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Evaluation of microbial community, modelling and optimization of processing parameters of a Maize-Cowpea blend weaning formulae.

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Abstract

Protein deficiency and malnutrition in locally processed infant formulae has been reported to cause stunted growth and preventable diseases. This study was carried out to determine the ideal processing conditions and microorganisms involved in the production of a weaning food made from maize and cowpea blend especially in the protein content responseA Box-Behnken Design method under Response Surface Methodology (RSM) was used to set up methodology for weaning food preparation. Evaluation of the microbial community responsible for fermentation was investigated using standard microbiological methods. Proximate composition was determined to ascertain the nutritional quality of the blended product. Sensory evaluation was conducted using the 9-point hedonic scale to determine the most acceptable combinations requirements by World Health Organization (WHO) standard for weaning foods. Statistical analysis of the data was carried out using Minitab (Version 17.0). The final product after processing yielded dry flour of maize and cowpea blend. *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium* sp and *Micrococcus* sp, *Lactobacillus lactis* and *Streptococcus faecalis* were predominant in the samples. Results from proximate composition analysis showed variances in moisture, ash, crude protein, and crude fiber content of the different samples. Although, Sample code WFC (cowpea substitution 35%, pH 5.5 and fermentation time 72 hours) was most acceptable for weaning mothers/babies, Sample code WFA (cowpea substitution in maize 15%, pH 3.5, fermentation time 48 hours) met the requirements of WHO standard for weaning food nutrient composition. The prospect of producing a traditional fortified weaning food of WHO standard can be achieved if these conditions are carefully explored.

Keywords: Weaning food, protein deficiency, fortification, optimization, response surface methodology.

Introduction

Aptly, weaning is the process of gradually introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's milk (Nwakalor & Obi, 2014; (MacDonald, 2016)). Weaning is an important process in the life of an infant because is confers many advantages to the infants such as; ensuring adequate energy intake; Bioavailability of nutrients such as iron, vitamin D, zinc, and copper; better neuromuscular coordination involving mouth movements such as biting, chewing etc; better speech developments resulting from better mouth and tongue movement and easier acceptance for variety of tastes and flavours.

To ensure that infant's nutritional needs are met, a careful selection of solid foods in the proper amount is essential. Formulated weaning foods must be soft, acceptable and must contain the essential nutrients in the correct proportion to ensure proper growth and development of the mammalian progeny. Many studies have looked into the improvement of weaning food with the hope of achieving the desired goal of a balanced nutrition for infants (Kulkarni *et al*., 1991; Nwakalor & Obi, 2014)). These above-mentioned studies were focused on different properties and characteristics. However, in general the weaning food was evaluated for functional properties such as viscosity, dispersibility, water absorption, water holding capacity, particle size and color (Kulkarni *et al*., 1991). Additionally, nutritive value such as vitamin C, minerals and amino acids (Kikafunda *et al.,* 1991). Other studies considered additional parameters such as flavor, nutrient density, storage stability, moisture content and acceptability (Bonsi *et al.,* 2014; Nwakalor & Obi, 2014).

In Nigeria, traditional weaning food are cereal gruels that are high in carbohydrates. To achieve a nutritionally balanced infant food, the cereal is fortified with a legume paste. These cereals after undergoing fermentation by microorganisms supply mainly carbohydrates. High carbohydrate intake by infants leads to slow growth rate due to malnutrition. Malnourished infants appear skinnier or bloated compared to their counterparts who consume nutritionally balanced weaning foods. Child malnutrition due to poor quality of complementary foods is a major cause of mortality among children in many sub-Saharan Africa. These complementary foods are made from starchy staple foods, which due to their heavy viscosity must be diluted with water before being given to children. Socially, parents who desire to improve the physical looks of their infants through proper nutrition desperately try different combinations of the cereals with proteins. However, proteins are not easily digestible thereby dousing the aim of this fortification project. These problems have been a source of concern to people especially in Africa among those who cannot afford the exorbitant prices of conventional standard weaning foods.

