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Research Article

Proximate, Minerals, Vitamins and Colour Parameters of Nutraceutical Products from of Soy Protein Isolate, Sorghum Pyrodextrin and Basil Leaf Instant Soluble Powder

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Abstract

Proximate, minerals, vitamins and colour parameters of nutraceuticals impacted by different levels of soy protein isolate, sorghum pyrodextrin and basil leaves instant soluble powder was evaluated. A central composite orthogonal experimental design (CCOD) was applied for the combination of the ingredients of nutraceutical products utilizing the independent parameters of their levels of inclusion while proximate, minerals, vitamins, and colour parameters were dependent factors. Both quadratic and linear fitting effects were identified from the response surface evaluation of the products' variables that were dependent ($P<0.05$).The formulated nutraceutical product had 5.45% moiture, 64.65% protein, 4.65% ash, 2.27% fat, 22.27% carbohydrate, 360.73 Kcal energy, 652.83 mg/g phosphorus, 8.07 mg/g iron, 112.17 mg/g calcium, 341.21 mg/g potassium, 28.45 mg/g, 1252.06 µg/100g β-carotene, 1.31 mg/100 g vitamin B1, 0.71 mg/100g vitamin B2, 1.29 mg/100g niacin, 11.88 mg/100g vitamin C, 3.15 g/g, L= 76.48, a = -0.59, b = 14.41, colour intensity = 171.45, delta chrome = 18.77, and hue angle = -90.16. Based on the product's observed and projected physicochemical qualities, a regression graph revealed a nicely fitted model with an R^2 value of 0.999.

Keywords: Nutraceuticals; Basil Instant Soluble Powder; Soy Protein Isolate; Sorghum Pyrodextrin

Abbreviation

CCOD: Central Composite Orthogonal Design; SPI: Soy Protein Isolates; SPD: Sorghum Pyrodextrin; W/V: Weight Per Volume; HCL: Hydrochloric Acid; NaOH: Sodium Hydroxide; rpm: Revolution Per Minute

Introduction

The adage, "Let food be your medicine and medicine be your food," which was first documented by Hippocrates more than 2500 years ago, is gaining a lot of attention these days as consumers and scientists become more aware of the numerous health advantages of foods. The idea that food may be used for reasons more than just sustenance is becoming more widely accepted in the scientific community and in the public sphere. Nutraceuticals are made of natural or health-promoting substances that are beneficial to the body's health [1].

There are already more than 470 functional food and nutraceutical products in the market with proven health advantages. The plant world is the source of many of these novel items that are being marketed as treatments for different ailments. This is a logical candidate since many plants create alkaloids, or secondary chemicals, to fend off infections, and because these ingredients may also help treat infections in humans [2].

It is now important for processed foods to have these bioactive compounds to meet the needs of customers who are health conscious. Nutraceuticals are now the area of the food sector that is expanding the quickest [3]. Soybeans, *Glycine max* (L.) Merr. (Leguminosae) are one of the most crucial legumes [4]. Numerous beneficial and useful phytochemicals, including isoflavones, phytic acids, saponins, and oligosaccharides, are also found in soybeans [5,6]. Owing to its elevated biological value and abundant essential amino acid composition, it has the potential to mitigate protein-calorie malnourishment in susceptible populations within the community [7]. According to several recent scientific research reported by Messina and Barne [8], eating traditional soy foods on a daily basis may help prevent breast, postrate, colon cancers, and menopausal issues in females [9]. Soybean helps prevent cancer by reducing the development of preexisting tumor cells and the risk of endometrial cancer since it contains phytoestrogen and isoflavones. Frequent use of soy products lowers blood pressure, total cholesterol, low density lipoprotein, and plaque accumulation in arteries (atherocleorosis), all of which contribute to disease prevention [10]. Soy protein isolate (SPI) is among the most crucial products in food processing and several other industrial applications, which is a protein powder derived from soybeans [11]. Its popularity is due to its capacity to improve the functional and nutritional characteristics of food items that it is added to, particularly those that include protein [12-14].

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical cereal grass. For a significant portion of the world's food insecure population, it is their sole source of sustenance [15]. Expanding the uses of sorghum by turning it into a nutraceutical product like dextrin will increase usage by providing new opportunities for the grain. By removing low molecular-weight fractions, dextrin may be made sugar-free, have a much better digestive tolerance, and have less hygroscopicity. A soluble dietary fiber that meets all the requirements for a desirable product would have complete and immediate water solubility, low viscosity, neutral flavor, high fiber content, high digestibility, great heat and acid stability, and ease of processing (good flow and direct compressibility). With all the qualities listed above, a new soluble dextrin fiber can overcome the stability and application restrictions of typical soluble fibers [16]. *Ocimum gratissimum* L. (Lamiaceae) is a perennial herbaceous plant that is widely recognized by its fragrant perfume as scent leaf. The genus *Ocimum*, well known by its common name Basil, is a member of the Labiatae plant family [17]. Alkaloids, tannins, flavonoids, and oligosaccharides have all been identified in the plant [18]. Many illnesses, including cancer [17], antinociceptive, anti-inflammatory [20], antidiahorreal [21], antibacterial [22], antifungal [23], wound healing [17], and nephroprotective [18], are treated with it. Many properties, including analgesic [25], antifungal [26], aphrodisiac [27], hepatoprotective [28], antioxidant [29,30], and anti-diabetic activity [31], have been demonstrated by its ethanolic extract. This study's specific goal was to evaluate proximate, mineral, vitamins and colour of nutraceutical products formulated from soybean, sorghum and basil.

Materials and Methods

Materials

Soybean and sorghum seeds were bought from Bodija market Ibadan. Freshly harvested basil leaves were purchased from Ojo market in Ibadan, Nigeria. The materials were transported to Food Technology Lab of the University of Ibadan for further processing.

Preparation of soy protein isolates (SPI)

Figure 1 illustrates the process of producing SPI. A technique of producing the defatted soybean meal was as reported by Olapade., *et al*. [32]. A total of twelve kilograms of soybean seeds were separated, cleaned, and properly cleansed using clean water. After washing, the seeds were steeped in a 1:5 water ratio for eight hours, after which they were drained and dehulled. After being dehulled, the seeds were dried, ground into fine flour with a hammer mill, and then the soxhlet extraction process was used to remove the fat. Using the technique described by Zhongjiang., *et al*. [33], the defatted meal was transformed into isolate. After being defatted and suspended in ten times as much water, 2 mol/L NaOH was used to bring the pH of the beans down to 7. Following an hour of stirring, the suspension underwent a 30-minute centrifugation at 8000 rpm. The pH was adjusted to 4.5 with 2 mol/L HCL, and the supernatant was then exposed to isoelectric precipitation. After centrifugation at 5,000 rpm for 30 minutes, the protein precipitate was recovered, resuspended in water, and its pH was adjusted to 7 using 2 mol/L NaOH. The protein solution was freeze-dried and crushed to produce SPI powder after a minor quantity of insoluble materials were removed by centrifuging at 10,000 rpm for 30 minutes.

