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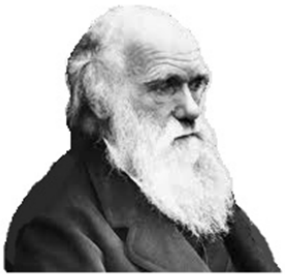

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Optimization of protein content of fish feed formulated using compositions of groundnut cake, milk powder, lemon grass and corn cob

Omotioma Monday[1,*], Obiora-Okafor Ifeoma Amaoge[2], Iloamuzor Fidelis Ejike[3], Adaku E. Okorafor-Nwosu[3], Nwobu Nneka Dymphna[3]

[1] Department of Chemical Engineering, Enugu State University of Science and Technology (ESUT), Agbani, Enugu State, Nigeria

[2] Department of Chemical Engineering, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Nigeria

[3] Department of Material and Energy Technology, Project Development Institute (PRODA), Emene, Enugu, Nigeria

*E-mail address: omorchem@yahoo.com , monday.omotioma@esut.edu.ng

ABSTRACT

This work presents the optimization of protein content of fish feed formulated using compositions of groundnut cake, milk powder, lemon grass and corn cob. The ingredients (precursors of the feed) were characterized in terms of proximate analysis, and they were used for the formulation fish feed. Functional groups of the feed were ascertained using Fourier transform infrared spectroscopy. The protein content of the feed was optimized using response surface methodology. From the analyses of the results, ash contents of 2.78 %, 6.74%, 3.53%, and 4.91% were recorded for groundnut cake, milk powder, lemon grass and corn cob respectively. It indicates high content of minerals. Also, there high protein contents, 21.69%, 35.13%, 31.02% and 3.40% for groundnut cake, milk powder, lemon grass and corn cob samples respectively, which show that the samples are suitable for the fish feed formulation. The major functional groups of the fish feed are; C-H stretch, O-H stretch, N-H symmetric, C-H stretch, ≡C-H stretch, N-H bend, $CH(CH_3)_2$, C-F stretch, C-O-C sym, =C-H bend and C-H bend. It revealed the presence of alkane and alkyls and carboxylic acids. Heteroatoms (O, N) are present in the feed. Quadratic equation represents the relationship between the feed protein content and the compositions of the feed raw materials. The predicted protein content (33.68%) compare significantly with the experimental protein content of 33.88%, with percentage deviation of 0.59% (< 5%). Thus, the

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feed's protein content obtained at appropriate quantities of groundnut cake, milk powder, lemon grass and corn cob was successfully optimized.

***Keywords*:** Fish feed, optimization, lemon grass, groundnut cake, corn cob

1. INTRODUCTION

Fish feed formulation is an area of great research interest in converting raw materials to useful products. As such, researchers have expressed the need for production of fish feed using locally sourced materials [1-4]. Fish is a source of protein, and can be processed for suitable meal [5]. The feed formulation process requires combinations of assorted raw materials. In most cases, various modifications of the feed's compositions are carried out to enrich the protein content of the fish feed. But there is need to optimize the optimization process for the enhancement of the feed protein content. This is so because animal feed requires high quality feeds with high protein content [6]. So, suitable feed is expected to contain complementary additives to keep the fish healthy [7]. Even when there are several ingredients for fish feed production, little or no efforts have been made in harnessing the raw materials for suitable fish feed formulation. It has been reported that operational production cost of fish feed ranged from 50% to 70% [8]. As such, emphasis is on formulating fish feed of optimum parameters. There is need to formulate optimum fish feed (using locally sourced agro-raw materials) for promoting fish farming. Thus, the aim of this study is to optimize protein content of fish feed formulated using compositions of groundnut cake, milk powder, lemon grass and corn cob.

2. MATERIALS AND METHOD

Materials for the feed formulation include; lemon grass, groundnut cake, corn cob, milk powder samples, Agar powder and garlic. All the chemicals used for this experiment are of analytical grade.

2. 1. Raw Materials' Proximate Analyses

Moisture content, crude fibre, protein content, fat content, ash content, carbohydrate of the sample were determined by standard official methods of analysis, AOAC [9].

2. 1. 1. Determination of protein content of the raw materials

As a standard method, Kjedahl method aided the evaluation of the protein content of the sample. It entails the quantification of the total nitrogen in the sample, and then converting the nitrogen to protein. It is based on the assumption that all the protein in the sample is in the form of nitrogen. Using a conversion factor of 6.25, the actual percentage of protein in the sample was calculated using Eq. 1.

