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Evaluation of the Essential Amino Acid Profile of Breakfast Cereals made from Millet, Malted Mungbean, and Defatted Tigernut Flours using Response Surface Methodology

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ABSTRACT

The essential amino acid profile of breakfast cereals made from millet, malted mungbean, and defatted tigernut flours was evaluated using response surface methodology. The acquired data were statistically evaluated using the RSM technique. The results were interpreted using three-dimensional model graphs and tables. The millet, malted mungbean, and tigernut flours have a significant ($p < 0.05$) effect on the essential amino acid profile of breakfast cereal. The most significant ($p < 0.05$) component in the preparation of breakfast cereal was revealed by millet, malted mungbean, and tigernut flours. A low coefficient of variation indicates great levels of accuracy in addition to experiment reliability. The model's suitability was validated by the non-significant ($p > 0.05$) lack of fit. The statistically analysed results were optimised to obtain a desirable blend of millet, malted mungbean, and tigernut flours for the production of breakfast cereal. This yielded 22.1g of millet flour, 33.2g of malted mungbean flour, and 44.7g of tigernut flour, with a desirability of 0.558. These ratios will therefore be crucial for the industrial preparation of breakfast cereal in addition to other food formulation systems.

Keywords: Millet, Mungbean, Tigernut, Essential Amino Acid, Simplex Centroid Experimental Design

1. INTRODUCTION

Sub-Saharan Africa has a higher prevalence of micronutrient malnutrition than other regions of the continent, particularly in children under five (Caulfield et al., 2006). The World Health Organisation has named zinc, iron, and vitamin A as the most restrictive nutrients in the diets of the underprivileged (Dewey, 2001). Today's consumers want an ever-expanding variety of more suitable light meals, calorie-free foods as well as high-nutrient food items. Possibility of creating inexpensive cereal for breakfast that gives the body the nutrients it needs Breakfast habits vary greatly between countries. It frequently consists of beverages, a protein source, occasionally dairy, and a carbohydrate item such as cereal, fruit, or vegetables (Rampersaud et al., 2005). Breakfasts that are mostly cereal-based give you energy and improve your productivity and mental performance; nevertheless, cereals are typically supplemented with other foods in order to make up for the deficits they contain (Enwere and Ntuen, 2005).

Breakfast cereals are prepared by puffing, crushing, flaking, rolling, or any other method of preparing cereal (Sharma and Caralli, 2004). One of the best options for a wholesome breakfast is ready-to-eat cereal. Due to their simplicity of preparation, ready-to-eat cereals promote independence by allowing kids and teenagers to prepare their own snacks (Jones, 2003). These foods can either be eaten raw right away, or they can be eaten after being reconstituted, heated in a vessel, or allowed to thaw if frozen (Okaka, 2005). Breakfast cereals are a staple on breakfast tables almost everywhere in the world. Today, consumers demand foods to come in a wide range of shapes, flavours, and colours as well as to adhere to strict nutritional standards. There are numerous ways in which eating breakfast is good for the body and the mind. The Body Mass Index (BMI) is lower in people who consume morning cereals compared to people who skip breakfast or opt for a different breakfast option (Hunty and Ashwell, 2006). In Nigeria, a lot more cereal is grown than is actually consumed. This is a result of cereal post-harvest losses; hence, there is a need to diversify the usage of cereals by turning them into a source of some year-round products.

Soluble and insoluble dietary fibres, Resistant starch, minerals, as well as antioxidants are all abundant in millet (Ragaee, Abdel-Aal, and Noaman, 2006). It has 2.1% ash, 92.5% dry matter, and 2.8% crude fibre. 13.6% crude protein, 63.2% starch, and 7.8% crude fat (Ali and EI-Tinay, 2003). Millet is therefore appropriate for significant use in the creation of food commodities such as breakfast meals due to the availability of these nutrients in millet. As a result of its difficult texture, lengthy preparation time, and inadequate knowledge about its nutritious makeup, mung bean (*Vigna radiata*) is currently underutilised as a food. It is only occasionally consumed. Mung beans have the capacity to significantly contribute to diets in terms of carbohydrates (630 g/kg), protein (240 g/kg), as well as different micronutrients (Anwar et al., 2007). The protein and carbs included in mungbeans are simpler to digest and cause less flatulence than those found in other legumes. Since the majority of diets based on cereal lack this essential amino acid, the mung bean's lysine level makes it a useful supplement (Baskaran et al., 2009). Mung beans are a great way to improve the quality of protein when mixed with the proteins from cereal grains since they have a high amount of lysine (Ashraf, 2006). Mung beans are an excellent source of protein, dietary fibre, iron, magnesium, and vitamins (thiamin and folate). Tigernut (Cyperus escullentum) tubers are an underutilised crop with a 38% Kcal (1635kg) value, 7.15% protein, 35% fat (oil), 46% starch, and 6% fibre content. In addition, tigernut contains significant amounts of vitamins E and C as well as minerals, particularly potassium and phosphorus (Belewu and Abodunrin, 2008; Oladele and Aina, 2007).

