International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 5; September 2017; Page No. 208-215

Compositing of neglected and underutilized legumes for theoretical minimum amino acid score in elastin

Vida Gyimah[*1], Isaac W Ofosu[2], Edward K Essuman[3]
[1, 2] Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[3] Department of Nutrition and Dietetics, University of Health and Allied Sciences, Ho, Ghana

Abstract

Elastin is restricted to few amino acids and its protein constitutes blood vessels in the body as well as tumors. It is believed that when the body is deprived of those amino acids that constitute elastin, little amount would be available to constitute those tumors. The study aims to obtain the score of amino acids that could theoretically supply minimum amino acids to build the protein elastin. This was achieved by compositing five underutilized legumes using Response Surface Methodology (RSM). The RSM revealed that there were strong interactions between the legumes in minimizing amino acids score. The optimized condition that gave a desirability of 1 was 15 % of *Vigna subterranean*, 10 % of *Cajanus cajan*, 20 % of *Phaseolus lunatus*, 21.50 % of *Canavalia ensiformis* and 33.50 % of *Mucuna pruriens*. The optimized legume composite gave an amino acid score in elastin (AASE) value (8.25 %) closer to the predicted value of 8.60 %.

Keywords: underutilized legumes, amino acids score, elastin, response surface methodology, composite

1. Introduction

Elastin is an essential part of various human tissues that depend on elasticity found in the form of fibres. They are found in membranes such as elastic ligaments, elastic blood vessels, and other compliant tissues such as lung, arteries and skin [1, 2, 3]. It is also essential for the formation of new blood vessels of all the organs in our bodies as well as cancers and tumors. The constituent amino acids found in elastin are: proline, leucine, isoleucine, valine and glycine; the latter comprising almost one quarter of the make-up of the elastin.

Underutilized legumes such as *Canavalia ensiformis* and *Cajanus cajan*, [4] are beneficial to man in agriculture, medicine and the food industry and have the potential to reduce poverty and alleviate hunger but necessary attention has not been given to their effective use. Underutilized legumes are not consumed in most regions of the world owing to the length of time needed to cook a legume-based meal, presence of flatulence and anti-nutritional factors [5]. Underutilized legumes, such as cowpea (*Vigna unguiculata*) and horse gram (*Macrotyloma uniflorum*) have been documented as potential sources of protein and other nutrients [6]. Recently, there has been a tendency to use exotic species such as soybean and peanut for food and for this, Pandey and Srivastava [7] supported the idea that alternative legumes should be searched for possible utilization. Unlike soybean, peanut, and other well-known legumes, the seeds of *Mucuna pruriens*, *Canavalia ensiformis*, *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna subterranea are* some of the local underutilized (unconventional) legumes in Ghana.

A lot of wild legumes have been identified but their use is limited due to absence of dietary information [8, 9]. Although the amino acid profile of *Phaseolus lunatus*, *Vigna subterranea*, *Mucuna pruriens*, *Cajanus cajan* and *Canavalia ensiformis* has been reported in several publications [10], little information is available on the protein or amino acid quality of their composite. A review of available literature reveals that more effort has been invested in nutritional and chemical evaluation of these legumes than the studies of their composited flour. Little is known regarding the calculation of amino acid score of elastin in food, especially in composited underutilized legume flour.

Assessing the amino acid score in elastin of some composited neglected and underutilized legumes (NULs) will enable recommendations to be made as to which of the composited NULs have lower amino acid score that can be included in the nation's food basket. This exploration may serve as an alternative source of treatment for cancer and diabetic patient. The aim of the study is to obtain the score of amino acids that could theoretically supply minimum amino acids to build the protein elastin required for building blood vessels.

2. Materials and Methods

2.1 Sources of Materials

Underutilized legumes such as Bambara groundnut (*Vigna subterranea*), Velvet bean (*Mucuna pruriens*), Jack bean (*Canavalia ensiformis*), Pigeon pea (*Cajanus cajan*) and Lima beans (*Phaseolus lunatus)* were sourced from a farm at *Anwomaso*, in the Ashanti Region of Ghana. Hexane was bought from Air Products Nederland BV (Utrecht, Nederland). Amino acid standards (2.5mol/L), sulphuric acid, phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich (Germany), 2, 4-dinitrofluorobenzene (DNFB), HPLC grade methanol and acetonitrile were purchased from MES Chemicals (Ghana). Other chemicals used were of analytical reagent grade. Water used for preparation of reagents was double distilled water.

2.2 Preparation of Sample

The legumes were sorted and thoroughly cleaned to remove the dust and other foreign materials. They were then weighed and milled into flour using MPE roller mills (Model GP-140 Grinder, Shanghai-China). They were further milled again by the use of Waring blender (Model: WPB05, USA) to reduce the particle size to 25µm. Each sample was stored in an airtight container for further analysis.