According to the procedure, 1g ground sample was put into the digestion flask. 1g of the catalyst mixture was added into the flask. Then, 15 ml conc. H_2SO_4 was added to the mixture. It was heated until a greenish clear solution appeared.

The digest was allowed to clear for 30 min. It was further heated for additional 30 min, and allowed to cool. 10 ml distilled H_2O was added so as to avoid caking. Then the digest was transferred with several washings into a 100 ml volumetric flask and made up to the mark with distilled H_2O. A 10 ml aliquot was collected from the digest and put into the flask. 100 ml receiver flask containing indicator solution (5 ml boric acid) was placed under the condenser of the distillation apparatus so that the tip was 2 cm inside the indicator. 10 ml of 40% NaOH solution was added to the digested sample through a funnel stop cork.

The distillation commenced by closing the steam jet arm of the distillation apparatus. Then, 35 ml distillate was collected in the receiver flask. Titration was done with 0.01M standard HCl to first pink colour.

% CP = %N x 6.25. (1)

where CP is the crude protein, and N is nitrogen.

$$\% \text{ N} = \frac{Titration\,vol. \times 0.014 \times M \times 100\,x}{wt.ofsample} \frac{100}{10} \quad (2)$$

where, M is the molarity of std HCl.

% crude protein =% N × 6.25 (3)

2. 1. 2. Determination of moisture content of the raw materials

The AOAC method [9] was used in the moisture content evaluation. In the method, 1g of each sample was put into weighed crucible and then put in an oven set at 105 °C for 3 hrs. The sample was removed from the oven at regular interval and then cooled and weighed. The continuous drying was observed until a constant weight was reached [10].

$$\% \text{ Moisture} = \frac{A-B}{A} \times \frac{100}{1} \quad (4)$$

The symbol '*A*' denotes initial weight of sample, and B represents the weight of dried sample.

2. 1. 3. Determination of crude fibre content of the raw materials

The AOAC [9] method was used. In this technique, 3g of the sample was weighed (w_1) into a 600 ml beaker and 150 ml of preheated 0.128M H_2SO_4 was added to it. This was heated for 30 min and filtered under suction and washed with hot distilled water until the washings were no longer acidic. The residue was then transferred to a beaker and boiled for 30 min with 150 ml of preheated KOH (0.223M). It was filtered and washed with hot water until the washings are no longer alkaline. The residue was washed three times with acetone and dried in an oven at 105 °C for 2 hrs. It was then cooled in a desiccator, weighed (W_2) and ashed in a muffle furnace at 500 °C for 4 hrs. The ash obtained was cooled in a desiccator and weighed (W_3)

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1} \quad (5)$$

where W_1 = weight of sample, W_2 = Weight of dry residue, and W_3 = Weight of ash.

2. 1. 4. Determination of Fat content of the raw materials

The AOAC [9] method was used in quantifying the fat content of each raw material. In this case, 3g of the ground sample was put in a rolled filter paper and then placed inside the extraction thimble. The thimble was placed inside the extractor. 300 ml petroleum ether was poured inside the extraction flask. The condenser and the flask were connected to the extractor. The whole unit was place on a heating mantle for 3 hrs after which the petroleum ether was recovered. The oil collected in the flask was dried in an oven. It was then weighed and the percentage fat was calculated using the equation:

$$\% \text{ Fat} = \frac{C - A}{B} \times \frac{100}{1} \quad (6)$$

C = weight of flask with oil, A = weight of empty flask, and B = weight of original sample.

2. 1. 5. Determination of Ash Content

The ash content is the residue remaining after all the moisture has been removed and the fats, proteins, carbohydrates, vitamins and organic acids burnt away by ignition at about 600 °C. AOAC [9] standard method was used in the determination of ash content. 3g of the finely ground sample was weighed into a clean porcelain crucible which has been washed, dried in an oven at 100 °C, cooled in a desiccator and weighed. It was then placed in a muffle furnace and heated at 600 °C for a period of 4hrs. Thereafter, the sample was removed from the furnace and cooled in a desiccator. The, the ash content was measured using Eq. 7.

$$\% \text{ Ash} = \frac{A - B}{C} \times \frac{100}{1} \quad (7)$$

A = Weight of crucible + ash, B = Weight of crucible and C = Weight of original sample.