Over the years, efforts have been made to improve the nutritive value of weaning food through fermentation processes of various cereal food (Adeyemo &Onilude 2018; Asres, D.T., Nana, A. & Nega, G., 2018). The fermentation processes have been shown to result in improved nutrient content, reduction in anti-nutritional factors such as phytate and tannin, enhanced digestibility and improved diarrhea inhibition food (Simango 1997; Adeyemo & Onilude 2018; Asres *et al*.,2018). However, there is a huge challenge, which involves the optimization of dynamic microbial changes during the fermentation process to avoid negative effect of fermented food on the infant. This project intends to use heterogeneous mixture of microorganisms as initial microflora for the cereal. Therefore, the microbial succession throughout the entire fermentation period is of paramount importance and will be used as an optimization factor.

Evaluation of the different controllable factors (variables) and the response needed during the fermentation process requires simple mathematical model such as response surface methodology (RSM) proposed by Zhang & Gao (2007).

This study reports onevaluation of microbial community, modelling and optimization of processing parameters of weaning food made from maize and cowpea blend

Materials and Methods

Weaning food preparation

The food materials used in this study include yellow Maize (*Zea mays*) and black eyed Cowpea(*Vigna unguiculata*) purchased at Ekeonunwa market located in Owerri municipal in Imo state. A Box-Behnken Design method under Response Surface Methodology was used for the design and the design was done using Minitab version 17.0. It involved varying three factors (pH, fermentation time, blend ratio) across three levels. The levels for pH are (3.5, 4.5 and 5.5), the levels of fermentation time are (24, 48 and 72hrs), while the blend ratios are (M M85C15; M65C35 and M50C50) where M-Maize and C- Cowpea (Table 1).A total of 15 samples was used. Each sample weighed a total of 1 kg (a combination of the Maize and Cowpea according to the design ratio) on the weighing scale. Milling was done whilst using the steeping water for the milling process. A blender of 1500 watts was used to wet mill to get a slurry.The slurry was then poured into a covered plastic bucket and left to ferment. The slurry was poured into flat trays and put oven dried.Some modifications used in this study to optimize the processing of this weaning wood includes; use of acidic pH water for steeping the Maize and Cowpea as well as during milling so as to limit the microorganisms to acidophiles. The plastic buckets used were closed to prevent entry of unwanted microorganisms.

Table 1: **Independent variables and the level used for the design**.

Independent variables (Factors)		Levels	
Blend ratio	M85C15	M65C35	M50C50
pH	3.5	4.5	5.5
Fermentation time (hrs)	24	48	72

Microbial isolation

Nutrient agar (NA) and Mann Rogosa and Sharpe (MRS) agar were prepared according to manufacturer's specifications (Cheesebrough, 2000). 10 g each of the weaning food sampleswas dispersed in 90 ml of sterile distilled water. The samples were serially diluted decimally by transferring 1 ml from each tube until the required dilution was obtained. One-tenth milliliter (0.1 ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried media, spread evenly and incubated at ambient temperature for 48 h. Colony counts obtained on the media were expressed as colony forming units per gram (CFU/g) to obtain total population. (Harrigan & McCance,2000).

Microscopic Characterization

The Gram staining technique, spore staining test and motility test was carried out as described by Cheesbrough (2000).

Biochemical characterization of bacteria isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough (2000).

Proximate Composition Analysis

The methods adopted for proximate composition was (AOAC, 2005) and (AOAC, 2006) with slight modifications.

Sensory Evaluation of Weaning Food

Sensory evaluation was based on a 9-point hedonic scale. The different sample formulations were given to 120 mothers. The formulation was prepared by mixing the dry powder with cold water to obtain a slightly thick consistency, then boiled hot water is added until it becomes a semi thick slurry. It was allowed to cool before serving the babies.

Statistical analysis for modelling the processing parameters

To obtain ideal models for the processing conditions, Minitab 17.0 version was employed. With the experimental data obtained from the proximate analysis, the regression coefficients and statistical significance of the model terms (ANOVA) were determined by fitting into the equation. Other parameters such as F-ratio were used to establish the significance of the model terms with the p-value < 0.05. The adequacy of the used model was ascertained using model analysis, coefficient of determination and lack of fit test.

Results and Discussion

Weaning food formulation

The final product of the weaning food formulation was a dry powder slurry. With different colours like white, creamy and light brown (Fig 1). The physical properties of the different sample slurries show that samples with higher maize content were softer and smoother. Also, they had a good aroma. While samples with higher cowpea content were thicker in consistency, grainier, and foamed during fermentation. They also had an awful smell.