Cold extraction method was used to defat each of the legume flour in separate containers. About 100 g of each legume flour was weighed and tied in a cheese cloth and soaked in hexane in the ratio of 1:10 w/v, with respect to flour to solvent ratio. Each of the containers were sealed and left for 72 h (3 days) at room temperature. The defatted flours were dried in a solar dryer to remove the residual solvent. They were stored in high density polyethylene bags at room temperature (25 °C) until when needed.

2.3 Mixture Design

Response Surface D-optimal, Mixture Design [11] was used in this study to composite the five different legume flours with the ranges as shown in Table 1. In all, there were a total of 45 runs based on the cubic design model in order to increase the flexibility of the design (Table 2).

Table 1: Design summary showing the upper and lower limit of the legumes.

Component	Name	Units	Low Actual	High Actual
A	BamGmt	%	15	55
B	CajCajan	%	10	50
C	PhaL	%	20	60
D	CanEs	%	10	50
E	Muc	%	5	45

Table 2: Experimental runs for compositing five legumes as designed by the D-Optimal Mixture Design of Response Surface Methodology.

Run	A: BamGrnt %	B: CajCajan %	C: PhaL %	D: CanEnsif %	E: Muc %
1	15.0	10.0	60.0	10.0	5.0
2	15.0	37.0	20.0	10.0	18.0
3	28.0	37.0	20.0	10.0	5.0
4	15.0	37.0	33.0	10.0	5.0
5	15.0	50.0	20.0	10.0	5.0
6	15.0	50.0	20.0	10.0	5.0
7	15.0	23.0	20.0	37.0	5.0
8	28.0	10.0	33.0	10.0	18.0
9	15.0	23.0	20.0	23.0	18.0
10	28.0	10.0	20.0	10.0	32.0
11	15.0	10.0	20.0	37.0	18.0
12	15.0	10.0	20.0	50.0	5.0
13	28.0	10.0	20.0	23.0	8.0
14	28.0	23.0	20.0	23.0	5.0
15	28.0	10.0	20.0	37.0	5.0
16	55.0	10.0	20.0	10.0	5.0
17	39.0	14.0	24.0	14.0	9.0
18	15.0	23.0	33.0	10.0	18.0
19	15.0	23.0	33.0	23.0	5.0
20	19.0	14.0	44.0	14.0	9.0
21	23.0	18.0	28.0	18.0	13.0
22	55.0	10.0	20.0	10.0	5.0
23	28.0	23.0	33.0	10.0	5.0
24	42.0	10.0	20.0	10.0	18.0
25	42.0	10.0	33.0	10.0	5.0
26	15.0	10.0	47.0	23.0	5.0
27	15.0	10.0	20.0	23.0	32.0
28	19.0	14.0	24.0	34.0	9.0
29	15.0	10.0	33.0	23.0	18.0
30	15.0	10.0	20.0	50.0	5.0
31	42.0	10.0	20.0	23.0	5.0
32	28.0	10.0	47.0	10.0	5.0
33	28.0	23.0	20.0	10.0	18.0
34	28.0	10.0	33.0	23.0	5.0
35	19.0	34.0	24.0	14.0	9.0
36	15.0	10.0	47.0	10.0	18.0
37	42.0	23.0	20.0	10.0	5.0
38	15.0	10.0	60.0	10.0	5.0
39	15.0	10.0	20.0	10.0	45.0
40	15.0	10.0	33.0	10.0	32.0
41	15.0	10.0	33.0	37.0	5.0
42	15.0	23.0	47.0	10.0	5.0
43	15.0	23.0	20.0	10.0	32.0
44	15.0	10.0	20.0	10.0	45.0
45	15.0	37.0	20.0	23.0	5.0

2.4 Extraction of Protein

Protein was extracted based on a method described by Gomez-Brenes *et al.* [12]. About 100 g of each composite from the runs was dispersed in 1000 ml of 0.01 M NaOH solution. Samples were agitated at room temperature for 1 h on an orbital shaker (Gallenkamp Orbital Shaker, London-UK) at 150 rpm. The resulting solutions were separated from insoluble material by centrifugation at 2000 rpm for 15 min at room temperature (25 ºC). The supernatant solutions produced were acidified to a pH range of 4.5-5.0 with 0.1 M HCl to precipitate the proteins. The precipitated proteins were thrice washed with distilled water and freeze dried (Heto power dry LL300 freeze dryer Thermo Fisher Scientific, USA).

2.5 Determination of Amino Acid Content

2.5.1 Acid hydrolysis

About 10 ml of a mixture of 6 N HCl and 5 % phenol was added to 50 mg of each of the protein isolate from the composite in a digestion tube. Then, 1 % of 2-mecaptoethanol was added to the mixture and sealed with aluminum foil to prevent air from entering. The digestion tube and its content were heated for 24 h at 110 ºC. The hydrosate was filtered and refrigerated at 4 ºC for further analysis.