2. 1. 6. Total Carbohydrate

Carbohydrate content was calculated by subtracting percentage values of protein, fat, crude fiber, ash and water contents from 100%. It was determined using Eq. 8.

Total carbohydrate =100 - (protein + fat + crude fiber + ash + water) (8)

2. 2. Formulation and characterization of the fish feed

Fish feed formulation was carried out by combining feed ingredients to form mixture that met the specific goals of the production. Standard method of feed formulation was adopted [11-15]. Groundnut cake was ground in to powder form and used as the main ingredient. Other

ingredients; ground corn cob, milk powder and ground lemon grass were added to the groundnut cake and mixed very well. Agar powder and garlic were added to the mixture. Distilled water was added to the mixture and then boiled at 80 °C for 45 minutes, and cooled at room temperature for 2 hrs. After cooling, cod liver oil was added. It was kept under refrigeration for 12 hrs. After 12 hrs it was squeezed over polythene sheet and dried at room temperature for 48 hrs. The dried nodules were ground using mortar and pestle, and then sieved to obtain fine particles (0.85 mm). To avoid fungal infection, the particles were sun dried for 3 days, weighed and stored in plastic bottle. The quantities of the ingredients used are as shown in Table 1. The formulated feed was characterized using Fourier transform infrared spectroscopy. The protein content was examined using the standard method as expressed in section 2.1.1.

Table 1. Precursors (ingredients) for the formulation of the fish feed

S/N	Ingredients	Qty	Function
i.	Lemon grass	3.0g	Source of protein
ii.	Milk powder	6.0g	Source of protein
iii.	Groundnut cake	3.0g	Source of fat
iv.	Corn cob	1.5g	Filler
v.	Agar powder	1.0g	binding agent
vi.	Garlic	0.5g	Antibiotics
vii.	Cod liver oil	1.5 ml	Source of the vitamins

2. 3. Optimization of the feed's protein content

Interactive effects of parameter composition (corn cob, milk powder and lemon grass and groundnut cake) on the protein content of the formulated fish field were examined using response surface methodology (RSM). Central composite design of design expert software 11 was employed in line with the procedure used by previous authors [16, 17]. The parameter compositions are shown in the experimental design, Table 2.

Table 2. Parameter composition design

Factor	Name	Units	Coded Low	Coded High	Mean	Std. Dev.
A	Corn cob	G	-1 ↔ 0.50	+1 ↔ 2.50	1.50	0.7878
B	Groundnut nut cake	G	-1 ↔ 1.00	+1 ↔ 9.00	5.00	3.15
C	Milk powder	G	-1 ↔ 2.00	+1 ↔ 10.00	6.00	3.15
D	Lemon grass	G	-1 ↔ 1.00	+1 ↔ 5.00	3.00	1.58

3. RESULTS AND DISCUSSION

3. 1. Proximate analyses of the samples

Proximate analyses of the lemon grass, groundnut cake, corn cob and milk powder samples are presented in Table 3. For all the samples, the moisture content is within the tolerable limit of less than 10% for long term storage [10, 18].

The low moisture content would enhance its storage stability and shelf life. Ash content of groundnut cake, milk powder, lemon grass and corn cob samples were 2.78, 6.74, 3.53, and 4.91% respectively. It was observed from the ash content results that the samples contain high content of minerals.

Protein content of groundnut cake, milk powder, lemon grass and corn cob samples were 21.69, 35.13, 31.02 and 3.40% respectively. It was observed from the protein content results that the samples are suitable for the fish feed formulation.

Table 3. Proximate analyses of the fish feed's precursors.

Parameter	Groundnut cake	Milk powder	Lemon grass	Corn cob
AH(%)	2.78	6.74	3.53	4.91
CFT(%)	25.43	6.01	16.98	4.42
CF(%)	7.71	10.46	6.29	3.18
MC(%)	8.08	4.95	8.74	7.62
PT(%)	21.69	35.13	31.02	3.40
CH(%)	34.31	36.71	33.44	76.47

AH – ash, CFT – crude fat, CF – crude fibre, MC – moisture content, PT – protein, CH - carbohydrate

3. 2. Fish feed's functional groups

The spectrum of fish feed's functional groups as determined by Fourier transform infrared spectroscopic analysis is presented in Figure 1. It depicts the transmittance – wave number relationship.