Response surface methodology is a statistical tool for the empirical modelling, design, and optimisation of procedures where the responses of interest are affected by a number of process variables (Freeny, Box and Draper, 1988; Gunst, Myers and Montgomery, 1996). Response surface methodology was used to study the associations connecting many independent and response variables using fewest resources and tests possible. The study is helpful in enhancing the dietetic value of cereals by supplementing their limiting amino acids with legumes. Additionally, findings would offer a way to use millet, malted mungbean, and tigernut, which are currently underutilised as raw resources for the commercial preparation of breakfast cereals. However, the essential amino acid score of most underutilised crops is yet to be determined. The study's product would significantly reduce consumers, particularly young people's, amino acid deficiency. For academics, health and nutrition policymakers, dietary counsellors, and households, the data from this study may be useful as a baseline or as reference material (Usman, 2009). The objective of this study is to evaluate the essential amino acid profile of breakfast cereals made from millet, malted mungbean, and defatted tigernut flours using response surface methodology.

2. MATERIALS AND METHODS

2.1 Materials

Green pearl millet and tigernut were procured from Relief Market, Imo State, Nigeria. Green mungbean was bought from Kingsway Market, Apapa, Lagos State. The Crop Science and Technology Department of FUTO identified millet, tigernut, and mungbean. The equipment and analytical-grade chemicals used were acquired from the Department of Food Science and Technology, Federal University of Technology, Owerri, Imo State; Polytechnic, Umuagwo-Ohji; Universities of Ilorin; Universities of Jos; and National Root Research Institute, Umudike.

2.2 Methods

2.2.1 Millet flour processing

The procedure described in Jideani (2005) was used to process millet flour. To get rid of dirt and any other impurities, two kilogrammes (2kg) of millet were washed and sorted. After being properly rinsed with distilled water, the cleaned and washed millet was steeped for 12 hours at room temperature. The millet was dried for six hours at 60ºC in an oven (DHG-9023A, Zenith Laboratory, China). It was then processed into flour using an attrition mill and 300-mm sieve to produce flour. The flour sample was preserved for subsequent examination and stored in an airtight container (Figure 1)

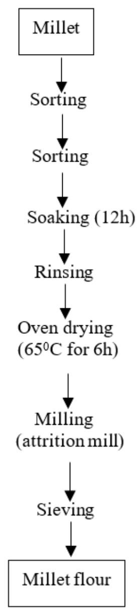

Figure 1: Millet flour processing flow diagram

Source: Jideani (2005)

2.2.2 Malted Mungbean flour Processing

Malted mung bean flour was processed according to Mubarak (2005). Mungbean seed (2kg) was cleaned, sorted, and washed in distilled water before being immersed in the liquid for 12 hours at room temperature in a clear container. The seeds were then spread out over an uncontaminated jute bag, covered with a muslin cloth, and stored at room temperature. The sample was left to malt for 24 hours. Distilled water was sprinkled on the white muslin fabric. The malted seeds were cleaned after 24 hours, dried in an air oven (DHG-9023A, Zenith Laboratory China) at 60ºC for 9 hours. It was milled to produce malted mungbean flour. The malted mungbean was packaged, and kept in an airtight container for additional testing (Figure 2)