2.5.2 HPLC analysis

HPLC system (Varian) consisting of a Varian ProStar 210/215/218/SD-1 Pumps, Varian ProStar 325LC Detector was used for analyzing the amino acid content. Galaxie software for data processing and Genini µL C18 110A 150 * 4.60 mm 5 micron 257052-7 analytical column was used for separation. The mobile phase consisted of mixture A (0.02 mol/L Na_2HPO_4 + 0.02 mol/L NaH_2PO_4) and mixture B (Methanol: Acetonitrile) in a ratio of 10: 90 (v/v)). Both mixtures were mixed in 70:30 ratios with a flow rate of 1.3 mL/min. The analysis was carried out at room temperature.

One ml of the prepared standard solution was taken and poured into 15 ml centrifuge tube. It was diluted with 2.0 ml of 0.2 M $NaHCO_3$. One ml of 1 % 1-fluoro-2, 4-dinitrobenzene dissolved in 100 ml of methanol was added to derivatize the mixture. This test tube was then placed on a water bath at 60 ºC for 40 min. All derivatization reactions were stopped by addition of 0.5 ml of 1M HCL. The resulting derivative was then filtered. All the other samples were treated in the same manner as described for the standard. The mixture was then injected into the HPLC. The amino acids peaks were then identified based on the retention time and their respective peak areas were measured and the respective amino acid quantified from the standard curve previously determined.

2.6 Statistical Method

2.6.1 Mixture design

The amino acid response data collected were loaded and run in the Design Expert [11]. The data was first analyzed in the fit summary section and then the model terms were examined to determine if the model best fit the data. Then the ANOVA table was carefully studied to examine the p-values of the legumes that interacted to produce effective amino acid score. With the optimization, each of the variables of the amount of legume in the composite that produces the final AASE score could either be set as ‘in range, maximum, minimum, target or equal to. Since the goal of the experiment was to achieve the minimum AASE, the amount of all the legumes were left ‘in range’, and the AASE was set to ‘minimum’ with importance point of 5. On the solution tab, the constrain table showing the goals of all the factors were shown, including the response goal and its importance ‘point’. More importantly, the solution to the ‘trade off’ set in the criteria section was ranked as the possible set of legumes ratios according to their ‘desirability’ points (0-1, with 1 maximum). This gave the most likely theoretical ratios of the legumes to be used to achieve the most minimum response as was selected by the software. Information on the point prediction was studied to give the limits permissible or acceptable when the experiment was re-run with the selected legumes.

3. Results and Discussion

Response surface methodology (RSM) is an experimental modeling technique that was used to estimate the relationship between a set of controllable experimental factors and observed results [13]. The data for dependent variable AASE and the independent variables; *Cajanus cajan, Canavalia ensiformis, Phaseolus lunatus, Mucuna pruriens* and *Vigna subterranea* were run in the Design Expert [11] package to obtain a regression model that predicted the response within the given data set as shown in Table 3.

Table 3: Sequential model sum of squares table showing the suggested model (*) of the highest order polynomial where the additional terms are significant and the model is not aliased for the Amino Acids Score in Elastin.

Source	Sum of Squares	df	Mean Square	F- Value	p-value Prob > F
Mean vs Total	15344.20	1	15344.20		
Linear vs Mean	97.50	4	24.37	0.89	0.4787
Quadratic vs Linear	206.34	10	20.63	0.69	0.7220
Sp Cubic vs Quadratic	425.57	10	42.56	1.88	0.1172
Cubic vs Sp Cubic*	289.94	10	28.99	1.97	0.1739
Sp Quartic vs Quadratic	448.99	14	32.07	1.17	0.3873
Residual	384.15	14	27.44		
Total	16481.17	43	383.28		

The model suggested both mean vs total and cubic vs special cubic. A p-value of 0.1739 was obtained for the cubic vs special cubic over the mean vs total but the design expert accepted cubic model based on the fact that it was the model that had the highest order polynomial where the additional terms are significant and the model is not aliased. The regression models were observed to trace the one that had the highest p-value. The p-value ranges from 0.1238 from the special quadratic to 0.3124 of the cubic regression. The model suggested the cubic with a p-value of 0.3124 as shown in

Table 4. It means that the amino acid score in elastin responses best fit into the cubic as compared to the others.

Table 4: Lack of fit test showing the suggested model (*) of the highest order polynomial with the biggest prob> F value for the Amino Acids Score in Elastin.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Linear	978.43	33	29.65	2.43	0.1629
Quadratic	772.09	23	33.57	2.75	0.1320
Special Cubic	346.51	13	26.65	2.18	0.1996
Cubic*	56.58	3	18.86	1.54	0.3124
Special Quartic	323.10	9	35.90	2.94	0.1238
Pure Error	61.05	5	12.21		

Studying the model summary statistics as shown in Table 5, the aim was to select a model with "*r-squared*” maximized and approaching 1. From Table 5, it suggested the cubic regression which had r-square of 0.897 which was approximately to 1 over the other models. It is clearly seen that there are progressions of the sum of squares from the linear regressions and maximizing with the cubic regressions.