Fig 1: Weaning food formulation

Microbial community

The predominant microorganisms were *Bacillus* sp, *Lactobacillus* sp *Micrococcus* sp, *Streptococuus faecalis* and *Corynebacterium* sp (Table 2). *Bacillus cereus* had the highest percentage of occurrence followed by *Bacillus subtilis (*Table 3). This can be attributed to the acidic pH environment. *Streptococcus* sp had the lowest occurrence. It grows symbiotically with *Lactobacillus* during fermentation and is a homofermenter. Lei and Jakobsen (2004) reported that microorganisms predominant in fermented cereals are *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* which are all acidophiles. Also, Obinna- Echem *et al*. (2013) reported that *Bacillus* sp is usually predominant in legume-based fermentation.

The LAB group of bacteria is usually regarded as safe. Knowledge of the microorganisms involved may be utilized in improving the organoleptic qualities of the final product. Fermented foods exhibit beneficial effects on health by reducing blood cholesterol levels, increasing immunity, protecting against pathogens, fighting carcinogenesis, osteoporosis, diabetes, diabetes, obesity, allergies and atherosceloris and alleviating the symptoms of lactose intolerance (Tamang & Kaliasapathy, 2010).

The microbial community present at the point of optimum production is of interest in this study. This is because identified isolates can be subjected to genetic manipulation to yield mutant strains with high production capacity. These isolates can be further produced massively and stored appropriately for use at any time. Furthermore, it will maximize production time frame and still yield a quality product. The *Bacillus* sp, *Lactobacillus* sp and *Corynebacterium* sp belong to this group.

Table 2: Microscopic and Biochemical Characteristics of Bacteria isolated from Different Blends

Spo	Mot	Gram rnx	Oxi	Cat	Coag	In	MR	VP	S	L	G	M	Identity of isolates
-	+	-R	-	+	-	+	-	+	+	+	+	-	*Corynebacterium* sp
-	-	-R	-	-	-	-	+	-	-	+	+	-	*Streptococcus* sp
-	-	+S	-	-	-	-	-	+	+	+	+	+	*Micrococcus luteus*
-	-	+R	-	-	-	-	+	-	+	+	+	+	*Lactobacillus* sp
+	+	+R	-	+	-	-	-	+	-	-	-	-	*Bacillus* sp
+	+	+R	-	+	-	-	-	+	-	-	-	+	*Bacillus* sp
-	-	+S	-	-	-	-	+	-	+	-	+	+	*Micrococcus roseus*
-	-	+S	-	-	-	-	+	-	+	-	_	-	*Enterococcus faecalis*
+	+	+R	-	+	-	-	+	-	+	+	+	-	*Bacillus megaterium*

Spo, Spore formation; Mot, Motility Test; Gram rnx, Gram reaction; Oxi, Oxidase; Cat, Catalase; Coag, Coagulase; In, Indole Test; MR, Methyl Red Test; VP, Voges Proskaeur Test; S, Sucrose; L, Lactose; G, Glucose; M, Maltose; +, Positive Test; -, Negative Test

Table 3: Percentage (%) Occurrence of Bacteria Isolated from Different sample blends.

Bacterial Isolates	Number of occurrence	% Occurrence
Bacillus cereus	23	18.4
Bacillus megaterium	8	6.4
Streptococcus sp	4	3.2
Enterococcus faecalis	6	4.8
Bacillus subtilis	28	22.4
*Corynebacterium*sp	18	14.4
Micrococcus roseus	10	8.0
Micrococcus luteus	14	11.2
Lactobacillus sp	18	14.4
Total number of isolates	**129**	100%

Bacterial count

Table 4 shows the average bacterial count on Nutrient agar/ MRS agar. Nutrient agar is a general purpose agar and will allow the growth of the desired acidophiles as well as mesophilic microorganisms which may be introduced as a result of contaminants at various points during production. De MannRogosa and Sharpe agar was used to favour the growth of *Lactobacillus*sp.