Preparation of sorghum pyrodextrin (SPD)

Isolation of starch from sorghum

Figure 2 illustrates the procedures used to extract sorghum starch according to Beta., *et al*. [34] and Sing., *et al*. [35]. Ten kilograms of sorghum grain were immersed in waste for twenty-four hours at five degrees Celsius in ten liters of NaOH (0.25%, w/v). After steeping, the grains were cleaned and blended for three minutes with an equal amount of water. The filtration of the slurry was done using 200-mesh screen. The residue was further rinsed out with water. Further grinding and filtering was done for 3 times to remove extract the soluble components. The residue was discarded after the filtering process. To settle, the filtrate was left for 1 hour. Centrifugation of the filtrate was done for 19 mins at the speed of

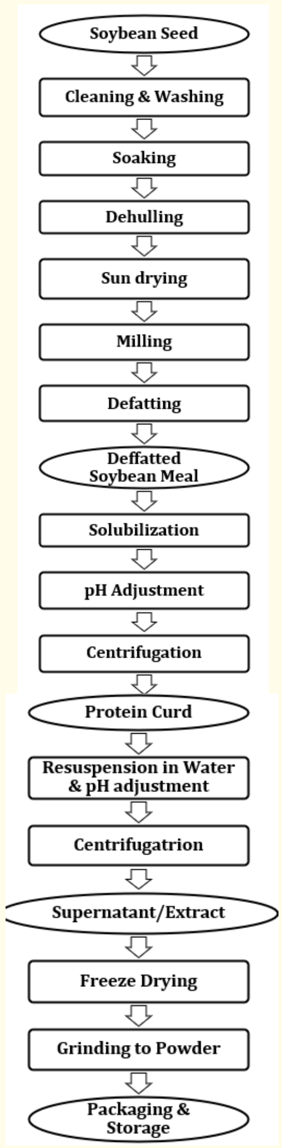

Figure 1: Preparation of soybean protein isolate (SPI) [32,33].

10,000 revolution per minute. After centrifugation and draining of supernatant, the protein grey layer was removed, with the aid of a spatula. The recovered residue was redissolved in excess water to resuspend the extracted matter. Centrifugation was repeated several times at the same speed for 5 mins, until the top starch layer was white. The recovered starch was dehydrated at 40 degrees Celsius for 24 hours.

Preparation of pyrodextrin from sorghum starch

The procedure reported by Sankhon., *et al*. [36] and Ghali., *et al*. [37] was used for the preparation of SPD. In summary, 2.2 M HCL was introduced to stainless trays containing 5 kg of starch. The w/v ratio of starch to HCL was 80:1. After the starch was covered with the acid, the combination was left to react at room temperature for sixteen hours. Following that, the mixture was dehydrated at 110 °C for three hours and subsequently ground to pass 100 µm sieve.

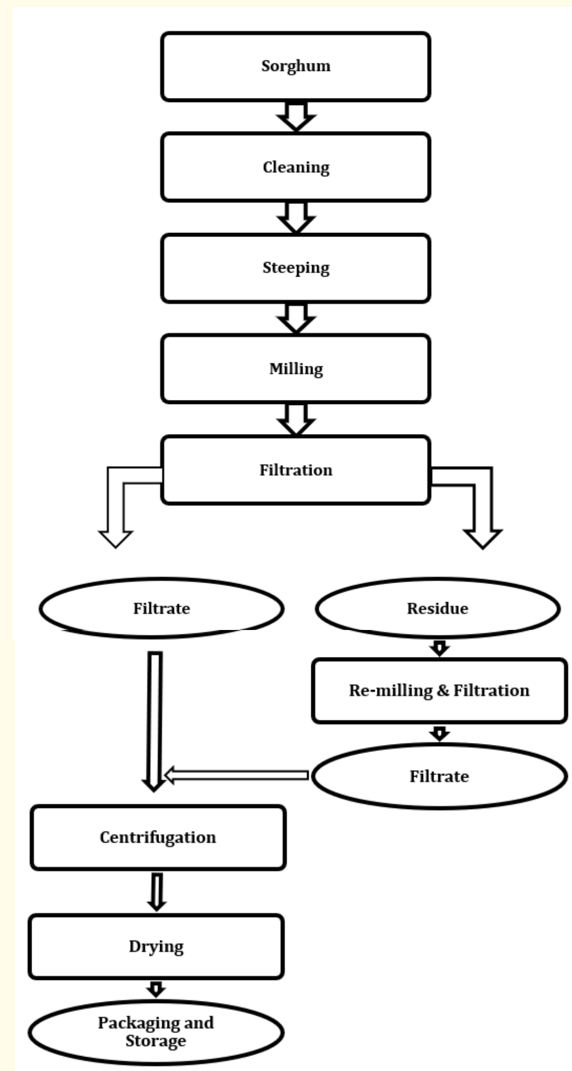

Figure 2: Preparation of pyrodextrin from sorghum.

Preparation of basil leaf instant soluble powder (BLISP)

As shown in Figure 3, newly chopped basil leaves were used to make the quick soluble basil powder. The technique of Sinija., *et al*. [38] and Sinija., *et al*. [39] was used to prepare basil leaf instant (soluble) powder. Shortly after the fresh basil leaves were plucked, they were steam-cooked to stop the fermentation process. The leaves were crushed, and the juice was manually extracted using cheesecloth. The extract (7-9% solid content) was freeze-dried, packaged in gas tight jar and store at refridgeration temperature before use.

Nutraceutical product preparation

Product formulation for nutraceuticals was done in a lab. Basil leaf instant soluble powder (BLISP), sorghum pyrodextrin (SPD), and soy protein isolates (SPI) were used to make each sample according to the experimental design in Table 1. After weighing each ingredient, they were thoroughly mixed with Kenwood mixer.

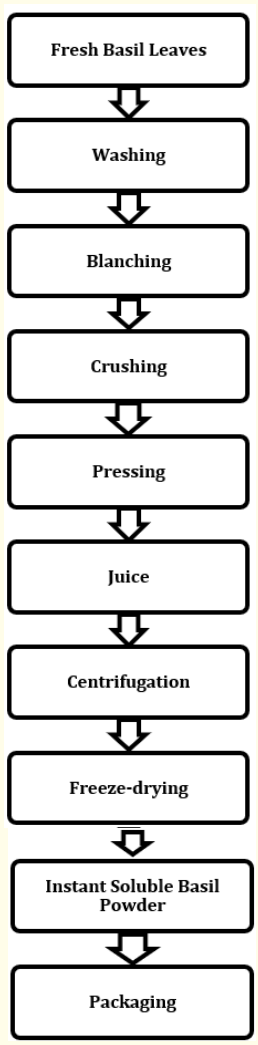

Figure 3: Preparation of basil leaf instant soluble powder.