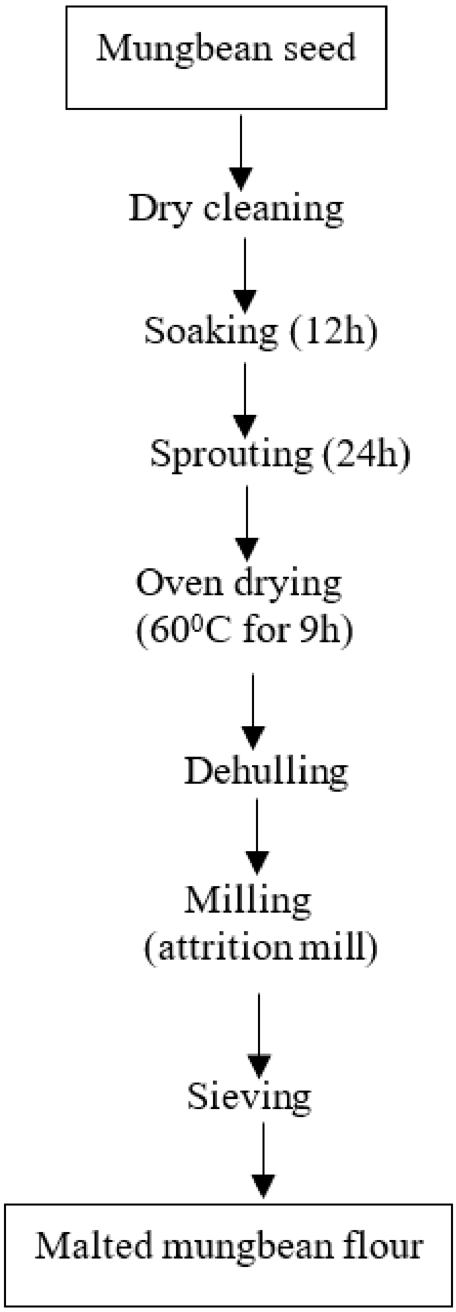

Figure 2: Malted mungbean flour processing flow diagram

Source: Mubarak (2005

2.2.3 Tiger nut flour processing

Fresh tigernuts weighing two kilogrammes (2kg) were properly cleaned, sorted, and rinsed to get rid of any impurities. Afterward, it was ground into a paste and homogenised in boiling water (100ºC). The paste was then placed on a cotton cloth and squeezed to extract the milk. The dried tigernut waste mash was placed in an airtight container for further examination after being dried in an oven (DHG-9023A, Zenith Laboratory China) for 8 hours at 60ºC (Figure 3) (Adejuyitan, 2011).

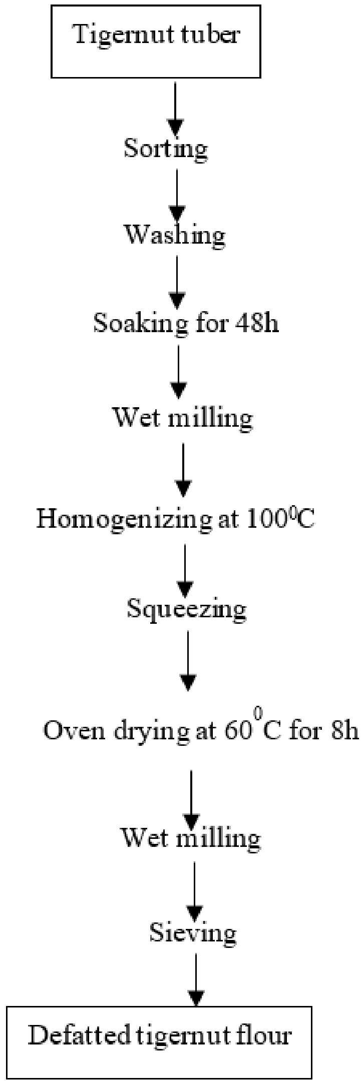

Figure 3: Defatted tigernut flour processing flow diagram

Source: Adejuyitan (2011)

2.2.4 Breakfast cereals production

The procedure described in Okafor and Usman (2014) was used to make breakfast cereals. Each of the three flours—millet, sprouted mungbean, and tigernut—was combined in quantities of 100 grammes (100g). 20 ml of distilled water, 0.5g of salt, 5 ml of vanilla flavour, and 2g of sugar were also added. They were combined and baked for 10 minutes to toast. Then it was chilled, ground, and packaged (Figure 4).