Table 5: Suggested model (*) of the highest order polynomial with the maximized r-squared value for the amino acids in elastin score.

	Std.	**R-Squared**	**Adjusted**	**Predicted**	
Source	Dev.		R-Squared	R-Squared	PRESS
Linear	5.23	0.086	-0.011	-0.147	1304.46
Quadratic	5.45	0.267	-0.100	-0.820	2069.07
Special Cubic	4.76	0.642	0.164	-3.631	5266.30
Cubic*	3.83	0.897	0.457	-22.30	26495.20
Special Quartic	5.24	0.662	-0.014		

The values of "P > F" less than 0.05 indicate model terms are significant

In this case BC, BE, ACD, BCD, and BCE are significant model terms (Table 6). Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve the model. There was an interaction between BC, BE, ACD, BCD and BCE.

Table 6: Significance of the factors and their interactions in the regression model that has been obtained for the amino acids score in elastin.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-Value**	**p-value Prob > F**
Model	862.62	19	45.40	3.81	0.0014
Linear Mixture	97.50	4	24.37	2.04	0.1216
BC	91.50	1	91.50	7.67	0.0109
BE	143.74	1	143.74	12.05	0.0021
ACD	83.67	1	83.67	7.01	0.0144
BCD	93.01	1	93.01	7.80	0.0103
BCE	146.07	1	146.07	12.25	0.0019

3.1 Optimization of Legume Quantities by Response Surface Methodology to Obtain Minimum AASE

In order to obtain the optimum quantities for the various legumes, constraints were set for all the legumes (Table 7). The AASE was also targeted at 8.57 in order to make available elastin to other organs of the body. The model suggested all the following mixtures as shown in Table 8 but selected number one as the best mixture.

Table 7: Constraints set for legumes for determining optimum condition.

Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
A:BamGrnt	is in range	15	55	1	1	3
B:CajCajan	is in range	10	50	1	1	3
C:PhaL	is in range	20	60	1	1	3
D:CanEs	is in range	10	50	1	1	3
E:Muc	is in range	5	45	1	1	3
AASE score	is target = 8.573	8	10	1	1	5

Table 8: Optimum condition predicted by the response surface methodology.

Number	**BamGrnt**	**CajCajan**	**PhaL**	**CanEs**	**Muc**	**AASE score**	**Desirability**	
1	15.000	10.000	20.000	21.514	33.486	8.573	1.000	Selected
2	15.000	10.000	20.000	18.165	36.835	8.573	1.000	
3	15.166	10.000	20.000	20.812	34.022	8.573	1.000	
4	15.270	10.000	20.000	19.736	34.994	8.573	1.000	

3.2 Interaction between B (*Cajanus cajan*) and C (*Phaseolus lunatus*)

The AASE ranges from 0.00 to 40.00 %, B from 10.00 to 12.00 % and C 20.00 to 22.00 % as shown in Figure 1. The quantities of *Vigna subterranea*, *Canavalia ensiformis* and *Mucuna pruriens* are held constant at 15.00, 19.50 and 33.50 % respectively while the quantities of *Cajanus cajan* and *Phaseolus lunatus* were varied. The interaction between BC did not contribute much to the change of the AASE. The AASE slightly increased when there was an increase in the *Phaseolus lunatus* from 20.00 to 22.00 % whiles that of *Cajanus cajan* decreased from 12.00 to 10.00 %.

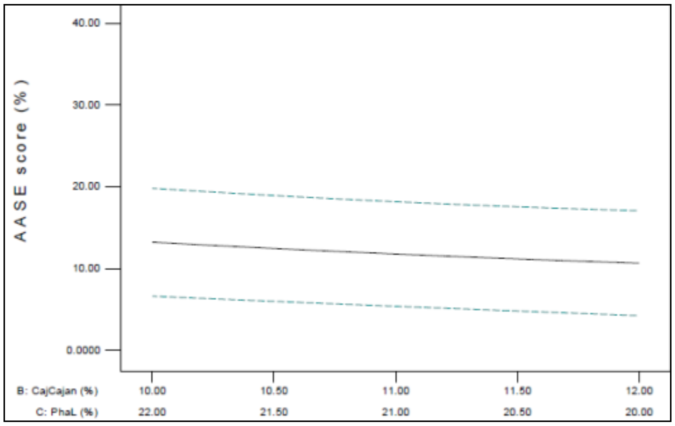

Fig 1: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual *Cajanus cajan* and *Phaseolus lunatus.*

It has been reported by Bracalello *et al*. [14] that the constituent amino acids found in elastin are valine, leucine, isoleucine, proline and glycine. According to Iheanacho [15], *Phaseolus lunatus* contains these amino acids that makes elastin; glycine (4.92 g kg^{-1}), proline (3.21 g kg^{-1}), leucine (7.04 g kg^{-1}), valine (5.41 g kg^{-1}) and isoleucine (4.51 g kg^{-1}). From Figure 1 it was evident that as the quantity of *Phaseolus lunatus* increased, the AASE also increased and vice versa. There was an increase in the AASE due to high amount of glycine that is contained in *Phaseolus lunatus*. Since glycine constitutes about one third of elastin, as the quantity of *Phaseolus lunatus* increased, there was a correspondence increase in the glycine which in tend increased the AASE.

