products.

RSM is a set of statistical and mathematical techniques useful for developing, improving and optimizing processes. It also has important applications in the design, development and formulation of new products as well as in improving the design of existing products [19] [20].

With this in mind, the objective of this present study was to propose a formulation of infant-type flour from local raw materials composed of Gamboma yam, pigeon peas and herring-type fish of adequate nutritional and sanitary quality, and acceptable by children of supplementation age.

2. Material and Methods

2.1. Material

Different biological materials of animal and plant origin (Figure 1) were used in this study. These are:

- Tubers of *Dioscorea cayenensis*, coming from the Plateaux department, precisely from Gamboma, and bought at the local market of Mati in the Texaco district in the north-east of Brazzaville;
- Pulses, in particular the Pigeon pea known under the scientific name *Cajanus cajan*, coming from the department of Bouenza and bought from the local market of Total in Bacongo, southwest of Brazzaville;
- Cereal grains of corn (*Zea mays*) coming from Loudima department of Bouenza in the south-west of Congo and bought from the Mati market of Texaco in the north-east of Brazzaville, which served us as malt;
- And fresh herring fish (*Clupea harengus*), bought in the city of Pointe-Noire in the department of Kouilou in the southwest of the country.

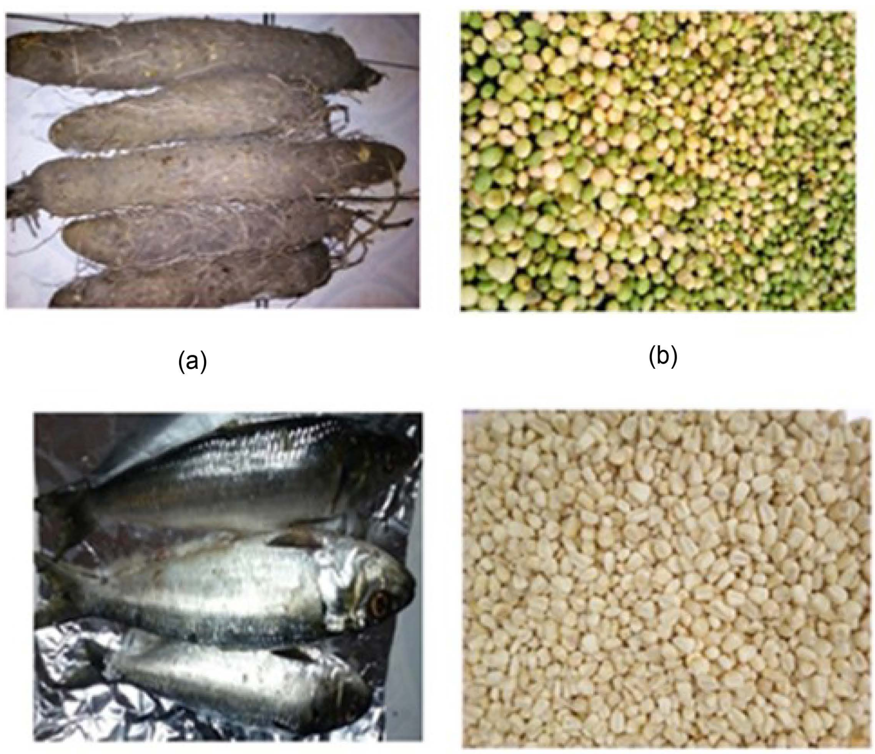

(a) (b) (c) (d)

Figure 1. *Diascorea cayenensis* (a); *Cajanus cajan* seeds (b); Fish (*Clupea harengus*) (c) and maize (*Zea mays*) seeds (d).

2.2. Methods

Before formulating the infant composite flours from the response surface by using the Box-Behnken matrix, we first proceeded separately to the elaboration of Yam, Pigeon pea and Herring flours following several unitary operations.

2.2.1. Preparation of Yam Flour

The yam tubers of the *cayenensis* species were manually sorted, weighed with a SUNRISE brand trade scale made in China (20 kg × 50 g), washed and cleaned with clean water. Then they were peeled, cut into pieces, weighed again on a precision scale of OHAUS Europe GmbH, Im Langacher 44, 8606 Greifensee, Switzerland, model PA423 (max: 210 g, d = 0.001 g), then, bleached at 70˚C for 15 min to facilitate drying and inactivate the enzymes. Afterwards, they were cut into 1 cm strips, dried in the Chinese DHP-9052 oven at 60˚C for 72 hours and in the boat type a solar dryer (a local manufacture) at a temperature close to 70˚C, crushed with a device of the type IKA-WERKE Gmbh-CO-KG, D-79219 Staufen, equipped with a 50 μm sieve to obtain a finer powder which will be stored away from air and insects.

2.2.2. Preparation of Pigeon Pea Flour

After reception, the pigeon peas were dehulled and/or sorted, weighed and dried in an air convection oven at 50˚C for 4 days and in a solar dryer for 3 to 4 days. Sorted and weighed again, they were soaked for 8 hours (3 V/Kg) to facilitate dehulling, drained, dehulled manually, and dried again in the oven at 40˚C to eliminate excess moisture, roasted for 30 min. The dried peas were then ground into flour, sieved through a 50 μm sieve and stored at room temperature in moisture-proof storage bags for the duration of the analysis.

2.2.3. Preparation of Herring Flour

After several chronological and manual steps of weighing, cleaning, washing with clean water, deheading, scaling, gutting, washing and weighing again; the herring fish was then filleted, oven dried at a temperature of 60˚C for 5 days. Once dry, the fish fillets were ground with a stainless steel electric grinder (Moulinex brand) to obtain the expected fish meal. The fish meal was then sieved with a 160 μm sieve, packaged and stored.

2.2.4. Preparation of the Sprouted Corn Flour

In order to obtain porridge of good caloric value and low viscosity, the corn kernels were malted. The malting consisted of soaking 2.5 kg of kernels in 20 L of clean, warm water for 24 hours to remove floating kernels, then soaking again in warm water for another 24 h before germinating. After removing water, we let the corn kernels germinate in a bag in the dark for 48 hours at room temperature. Then, the germinated grains were air-dried for 3 days, and their rootlets were removed before being finely ground with an electric hammer mill. The flour obtained is then packaged and stored.

2.3. Experimental Design

The optimization of the formulation conditions of infant flours was performed with the response surface method (RSM). A three-level Box-Behnken matrix was employed in this regard. Yam flour (X_1), herring flour (X_2) and pigeon pea flour (X_3) were chosen for the independent variables. The coded and uncoded independent variables and their levels are presented in **Table 1**. The independent variables and their ranges were selected on the basis of screening against chemical composition.