Experimental design for the nutraceutical product formulation

The best performing nutraceutical product was determined using central composite orthogonal design. comprising of 20 runs. The 20 runs were made generated from five level, three factor mixture design of response surface methodology. The range for SPI, SPD and BLISP were 54.75 - 85.25, 14.75-45.25g and 0.95-7.05g, respectively. The range of the $-\alpha$, -1, 0, +1, $+\alpha$ variables for each sample is presented in table 1. In summary, the accurate quantities of the ingredients were carefully weighed, mixed thoroughly in a laboratory mixer to ensure even distribution.

Analyses of the nutraceutical products

Proximate, minerals, vitamins, and colour parameters of the products were analysed using standard methods. All analysis were carried out in triplicates and the average and standard deviation reported.

Proximate analysis

The proximate composition was determined by the methods of [40]. Moisture content, crude fiber, fat content, total ash, and carbohydrate were analysed. The analysis was carried out in triplicates and average results reported.

Total energy (calorific value)

Using the Atwater factors, energy was computed as per Osborne and Voogt's [41] description: 1 gram of carbohydrates and protein (P.), gives 4 kcal. each, while 1 gram of fat (f.) contribute 9 Kcal [42].

Mineral content analysis

Determination of minerals like Fe and Ca, P, Mg, K were carried out according to AOAC [43].

Determination of vitamins

The method of [44] was used to determine vitamin A content in the form of beta-carotene, the method of Kalia [45] was employed for the determination of vitamin C. Analysis of vitamin B1, B2, and niacin was done using methods of Snell and Snell [46].

Colour measurements

A handheld colorimeter (CR-300 Minolta Meter) was used to test the samples' colour parameters. The *L**, *a**, *b**, colour intensity, delta chrome and hue angle were generated in triplicates, and average result reported [32].

Statistical analysis

Version 7.0 of the Statease Design Expert Software Package was used for all statistical analysis and graphical displays. The statistical confidence level was set at 95%

	Soy Protein Isolate (g)		Sorghum Pyrodextrin (g)		Basil Leaf Instant Soluble Powder (g)	
Runs	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded
1	-1.52465	54.75	0	30.00	0	4.00
2	0	70.00	0	30.00	0	4.00
3	0	70.00	0	30.00	0	4.00
4	0	70.00	+1.52465	45.25	0	4.00
5	-1	60.00	+1	40.00	-1	2.00
6	-1	60.00	-1	20.00	-1	2.00
7	+1	80.00	-1	20.00	-1	2.00
8	+1.52465	85.25	0	30.00	0	4.00
9	-1	60.00	+1	40.00	+1	6.00
10	0	70.00	0	30.00	-1.52465	0.95
11	0	70.00	0	30.00	0	4.00
12	+1	80.00	+1	40.00	+1	6.00
13	0	70.00	0	30.00	0	4.00
14	0	70.00	-1.52465	14.75	0	4.00
15	0	70.00	0	30.00	+1.52465	7.05
16	0	70.00	0	30.00	0	4.00
17	0	70.00	0	30.00	0	4.00
18	+1	80.00	+1	40.00	-1	2.00
19	-1	60.00	-1	20.00	+1	6.00
20	+1	80.00	-1	20.00	+1	6.00

Table 1: Levels of dependent and independent variables.

Results and Discussions

Effects of independent variables on the proximate composition of the nutraceutical products

The effects of changes in concentration of SPI, SPD, and BLISP on the proximate composition of the nutraceutical products is presented in Table 2. The result of the proximate composition of the nutraceutical products showed that moisture content ranged between 4.20% and 6.10%, crude protein content, 52.08% and 70.98%, ash content, 3.86% and 4.87%, crude fat, 0.61% and 3.50%, carbohydrate, 15.60% and 37.27%, while total energy ranged between 360.81Kcal and 437.40Kcal.

The response fitted model of the effect of independent variables on the proximate composition of the nutraceutical products is shown by the regression analysis equations presented in Table 3. The equations revealed that while moisture content, crude protein, ash content, crude fat and total energy were affected linearly and quadratically by the independent variables, carbohydrate.

Content was only showed linear relationship. Also, the 3D response surface plots for the effects of independent variables on the proximate composition of the nutraceutical products is presented in figure 4.

The low moisture content obtained implies that the products would be shelf stable since moisture content plays critical role in determining the rate of spoilage mechanisms of foods. The plots showed that increased SPI resulted to increase in the moisture content of the nutraceutical products. This could be attributed to the fact that SPI contributed to an integral part of the product.

The high protein contents of the nutraceuticals would meet the daily required intake of the consumers as the required daily intake for adults and children would be within the region. There was increase of SPI resulted to increase in the protein content of the nutraceutical products while as the SPD was increased, the crude protein content decreased. BLISP had no marked effect on the overall protein content of the crude protein. This could be as result of the fact that SPI is about 90 percent crude protein unlike SPD and BLISP which contains little quantity of crude protein.

On the other hand, the relatively high ash content could be attributed to the effect of incorporation of BLISP on the nutraceutical products. Basil leaf had been found to be rich in mineral, hence remaining integral toward provision of the body's minerals. Increasing SPI resulted to increase in the ash contents of the products while reverse was the case for SPD. Increasing BLISP, resulted to increase in the ash content of the products.

The relatively low crude fat contents observed in all the sample is desirable as fat rancidity is one of the major causes of spoilage of foods. Therefore, the relative low-fat content will ensure increased shelf stability of the nutraceutical products. SPD and BLISP had little effect on the overall crude fat contents while increase in SPI resulted to a corresponding increase in the crude protein contents of the samples.

Also, the relatively low carbohydrate content is desirable as part of the aim of the research was to combat dietary related diseases such as diabetes, which have been known to be aggravated by high carbohydrate foods. The plots showed that the increase in the pyrodextrin content of the sample lead to a corresponding increase in the carbohydrate content of the product. Reverse was the case for SPI while BLISP had no marked effect on the carbohydrate content of the products. This could be explained from the point of view of the fact that SPD is relatively high in carbohydrate unlike SPI and BLISP.

SPI and SPD as well as BLISP had positive effect on the increasing the total energy content of the samples, while BLISP had no significant effect on the total energy content of the products.

The developed nutraceutical products developed in conformance with regulation of Food and Agricultural Organization of the United Nations [47]. The results reported by Almoselhy [48] for novel nutraceutical made from whey protein isolates, skim milk powder and other additives, and flavoured with vanilla, cinnamon, coffee and chocolate is comparable to proximate composition results obtained for this study. Also, the crude protein, ash content, crude fat, carbohydrate and total energy obtained for this study is comparable with the ones reported by Taiwade [49] for nutraceutical rejuvenator for sport persons.