The wave numbers represent the fish feed's functional groups, which were identified using infrared (IR) chart (Onukwuli and Omotioma, 2016).

As shown in Table 4, the major functional groups of the fish feed are; C-H stretch, O-H stretch, N-H symmetric, C-H stretch, ≡C-H stretch, N-H bend, $CH(CH_3)_2$, C-F stretch, C-O-C sym, =C-H bend and C-H bend. It revealed the presence of alkane and alkyls and carboxylic acids [6, 7].

The formulated fish feed is rich in protein; suitable nutrient [2-4, 19]. Heteroatoms (O, N) are present in the feed, which signifies nutritious fish feed.

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Sample ID:-CC
Sample Scans:32
Background Scans:16
Resolution:8
System Status:Good
Method Name:Transmittance
User:Admin
Date/Time:2024-06-15T19:56:09.062-07:00
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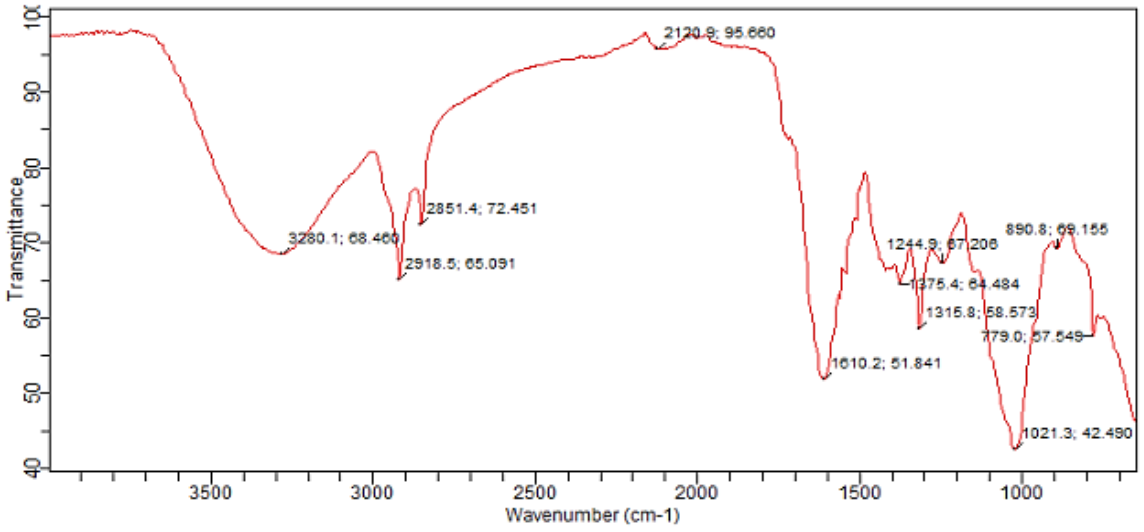

Figure 1. Spectrum of fish feed's functional groups.

Table 4. Fish feed's functional groups

Peaks	Intensity	Functional Group	Class of Compound
3280.1	08.400	C-H stretch O-H stretch N-H symmetric	Alkynes. Carboxylic Acid. Amides (R-$C_{(O)}$—NH_2
2918.5	05.091	C-H stretch. O-H stretch	Alkane and Alkyls. Carboxylic Acids
2851.4	72.451	C-H stretch. O-H stretch	Alkane and Alkyls. Carboxylic Acids
2120.9	95.660	≡C-H stretch	Alkynes(R-C≡C-H)
1610.2	51.841	N-H bend	R-NH_2

1375.4	64.484	$CH(CH_3)_2$	Alkane and Akyls
1244.9	67.208	C-F stretch C-O-C sym	R-F (Alkyl halides) Ar-O-R
1021.3	42.490	C-F stretch C-O-C Sym	R-F (Alkyl halides) Ar-O-R
890.8	09.155	=C-H bend	Alkenes **(**$RR'C=CH_2$)
779.0	57.549	C-H bend	m-disubstituted

3. 3. RSM result of the protein content optimization

As determined by response surface methodology of the optimization process, interactive influence of the feed precursors on the protein content is presented in Table 5. It was observed that the protein content varied with factors of the feed compositions. Maximum protein yield of 33.88% was attained at the compositions of corn cob (1.5g), groundnut cake (5g), milk powder (6g) and lemon grass (3g). It competes favourably with previous report [2], where it was stated that fermentation process increased protein content from 19.37% to 23.47%. The relationship between the protein content and the composition of the precursors is shown in Equation 9.