Experiments were designed according to the Box-Benhken centered matrix using Minitab software (Minitab, version 2011, n˚17.3.1.). The entire design consisted of 15 separate trials where the experimental conditions varied in a programmed manner in a completely randomized order (**Table 2**). Three replicates (experiments 13 - 15) in the center of the domain were conducted to determine the experimental error (analysis of variance), which error is incorporated in the calculation of the factor effects estimate. Taking into account the standard nutritional composition per 100 g of complement food [21] cited by Nago [22], eight (08) dependent variables or response noted Y contents of water (Y_1), ash (Y_2), oil (Y_3), protein (Y_4), sugar (Y_5), amount of total energy (Y_6), fiber (Y_7) and finally water absorption capacity (Y_8) were considered to evaluate the influence of independent variables on the composition of infant flour.

It should be noted that in the 15 distinct formulations, ten grams (10 g) of corn malt was incorporated in order to make the composite flour caloric and digestible, better yet infant.

A second order polynomial equation was used to express the different responses as a function of the independent variables as follows:

$$\begin{aligned} Y = {} & a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 \\ & + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 \end{aligned} \quad (1)$$

where Y represents the response variables; a_0 is a constant that fixes the response at the center point of the experiment; a_1, a_2, and a_3 represent the regression coefficients for the linear effect terms; a_1^2, a_2^2, and a_3^2 are the quadratic effect terms; and a_{12}, a_{13}, and a_{23} are the interaction effect terms, coded independent variables, respectively.

Minitab software (Minitab, version 2011, n˚17.3.1.) was used to determine the

Table 1. Independent variables and their coded and actual values used for the optimization.

Independent variables	Symbols	Coded variables		
		−1	0	1
Yam flour (g)	X_1	40	45	50
Herring meal (g)	X_2	10	15	20
Pigeon pea flour (g)	X_3	20	25	30

Table 2. Coded and uncoded values of the independent variables of the Box-Benhken centered matrix design for the optimization of infant flour formulation.

		Independent variables					
		Coded			Not coded		
(a)Order Statistics	(b)Test order	X_1 (g)	X_2 (g)	X_3 (g)	X_1 (g)	X_2 (g)	X_3 (g)
14	1	0	0	0	45	15	25
4	2	1	1	0	50	20	25
6	3	1	0	−1	50	15	20
2	4	1	−1	0	50	10	25
15	5	0	0	0	45	15	25
12	6	0	1	1	45	20	30
1	7	−1	−1	0	40	10	25
9	8	0	−1	−1	45	10	20
10	9	0	1	−1	45	20	20
8	10	1	0	1	50	15	30
7	11	−1	0	1	40	15	30
13	12	0	0	0	45	15	25
3	13	−1	1	0	40	20	25
5	14	−1	0	−1	40	15	20
11	15	0	−1	1	45	10	30

analysis of variance (ANOVA) and coefficient of determination (R^2) to estimate model fit.

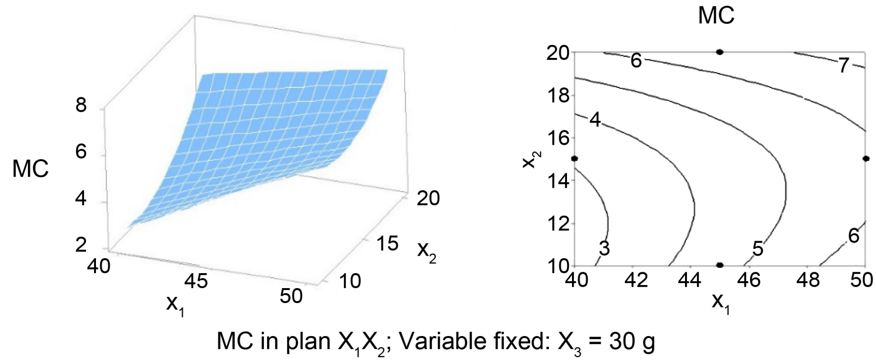
The significance of each coefficient was determined using the F-test and the value ($p < 0.05$) and the non-significant coefficients were removed to obtain a reduced model. The corresponding variables would be more significant if the absolute F-value becomes higher and the *p*-value becomes smaller [23] [24].

The relationship between the independent variables (X_1, X_2, and X_3) and the variable response (Y_1 to Y_8) was demonstrated by graphical representations of the 3- and 2-D response surface plots and isoresponse curves (**Figure 2**), respectively, generated by Minitab software, in order to derive the optimal conditions.

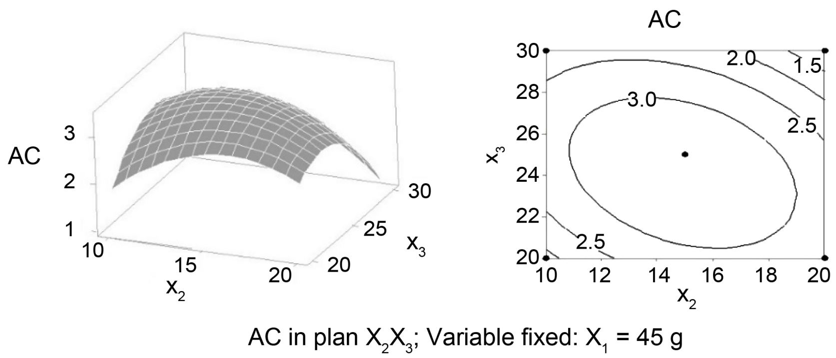
2.4. Analytical Methods for Basic and Composite Infant Flours

The analyses carried out were related to:

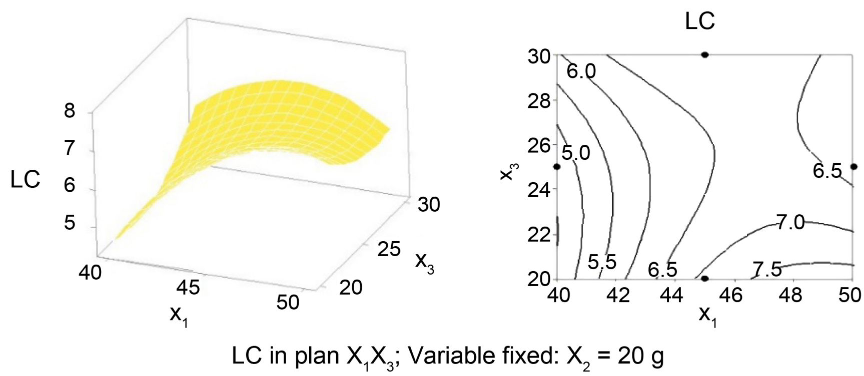
- Dry matter: where it was determined after drying by placing 5 g of sample in an oven at 105˚C for 24 hours [25];
- Total ash: where it was obtained after incineration of the powder sample in a muffle furnace heated to 450˚C - 550˚C for 8 h [25];