Independent Variables (Factors) Proximate Composition (Responses)										
Run	Type	Soy Protein Isolate (g)	Sorghum Pyrodextrin (g)	Basil Instant Powder (g)	Moisture Content (%)	Crude Protein (%)	Ash Content (%)	Crude Fat (%)	Carbohydrate Content (%)	Total Energy (Kcal)
1	Fact	80.00(1.0)	40.00(1.0)	2.00(-1.0)	5.60	59.34	3.87	2.97	28.22	376.97
2	Axial	70.00(0.0)	30.00(0.0)	7.05(1.525)	4.20	67.64	4.60	3.38	20.18	437.40
3	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	4.99	60.17	4.27	3.00	27.57	377.96
4	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	4.78	61.35	4.00	2.31	27.56	376.43
5	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	5.30	61.35	3.90	2.61	26.84	376.25
6	Fact	60.00(-1.0)	20.00(-1.0)	2.00(-1.0)	5.50	66.34	4.29	3.15	20.72	376.59
7	Axial	70.00(0.0)	30.00(0.0)	0.95(-1.525)	4.89	63.00	3.91	2.52	25.68	377.40
8	Fact	60.00(-1.0)	40.00(1.0)	2.00(-1.0)	5.23	53.33	3.87	2.13	35.44	374.25
9	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	5.50	61.35	4.10	0.61	28.44	364.65
10	Fact	80.00(1.0)	20.00(-1.0)	6.00(1.0)	5.95	70.00	4.87	3.50	15.68	374.22
11	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	5.40	61.35	4.30	1.97	26.98	371.05
12	Fact	80.00(1.0)	40.00(1.0)	6.00(1.0)	5.20	58.09	4.25	3.20	29.26	378.20
13	Fact	60.00(-1.0)	40.00(1.0)	6.00(1.0)	5.33	52.08	4.11	1.21	37.27	368.29
14	Fact	60.00(-1.0)	20.00(-1.0)	6.00(1.0)	5.09	64.19	4.83	2.42	23.47	372.42
15	Axial	70.00(0.0)	14.75(-1.525)	4.00(0.0)	5.32	69.59	4.83	0.99	19.27	364.35
16	Axial	54.75(-1.525)	30.00(0.0)	4.00(0.0)	6.00	56.42	4.14	2.89	27.28	360.81
17	Center	70.00(0.0)	30.00(0.0)	4.00(0.0)	5.11	61.35	3.80	1.57	28.17	372.21
18	Axial	85.25(1.525)	30.00(0.0)	4.00(0.0)	4.99	65.01	4.36	2.60	23.04	375.60
19	Fact	80.00(1.0)	20.00(-1.0)	2.00(-1.0)	6.10	70.98	4.43	2.89	15.60	402.09
20	Axial	70.00(0.0)	45.25(1.525)	4.00(0.0)	5.05	53.50	3.86	2.12	35.47	374.96

Table 2: Proximate composition of the nutraceutical product formulations.

Variable	Model	R^2
Moisture Content	$+15.56500 - 0.31020*A + 0.011226*B + 0.27915*C - 1.52500E-003*A*B - 1.50000E-003*A*C + 1.62500E-003*B*C + 2.59443E-003*A^2 + 1.26084E-003*B^2 - 0.037309*C^2$	0.4996
Crude Protein	$+ 45.72817 + 0.88822*A - 0.64814*B - 3.54186*C + 1.96250E-003*A*B + 7.31250E-003*A*C + 3.93750E-003*B*C - 4.96547E-003*A^2 - 1.39490E-003*B^2 + 0.37112*C^2$	0.9635
Ash Content	$+8.72670 - 0.095273*A - 0.085320*B + 6.30686E-003*C - 5.00000E-005*A*B + 2.50000E-004*A * C - 2.25000E-003*B*C + 7.21105E-004*A^2 + 1.12979E-003*B^2 + 0.018565*C^2$	0.9021
Crude Fat	$+27.34349 - 0.61083*A - 0.073891*B - 1.85131*C + 2.51250E-003*A*B + 0.015563*A*C - 3.56250E-003*B*C + 3.56121E-003*A^2 - 1.55804E-003 *B^2 + 0.11108*C^2$	0.4856
Carbohydrate Content	$+ 26.84577 - 0.27357*A + 0.62786*B - 0.10616*C$	0.8773
Total Energy	$+122.08543 + 6.81311*A + 2.43177*B - 21.70949*C - 0.018337*A*B - 0.10319*A*C + 0.17069*B*C - 0.038259*A^2 - 0.032021*B^2 + 3.24486*C^2$	0.5599

Table 3: Regression equations of the proximate composition of the nutraceutical product formulations.