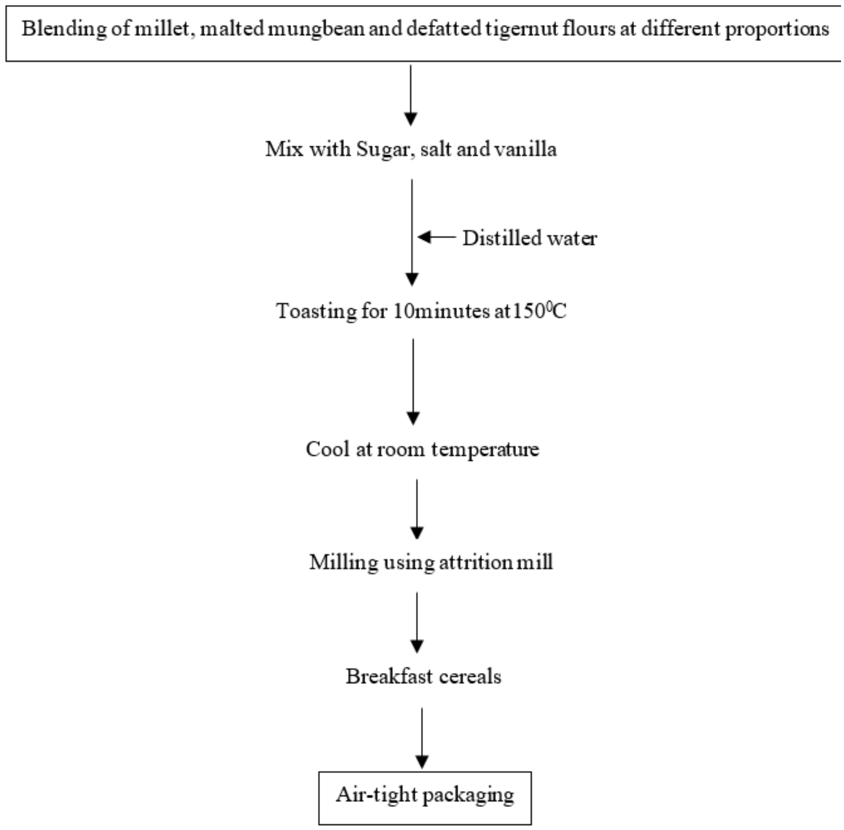

Figure 4: Breakfast cereal production

Source: Okafor and Usman (2014)

2.3 Essential Amino Acid Profile Determination

The essential amino acid profile was determined using the techniques detailed in Benitez (1989). After being dried to a fixed weight, the known sample was defatted. It was hydrolyzed, evaporated in a rotary evaporator, and put into the Applied Biosystems PTH Amino Acid Analyzer (Model 120A, Serial No. 704520, Patent: U.S.A. 4347131). A 2:1 combination of chloroform and methanol was used to defatten the material. Two-thirds of the material, 2.0 g, was placed in an extraction thimble and extracted using a soxhlet device for 15 hours (AOAC, 2015).

2.4 Experimental Design and Statistical Analysis

A three-component augmented simplex centroid experimental design of response Surface Methodology (RSM) (Scheffé, 1963) was employed to evaluate the essential amino acid profile of breakfast cereals made from millet, malted mungbean, and tigernut flours using response surface methodology. Fourteen standard points were generated from the design. Four standard points were repeated to assess the internal error in the design (Table 1). Statistical analysis including the ANOVA was carry out using Design-Expert software version 12.0.6.2. Three-dimensional response surface plots were generated as well. Non-significance ($p > 0.05$) lack of fit and coefficient of variation were determined from the analysis. Special quartic model was implemented as stated below:

$$y = \sum_{i=1}^{q} \beta_i x_i + \sum_{i \neq j}^{q} \beta_{ij} x_i x_j + \sum_{i \neq j \neq k}^{q} \beta_{ijk} x_i x_j x_k + \varepsilon_{ijk} \qquad (1)$$

Where, β_i are the main effect, β_{ij} are the binary blends effects, β_{ijk} are the ternary blends effects. Y is the predicted response, q is the number of process parameters (q = 3), ε_{ijk} is error estimate

Table 1: Three component augmented simplex centroid experimental design

Standard	Run	Millet flour (g)	Malted Flour (g)	Mungbean	Tiger Nut Flour (g)
1	10	100	0		0
2	12	0	100		0
3	6	0	0		100
4	1	50	50		0
5	14	50	0		50
6	9	0	50		50
7	5	33.33	33.33		33.33
8	7	66.67	16.67		16.67
9	4	16.67	66.67		16.67
10	8	16.67	16.67		66.67
11	3	100	0		0
12	2	0	100		0
13	11	0	0		100
14	13	50	50		0