Sample code WFG had the highest average colony counts on the two different media while sample WFA had the lowest colony count. Samples with high colony counts is an indication of the diversity of microorganisms present. Since sample code WFG has a high colony count, it may be attributed to contamination during the production process whereas sample code WFA had low colony counts because the modalities put in place as discussed above limited entry of unwanted microorganisms.

Table 4: Average bacterial count on Nutrient agar/ MRS agar

Sample codes	Colony Counts (CFU/g)
WFA	1.81×10^5
WFB	9.58×10^4
WFC	$3.1\ 3 \times 10^5$
WFD	2.47×10^5
WFE	8.65×10^4
WFF	3.71×10^5
WFG	9.66×10^4
WFH	5.57×10^5
WFI	9.16×10^4
WFJ	6.06×10^5
WFK	2.01×10^5
WFL	2.91×10^5
WFM	5.11×10^5
WFN	9.06×10^4
WFO	3.67×10^5

Sensory evaluation

A 9- point hedonic scale was used to compute the responses of participants to the evaluation. In agreement with other workers (Abubakar & Ziglar, 2021), It was observed from the results in table 4.5 that the formulations were generally accepted by the mothers for their babies as the samples were presented to mothers who in turn fed their babies. The weaning food was reconstituted with cold water to get a light consistency and then hot water was added to get a thick slurry. Sample code WFC scored the highest with 95% acceptance. It was the most acceptable among the mothers who fed their babies with it. in other words, they were satisfied with the taste, colour, aroma and texture. However, since this study is primarily concerned with protein fortification, sample WFC falls short of WHO recommendation for protein. It had protein content of 14% while WHO standard for weaning food is at least protein content of 20%. Although taking into cognizance the specific processing parameters at play for this sample order WFC, it is safe to say that holding pH at 5.5, blend ratio of M65C35, fermentation time at 72 hours with a controlled fermentation culture, the final product will be of great commercial value since acceptability is very high. Conversely, sample order WFA fit into the standard recommendation of WHO as can be seen in Table 5. Further research will be on enhancing and harmonizing the processing parameters of the above sample codes to obtain not just an acceptable but a fortified weaning food as well.

Table 5: Values for sensory evaluation Sensory evaluation (%)

Sample order	Colour	Taste	Texture	Flavour	Aroma	Acceptance
WFA	75	79	90	87	93	80
WFB	60	90	75	72	81	80
WFC	95	85	87	90	85	95
WFD	90	80	87	89	76	76
WFE	95	75	74	70	65	75
WFF	65	71	89	80	75	90
WFG	95	87	90	67	89	78
WFH	75	90	65	87	65	76
WFI	68	90	87	75	68	66
WFJ	95	76	76	80	87	76
WFK	75	76	65	87	87	68
WFL	95	77	74	78	96	88
WFM	63	78	77	68	65	88
WFN	75	65	78	86	71	83
WFO	95	65	72	88	90	88

Proximate Composition

The different processing conditions and their effects on the quality characteristics of cowpea and maize blend are shown in(Table 6). The independent or explanatory variables are pH, cowpea and maize blend and fermentation time. The dependent or response variable are crude protein, fat, ash, crude fiber, moisture and NFE.

The protein content ranged from 10.7 to 21.2 %. WFA had the highest protein content while WFL had the lowest protein content. The presence of protein can be attributed to the addition of cowpea to the maize (Adeyanju *et al.,* 2022). Temple &Bassa (1991) reported that adding legumes to cereals improved protein intake. With the presence of protein, the objective of a fortified weaning food has been achieved. Protein is required for tissue replacement, the deposition of lean muscle mass, and growth. (Adeyanju*et al*,.2022. Further improvements will be on adjustments to be in line Recommended Daily Allowance for infants.

The fat content of 3.5 to 5.7% is within the range of WHO standard of 6%. High levels of fat may cause rancidity and odorous smell as reported by Ihekoronye&Ngoddy(1985). Fat enhances taste and increases acceptance as well as adds to shelf life. It is therefore recommended that fat containing additives should be added to this weaning food to achieve a good weaning diet for infants.