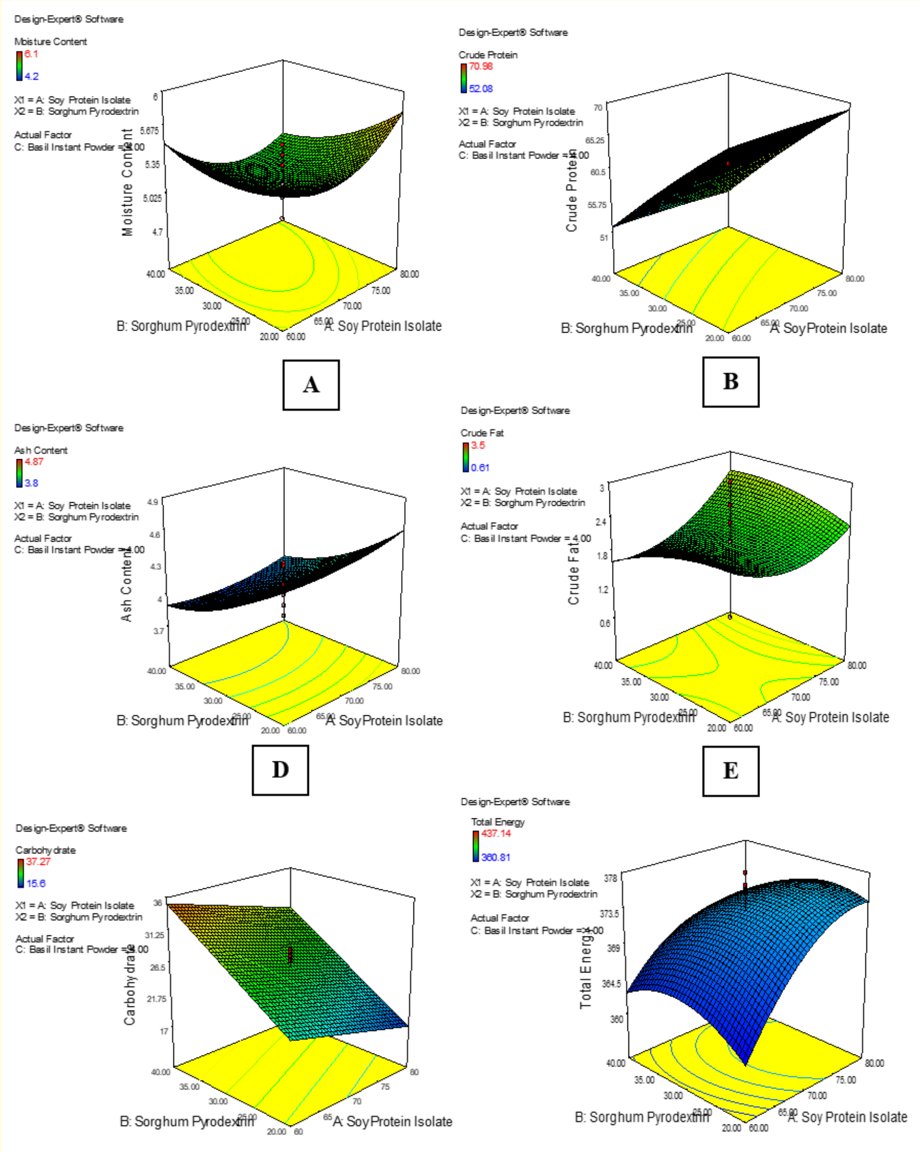

Figure 4: 3D Response surface plots of the effects of independent factors on the proximate composition of the nutraceutical products. *A= moisture contents, B= crude protein, C= Ash contents, D= Crude fat, E= Carbohydrate content, F= Total energy

Effects of independent variables on the minerals and vitamins contents of the nutraceutical products

Table 4 showed the effects of SPI, SP and BLISP on the minerals and vitamins contents of the nutraceutical products. The phosphorus content ranged between 536.62 mg/g and 677.90 mg/g, iron, 5.38 mg/g and 10.43 mg/g, calcium, 90.55 mg/g and 123.62 mg/g, potassium, 221.0 mg/g and 395.18 mg/g, magnesium, 26.47 mg/g and 29.24 mg/g, β-carotene, 577.61 mg/g and 1418.60 mg/g, Vitamin B1, 1.21130 μg/100g and 1.43396 μg/g, vitamin B2, 0.63 mg/100g and 0.77 mg/100g, niacin, 1.02 mg/100g and 1.35 mg/100g, Vitamin C, 9.78 mg/100g and 12.33 mg/100g.

The regression equations showing the linear and quadratic effects of independent variables on the minerals and vitamins composition of the nutraceutical products is shown in table 5. While SPI, SPD and BLISP had only linear effects on iron and phosphorus contents of the nutraceuaticals, their effects were both linear and quadratic for calcium, potassium, magnesium, β-carotene, vitamin B1, vitamin B2, niacin and Vitamin C.

The 3D response surface plots of the effects of SPI, SPD and BLISP on the mineral and vitamin contents of the nutraceutical products are depicted in Figure 5. From the plots, phosphorus contents were affected mostly by SPD. Increase in SPD resulted to increase in the phosphorus contents of the samples while, both SPI and BLISP had minimal effect on the phosphorus contents of the samples.

On the other hand, SPI affected immensely the iron contents of the samples possessing direct variation relationship. As the SPI inreased, the iron content of the samples equally increased. This was not true for instant soluble powder and SPD as both variables had inverse relationship with the iron contents of the sample.

The plots showed that BLISP did not affect markedly the calcium content of the sample. However, increase in SPI resulted to a corresponding increase in calcium content of the samples. The reverse was the case for SPD, as its' increase led to a decrease in the calcium contents of the samples.

The BLSIP and SPD showed negative relationship with the potassium contents of the samples. Increase in the afore-mentioned two factors lead to a decrease in the potassium contents of the samples. On the other hand, increase in SPI gave rise to increase in the potassium content of the samples.

For magnesium content, BLISP affected markedly the magnesium contents of the sample. Increase in BLISP resulted to increase in the sorghum SPD of the sample. On the contrary, increase in SPD resulted to a decrease in BLISP. SPI had little or no marked effect on the magnesium contents of the samples.

From the plot, SPD and SPI had no marked effects on the β-Carotene contents of the samples. on the other hand, increase in BLISP resulted to a commensurate increase in the β-Carotene contents of the samples.

Also, increased incorporation of SPI resulted to a decreased vitamin B1 contents of the samples. On the other hand, both increase in SPD and BLISP lead to increase the thiamin contents of the products.

Both increase in the duo of BLISP and SPD results to increase in vitamin B2 contents of the samples. Reverse was the case for the SPI which increased with decrease in vitamin B2 contents of the samples.

Also, both SPI and BLISP had a positive effect on the niacin contents of the samples. Increase in both factors resulted to an increase in the niacin contents of the sample. the reverse is the case for SPD, which decreased the niacin contents of the samples as it was increased.

For vitamin C content, while SPI had little or no marked effect on the vitamin C contents of the samples, SPD had an inverse variation with the vitamin C contents of the samples. As SPD increases, vitamin C content decreases and vice versa. However, increase in BLISP resulted to an increased vitamin C content of the sample. Vitamin C content of the nutraceutical products is comparable with that obtained for nutraceutical products from fresh onion, tomato, garlic, ginger, watermelon, and palm oil [50]. The mineral parameters reported by Gironés-Vilaplana [51] for new isotonic drinks made from berries and lemon juice is comparable with the results obtained for this research.

Effects of independent variables on the colour parameters of the nutraceutical products

Colour is one of the most vital attributes of any foods product due to consumer acceptability considerations. Colour parameters of the nutraceutical products as affected by the independent variables of SPI, SPD and BLISP is presented in Table 6. The lightness (*L*) of the samples ranged between 69.47 and 92.72, redness-greenness index (*a*), -0.73 and 0.62333, yellowness-blueness index (*b*), 13.1167 and 18.3767, colour intensity (ΔE),163.417 and 187.121, delta chrome (ΔC), 17.4326 and 22.7716, and hue angle (h^o), -89.265 and 89.973.

The regression equations showing the effects of the SPI, SPD and BLISP on the colour parameters of the nutraceutical products is shown in table 7. From the regression equation, all the colour parameters measured where linearly and quadratically affected by the independent variables of SPI, SPD and BLISP.

The 3D response surface plots showing the colour parameters as they are affected by the different proportions of SPI, SPD and BLISP is depicted in figure 6.

The plots showed that both increase on the SPI and SPD of the sample resulted to an increase in the lightness index, while the reverse was the case for BLISP. This could be as result of the fact that BLISP produced was dark.

SPD had no effects on the *a* index. Increasing SPI resulted to tilting of the colour of the sample toward redness, while BLISP led to increase in the greenness.

As depicted in the plots, while SPD had little or no effect on the yellowness-blueness index, increase in BLISP led to a decrease in the degree of yellowness of the samples. On the other hand, increasing SPI equally increased the yellowness of the samples. It could be because SPI is yellowish, while BLISP is dark greened.

it can be inferred that both SPI and SPD affected positively the colour intensity of the samples. Increase in both independent variables lead to an increase in the colour intensity of the samples. The reverse was the case for BLISP. As BLISP increased, the colour intensity of the samples decreased.