Cajanus cajan contains 3.07 g kg^{-1} of glycine, 3.17 g kg^{-1} of proline and 3.47 g kg^{-1} of isoleucine but higher quantities of leucine (6.78 g kg^{-1}) and valine (5.58 g kg^{-1}) [16]. As the quantity of *Cajanus cajan* increased, AASE also decreased due to smaller amount of glycine in *Cajanus cajan* as compared to *Phaseolus lunatus.* Although *Cajanus cajan* has higher amount of leucine and valine, these amino acids did not contribute enough to increase the elastin score because their percentages in elastin are smaller; 2.4 % and 2.2 % respectively. The percentage abundance of glycine (32.9 %), proline (12.6 %) and alanine (10.9 %) are comparably higher than leucine (2.4 %), valine (2.2 %), and isoleucine (1.1%) [17] and therefore have impact on the increase in the AASE.

3.3 Interaction between B (*Cajanus cajan*) and E (*Mucuna pruriens*)

There was a strong interaction between B and E. Figure 2 shows a quadratic curve which goes through two minimum ends and an upper middle part. As *Canavalia ensiformis*, *Vigna subterranea* and *Phaseolus lunatus* are kept at optimum percentages of 21.50, 15.00 and 20.00 % respectively, the amount of *Cajanus cajan* ranged from 10.00-38.50 % while *Mucuna pruriens* ranged from 5.00-33.50 %. Amino Acid in Elastin Score was at a minimum when the quantity of *Cajanus cajan* was low (10.00 %) and that of *Mucuna pruriens* was also high (35.50 %). Amino Acid in Elastin Score was also at a minimum when *Cajanus cajan* was also high (38.50 %) and *Mucuna pruriens* was also low (5.00 %). Amino Acid in Elastin Score was at maximum when almost equal quantities of B (24.25 %) and E (19.25 %) were added.

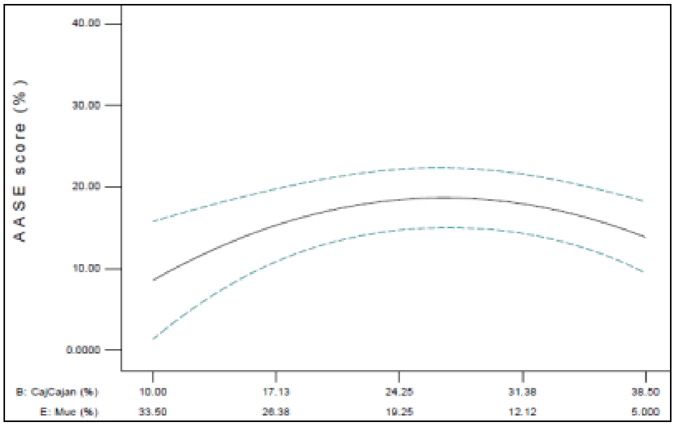

Fig 2: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual *Cajanus cajan* and *Mucuna pruriens.*

It was expected that AASE would increase as the quantity of *Mucuna pruriens* was increasing but the converse was observed. The constituents of amino acids in elastin in *Mucuna pruriens* are as follows; glycine (5.92 g kg^{-1}), proline (2.80 g kg^{-1}), leucine (7.24 g kg^{-1}), valine (3.90 g kg^{-1}) and isoleucine (5.94 g kg^{-1}) [18]. With the exception of proline and valine, *Mucuna pruriens* had higher glycine, leucine and isoleucine than *Cajanus cajan.*

Apart from the glycine that contributed to 32.9 % in elastin, proline is the second amino acid with 12.6% in elastin [17]. The difference in the amount of proline in these legumes contributed to the difference in the two minimum ends. The higher proline content in *Cajanus cajan* contributed to the increase in the AASE. Moreover, the aggregate of other amino acids in the legumes could also influence the increase in AASE. The maximum part of the quadratic graph was as a result of each of the legumes with almost the same quantity; *Cajanus cajan* (24.25 %) and *Mucuna pruriens* (19.25 %) contributing higher amino acid content of valine, glycine, proline, isoleucine, leucine and alanine. The amino acids in both legumes did influence the increase in AASE.

3.4 Interaction between C (*Phaseolus lunatus*) and E (*Mucuna pruriens*)

From Figure 3, as *Canavalia ensiformis*, *Vigna subterranea* and *Cajanus cajan* are kept at optimum percentages of 21.50, 15.00 and 10.00 % respectively. The amount of *Phaseolus lunatus* ranged from 20.00-48.50 % while *Mucuna pruriens* ranged from 5.00-33.50 %. AASE was at a minimum when the quantity of *Phaseolus lunatus* was 20.00 % and that of *Mucuna pruriens* was 35.50 %. The amount of AASE increased when the amount of *Phaseolus lunatus* proteins increased from 20.00-48.50 %. AASE was at maximum when quantities of *Mucuna pruriens* and *Phaseolus lunatus* proteins were 19.25 and 34.25 % respectively.