Moisture content varied between 5.2- 17.1%. Moisture content measures the amount of water that a food contains thereby showing its storage capacity. Sample WFB had the highest moisture content, this may be as a result of its Maize-Cowpea blend ratio (M50:C50) since the legume is of equal amount. The foaming from the legume increased the moisture of the formulation. Compared to WFG which had the lowest moisture content closer to recommendation by PAG (1971) where recommended moisture value should be 5-10%. Variations in moisture may also be attributed to the amount of water squeezed out from the slurry, this is in line with Obinna-Echem*et al*., 2013).

The ash content of the samples was between 1.2 - 3.7%. Ash content measures the amount of ash left over after burning. Low ash content is an indicator of higher quality of food. This is because processed foods have high ash content which is a pointer that a large portion of the food is not useful to the consumer. Since weaning should be a continuum of breastmilk, it is expected that a large portion if not all should be useful to the infant for proper growth

Crude fibre values ranged from 0.7 to 2.9%. Fibre is the indigestible part of food. Cereals and legumes have been reported to contain fibre. The benefits of fibre as revealed by (Maćkowiak*etal*.,2016) includes: reducing the risk and lowering the incidence of numerous diseases. However, in weaning foods, NHS (National Health Service) suggests minimal amount of fibre in weaning foods because a lot of fibre can fill up small tummies leaving little room for other foods. So, the samples had the adequate amount of fibre for a weaning food.

Nitrogen free extract (NFE). This is calculated by adding other nutrient values and then subtracting the total from 100. It consists of carbohydrates,sugars, starches,and a major portion of materials classified as hemicellulose in feeds. The Nitrogen free extract ranged from 57-72%.

Table 6: Proximate Composition analysis result

Trial	Independent variables/ Explanatory Variables			Dependent variables/response variables					
	pH	CM (%)	Ft (hrs.)	Crude protein	Fat	Ash	Crude fiber	Moisture	NFE
WFA	3.5	15	48	21.2	4.2	1.6	2.9	6.5	63.6
WFB	4.5	50	24	16.5	4.6	2.8	1.2	17.1	57.7
WFC	5.5	35	72	14.7	4.2	2.6	0.8	6.6	71.0
WFD	3.5	35	24	12.6	4.8	2.3	0.7	6.9	72.8
WFE	4.5	35	48	16.9	3.5	1.6	2.4	6.1	69.6
WFF	4.5	50	72	17.1	3.6	2.5	1.5	14.0	61.3
WFG	4.5	35	48	17.3	3.8	2.8	3.9	5.2	67.0
WFH	4.5	15	24	17.0	3.6	2.0	1.9	9.9	65.6
WFI	3.5	50	48	12.1	4.5	1.7	0.8	9.1	71.8
WFJ	4.5	35	48	14.3	4.1	2.2	1.0	8.6	69.8
WFK	4.5	15	72	11.4	5.6	1.2	1.3	8.8	71.8
WFL	5.5	35	24	11.1	5.1	2.6	1.0	8.1	72.1
WFM	5.5	50	48	15.1	4.4	3.0	3.3	9.7	64.5
WFN	5.5	15	48	10.7	4.8	3.2	0.9	9.5	70.7
WFO	3.5	35	72	15.4	5.7	2.7	2.9	11.2	62.0

Statistical analysis of crude protein response

The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used. The model terms such as pH and pH*blend ratio are significant for the crude protein model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the crude protein content model is shown to be 0.72 and 0.21 while the standard deviation is 2.5 (Table 4.9). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

Table 7: Analysis of variance on response surface for crude protein content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	85.485	9.4983	1.44	0.360
Linear	3	68.013	22.6711	3.43	0.109
pH	1	58.193	58.1930	8.81	0.031
Blend ratio	1	0.397	0.3967	0.06	0.816
Fermentation time	1	9.424	9.4236	1.43	0.286
Square	3	14.384	4.7946	0.73	0.579
pH*pH	1	10.535	10.5352	1.60	0.262
Blend ratio*Blend ratio	1	0.436	0.4359	0.07	0.807
Fermentation time*Fermentation time	1	3.659	3.6585	0.55	0.490
2-Way Interaction	3	59.139	19.7130	2.99	0.135
pH*Blend ratio	1	46.915	46.9147	7.11	0.045
pH*Fermentation time	1	0.179	0.1785	0.03	0.876
Blend ratio*Fermentation time	1	12.046	12.0460	1.82	0.235
Error	5	33.013	6.6025		
Lack-of-Fit	3	27.677	9.2258	3.46	0.232
Pure Error	2	5.335	2.6675		
Total	14	118.497			