For delata chrome (ΔC), while BLISP affected delta chrome negatively, SPI had positive effect on the delta chrome. Increase in BLISP, gave rise to a decrease in the delta chrome, while increase in SPI results to increase in the delta chrome. SPD had no marked effect on the delta chrome of the samples.

In addition, both SPI and SPD had no marked effects on the hue angle of the samples. On the other hand, increase in BLISP resulted to an increase in the hue angle of the samples.

The colour parameters reported by Gironés-Vilaplana [51] for new isotonic drinks made from berries and lemon juice is comparable with the results obtained for this research.

Run	Phosphorus (mg/g)	Iron (mg/g)	Calcium (mg/g)	Potassium (mg/g)	Magnessium (mg/g)	B-Carotene µg/100g	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100)	Niacin (mg/100g)	Vitamin C (mg/100g)
1	575.02	7.26	100.29	328.16	27.0492	655.74	1.34426	0.688525	1.09836	10.0000
2	606.79	7.39	105.90	328.13	28.5146	1342.28	1.34610	0.738899	1.23446	12.0735
3	602.00	7.52	105.50	337.20	27.4600	971.00	1.35000	0.701923	1.18269	10.9615
4	620.00	8.01	110.01	283.00	27.8800	972.00	1.33000	0.702000	1.19000	10.8000
5	605.00	6.99	107.60	277.00	27.6700	970.00	1.32000	0.700000	1.17900	10.9000
6	676.00	8.12	112.60	366.20	28.0488	804.88	1.26829	0.658537	1.21951	10.4878
7	625.08	7.66	105.12	346.89	27.2166	577.61	1.30659	0.662714	1.12780	9.78235
8	570.42	6.53	90.550	294.47	26.4706	666.67	1.41176	0.725490	1.01961	10.0000
9	618.00	10.43	105.00	323.53	27.8846	971.15	1.32692	0.704000	1.18000	10.9490
10	666.09	8.47	119.89	378.38	29.2453	1264.15	1.24528	0.679245	1.33962	11.8868
11	599.78	6.09	105.50	221.00	27.8846	971.09	1.32692	0.712000	1.18340	10.9500
12	556.89	7.13	100.86	318.32	27.9000	1079.37	1.36508	0.730159	1.15873	11.2698
13	555.54	5.38	91.580	284.04	27.3585	1169.81	1.43396	0.773585	1.09434	11.5094
14	665.98	7.88	112.88	350.09	29.0698	1418.60	1.30233	0.720930	1.30233	12.3256
15	670.00	8.95	123.62	395.18	29.2391	1120.80	1.21130	0.650718	1.35150	11.4703
16	616.78	7.05	97.850	309.26	27.5212	1034.91	1.38308	0.736610	1.12818	11.1267
17	602.00	9.2	115.75	292.74	27.7800	974.00	1.32692	0.722000	1.19200	10.9900
18	620.05	8.06	111.19	358.07	28.1551	923.70	1.28512	0.676106	1.22326	10.8386
19	677.90	8.69	119.96	392.51	28.4314	764.71	1.21569	0.627451	1.27451	10.3922
20	535.62	6.66	92.010	294.10	26.8765	859.77	1.41298	0.740034	1.05705	10.5829

Table 4: Minerals and vitamins contents of the nutraceutical products.

Variable	Model	R^2
Phosphorus	+ 769.28797 + 0.10234*A - 5.00416 *B - 3.27003*C	0.9606
Iron	+ 7.60222 + 0.040951*A - 0.081835*B - 0.085052*C	0.5051
Calcium	+ 49.57113 + 2.20215* A - 1.49399*B + 2.11983*C + 5.81250E-003*A*B - 5.06250E-003*A*C + 8.68750E-003*B*C - 0.013796*A^2 + 3.78263E-004*B^2 - 0.23844*C^2	0.9485
Potassium	+ 1342.58291 - 20.53827*A - 16.77021*B - 40.18621*C + 0.016713*A*B + 0.016063*A*C + 0.062313*B*C + 0.15379*A^2 + 0.20100*B^2 + 4.25816*$C^{2'}$	0.7565
Magnesium	+31.31757 - 0.033399*A - 0.19637*B + 0.26454*C +7.02492E-004*A*B -1.52599E-003*A*C - 6.01096E-004*B*C + 2.80971E-004*A^2 +1.22583E-003*B^2 +9.97469E-003 *C^2	0.9858
β-carotene	+ 960.84687 -7.02178*A -16.87194*B + 256.41725*C + 0.11656*A*B -1.21124*A*C - 1.16500*B*C + 0.033495*A^2 +0.080738*B^2 -1.24498*C^2	0.9998
Vitamin B1 (Thiamin)	+1.25169 - 4.69963E-003*A + 0.013927*B + 0.015794*C - 3.34204E-005 *A*B - 3.63540E-005*A*C - 1.28844E-004*B*C + 1.94417E-005*A^2 - 7.50446E-005*B^2-3.48485E-004*C^2	0.9924
Vitamin B2 (Riboflavin)	+0.70052 -1.94014E-003*A +2.90749E-003*B +0.012652*C	0.9683
Niacin	+ 1.15456 +6.96919E-003*A - 0.018923*B +0.036636*C +6.35608E-005*A*B -2.00393E-004*A*C - 8.01559E-005*B*C - 3.61937E-005*A^2 + 8.66385E-005*B^2 -3.23477E-004 *C^2	0.9989
Vitamin C (Acorbic acid)	+10.32640 -6.08035E-003*A - 0.040953*B +0.73833*C +3.68555E-004*A*B -3.64209E-003*A*C -3.45711E-003*B*C	0.9944

Table 5: Regression equations of minerals and vitamins contents of the nutraceutical product formulations.

Run	L*	a*	b*	Hue Angle (h^o)	Delta Chrome (ΔC)	Colour Intensity (ΔE)
1	92.7200	0.5633	18.3767	88.244	22.7716	187.121
2	73.7767	-0.5967	13.4933	-87.468	17.8128	167.735
3	73.2867	-0.2800	13.9467	-88.849	18.2858	167.299
4	75.4467	-0.3100	14.4567	-88.771	18.7924	169.502
5	74.5267	-0.2933	14.2067	-88.817	18.5442	168.560
6	73.0400	0.1100	14.6700	89.570	19.0388	167.138
7	81.3167	0.6233	16.5967	87.849	21.0068	175.588
8	78.3100	0.0067	14.6900	89.973	19.0496	172.376
9	74.7300	-0.4033	14.0500	-88.355	18.3803	168.744
10	69.4700	-0.6200	13.1533	-87.301	17.4720	163.417
11	75.8300	-0.2767	14.6367	-88.917	18.9743	169.903
12	77.0100	-0.5733	13.9233	-87.642	18.2434	170.996
13	75.7267	-0.6700	13.1167	-87.075	17.4326	169.635
14	75.2800	-0.7300	13.8233	-86.977	18.1352	169.264
15	73.7600	-0.3533	14.9733	-88.648	19.3049	167.884
16	80.1833	-0.5433	14.5600	-87.862	18.8807	174.219
17	75.0567	-0.4733	13.9933	-88.062	18.3193	169.062
18	80.0667	-0.2000	15.6067	-89.265	19.9473	174.222
19	80.7800	0.4600	17.0167	88.451	21.4080	175.104
20	79.1960	-0.4867	14.2200	-88.039	18.5447	173.202

Table 6: Colour parameters of the nutraceutical formulations.