3. RESULTS AND DISCUSSION

3.1 Essential amino acid of breakfast cereal

Table 2 showed that there were variations in the essential amino acid contents of breakfast cereal. These variations could be attributed to the constituents, the processing methods, and the blending ratios of millet flour, malted flour, and tigernut flour. Figure 5 showed the interaction of millet, malted mungbean, and tigernut increased the leucine content, while the interaction of millet and mungbean, as well as the interaction of millet and tigernut flour, decreased the leucine content of breakfast cereals. According to Jennifer (2019), a diet high in leucine promotes bone and muscular growth as well as blood sugar control. Fatigue, rashes on the skin, and hair loss are symptoms of leucine insufficiency. Figure 6 indicated that blending millet flour and malted mungbean significantly ($p < 0.05$) increased the lysine level of breakfast cereal. Furthermore, the blending of millet flour and tigernut flour, as well as the blending of malted mungbean flour and tigernut flour mungbean, significantly ($p < 0.05$) decreased the lysine level of breakfast cereal (Figure 6; Table 3). Lysine is essential for preserving bone density, developing muscle, speeding up healing from surgery or injury, and controlling hormones, antibodies, and enzymes. Anxiety brought on by stress is caused by a reduction in lysine (Jennifer, 2019). In figure 7 and table 3, blending of millet flour and malted mungbean, blending of millet flour and tigernut flour, and blending of malted mungbean flour and tigernut flour significantly ($p < 0.05$) increased the isoleucine level of breakfast cereal. Isoleucine supports hormone production, blood sugar control, immunity, and wound healing. Energy levels are regulated by it, which is largely found in muscle tissue (Jennifer, 2019). Figure 8 showed that the blending of malted mungbean flour and tigernut flour significantly increased the Phenylalanine levels of breakfast cereal. According to Jennifer (2019), the body transforms phenylalanine into tyrosine, which is required for particular brain processes. Phenylalanine aids the body's utilisation of proteins, enzymes, and other amino acids. When phenylalanine levels drop, babies struggle to gain weight, while adults experience dermatitis, exhaustion, and memory issues. It was observed in figure 9 that the combination of millet flour and malted mungbean flour significantly increased the Tryptophan content of the breakfast cereal. According to Jennifer (2019) tryptophan is a precursor to the neurotransmitters serotonin and melatonin and is essential for newborns' healthy development. A neurotransmitter called serotonin controls pain, emotion, appetite, and sleep. Additionally, melatonin controls sleep. Mohajeri et al., (2015) showed that tryptophan supplementation enhanced mental acuity and emotional processing in healthy females. Pellagra, a disorder caused by a tryptophan deficit, can result in dementia, skin rashes, and digestive problems. The blending of millet flour, malted mungbean flour, and tigernut flour significantly increased the Valine content of breakfast cereals (Figure 10, Table 3). Mental clarity, physical coordination, and emotional stability were all dependent on valine. Insomnia and lowered mental capacity were caused by a decrease in valine (Jennifer, 2019). Figure 11 indicated that the interaction of millet, malted mungbean, and tigernut flours significantly increased the histidine content of the breakfast cereals. Histidine aided in tissue repair, blood cell production, and growth. It supported the preservation of the unique protective layer around nerve cells. Anaemia and low blood levels were brought on by a decrease in histidine (Jennifer, 2019). A low coefficient of variation indicates high levels of accuracy and experiment reliability. The model's suitability was validated by the non-significant ($p > 0.05$) lack of fit (Table 3). Table 4 shows the criteria for the numerical optimisation of the essential amino acid. The statistically analysed results were optimised to obtain a desirable blend of millet, malted mungbean, and tigernut flours for the production of breakfast cereal. This yielded 22.1g of millet flour, 33.2g of malted mungbean flour, and 44.7g of tigernut flour, with a desirability of 0.558 (Figure 12). These ratios will therefore be crucial for the industrial preparation of breakfast cereal in addition to other food preparation systems.