Table 8. Crude protein content model summary

S	R-sq	R-sq(adj)
2.5	72.14%	21.99%

3D surface plots of protein response

The 3D contour plots show that there is an increase in crude protein when pH is around 3.5 and blend ratio close to 15% with fermentation time held at 48. With blend ratio held at 2, there is an increase in crude protein content when fermentation time is 24 hours and pH of 3.5. Furthermore, when the pH is held at 4.5, the crude protein content increases as the fermentation time is approximately 24 hours and blend ratio around 15%. These plots are graphical representation of regression equation.

To the extent of this study, to achieve maximum protein content in Maize and Cowpea weaning food formulation, the following conditions are ideal; pH 3.5. blend ratio 15% cowpea substitution, fermentation time 24 hours. It can be seen in the surface plots below.

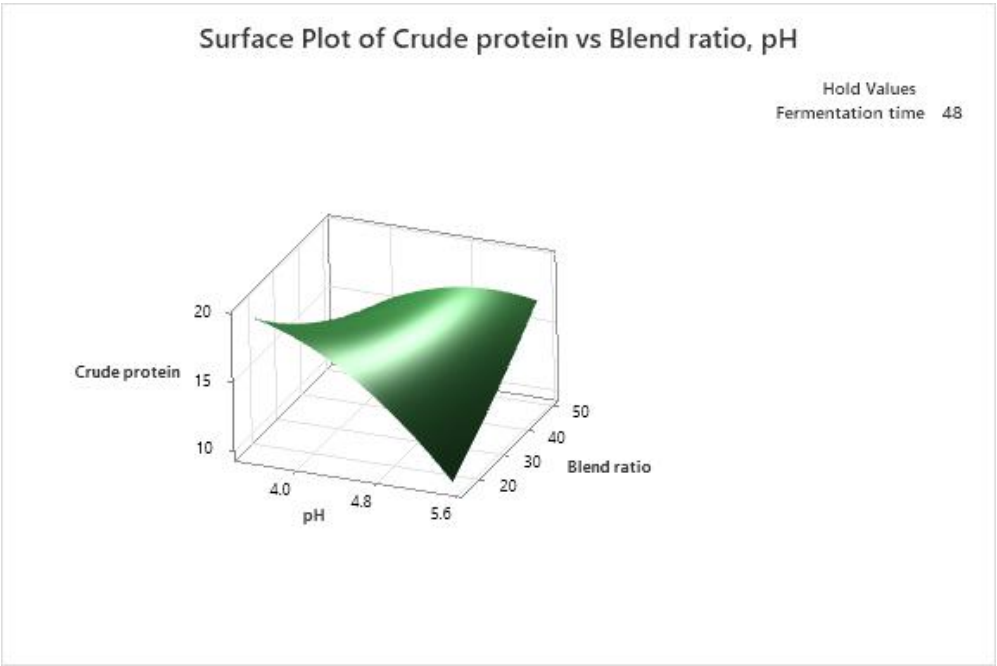

Fig 2: Surface plot of Crude protein vs Blend ratio, pH