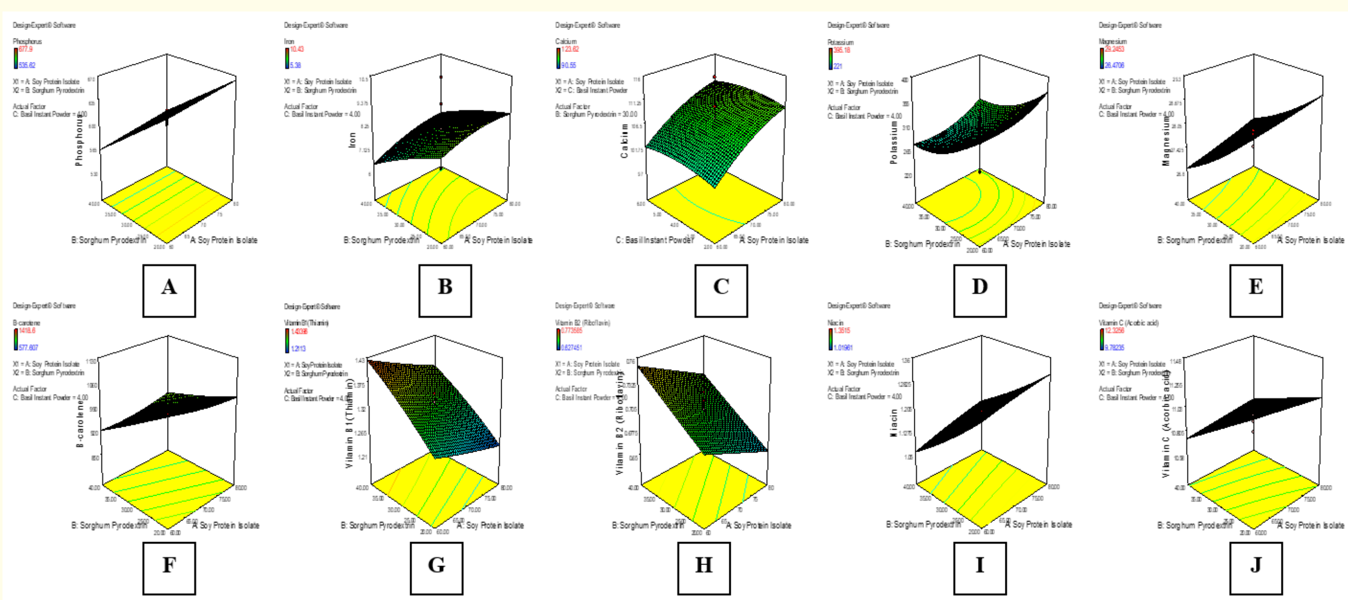

Figure 5: 3D Response surface plots of the effects of independent factors on the minerals and vitamins composition of the nutraceutical products.

*A= phosphorus. B= Iron, C= Calcium, D= Potassium, E= Magnessium, F= β-carotene, G= Vitamin B1, H= Vitamin B2. I= Niacin, J= Vitamin C

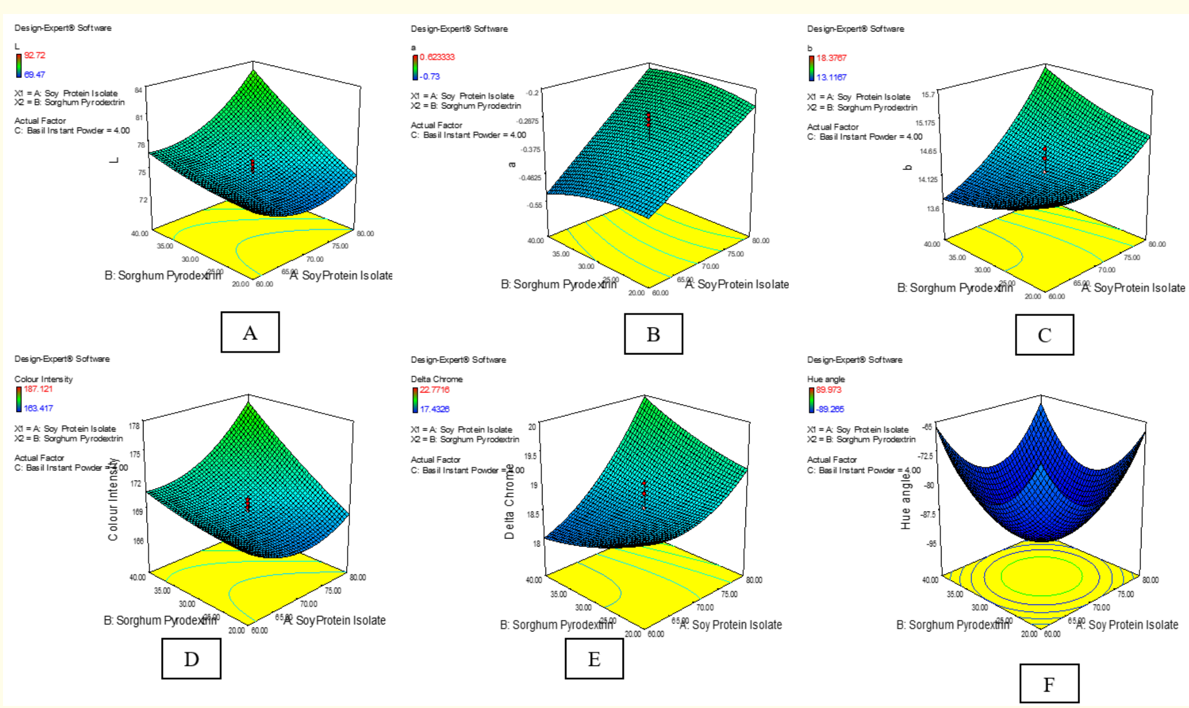

Figure 6: 3D Response surface plots of the effects of independent factors on the colour parameters of the nutraceutical products.

* A= lightness index (L), B= Redness-greenness index (a), C= Yellowness-blueness index (b), D= Colour intensity (ΔE), E= Delta

Optimization and validation of nutraceutical product formulation

The zones of acceptability for each attribute in the contour plot were layered from these authors' earlier work to get the best possible formulation. Desirability, a multiple response technique, served as the foundation for optimization. During this process, the values on each dependent variable were converted into desirability scores, which might vary from 0.0 for highly undesired to 1.0 for very desirable. Considering the strategy, the goal was to maintain within range color criteria, limit moisture content, and increase the contents of minerals, vitamins, crude protein, and ash.