MC
X1
X2
MC in plan X1X2; Variable fixed: X3 = 30 g

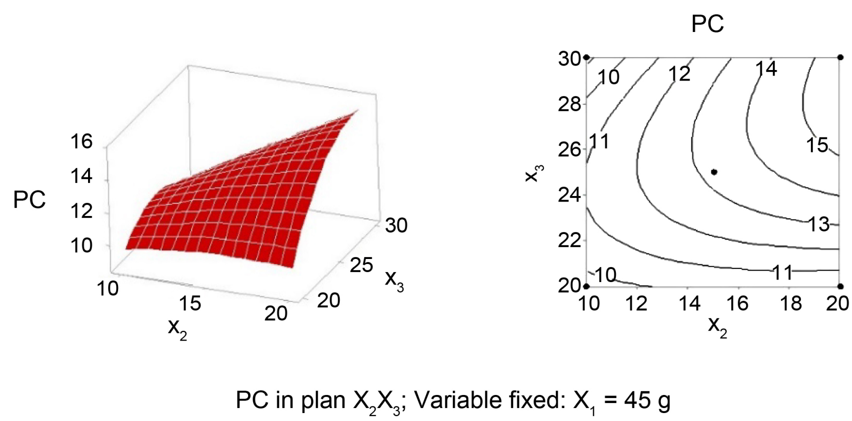
(a)


AC
X2
X3
AC in plan X2X3; Variable fixed: X1 = 45 g

(b)


LC
X1
X3
LC in plan X1X3; Variable fixed: X2 = 20 g

(c)


PC
X2
X3
PC in plan X2X3; Variable fixed: X1 = 45 g

(d)

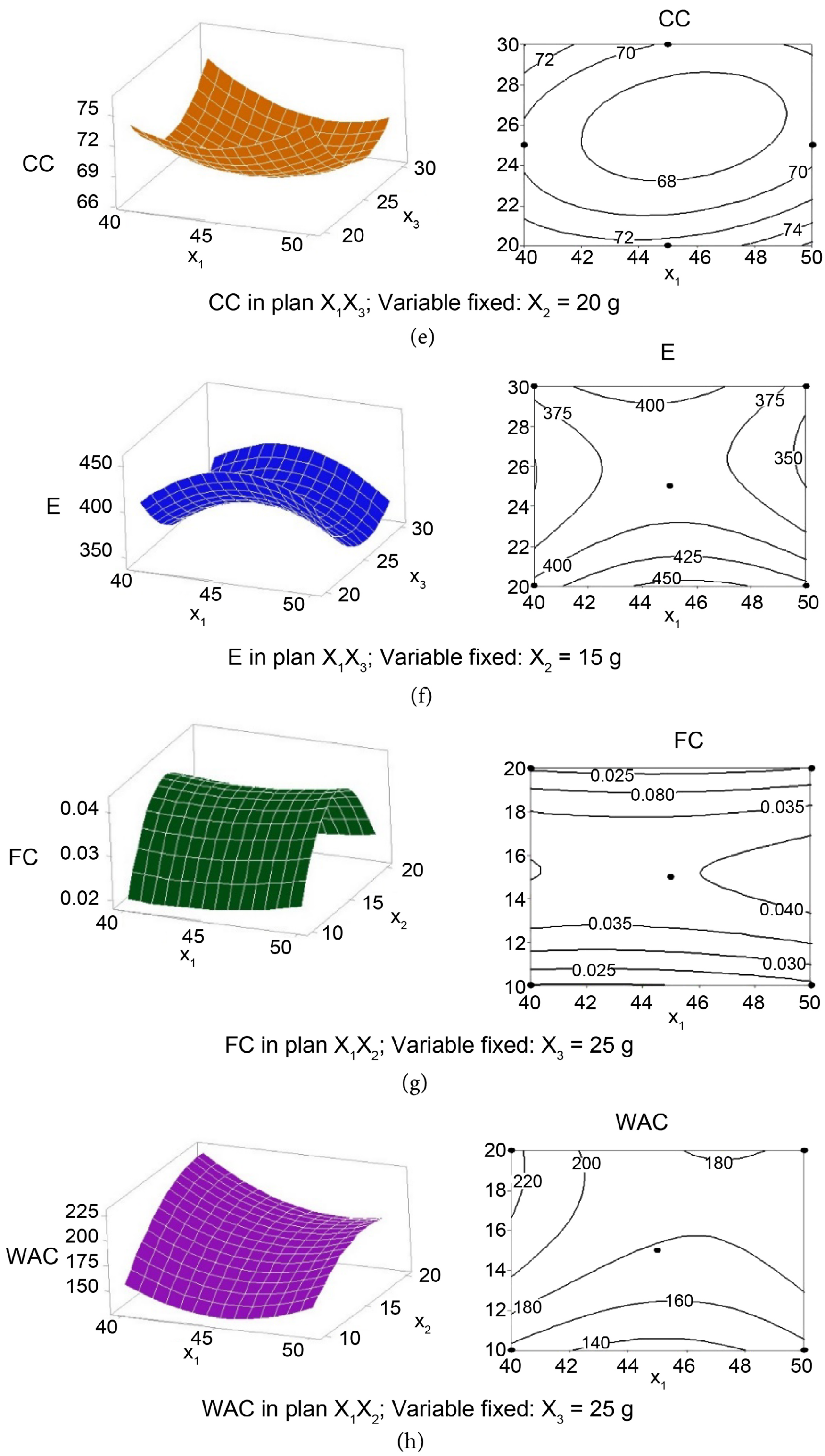


Figure 2. Response surface plots (3-D) and contour plots of (a) MC, (g) FC and (h) WAC as a function of the amount of yam and Herring flours for a fixed amount of pigeon pea flour at 30, 25 and 25 respectively; (b) AC and (d) PC as a function of the quantity of herring and pigeon pea flours for a fixed quantity of yam flour at 45; (c) LC, (e) CC and (f) E as a function of the quantity of yam and pigeon pea flours for a fixed quantity of herring flour at 20, 20 and 15 respectively.

- Lipid content: where this is obtained after extraction by soxhlet with hexane for 6 h [25];
- Protein content: it is determined by measuring the total nitrogen present in the samples according to the Kjeldahl method [26];
- Total carbohydrate content: which was deduced by difference by the method of Egan *et al.* [27] according to the formula:

$$\text{Carbohydrate rate}(\%) = 100 - \left[\text{P}(\%) + \text{L}(\%) + \text{Te}(\%) + \text{C}(\%)\right] \quad (2)$$

- Energy value: To determine the theoretical caloric value of infant formula, the products of the major constituents (carbohydrates, proteins, lipids) were summed with their Atwater heat coefficients [28] according to the formula:

$$\begin{aligned}&\text{Energy value}(\text{Kcal/100 g})\\&= (\%\text{Carbohydrates} \times 4) + (\%\text{Proteins} \times 4) + (\%\text{Lipids} \times 4)\end{aligned} \quad (3)$$

- Determination of dietary fiber: it was done by a chemical method adapted to the laboratory. We have weighed one gram (1 g) of flour in a flask to which 100 mL of sulfuric acid 0.25 N were poured. The mixture was boiled under reflux for 30 min. Then 100 mL of 0.31 N sodium hydroxide was added and heated for 30 min. The extract obtained is filtered through a Whatman filter paper. Once recovered, it is dried in an oven at 130˚C for 2 h. The dry residue is then incinerated in a muffle furnace at 600˚C for 30 min and weighed for determination of the crude fiber content.
- Water absorption capacity (WAC) of the flours: it was evaluated according to the method described by Phillips R. D *et al.* [29].