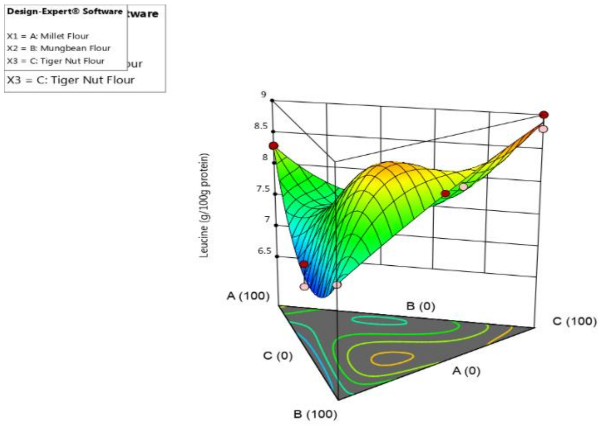

Figure 5: 3D model graph of leucine content of breakfast cereal

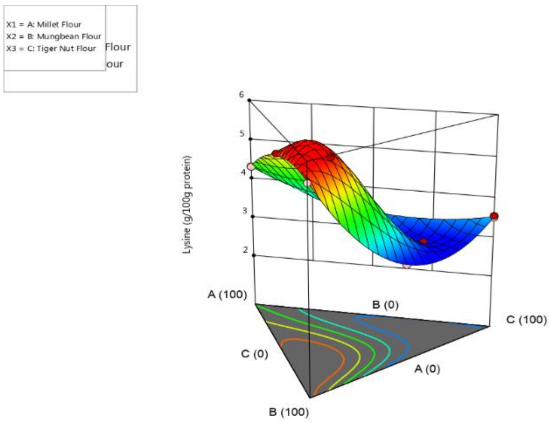

Figure 6: 3D model graph of lysine content of breakfast cereal

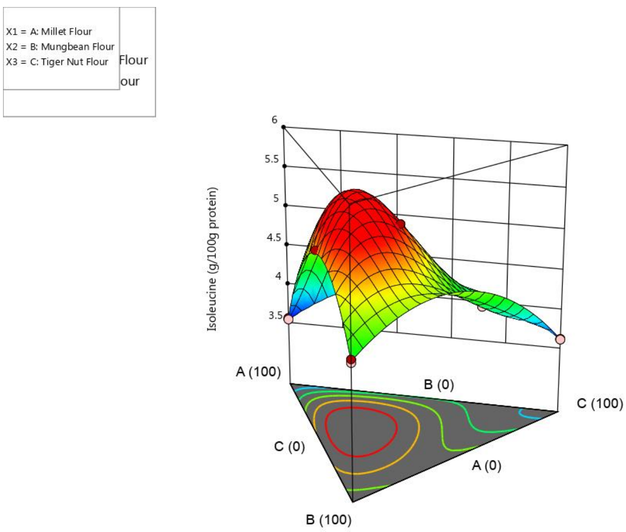

Figure 7: 3D model graph of isoleucine content of breakfast cereal

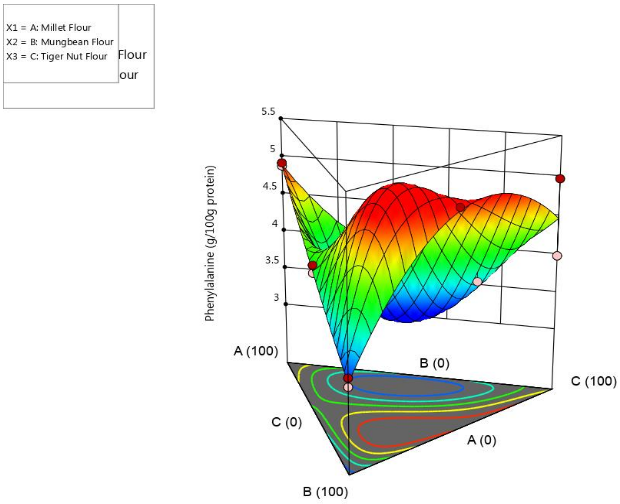

Figure 8: 3D model graph of phenylalanine content of breakfast cereal

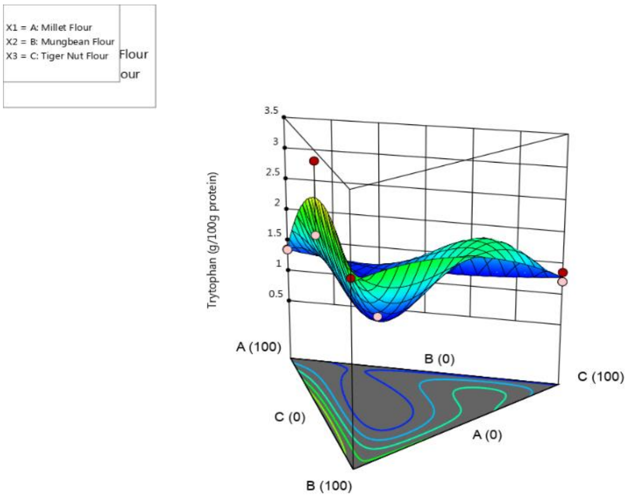

Figure 9: 3D model graph of tryptophan content of breakfast cereal

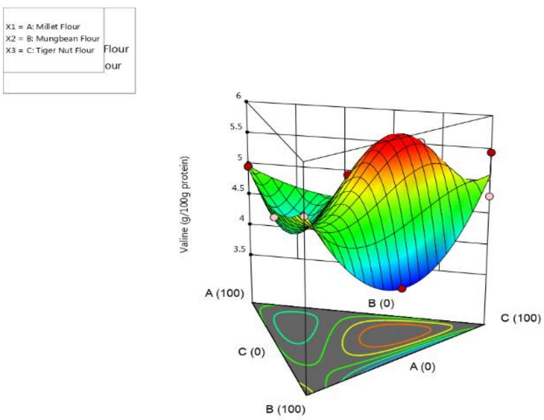

Figure 10: 3D model graph of valine content of breakfast cereal

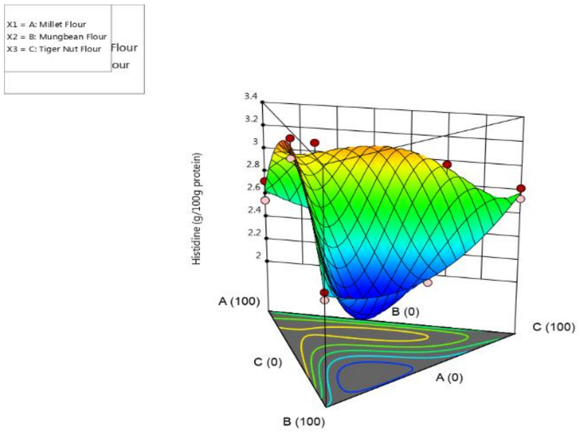

Figure 11: 3D model graph of histidine content of breakfast cereal

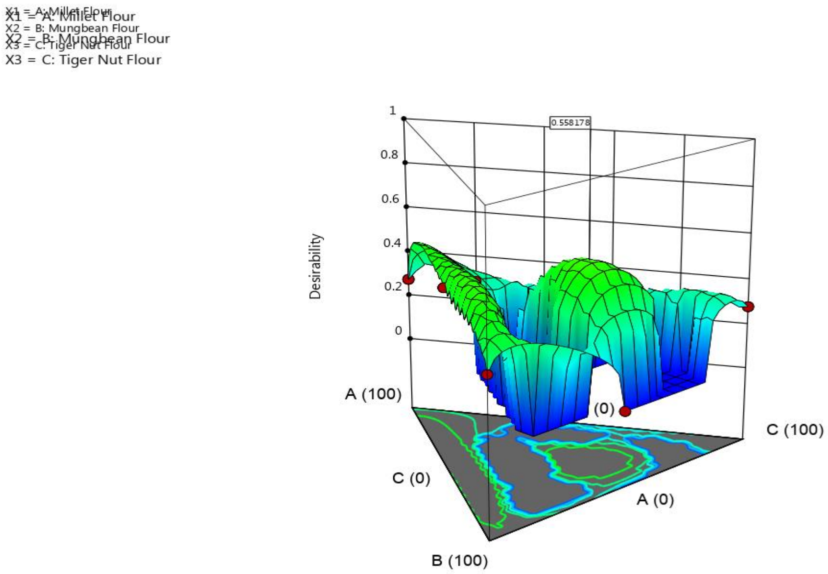

Figure 12: 3D model graph of desirability of the optimized blend proportion.

Table 2: Essential amino acid of breakfast cereal

		Independent Factors			Essential Amino Acid								