Table 5. Interactive influence of the fish feed precursors on the protein content

S	R	A, Corn cob (g)	B, Groundnut cake (g)	C, Milk powder (g)	D, lemon grass (g)	Protein content %
25	1	1.5	5	6	3	33.88
23	2	1.5	5	6	1	31.13
26	3	1.5	5	6	3	33.88
20	4	1.5	9	6	3	32.85
22	5	1.5	5	10	3	33.04
24	6	1.5	5	6	5	33.17
8	7	2.5	9	10	1	29.87
17	8	0.5	5	6	3	29.02
30	9	1.5	5	6	3	33.88
2	10	2.5	1	2	1	21.32
5	11	0.5	1	10	1	21.93
27	12	1.5	5	6	3	33.88

13	13	0.5	1	10	5	20.43
15	14	0.5	9	10	5	23.89
14	15	2.5	1	10	5	26.33
9	16	0.5	1	2	5	20.57
11	17	0.5	9	2	5	21.94
29	18	1.5	5	6	3	33.88
16	19	2.5	9	10	5	33.29
3	20	0.5	9	2	1	20.68
12	21	2.5	9	2	5	30.75
18	22	2.5	5	6	3	33.45
1	23	0.5	1	2	1	19.79
4	24	2.5	9	2	1	23.09
7	25	0.5	9	10	1	26.21
21	26	1.5	5	2	3	28.77
19	27	1.5	1	6	3	27.36
28	28	1.5	5	6	3	33.88
6	29	2.5	1	10	1	23.77
10	30	2.5	1	2	5	23.46

$$\text{Protein content} = +33.68 + 2.27A + 2.09B + 1.58C + 0.8911D + 0.7575AB + 1.10AD + 0.5925BC - 0.6050CD - 2.24A^2 - 3.37B^2 - 2.57C^2 - 1.32D^2 \quad (9)$$

The equation in terms of coded factors can be used to forecasts the protein content for given levels of each factor. Obtained protein content's coded quadratic equation is beneficial for recognizing the relative influence of the precursors' composition by comparing the corresponding coefficients. The equation is acceptable based on the analysis of variance (ANOVA) of Table 6. The ANOVA revealed the Model F-value of 100.49, which suggests that the model is significant [20]. In addition, the suitability of the quadratic equation is supported by the recorded adequate precision of 26.029. In addition, the predicted correlation of determination (R^2) of 0.9229 is in reasonable agreement with the adjusted R^2 of 0.9796.

The coefficient estimate in terms of the coded factors of the equation is presented in Table 7. It represents the expected change in response per unit change in factor value when all remaining factors are held constant. When the factors are orthogonal, the variance inflation factors (VIFs) are 1. Multi-colinearity exists in a situation where VIFs is > 1. So, higher VIF warrants severe correlation of the considered factors. As an estimated criterion, VIFs < 10 are tolerable. Thus, the quadratic equation is acceptable for describing the relationship between the

protein content and feed precursor compositions. The VIF ranged from 1 – 2.78. Detained diagnostic report of the protein content is presented in Table 8. There is significant and close relationship between the values of the predicted and actual protein content. This is based on the recorded values of the Cook's distance and the difference in fits (DFFITS).

Table 6. ANOVA of the protein content's quadratic equation.

Source	Sum-of-Squares	Df	Mean Square	F-value	p-value	
Model	792.41	14	56.60	100.49	< 0.0001	significant
A-Corn cob	92.80	1	92.80	164.76	< 0.0001	
B-Groundnut nut cake	78.58	1	78.58	139.52	< 0.0001	
C-Milk powder	44.78	1	44.78	79.50	< 0.0001	
D-Lemon grass	14.29	1	14.29	25.38	0.0001	
A*B	9.18	1	9.18	16.30	0.0011	
A*C	1.66	1	1.66	2.95	0.1062	
A*D	19.27	1	19.27	34.22	< 0.0001	
B*C	5.62	1	5.62	9.97	0.0065	
B*D	2.28	1	2.28	4.05	0.0625	
C*D	5.86	1	5.86	10.40	0.0057	
A^2	12.97	1	12.97	23.03	0.0002	
B^2	29.38	1	29.38	52.16	< 0.0001	
C^2	17.08	1	17.08	30.32	< 0.0001	
D^2	4.53	1	4.53	8.04	0.0125	
Residual	8.45	15	0.5632			
Lack-of-Fit	8.45	10	0.8449			
Pure-Error	0.0000	5	0.0000			
Cor-Total	800.86	29				
Std.-Dev.	0.7505		R^2		0.9895	
Mean	27.98		Adjusted R^2		0.9796	