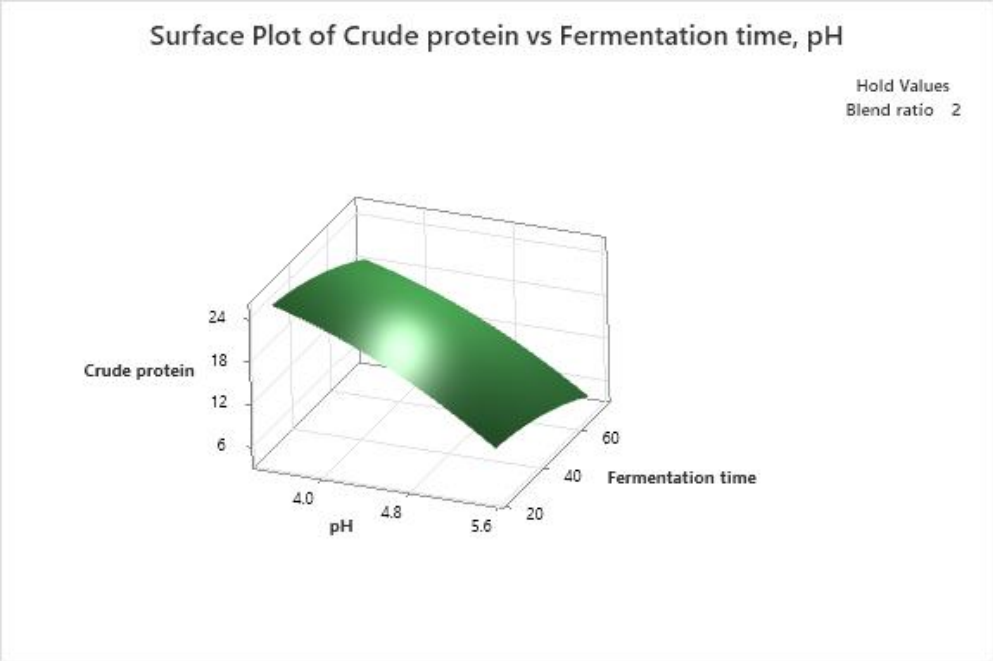

Fig 3: Surface plot of Crude protein vs Fermentation time, pH

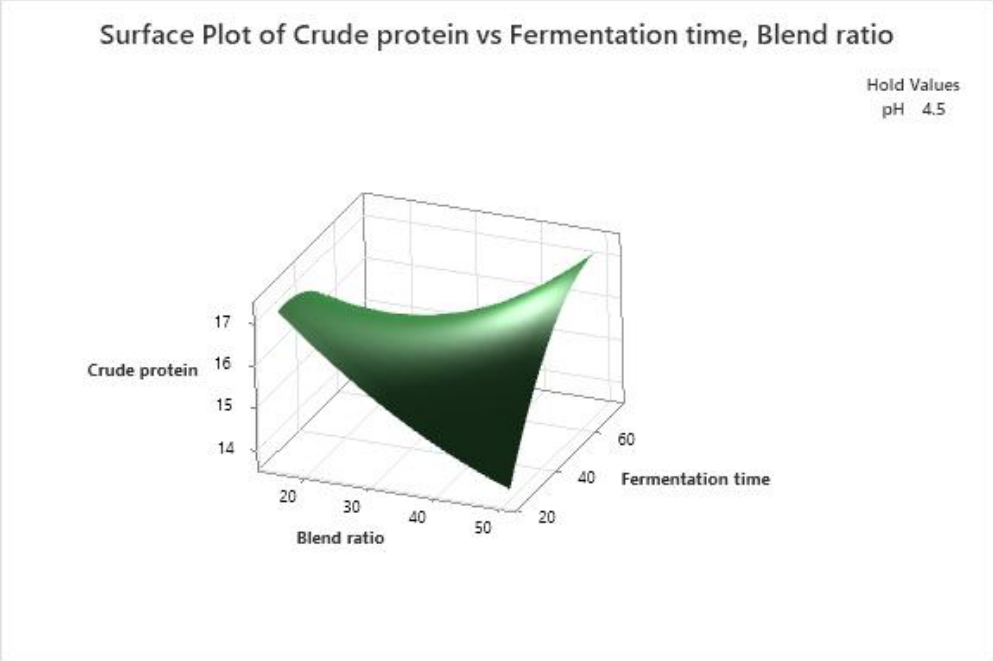

Fig 4: Surface plot of Crude protein vs Fermentation time, Blend ratio

Table 9: Response optimization: Crude protein

Variable	Setting
pH	3.5
Blend ratio	15
Fermentation time	24.9

Response	Fit	SE Fit	95% CI	95% PI
Moisture	20.50	3.03	(12.71, 28.29)	(10.29, 30.72)

Conclusion

To the extent of this study, it has been revealed that a Maize and Cowpea blend will yield a fortified weaning formulae. Evaluation of microbial isolates will enhance the choice of starter culture to be used for production. Also, processing parameters set at pH 5.5, blend ratio M65: C35, fermentation time 72 hours were ideal conditions in this work. It is recommended that these model parameters coupled with the best strains of the isolates will serve as a production design as well as a guide.

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