The best regions for formulating nutraceutical products were obtained by superimposing contour plot zones of interest, where each characteristic earned ratings more than or equal to 3.0. The ideal ingredient (independent variable) values that were determined were 60.0 g of SPI, 20.0 g of SPD, and 4.83 g of instant soluble basil powders, as shown by the overlaid plots. This formulation has a computed desirability of 0.679, which produced a high-quality nutraceutical product.

The nutraceutical product with the optimal ingredient level was examined, and the outcomes were statistically compared to the expected values of the mathematical model to confirm the ideal formulation. At a 95% confidence level, there was no statistically significant difference seen between the anticipated and actual response values for the improved products. Both values fell within the range. Table 8 presents the expected and observed reactions of the created nutritional supplement.

Considering the correlation coefficient, or R2, between the experimental and modeled data, the matching quality of the data acquired by the suggested optimization model was assessed. After those numbers were mathematically adjusted, the model's R2 score was 0.999, indicating that it was a strong statistical model because it could not account for just 1% of the total impacts. The observed and predicted values have a good correlation, as seen by the parity plot (Figure 7).

Variables	Model	R^2
Lightness (L)	+ 148.94000 - 2.45412*A - 0.94323*B + 10.16439*C + 0.017204*A*B - 0.16673*A*C -0.057646*B*C + 0.019592*A^2 + 3.90407E-003*B^2 +0.21250*C^2	0.9306
Redness-Greenness Index (a)	- 0.44595 + 0.021825*A - 8.45682E-003*B - 0.26805*C + 2.41667E-004*A*B - 4.37500E-003*A*C + 6.66667E-004*B*C + 9.75234E-006*A^2 - 1.98173E-004*B^2 +0.041650*C^2	0.9775
Yellowness-Blueness Index (b)	+25.17509 -0.31940*A - 0.26670*B + 1.64453*C + 3.52083E-003*A*B - 0.036854*A*C -8.22917E-003*B*C + 3.01844E-003*A^2 + 9.24849E-004*B^2 + 0.071338*C^2	0.9415
Colour Intensity (ΔE)	+ 243.71837 - 2.47415*A - 0.96733*B + 10.28629*C + 0.017485*A*B -0.16998*A*C - 0.058159*B*C + 0.019812*A^2+3.98961E-003*B^2 + 0.22003*C^2	0.9307
Delta Chrome (ΔC)	+ 29.49564 - 0.31728*A - 0.26707*B +1.61914*C +3.53354E-003*A*B -0.037147*A*C - 8.16028E-003*B*C + 3.01460E-003*A^2 + 9.10111E-004*B^2 + 0.074911*C^2	0.9432
Hue Angle (h^o)	+1081.54748 - 19.59516*A - 8.33160*B - 143.44438*C - 1.06625E-003*A*B + 0.012231*A*C - 3.96875E-003*B*C + 0.13951*A^2 + 0.14046*B^2 +13.03308*C^2	0.9229

Table 7: Regression equations of colour parameters of the nutraceutical product formulations.

	Proximate composition					Minerals					
Responses	Moisture Content (%)	Crude Protein (%)	Ash content (%)	Crude Fat (%)	Carbohydrate (%)	Energy (Kcal)	Phosphorus (mg/g)	Iron (mg/g)	Calcium (mg/g)	Potassium (mg/g)	Magnesium (mg/g)
Predicted	5.3918	64.0116	4.6082	2.2426	22.4939	364.3780	659.4290	8.14660	113.2990	344.6570	28.7370
Observed	5.4457	64.6517	4.6543	2.2650	22.2689	360.7342	652.8347	8.0651	112.1660	341.2104	28.4496

	Vitamins					Colour Parameters					
Response	B-Carotene (μg/100g)	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)	Niacin (mg/100g)	Vitamin C (mg/100g)	L	A	B	Colour intensity	Delta Chrome	Hue angle
Predicted	1239.6600	1.2935	0.7034	1.2780	11.7582	75.7232	-0.5856	14.2693	169.7540	18.5872	-89.2650
Observed	1252.0570	1.3064	0.7104	1.2908	11.8758	76.4804	-0.5915	14.4119	171.4515	18.7731	-90.1577

Table 8: Predicted and Observed values for the physicochemical properties of the nutraceutical products.

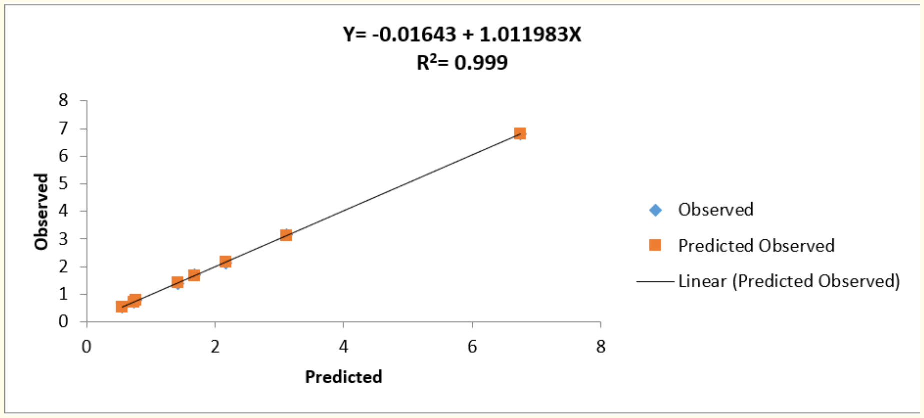

Figure 7: Graph of experimental versus predicted values.

Conclusion

Optimization of the creation of a soy-sorghum-basil nutraceutical product was achieved through the effective use of Response Surface Methodology (RSM). Soy-sorghum-basil significantly affected the proximate, mineral, vitamins and colour properties of the products.

The three variables employed in the study had a significant effect on the proximate, minerals, vitamins, functional, antinutritional and colour parameters of the nutraceutical products. The creation of practical regression equations for widespread use to forecast the behaviors of the products under various factor combinations was made possible by the modeling of experimental data.

Incorporation of SPI caused increase crude protein, moisture, ash, crude fat, iron, calcium, potassium contents, and lightness index. Sorghum pydrodextrin caused a decrease in ash content, and increase in carbohydrate content, phosporus, vitamin B_1, and B_2, and lightness index. Both SPI and BLISP had negative contribution to carbohydrate content. On the other hand, addition of BLISP led to increase in ash content, magnesium content, beta-carotene content, vitamin B1 and B2, vitamin C of the products, while BLISP and SPD had no significant impact on crude fat content. All the three ingredients had positive effects on the energy content of the nutraceutical products.

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