C.V. %	2.68		Predicted R^2	0.9229
			Adequate Precision	26.0294

Table 7. Coefficients estimates

Factor	CE	DF	SE	95% CI Low	95% CI High	VIF
Intercept	33.68	1	0.2331	33.18	34.17	
A-Corn cub	2.27	1	0.1769	1.89	2.65	1.00
B-Groundnut nut cake	2.09	1	0.1769	1.71	2.47	1.00
C-Milk powder	1.58	1	0.1769	1.20	1.95	1.00
D-Lemon grass	0.8911	1	0.1769	0.5141	1.27	1.00
A*B	0.7575	1	0.1876	0.3576	1.16	1.00
A*C	0.3225	1	0.1876	-0.0774	0.7224	1.00
A*D	1.10	1	0.1876	0.6976	1.50	1.00
B*C	0.5925	1	0.1876	0.1926	0.9924	1.00
B*D	0.3775	1	0.1876	-0.0224	0.7774	1.00
C*D	-0.6050	1	0.1876	-1.00	-0.2051	1.00
A^2	-2.24	1	0.4663	-3.23	-1.24	2.78
B^2	-3.37	1	0.4663	-4.36	-2.37	2.78
C^2	-2.57	1	0.4663	-3.56	-1.57	2.78
D^2	-1.32	1	0.4663	-2.32	-0.3285	2.78

CI – Confidence interval, VIF - variance inflation factors, CE - Coefficient Estimate, SE - Standard Error, DF – degree of freedom

Table 8. Detained diagnostic report of the protein content

AV	PV	R	L	Internally Studentized Residuals	Externally Studentized Residuals	CD	Influence on Fitted Value DFFITS
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090

31.13	31.46	-0.3327	0.485	-0.618	-0.605	0.024	-0.587
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090
32.85	32.40	0.4517	0.485	0.839	0.830	0.044	0.806
33.04	32.69	0.3539	0.485	0.657	0.644	0.027	0.626
33.17	33.24	-0.0750	0.485	-0.139	-0.135	0.001	-0.131
29.87	30.03	-0.1606	0.659	-0.366	-0.355	0.017	-0.494
29.02	29.17	-0.1483	0.485	-0.275	-0.267	0.005	-0.259
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090
21.32	20.08	1.24	0.659	2.823	3.983	1.025	5.532
21.93	22.43	-0.5006	0.659	-1.142	-1.154	0.168	-1.603
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090
20.43	20.05	0.3771	0.659	0.860	0.852	0.095	1.184
23.89	24.66	-0.7667	0.659	-1.749	-1.893	0.393	-2.630
26.33	25.92	0.4110	0.659	0.937	0.933	0.113	1.296
20.57	19.94	0.6316	0.659	1.440	1.499	0.267	2.082
21.94	22.17	-0.2323	0.659	-0.530	-0.517	0.036	-0.718
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090
33.29	33.55	-0.2629	0.659	-0.599	-0.586	0.046	-0.814
20.68	20.62	0.0599	0.659	0.137	0.132	0.002	0.184
30.75	29.78	0.9716	0.659	2.216	2.610	0.631	3.625
33.45	33.71	-0.2594	0.485	-0.482	-0.469	0.015	-0.456
19.79	19.90	-0.1062	0.659	-0.242	-0.234	0.008	-0.326
23.09	23.84	-0.7462	0.659	-1.702	-1.830	0.372	-2.542
26.21	25.52	0.6855	0.659	1.563	1.651	0.314	2.293
28.77	29.53	-0.7616	0.485	-1.415	-1.468	0.126	-1.426
27.36	28.22	-0.8594	0.485	-1.596	-1.693	0.160	-1.644
33.88	33.68	0.2039	0.096	0.286	0.277	0.001	0.090

23.77	23.91	-0.1367	0.659	-0.312	-0.302	0.013	-0.420
23.46	24.51	-1.05	0.659	-2.405	-2.964	0.744	-4.117