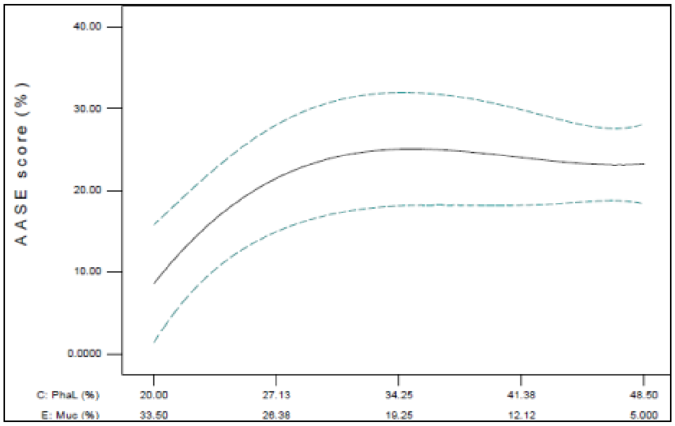

Fig 3: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual *Phaseolus lunatus* and *Mucuna pruriens.*

It was expected that AASE would increase as the quantity of *Mucuna pruriens* was increasing but it was rather decreasing. However, an increase in *Phaseolus lunatus* increased the AASE. The constituent amino acids of elastin in *Mucuna pruriens* were higher than *Phaseolus lunatus* except proline and valine. The difference in the amount of proline in *Phaseolus lunatus* (3.2 g kg^{-1}) and *Mucuna pruriens* (2.80 g kg^{-1}) has contributed to the increase in the AASE. Proline, which represents 12.6 % of the elastin was the second highest and was able to increase the elastin score.

3.5 The interaction between A (*Vigna subterranea*), C (*Phaseolus lunatus*) and D (*Canavalia ensiformis*)

There was an interaction between these legumes with a p-value of 0.0144 (Table 6). The quantities of *Vigna subterranea*, *Phaseolus lunatus* and *Canavalia ensiformis* were varied while that of *Cajanus cajan* and *Mucuna pruriens* were kept at optimum percentages of 10.00 and 35.50 % respectively as shown in Figure 4. The range of the AASE was from 8.57 to 32.00 %. The amount of *Vigna subterranea* ranged from 15.00-26.50 %, *Canavalia ensiformis* at 10.00-21.50 % and *Phaseolus lunatus* at 20.00-31.50 %.

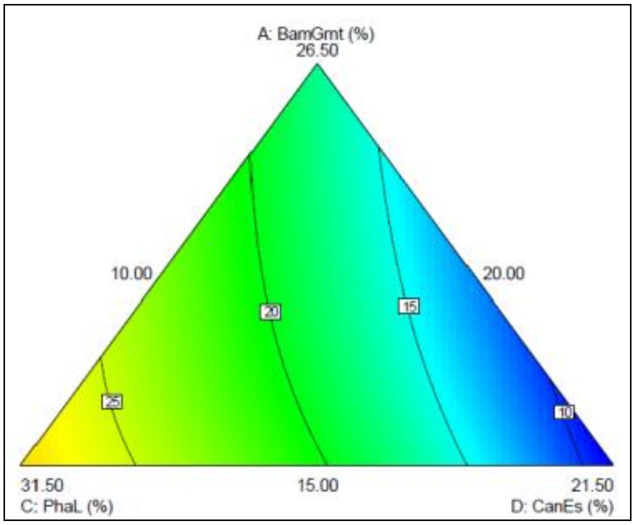

Fig 4: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual *Cajanus cajan, Phaseolus lunatus* and *Canavalia ensiformis*.

AASE increased with an increase in the quantity of *Vigna subterranea* and *Phaseolus lunatus* because of higher content of amino acids in *Vigna subterranea* [19] and *Phaseolus lunatus* [15]. However, an increase in the quantity of *Canavalia ensiformis* did not increase the AASE. Their amino acid contents were comparably higher than that of *Canavalia ensiformis* and was able to increase the AASE. There was a decrease in the AASE as the quantity of *Canavalia ensiformis* increased due to lower amount of relevant amino acids that were present (Table 9). Thus, their amino acids; glycine, proline, valine and leucine in *Canavalia ensiformis* were in their minimum ranges.

Table 9: Predicted values ACD (*Vigna subterranea*, *Phaseolus lunatus* and *Canavalia ensiformis*).