3. Results and Discussion

3.1. Preliminary Study

A Box-Behnken matrix (BBD) design was used to elucidate the main effects and interactions of the parameters involved in the formulation of infant flours: influence of the amount of yam flour (X_1: 40 - 50 g), herring flour (X_2: 10 - 20 g) and pigeon pea flour (X_3: 20 - 30 g).

The eight responses of interest and the results of 15 experimental trials using the BBD design (**Table 3**), which include part of the design and observed experimental responses. The water and ash contents range from 3.364% - 10.214% and 0.789% - 4.668% respectively. The highest water content (10.214%) was obtained under the experimental conditions of X_1 = 40 g, X_2 = 20 g and X_3 = 25 g; while the lowest ash content (0.789%) was obtained under the conditions X_1 = 20 mL/g, X_2 = 55˚C and X_3 = 100 min. The results of **Table 3** indicate that according to the standard of the global composition of infant flours (WC fixed at 5% and AC at 2%), only experiences 6, 7 and 11 for MC; 1, 3, 12 and 14 for AC meet the standard. In the same spirit of analysis, the values of LP, PC, CC, E, FC and WAC vary respectively from: 3.311% - 7.725%.; 8.400% - 16.713%; 66.864% - 78.216%; 359.598 - 630.077 Calories; 0.007% - 0.065% and finally 102 - 283 (no

Table 3. Experimental data obtained for the dependent variables.

[(a)]Order Statistics	[(b)]Test order	MC (%)	AC (%)	LC (%)	PC (%)	CC (%)	E (Cal)	FC (%)	WAC (%)
14	1	8.409	1.964	7.725	13.825	66.864	392.284	0.040	229.167
4	2	8.494	1.577	7.551	13.563	68.428	395.925	0.026	189.583
6	3	4.928	4.418	5.457	10.719	77.319	401.262	0.007	283.333
2	4	9.016	2.262	2.667	11.200	72.699	359.598	0.021	108.333
15	5	7.471	1.185	5.792	12.600	71.876	390.030	0.041	138.889
12	6	6.204	2.082	6.945	16.713	68.953	405.170	0.014	102.381
1	7	8.352	1.717	3.311	8.400	77.854	374.817	0.019	150.000
9	8	8.776	3.872	4.000	8.400	77.107	378.028	0.016	182.500
10	9	7.909	2.868	6.271	8.803	73.145	630.077	0.015	210.294
8	10	6.327	2.240	4.183	11.375	75.247	384.131	0.065	134.812
7	11	3.364	4.668	7.430	8.750	78.216	414.734	0.055	147.849
13	12	7.741	1.195	4.678	13.210	69.703	373.750	0.038	166.667
3	13	10.214	2.289	4.333	13.384	70.874	362.650	0.027	270.833
5	14	7.926	0.789	5.773	9.734	74.278	388.006	0.009	230.208
11	15	4.345	0.789	6.847	10.063	77.957	413.697	0.013	130.000

MC: Moisture content; AC: Ash content; LC: Lipid content; PC: Protein content; CC: Carbohydrates content; E: Energie; FC: Fiber content; WAC: Water absorption capacity.

unit). Still in relation to the standard norm in LC (≥7%), PC (≥13%), and E (~400 Cal), five experiments for each and respectively responses (1, 4, 7, 12, and 14); (3, 4, 12, 13, and 14); (4, 6, 12, 14, and 14) are in agreement. As for the CC (norm equal to 68%) and FC (norm 5%) respectively four (4, 12, 13, and 14) and all 15 experiments obey this same norm. On the other hand, there is no norm for the WAC. Therefore, an optimization process was highlighted, in order to obtain the formulation meeting the desirable criteria of infant flours.

3.2. Model Fitting and Interpretation of Response Surfaces

Tables 4(a)-(c) summarizes the ANOVA results, including the coded regression coefficients for the second-order polynomial equation, coefficients of determination (R^2), and F and p-values for the response variables MC (Y_1), AC (Y_2), LC (Y_3), PC (Y_4), CC (Y_5), E (Y_6), FC (Y_7), and WAC (Y_8) under different formulation conditions. Most of the R^2 values of the response variables being ≥ 0.6; the developed quadratic models can be considered as valid or adequate models [30] [31]. This indicates that the regression model well explains the influence of the independent variables on the responses of the formulated infant flours. The quadratic models adjusted for the eight responses in coded variables are given in

Table 4. (a): Analysis of variance and regression coefficients of water (MC), ash (AC) and lipid (LC) content responses of flours. (b): Analysis of variance and regression coefficients of protein (PC), ash content (CC) and energy (E) responses of flours. (c): Analysis of variance and regression coefficients of fiber content (FC) and water absorption capacity (WAC) responses of flours.

(a)

Term									
Variables	MC			AC			LC		
	Coefficients	Value of *F*	Value of *p*	Coefficients	Value of *F*	Value of *p*	Coefficients	Value of *F*	Value of *p*
Constant (a_0)	7.874			3.369			6.065		
Linear									
a_1	−0.136	0.43	0.540	0.378	0.50	0.513	−0.124	0.050	0.832
a_2	0.291	1.98	0.219	−0.099	0.03	0.861	1.035	3.490	0.121
a_3	−1.162	31.46	0.002	−0.297	0.31	0.604	0.488	0.780	0.418
Quadratic									
a_1* a_1	−0.014	0.00	0.966	−0.300	0.14	0.719	−0.952	1.370	0.295
a_2* a_2	1.159	14.44	0.013	−0.654	0.69	0.446	−0.647	0.630	0.463
a_3* a_3	−2.224	53.17	0.001	−0.824	1.09	0.344	0.598	0.540	0.496
Interaction									
a_1* a_2	−0.596	4.13	0.098	−0.392	0.27	0.628	0.966	1.520	0.272
a_1* a_3	1.490	25.87	0.004	0.335	0.20	0.677	−0.733	0.880	0.392
a_2* a_3	0.682	5.41	0.068	−0.440	0.34	0.587	−0.543	0.480	0.519
R^2	96.59%			40.10%			66.30%		

a_0 is the constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients of the polynomial quadratic equation, respectively; R^2: Coefficient of determination; F: Fisher's test of equality of two variances; p: probability of obtaining the results of a test (limit value of $p = 0.05$).