DFFITS – difference in fits, R – Residual, L – Leverage, AV - Actual Value, PV - Predicted Value, CD - Cook's distance

3. 4. Graphical connotation of the RSM results

In Figure 2, plot of predicted versus actual protein content depicts straight line graph. The line of best fit (with points clustered along it) indicates that the quadratic equation is a suitable model for describing the correlation between the protein content and the precursor factors. On the three-dimensional coordinates, parabolic curves explain the interactive influence of the feed precursors on the protein content (Figure 3 a, b, c, d). It is a four – in – one, where Figure 3a stands for the influence of corn cob and groundnut cake on the protein content. It reveals positive interactive effects of the two precursors on the protein content. The relationship has a probability value of 0.0011 as reflected in the previous section of Table 6. Similarly, Figure 3b shows the interactive influence of corn cob and lemon grass on the protein content. Furthermore, Figure 3c displays the influence of groundnut cake and milk powder on the protein content, while Figure 3d showcase the influence of milk powder and lemon grass on the protein content. In all the cases, optimum (predicted) protein content of 33.68% was revealed, which was obtained at the compositions of 1.5g corn cob, 5g groundnut cake, 6g milk powder and 3g lemon grass. The optimum protein compare favourably with the reports of previous studies [11, 21-24]. The predicted protein content (33.68%) compare significantly with the experimental protein content of 33.88%, with percentage deviation of 0.59% (< 5%). Thus the RSM adequately optimized the protein content of the formulated fish feed.