Std	Run	MiF (g)	MMF (g)	TNF (g)	LEU (g/100g protein)	LYS (g/100g protein)	ISO (g/100g protein)	PHE (g/100g protein)	TRY (g/100g protein)	VAL (g/100g protein)	MET (g/100g protein)	HIS (g/100g protein)	THR (g/100g protein)
1	10	100	0	0	8.29	4.34	3.56	4.92	1.36	4.96	2.76	2.55	3.77
2	12	0	100	0	7.34	5.60	4.32	3.36	2.29	5.26	2.33	2.39	3.55
3	6	0	0	100	9.00	3.53	3.61	4.96	1.20	5.44	2.56	2.72	3.44
4	1	50	50	0	7.00	5.35	4.94	4.01	3.34	4.69	3.62	3.18	3.22
5	14	50	0	50	7.60	3.40	4.23	3.76	1.21	4.56	3.39	2.43	4.05
6	9	0	50	50	8.20	3.02	4.65	4.96	2.11	3.76	3.67	2.26	3.05
7	5	33.33	33.33	33.33	8.22	4.49	5.21	4.29	1.29	5.25	4.15	2.65	3.58
8	7	66.67	16.67	16.67	7.53	4.35	5.14	3.43	1.21	4.25	4.21	3.16	3.56
9	4	16.67	66.67	16.67	8.30	5.65	5.01	4.96	1.31	4.83	4.67	2.17	3.61
10	8	16.67	16.67	66.67	8.00	3.02	4.12	3.72	1.94	5.67	4.33	3.05	3.89
11	3	100	0	0	8.30	4.33	3.54	4.88	1.37	4.97	2.76	2.72	3.69
12	2	0	100	0	7.35	5.54	4.28	3.25	2.18	5.39	2.68	2.45	3.91

13	11	0	0	100	8.79	3.49	3.62	3.97	1.35	4.76	2.41	2.81	3.34
14	13	50	50	0	6.65	5.41	4.98	4.11	2.24	4.89	1.24	3.34	3.47

Key: MiF- Millet Flour; SMF - Malted Mungbean Flour, TNF - Tiger Nut Flour; LEU – Leucine; LYS – Lysine;