(b)

Term									
Variables	PC			CC			E		
	Coefficients	Value of *F*	Value of *p*	Coefficients	Value of *F*	Value of *p*	Coefficients	Value of *F*	Value of *p*
Constant (a_0)	13.212			69.48			385.4		
Linear									
a_1	0.823	2.080	0.209	−0.941	1.490	0.276	0.100	0.000	0.996
a_2	1.800	9.920	0.025	−3.027	15.430	0.011	33.500	3.960	0.103
a_3	1.156	4.090	0.099	−0.185	0.060	0.820	−22.500	1.780	0.239
Quadratic									
a_1* a_1	−1.212	2.080	0.209	2.480	4.780	0.081	−35.900	2.100	0.207
a_2* a_2	−0.363	0.190	0.684	0.500	0.200	0.675	23.800	0.920	0.381
a_3* a_3	−1.855	4.860	0.079	4.310	14.410	0.013	47.600	3.690	0.113
Interaction									
a_1* a_2	−0.655	0.660	0.454	0.680	0.390	0.562	12.100	0.260	0.632
a_1* a_3	0.410	0.260	0.633	−1.500	1.900	0.226	−11.000	0.210	0.664
a_2* a_3	1.562	3.730	0.111	−1.260	1.340	0.300	−65.100	7.500	0.041
R^2	84.50%			88.54%			80.65%		

a_0 is the constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients of the polynomial quadratic equation, respectively; R^2: Coefficient of determination; F: Fisher's test of equality of two variances; p: probability of obtaining the results of a test (limit value of $p = 0.05$).

(c)

Term						
Variables	MC			AC		
	Coefficients	Value of *F*	Value of *p*	Coefficients	Value of *F*	Value of *p*
Constant (a_0)	0.040			178.200		
Linear						
a_1	0.001	0.030	0.861	−10.400	0.320	0.595
a_2	0.002	0.070	0.801	25.300	1.920	0.224
a_3	0.013	4.190	0.096	−48.900	7.190	0.044
Quadratic						
a_1* a_1	0.002	0.030	0.870	22.100	0.680	0.448
a_2* a_2	−0.018	4.000	0.102	−20.700	0.590	0.477
a_3* a_3	−0.007	0.640	0.459	−1.300	0.000	0.964
Interaction						
a_1* a_2	−0.001	0.010	0.934	−9.900	0.150	0.717
a_1* a_3	0.003	0.120	0.742	−16.500	0.410	0.550
a_2* a_3	0.001	0.000	0.956	−13.900	0.290	0.614
R^2	64.26%			69.98%		

a_0 is the constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients of the polynomial quadratic equation, respectively; R^2: Coefficient of determination; F: Fisher's test of equality of two variances; p: probability of obtaining the results of a test (limit value of $p = 0.05$).

Equations (4)-(11) as follows:

$$Y_1 = 7.874 - 0.136X_1 + 0.291X_2 - 0.162X_3 - 0.596X_1X_2 + 1.490X_1X_3 + 0.682X_2X_3 - 0.014X_1^2 + 1.159X_2^2 - 2.224X_3^2 \quad (4)$$

$$Y_2 = 3.369 + 0.378X_1 - 0.099X_2 - 0.297X_3 - 0.392X_1X_2 + 0.335X_1X_3 - 0.440X_2X_3 - 0.300X_1^2 - 0.654X_2^2 - 0.824X_3^2 \quad (5)$$

$$Y_3 = 6.065 - 0.124X_1 + 1.035X_2 - 0.488X_3 + 0.966X_1X_2 - 0.733X_1X_3 - 0.543X_2X_3 - 0.952X_1^2 - 0.647X_2^2 + 0.598X_3^2 \quad (6)$$

$$Y_4 = 13.212 + 0.823X_1 + 1.800X_2 + 1.156X_3 - 0.655X_1X_2 + 0.410X_1X_3 + 1.562X_2X_3 - 1.212X_1^2 - 0.363X_2^2 - 1.855X_3^2 \quad (7)$$

$$Y_5 = 69.48 - 0.941X_1 - 3.027X_2 - 0.185X_3 + 0.68X_1X_2 - 1.50X_1X_3 - 1.26X_2X_3 + 2.48X_1^2 + 0.50X_2^2 + 4.31X_3^2 \quad (8)$$

$$Y_6 = 385.4 + 0.100X_1 + 33.500X_2 - 22.500X_3 + 12.100X_1X_2 - 11.000X_1X_3 - 65.100X_2X_3 - 35.900X_1^2 - 23.800X_2^2 + 47.600X_3^2 \quad (9)$$

$$Y_7 = 0.040 + 0.001X_1 + 0.002X_2 + 0.013X_3 - 0.001X_1X_2 + 0.003X_1X_3 + 0.001X_2X_3 + 0.002X_1^2 - 0.018X_2^2 - 0.007X_3^2 \quad (10)$$

$$Y_8 = 178.2 - 10.400X_1 + 25.300X_2 - 48.900X_3 - 9.900X_1X_2 - 16.500X_1X_3 - 13.900X_2X_3 + 22.100X_1^2 - 20.7000X_2^2 - 1.300X_3^2 \quad (11)$$

To visualize the effect of the independent variables on the dependent variables, contour curve plots (2-D) and response surface plots (3-D) (**Figure 2**) of the quadric polynomial models were generated by varying two of the independent variables in the experimental range while holding one of the variables constant at its respective lowest, middle, and highest level.

The response surface method effectively identifies the optimal values of the independent variables so that the responses are maximized by taking into account the nutritional value of the components of the supplemental infant flours following the codex alimentarius recommended standard [32] and the standard norm Nago [22] cited by Adjadogbedji [33].

3.2.1. Moisture Content (MC)

For this response (**Table 4(a)**), significant effects were observed for only one linear term ($p < 0.01$) at the level of the factor X_3 (pigeon pea flour), and at the level of two quadratic terms X_2^2 ($p < 0.01$) and X_3^2 ($p < 0.001$) respectively. Pigeon pea flour (X_3) showed a negative effect on the linear term (−1.162) but showed a positive effect (1.159) and a negative effect (−2.224) on its quadratic terms. This indicates that the relationships between herring meal (X_2) and MC, pigeon pea meal (X_3) and MC follow a curved line. The interaction effect between factor X_1 (yam flour) and factor X_3 (pigeon pea flour) was significant ($p < 0.004$) and its effect was positive (1.490) on MC. This means that the effect of Yam flour on the moisture content of the composite flour depends on the pigeon pea flour. The latter has a negative influence on the water content, which has a mean value of 7.874%, which is higher than 5%, the value recommended by the standard norm for infant flours. The coefficient of determination (R^2) is of the order of 96.59%, indicating that the model explains more than 96% of the variation in the observed data. To visualize the effect of the independent variables on MC, the objective is to target its value at 5% (standard infant formula) to obtain an adjusted response.