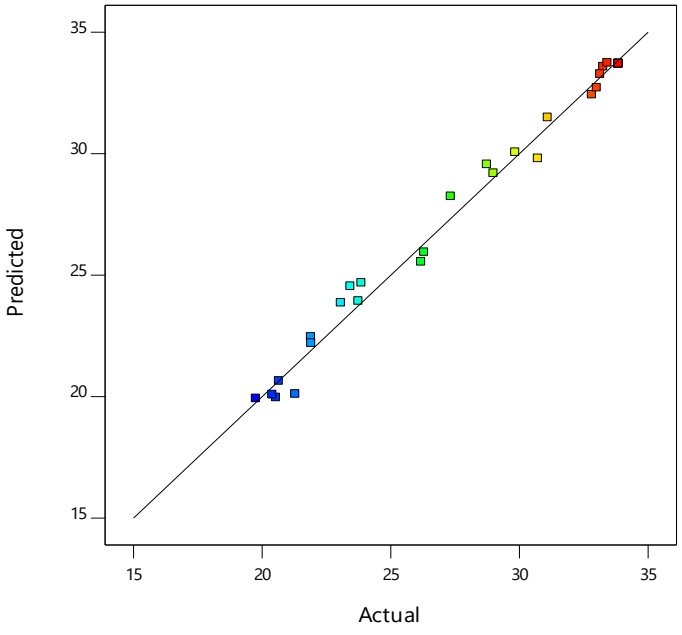

Figure 2. Diagnostic plot in terms of predicted against actual protein content

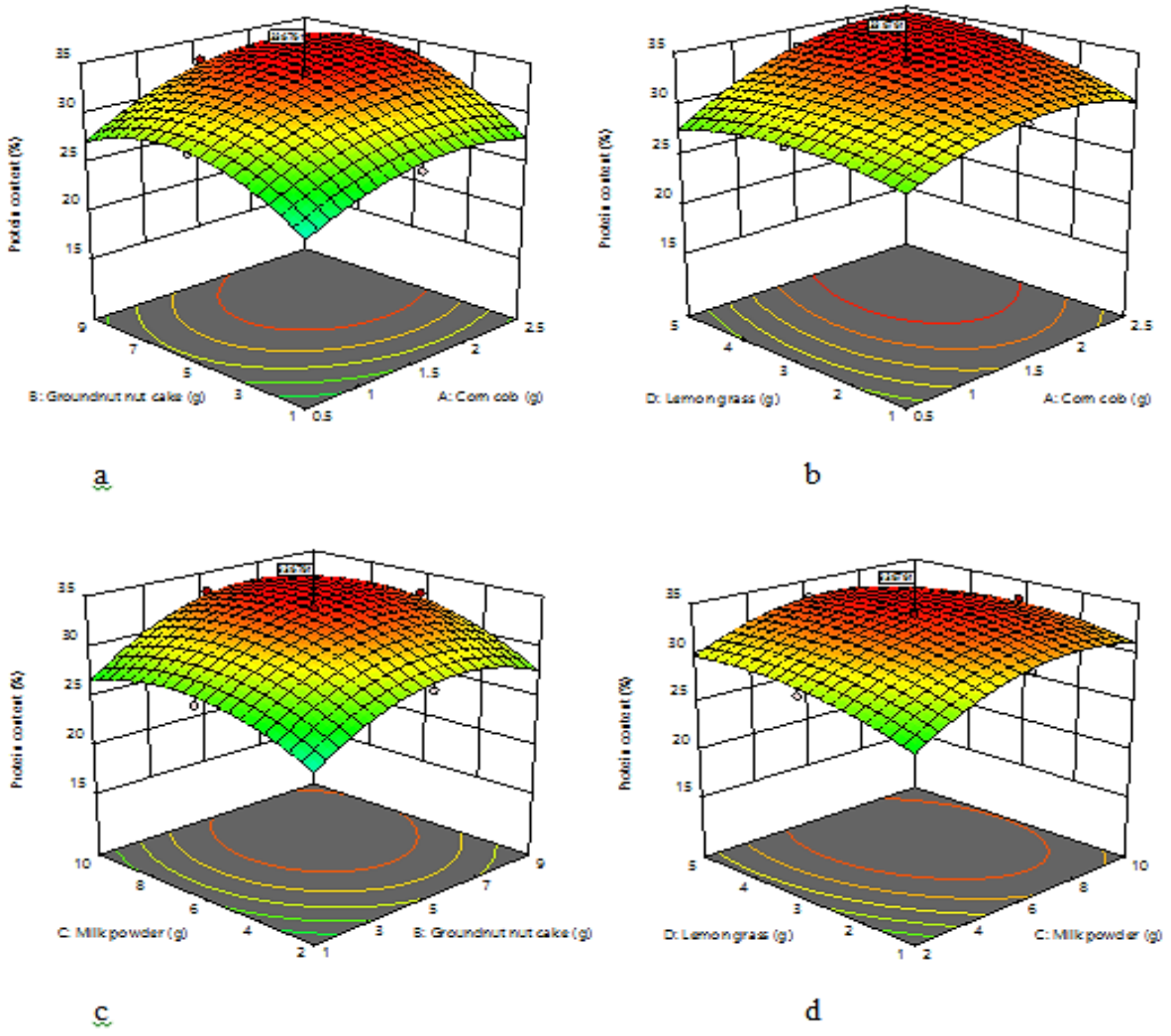

Figure 3. Interactive influence of the precursor's compositions on the protein yield a - Influence of corn cob and groundnut cake on the protein content; b - Influence of corn cob and lemon grass on the protein content; c - Influence of groundnut cake and milk powder on the protein content; d - Influence of milk powder and lemon grass on the protein content.

4. CONCLUSIONS

The following conclusions emanated from the analysis of the experimental results:

- Ash contents of 2.78%, 6.74%, 3.53%, and 4.91% were recorded for groundnut cake, milk powder, lemon grass and corn cob respectively. This is an indication of high content of minerals. Also, high protein content of (21.69, 35.13, 31.02 and 3.40)% for groundnut cake, milk powder, lemon grass and corn cob samples respectively, showed that the samples are suitable for the fish feed formulation.
- The major functional groups of the fish feed are; C-H stretch, O-H stretch, N-H symmetric, C-H stretch, ≡C-H stretch, N-H bend, $CH(CH_3)_2$, C-F stretch, C-O-C sym,

=C-H bend and C-H bend. It revealed the presence of alkane and alkyls and carboxylic acids. Heteroatoms (O, N) are present in the feed, which signifies nutritious fish feed.

- Quadratic equation represents the relationship between the feed protein content and the compositions of the feed precursors. The ANOVA revealed the equation F-value of 100.49, which suggests that the model is significant. In addition, the suitability of the quadratic equation is supported by the recorded adequate precision of 26.029. In addition, the predicted correlation of determination (R^2) of 0.9229 is in reasonable agreement with the adjusted R^2 of 0.9796. The predicted protein content (33.68%) compare significantly with the experimental protein content of 33.88%, with percentage deviation of 0.59% (< 5%). Thus the RSM adequately optimized the protein content of the formulated fish feed.

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