ISO- Isoleucine; PHE – Phenylalanine; TRY – Tryptophan; VAL – Valine; MET – Methionine; HIS – Histidine; THR - Threonine

Table 3: Coefficient table

EAA	A	B	C	AB	AC	BC	ABC	A^2BC	AB^2C	ABC^2	LOF	CV
LEU	**8.29**	**7.34**	**8.89**	**-3.98**	**-4.00**	0.29		-1.93	**93.82**	-22.13	0.72	1.66
p-values	**0.0001**	**0.0001**	**0.0001**	**0.0003**	**0.0010**	0.6328		0.8876	**0.0008**	0.1535		
LYS	**4.33**	**5.57**	**3.51**	**1.71**	**-2.06**	**-6.05**		-6.31	**103.30**	**-35.41**	0.21	0.84
p-values	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**<0.0001**	**<0.0001**		0.1450	**<0.0001**	**0.0002**		
ISO	**3.54**	**4.29**	**3.61**	**4.13**	**2.57**	**2.75**		**61.07**	4.912	**-40.19**	0.24	0.53
p-values	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**<0.0001**	**<0.0001**		**<0.0001**	0.0857	**< 0.0001**		
PHE	4.88	3.29	4.45	-0.21	-3.81	**4.17**		-68.35	**111.10**	-53.02	0.45	0.82
p-values	0.1194	0.1194	0.1194	0.8656	0.0547	**0.0412**		0.0999	**0.0220**	0.1838		
TRY	**1.36**	**2.23**	**1.27**	**3.94**	-0.49	1.36		-43.77	**-96.69**	63.13	0.75	0.20
p-values	**0.0347**	**0.0347**	**0.0347**	**0.0245**	0.7677	0.4313		0.2707	**0.0410**	0.1386		
VAL	4.96	5.32	5.09	-1.42	-1.90	**-5.82**		-57.77	20.70	**127.86**	0.92	4.65
p-values	0.4887	0.4887	0.4887	0.1313	0.1203	**0.0023**		0.0505	0.4006	**0.0025**		
MET	2.78	2.59	2.47	-0.56	3.19	5.21	36.23				0.63	0.24
p-values	0.9297	0.9297	0.9297	0.8388	0.3811	0.1714	0.170					
HIS	**2.64**	**2.42**	**2.77**	**2.95**	-0.99	-1.24		26.84	**-66.21**	**45.79**	0.10	4.36
p-values	**0.0324**	**0.0324**	**0.0324**	**0.0008**	0.1159	0.0635		0.0697	**0.0024**	**0.0118**		
THR	3.69	3.74	3.42	-1.58	1.96	-1.70	5.642				0.24	5.45
p-values	0.4477	0.4477	0.4477	0.0509	0.0552	0.0869	0.373					

EAA – Essential Amino acid; LEU – Leucine; LYS – Lysine; ISO- Isoleucine; PHE – Phenylalanine; TRY – Tryptophan; VAL – Valine; MET – Methionine; HIS – Histidine; THR – Threonine; LOF – Lack of Fit; CV - Coefficient of Variation; A – millet flour; B – Malted mungbean flour; C – Tigernut flour; p – value shading: $p < 0.05$

Table 4: Numerical optimization criteria

Factor	**Target**	**Lowest Limit**	**Highest Limit**	**Lowest Weight**	**Highest Weight**	**Importance**
Millet Flour	range	0	100	1	1	3
Malted Mungbean Flour	range	0	100	1	1	3
Tiger Nut Flour	range	0	100	1	1	3
Response						
Leucine	maximize	6.65	9	1	1	3
Lysine	maximize	3.02	5.65	1	1	3
Isoleucine	maximize	3.54	5.21	1	1	3
Phenylalanine	maximize	3.25	4.96	1	1	3
Tryptophan	maximize	1.2	3.34	1	1	3
Valine	maximize	3.76	5.67	1	1	3
Methionine	maximize	1.24	4.67	1	1	3
Histidine	maximize	2.17	3.34	1	1	3
Threonine	maximize	3.05	4.05	1	1	3

4. Conclusion

The essential amino acid profile of breakfast cereals made from millet, malted mungbean, and defatted tigernut flours was evaluated using response surface methodology. The three independent factors were shown to have a significant ($p < 0.05$) effect on the response variables. The most significant ($p < 0.05$) constituents in the preparation of breakfast cereal were millet, malted mungbean, and defatted tigernut flours. RSM is capable of predicting the interaction-blending effect of three variables on responses, which is difficult to achieve with conventional methods. Millet, malted mungbean, and defatted tigernut flours can be blended for use by food manufacturing businesses.

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