The different plans were defined as follows: Plan X_1 and X_2 (fixed variable X_3 = 30 g); Plan X_1 and X_3 (fixed variable X_2 = 15 g); Plan X_2 and X_3 (fixed variable X_1 = 45 g).

The 3D response surfaces and contour plot **Figure 2(a)** showed the changes in MC values as a function of the Yam flour and Herring flour factors. The results show that in the X_2 (10 - 20 g) and X_3 (20 - 30 g) plane where X_1 at its average level of 45 g (figure not shown), higher MC values (5% - 9%) are obtained, compared to the X_1 (40 - 50 g) and X_2 (10 - 20 g) plane where X_3 at its upper level of 30 g (**Figure 2(a)**) and where MC is between 3% - 7%.

Almost similar values (4% - 8%) are obtained when these two parameters X_1 and X_3 are varied in the plane and X_2 is fixed at 15 g (figure not shown). It can be concluded that for the moisture content to reach its target value, we would have to quantify the yam flour at 47 g and the herring flour at 15 g.

3.2.2. Ash Content (AC)

Analysis of the regression coefficients (**Table 4(a)**) showed that for the AC response, the linear (X_1, X_2, and X_3), quadratic (X_1^2 , X_2^2 , and X_3^3), and interaction (X_{12}; X_{13}; and X_{23}) terms had no significant effect ($p > 0.05$).

The linear terms X_1, X_2 and X_3 have *p*-values of the order of 0.513, 0.861 and 0.604 respectively. The quadratic terms X_1^2, X_2^2 and X_3^3 also have *p*-values of 0.719; 0.446 and 0.344 respectively. This indicates that the relationships between yam, herring and pigeon pea flours and AC follow a straight line. For the interaction terms X_{12}; X_{13} and X_{23}, the *p*-values are 0.628; 0.677; 0.580 respectively. All of these results allow us to suggest that the three factors have no influence on the ash content. The coefficient of determination (R^2) is of the order of 40.10%, indicating that the model explains only 40% of the variation in the observed data. This does not suggest a good fit to the mathematical model Equation (3). The standard for infant formula recommends that the ash content be 2%. The target ash value is only reached if the yam and herring flours are quantified around 45 and 15 g respectively in the X_1 and X_2 versus X_3 30 g plane (figure not shown); 27 and 50 g respectively for pigeon pea and yam flours in the plane where the herring flour factor is kept at its upper level of 20 g (figure not shown); finally 20 and 28 g respectively for herring and pigeon pea flours (**Figure 2(b)**) when factor X_1 (yam flour) is fixed at its average level of 45 g (figure not shown). Since all three factors (X_1, X_2 and X_3) were not significant in the response evaluated, it is specified that it is in the X_2 and X_3 versus X_1 = 45 g (fixed variable) design that larger AC values between 1.5% - 3.0% are obtained compared to the other two designs where the values are between 1.0% - 2.5%.

3.2.3. Lipid Content (LC)

As for the ash content, the analysis of the regression coefficients (**Table 4(a)**) showed no significant effect ($p > 0.05$) for the LC response for all the linear (X_1, X_2 and X_3), quadratic (X_1^2 , X_2^2 and X_3^3) and interaction (X_{12}; X_{13} and X_{23}) terms. All these results allow us to suggest that the three factors have no influence on the lipid content. Since there are no significant quadratic effects, this indicates that the relationships between yam, herring and pigeon pea flours and LC follow a straight line. The coefficient of determination (R^2) is about 66.30%, indicating that the model explains only 66% of the observed data variation.

The plots of the area diagrams and isoresponse curves in the experimental designs X_1 (40 - 50 g) and X_2 (10 - 20 g) versus X_3 = 25 g (figure not shown); X_1 and X_3 (20 - 30 g) versus X_2 = 20 g (**Figure 2(c)**); X_2 and X_3 versus X_1 = 50 g (figure not shown), reveal to us the set of possible responses to be obtained. The results show that the X_1 (40 - 50 g) and X_3 (20 - 30 g) plan versus X_2 maintained at its higher level of 20 g would give a higher LC yield (5% - 7.5%) compared to the other plans, although all of them allow to reach the target value of 7%, which is the recommended value for the standard nutritional composition for 100 g of weaning feed. In this case, we would have to quantify the yam meal at about 46 g and 22 g of pigeon pea meal.

3.2.4. Protein Content (PC)

Table 4(b) indicates that the variable with the largest linear effect on PC was the amount of herring meal (X_2) with $p = 0.025$ (0.05); the other two linear terms X_1 (amount of yam meal) and X_3 (amount of pigeon pea meal) did not show a significant effect ($p > 0.05$). However, all three quadratic terms did not show a significant effect ($p > 0.05$) on the response, thus indicating that the relationships between flours and protein content follow a straight line. The interaction terms X_{12}; X_{13} and X_{23} had p-values of 0.454; 0.633 and 0.111 respectively; also did not show significant effects ($p > 0.05$) on the response. The values of the protein content response did not depend on the effect of one factor on another. The coefficient of determination (R^2) is about 84.50%, indicating that the model is adequate to explain more than 84% of the observed variation in the data.

In the case of protein content, the observations made on the graphs show that when the pigeon pea flour (X_3) and herring flour (X_2) factors are maintained at their upper level of 30 and 20 g respectively, and the yam flour factor (X_1) is maintained at its average level of 45 g, the recommended limit value of 13% is reached. The highest values are obtained in the X_2, X_3 and $X_1 = 45$ g (fixed variable) design by quantifying 15 g and 25 g of herring and pigeon pea flours respectively (**Figure 2(d)**).

3.2.5. Carbohydrate Content (CC)

For this response (**Table 4(b)**), there is only one negative significant effect (−3.027) in the linear term X_2 ($p < 0.01$). Two square terms (X_1^1 and X_2^2) have p-values greater than 0.05 (0.081 and 0.675 respectively), which is equivalent to saying that there are no significant effects. While for the squared effect X_3^3 with p-value less than 0.05 ($p = 0.013$), shows a positive significant effect (4.31). The relationship between pigeon pea flour and carbohydrate content follows a curved line rather than a straight line. No interaction was observed between the factors for this response as the p-values were 0.562; 0.226; 0.300 for X_{12}; X_{13} and X_{23} respectively, thus greater than 0.05. This means that there are no significant interaction effects and the influence of one factor on the CC response does not depend on any other factor. The coefficient of determination (R^2) is of the order of 88.54%, indicating that the model is valid and explains more than 88% of the variation in the observed data.