Predicted values for AASE (%)	Predicted values for *Vigna subterranea* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values for *Canavalia ensiformis* (%)
10.00	16.02	20.35	20.13
15.00	19.48	21.78	15.24
20.00	19.70	24.42	12.38
25.00	16.88	28.87	10.75

3.6 Interaction between B (Cajanus cajan), C (Phaseolus lunatus) and D (Canavalia ensiformis)

Cajanus cajan, *Phaseolus lunatus* and *Canavalia ensiformis* were varied whiles *Vigna subterranea* and *Mucuna pruriens* were kept at optimum percentages of 15.00 % and 33.50 % respectively. Form Figure 5, AASE ranged from 8.57 to 32.00 %. The amount of *Canavalia ensiformis* ranged from 10.00 to 21.50 % *whiles Cajanus cajan* ranged from 10.00 to 21.50 % and *Phaseolus lunatus* at 20.00 to 31.50 %. It was observed that increasing the quantity of *Canavalia ensiformis* and *Phaseolus lunatus* did not have much influence on the AASE but increasing *Cajanus cajan* increases the AASE. *Cajanus cajan* was kept at minimum to observe a low AASE.

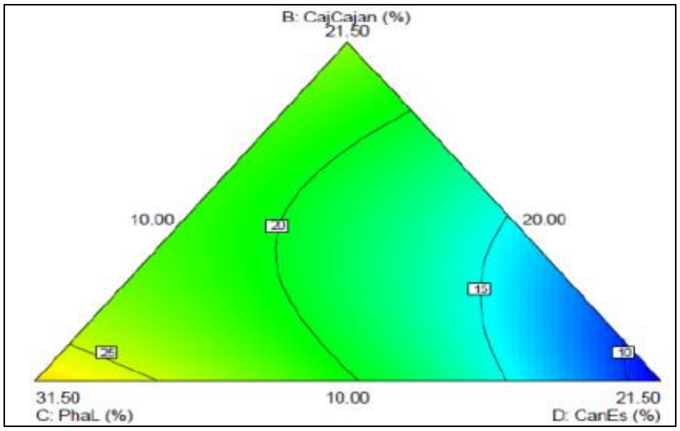

Fig 5: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual *Cajanus cajan, Phaseolus lunatus* and *Canavalia ensiformis.*

The higher content of glycine, leucine, proline and valine [15, 16] in *Cajanus cajan* and *Phaseolus lunatus* resulted in an increase in AASE. Increasing the quantity of *Canavalia ensiformis* did not increase AASE due to lower amount of relevant amino acids that were present (Table 10).

Table 10: Predicted values for BCD (*Cajanus cajan*, *Phaseolus lunatus* and *Canavalia ensiformis*).

Predicted values for AASE (%)	Predicted values for *Cajanus cajan* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values for *Canavalia ensiformis* (%)
10.00	10.53	20.39	20.58
15.00	12.08	22.08	16.96
20.00	15.37	24.30	11.83
25.00	10.08	29.34	12.05

3.7 Interaction between B (Cajanus cajan), C (Phaseolus lunatus), E (Mucuna pruriens)

From Figure 6, the quantities of *Cajanus cajan, Phaseolus lunatus* and *Mucuna pruriens* were varied as that of *Vigna subterranea* and *Canavalia ensiformis* were kept at optimum percentages. The value for *Mucuna lunatus* ranged from 5 to 33.50 %, *Cajanus cajan* ranged from 10 % to 38.50 % and *Phaseolus lunatus* ranged from 20 to 48.50 %.

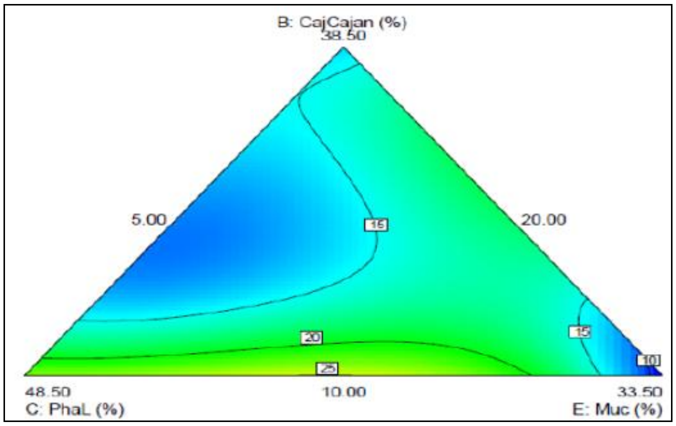

Fig 6: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual *Cajanus cajan, Phaseolus lunatus* and *Mucuna pruriens.*

Amino acid in elastin (AASE) increased with increase in the quantity of *Cajanus cajan* and *Phaseolus lunatus* but a decrease in *Mucuna pruriens.* It was expected that, with an increase in the quantity of *Mucuna pruriens*, AASE should increase but there was a decrease rather due to higher values of glycine (5.95 %) and alanine (4.24 %) [18]. The amount of proline in *Mucuna pruriens* was 2.80 g kg^{-1}which was lower as compared to the amounts in *Cajanus cajan* and *Phaseolus lunatus.* The higher values of proline in *Cajanus cajan* and *Phaseolus lunatus* have contributed to the increase of AASE (Table 11).