Referring to the standard norm of infant flours, we targeted the value of carbohydrate content at 68% to exploit the optimization graphs in all designs X_1 (40 - 50 g) and X_2 (10 - 20 g) versus $X_3 = 25$ g (figure not shown); X_1 and X_3 (20 - 30 g) versus $X_2 = 20$ g (**Figure 2(e)**); X_2 and X_3 versus $X_1 = 50$ g (figure not shown). It was found after exploitation of the results, that in all the designs, the CC values vary from 68% - 75%.

In order for the response to be at its adjusted value of 68%, the formulation would have to be composed with 45 g of yam flour, 18 g of herring flour and 25 g of pigeon pea flour. It can also be formulated by quantifying 24 - 28 g pigeon pea flour, 42 - 49 g yam flour when the herring flour is fixed at 20 g. This is also the case under conditions where the amount of yam flour is set at its highest level (50 g), only if herring and pigeon pea flour are quantified at 18 g and 25 g respectively.

3.2.6. Total Energy (E)

The analysis of the regression coefficients (**Table 4(b)**) showed that for the response E, the linear (X_1, X_2 and X_3) and quadratic (X_1^2, X_2^2 and X_3^3) terms do not present any significant effect ($p > 0.05$). On the other hand, for the effects of the interactions (X_{12}, X_{13} and X_{23}), the model contains only one significant effect ($p = 0.041$) and negative (−65.10) for the term X_{23}. This result indicates that the effect of herring meal on energy depends on pigeon pea meal. The coefficient of determination (R^2) obtained indicates that the model is valid and is of the order of 80.65%. Taking into account the standard norm and the Codex Alimentarius norm [34], the value of energy intake was targeted at 400 calories. The best results are obtained when the herring meal factor is maintained at its average level of 15 g, and/or the quantities of pigeonpea and yam meal should be quantified around 23 and 45 g respectively (**Figure 2(f)**). Nevertheless, the target value can also be reached under the following conditions: X_1 (41 and 49 g) and X_2 (12 and 14 g) for X_3 = 20 g; X_2 (17 g) and X_3 (25) for the fixed variable X_1 = 45 g.

3.2.7. Fiber Content (FC)

The ANOVA (**Table 4(c)**) for the FC response indicates that all linear (X_1, X_2 and X_3), quadratic (X_1^2, X_2^2 and X_3^3) and interaction (X_{12}; X_{13} and X_{23}) terms show no statistically significant effect ($p > 0.05$). Given the lack of interaction effect, the influence of one factor on the fiber content response does not depend on any other factor. The coefficient of determination (R^2) is about 64.26%; indicating that the model can be considered adequate and explains 64% of the observed data variation. **Figure 2(g)** shows the 2-D and 3-D plot for FC as a function of yam and herring flours, with 25 g of pigeon pea flour. According to the standard norm of infant flours, the value of fiber content should be less than 5%. Our results were not satisfactory where the values were well below the standard; which therefore led us to maximize the response where the formulation conditions could be as follows: X_1 (46 g) and X_2 (15 g) for X_3 = 25 g (fixed variable); X_1 (50 g) and X_3 (26 g) for X_2 = 20 g (fixed variable), finally X_2 (15 g) and X_3 (25) for the fixed variable X_1 = 45 g.

3.2.8. Water Absorption Capacity (WAC)

The WAC response was negatively influenced by a significant linear effect (−48.9) at the level of factor X_3 according to the results given in **Table 4(c)**, while all other factors were statistically insignificant for the response ($p > 0.05$). These are the two linear terms (X_1, and X_2), all quadratic terms (X_1^2, X_2^2 and X_3^3) and interactions (X_{12}; X_{13} and X_{23}). These results indicate that the relationships between flours and fiber content follow a straight line, and that the effect of one factor on this response does not depend on another factor. The coefficient of determination (R^2) obtained indicates that the model is valid and is of the order of 69.98%. As far as the WAC is concerned, there are no standards. Our results showed that the WAC varies between 102.381 and 283.333. Only, the literature states that when a flour has a high WAC, it has good functional properties such as viscosity, workability but also can preserve against protein dissolution. In relation to the results obtained, we prefer to minimize the value of WAC. This leads us to propose the following formulation conditions: X_1 (45 g) and X_2 (10.5 g) for X_3 = 25 g (fixed variable) (**Figure 2(h)**); X_1 (50 g) and X_3 (29 g) for X_2 = 20 g (fixed variable), finally X_2 (20 g) and X_3 (29) for the fixed variable X_1 = 50 g.

3.3. Optimization of the Response

Optimizing the conditions composite infant flour production was achieved using a multiple response method called desirability [35] [36]. This method involves transforming each response variable (Y_i) into an individual desirability function (d_i). This method looks for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. The scale of the desirability function ranges from 0 to 1; $d = 0$, for a totally undesirable response, to $d = 1$ for a fully desired response above which further improvements would not matter [37].

Individual desires (d) for each response were obtained by specifying the goals and limits required for each response. There were three goals to choose from: minimize the response, target the response, or maximize the response. The upper and lower bounds for each objective should also be specified. A weighting factor, which defines the shape of the desirability function for each response, was then assigned for each response. The weights should be between 0.1 and 10, with higher weights corresponding to more important responses and lower weights corresponding to less important responses. Once the individual desirabilities were calculated for each response, they were combined to provide a composite desirability measure of the multiple response system. This composite desirability measure is the weighted geometric mean of the individual desirabilities or responses [38].

In order to optimize the response, and with reference to the standard nutritional composition for 100 g of complement flour, the objectives were to individually target the values of the MC, AC, LC, PC, CC, and E responses relative to this standard; to maximize FC and then minimize WAC. Goal setting, lower and upper limits, weights, and individual importance are shown in **Table 5**. For MC, the goal is to obtain a value equal to or close to the 5% target value. Values below 3.364 or above 10.214% are not acceptable. For AC, the goal is to obtain a value equal to or close to the 2% target value. Values below 0.789 or above 4.668% are not acceptable. The objectives for LC, PC, CC, and E were to obtain target values or close to 7%, 13%, 68%, and 400 calories, respectively. For each of the latter responses, values below 2.667 or above 7.725%; below 8.400 or above 16.712%; below 66.864 or above 78.216%; and below 359.598 or above 630.077 calories, respectively, were not acceptable. The objective for FC is to maximize the value where 0.065% or more is considered excellent, while those below 0.007% are not acceptable. Finally, the objective for the WAC is to minimize the value. A value

Table 5. Responses optimization parameters.

Responses	Objective	Lower	Target	Higher	Weighting	Importance
MC	Target	3.364	5.000	10.214	1	1
AC	Target	0.789	2.000	4.668	1	1
LC	Target	2.667	7.000	7.725	1	1
PC	Target	8.400	13.000	16.712	1	1
CC	Target	66.864	68.000	78.216	1	1
E	Target	359.598	400.000	630.077	1	1
FC	Maximum	0.007	0.065		1	1
WAC	Minimum		102.381	283.333	1	1

Table 6. Optimal plan and predicted responses.