Table 11: Predicted values for BCE (*Cajanus cajan, Phaseolus lunatus* and *Mucuna pruriens).*

Predicted values for AASE (%)	Predicted values for *Cajanus cajan* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values for *Mucuna pruriens* (%)
10.00	10.59	20.35	32.57
15.00	13.52	21.92	28.07
20.00	12.45	34.94	16.10
25.00	10.06	36.41	17.32

4. Conclusion

The optimized condition that gave a desirability of 1 was 15 % of *Vigna subterranean*, 10 % of *Cajanus cajan*, 20 % of *Phaseolus lunatus*, 21.50 % of *Canavalia ensiformis* and 33.50 % of *Mucuna pruriens*. The optimized legume composite gave an AASE value (8.25 %) closer to the predicted value of 8.60 %. The protein quality of foods for human consumption through the use of amino acid profiles and amino acid score in elastin can provide added value to the national food composition tables and international food databases. It may also ensure that extensive research is conducted on them to improve their quality for both human and animal consumption.

5. References

1. Vrhovski B, Weiss AS. Biochemistry of tropoelastin. Eur J Biochem, 1998, 258:1-18.
2. Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. Faseb J, 1993; 7:1208-1218.
3. Uitto J, Christiano AM, Kahari VM, Bashir MM, Rosenbloom J. Molecular biology and pathology of human elastin. Biochem Soc Trans, 1991; 19:824-829.
4. Bhat R, Karim AA. Exploring the nutritional potential of wild and underutilized legumes. Compr Rev Food Sci Food Safety, 2009; 8:305-331.
5. Adsule RN, Lawande KM, Kadam SS, Pea In: DK Salunkhe and SS Kadam Eds. Handbook of world legumes: Nutritional chemistry, processing technology and utilization Florida: CRC Press, 1989; 3:215-251.
6. Sreerama YN, Sashikala VB, Pratape VM, Singh V, Nutrients. antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chem, 2012; 131:462-468.
7. Pandey VN, Sirvastava AK. Seed protein yield from some Crotalaria spp. and in vitro nutritional quality of that from C. juncea. Plant Foods Hum Nutr, 1990; 40:195-200.
8. Viano J, Masotti V, Gaydou EM, Bourreil PJL, Ghiglione GM. Compositional characteristics of 10 wild plant legumes from Mediterranean French pastures. J Agr Food Chem, 1995; 43:680-683.
9. Vijayakumari K, Siddhuraju P, Janardhanan K. Nutritional assessment and chemical composition of the lesser known tree legume, Acacia leucophloea. Food Chem, 1994; 50:28-58.
10. Ezeagu IE, Gopalakrishna AG, Khatoon S, Gowda LR. Physico-chemical characterization of seed oil and nutrient assessment of Adenanthera pavonina: An underutilized tropical legume. Ecol Food Nutr, 2004, 43:295-305.
11. Design-Expert, packaged with Montgomery DC, Design and analysis of experiments, 6th ed., Wiley-Interscience, New York, 2007.
12. Gomez-Brenes RA, Nunez EI, Bressani RB, Effect of various solvents on the extraction of protein fractions of beans Phaseolus vulgarus. Arch Latinoam Nutr, 1983; 33:503-518.
13. Gao Y-L, Ju X-R, Jiang H-H, Studies on inactivation of Bacillus subtilis spores by high hydrostatic pressure and heat using design of experiments. J Food Eng, 2006; 77:672-679.
14. Bracalello A, Santopietro V, Vassalli M, Marletta G, Del Gaudio R, Bochicchio B and Pepe A, Design and production of a chimericresilin-, elastin-, and collagen-like engineered polypeptide. Biol Macromol, 2011; 12:2957-2965.
15. Iheanacho KME, Comparative studies of the nutritional composition of soy bean glycine max and Lima bean Phaseolus lunatus. Scientia Afr, 2010; 9(2):29-35.
16. Akande SR, Fasoyiro SB, Arowora KA, Sodeko OO, Sulaiman PO, Olapade CO and Odiri CE, Physico-chemical and sensory properties of pigeon pea Cajanus cajan flours. Afr J Food Sci. 2010; 4(3):120-126.
17. Szpak P. Fish bone chemistry and ultrastructure: Implications for taphonomy and stable isotope analysis. J Arch Sci. 2011; 38(12):3358-3372.
18. Fathima KR, Tresina Soris P, Mohan VR, Nutritional and Antinutritional Assessment of Mucuna pruriens L. DC var. pruriens an Underutilized Tribal Pulse. Adv Biores. 2010; 1(2):79-89.
19. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE, Doma UD, Fabiyi EF. Nutrient composition and uses of bambara groundnut Vignia substerranea L. Verdcourt. Contin J Food Sci Technol, 2009; 3:8-13.