Responses				MC	AC	LC	PC	CC	E	FC	WAC	
	A	B	C	Va	Va	Va	Va	Va	Va	Va	Va	
Solution	46.263	15.859	30	5.004 I D 0.999	2.280 I D 0.895	6.981 I D 0.996	13.285 I D 0.923	72.451 I D 0.564	401.237 I D 0.995	0.0459 I D 0.671	123.580 I D 0.883	DC 0.850

I D: Individual Desirability; Va: adjusted value; DC: Desirability Composit.

of 102.381% or less is considered excellent, while those above 283.333% are not acceptable.

All responses have the same importance value of 1 on the composite desirability. In other words, the quality of the flour is equally dependent on all evaluated responses.

From the optimization parameters, the optimal conditions for the composite infant flour formulation are obtained along with the predicted response values shown in Table 6. The individual desirability of MC, AC, LC, PC, E and WAC is 0.999; 0.895; 0.995; 0.923; 0.995 and 0.883 respectively, whereas individual desirability of CC and FC were much lower at 0.564 and 0.671 respectively. The composite desirability was evaluated at 0.850. The optimal formulation conditions for infant-type flour correspond to 46.263 g of yam flour (X_1), 15.859 g of herring flour (X_2) and 30.00 g of pigeon pea flour (X_3).

Figure 3 shows predicted responses generated from the optimized factors. Each column and row of the graph corresponds to a factor and a response variable, respectively. The upper and lower limits of the factors and the current factor setting in parentheses or square brackets are displayed in the top row. The response objective, the expected response to the current factor settings, y, and the individual desirability, d, are shown in the left column.

The vertical and horizontal lines on the graph represent the current factor parameters and response values, respectively. In general, when increasing the amount of yam flour, the MC, AC, PC and FC responses increase, while LC, CC,

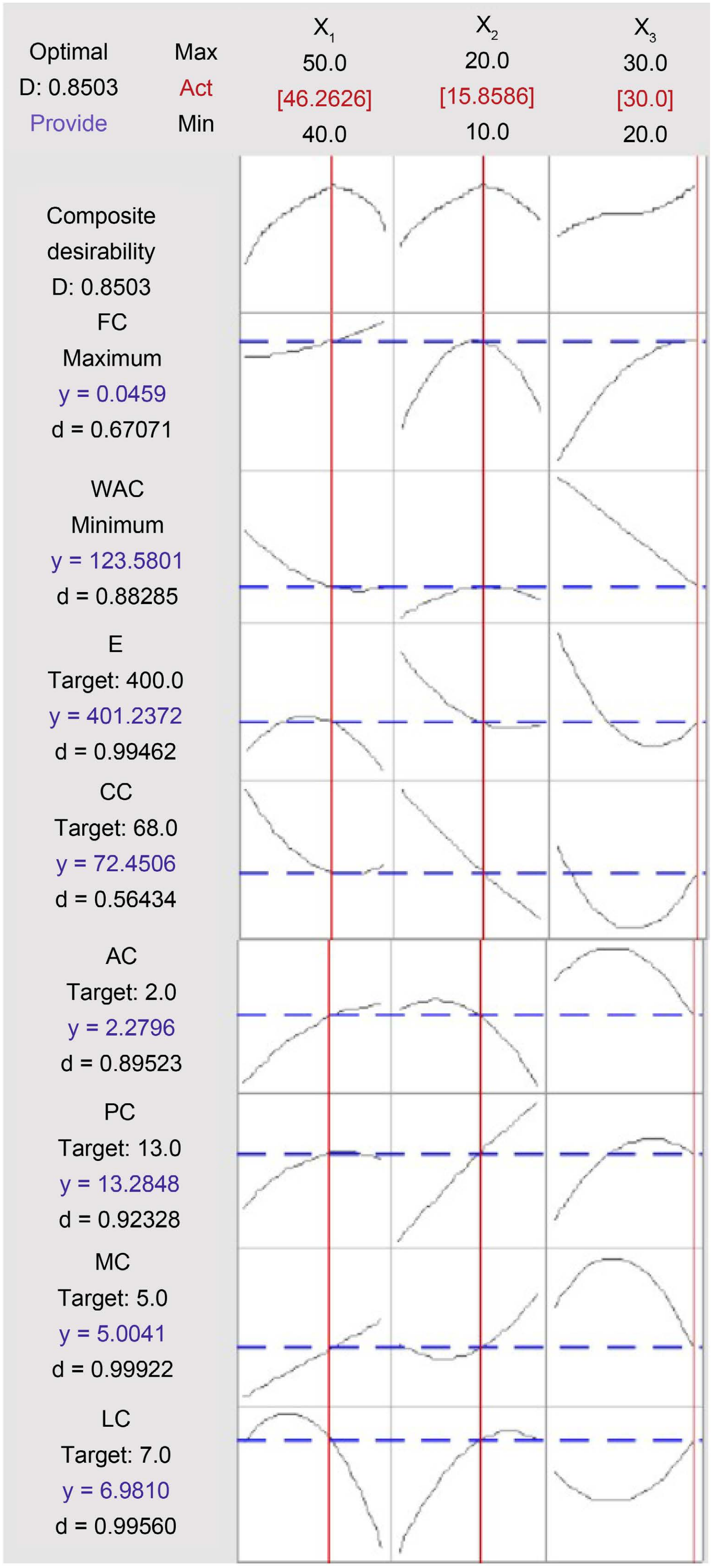


Figure 3. Simultaneous optimization of infant composite flour formulation conditions based on independent variables, predicted response variables, and desirability profile.

E and WAC decrease. Increasing herring meal decreased energy, ash, carbohydrate, while the other responses increased. There was only an increase in fiber, protein and lipids as the pigeon pea meal load increased while the other responses decreased.

4. Conclusion

The purpose of this study was achieved. The RSM method used to determine the optimal conditions for the formulation of a composite infant flour from local products was carried out using several quadratic models. These models were developed from the coefficients obtained from regression analysis of the experimental data. All models were able to predict the response performance to at least 60% accuracy. The graphical optimization method was adopted to determine the best mixing conditions. Using the Box-Behnken design of experiment, an optimal flour blend formulation was obtained by setting target values, while minimizing and maximizing response values with the best definition of each quality response of the composite infant flour. The optimal conditions based on both individual and combined responses yielded: 46.263 g of yam flour (X_1), 15.859 g of herring flour (X_2) and 30.00 g of pigeon pea flour (X_3).

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Conflicts of Interest

The authors declare that we do not have any conflict of interest.

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Nomenclature

RSM: Response Surface Method.

BBD: Box-Bohnken Matrix.

WAC: Water absorption capacity.

MC: Water content.

AC: Ash content.

LC: Fat content.

CC: Carbohydrate content.

PC: Protein content.

FC: Fiber content.

E: Energy.