KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

COMPOSITING NULs PROTEINS FOR THEORETICAL MINIMUM AMINO ACID SCORE IN ELASTIN

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY

BY

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CERTIFICATION PAGE

I hereby declare that this submission is my own work toward the MSc. and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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NOTATION

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DEDICATION

I dedicate this work to my dear husband, Mr. Maxwell Osei Boadu and children, for the relentless and keen interest they have demonstrated throughout the course of the study.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The nutritional value of legumes as sources of protein and carbohydrates in the diet is undeniable, not only for vegetarians but more especially in developing countries where large segments of the population suffer from protein malnutrition and where legumes are of utmost importance. As a consequence of this, there is an increasing need to identify and evaluate new potential food sources. Legumes are target crops in this regard because they offer rich and abundant sources of protein. Many of the legumes have protein contents between 20 % and 40 % and a few range between 40 % and 60 % (Lawal and Adebowale, 2006).

Research efforts are being directed towards the study of underexploited legumes that are well adapted to adverse environmental conditions and highly resistant to disease and pests. Unlike soybean, peanut and other well-known legumes, the seeds of *Phaseolus lunatus*, *Vigna subterranea*, *Mucuna pruriens*, *Cajanus cajan* and *Canavalia ensiformis* indigenous to Ghana, are among the wild legumes relatively underutilized. In the local markets, these seeds are available throughout the year. Even though widely consumed, the use of this legume is limited to utilization for traditional purposes as a soup ingredient for therapeutic purposes such as curing dropsy, relieving diarrhea and a tonic to the viscera (Li, 1973; Salami *et al*, 2009). However, other nutrient information such as amino acid profiles of the composite is still lacking.

While soybeans have had a competitive advantage over other legume seeds, there is a need to develop other sources of concentrated plant proteins (Vose, 1980; Thissen, 2004) which ideally should be crops that are widely grown in tropical countries. Moreover, the availability of animal proteins is hindered due to their inadequate supply accompanied by escalating costs, thus highlighting the need to exploit alternative sources to meet the current protein requirements (Bhat and Karim, 2009; Khattab *et al*., 2009). Even though large number of legumes exists world over, the utilization of many of these legumes is mainly on kidney beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), and cowpea (*Vigna unguiculata*) (Khattab *et al*., 2009). Thus a wide gap exists in exploring some of the underutilized legumes which are confined to localized regions of the world. Previously, studies have been conducted on some of the underutilized legumes from different parts of the world such as on *Mucuna pruriens*, *Canavalia ensiformis*, Sesbania and others (Bhat and Karim, 2009).

A study by Agunbiade and Ojezele (2010) supported by Omueti and Agbaje (1996), identified that as a worldwide food, it is mainly considered as an important protein food source for low income earners. Rochfort and Panozzo (2007) explained that legumes together with cereals are the main plant source of protein. Most legumes especially beans are generally rich in high quality proteins, dietary fibers, carbohydrates as well as a low content of saturated fats. This makes legumes have several beneficial health effects that can be obtained from their consumption. The presence of some minor compounds such as certain lipids, polyphenols and bioactive peptides and fibers improve digestion and cell activity upon consumption (Campos-Vega *et al*., 2013).

However, the use of legume flours in various food formulations is dependent on their amino acid composition. A high quality protein diet contains all nine of the essential amino acids, in their correct proportion as required by the body. Many plants do not contain the essential amino acid in their right quantity and hence it is important to monitor the levels of the amino acids to determine the protein quality of the meal (Reeds, 2000).

1.2 Statement of the Problem

The consumption of legumes has been associated with the poor and low income earners, as such less research has been conducted on them. This clearly shows that after a few decades, these useful legumes face extinction as a result of genetic erosion (Duranti, 2006). This will have adverse effects on the economy as many people will be deprived of their daily source of protein foods as well as several farmers especially women will be out of job as producers and marketers.

The current trend in population growth shows that the protein gap may continue to increase in future unless well-planned measures are taken to tackle the situation (Iqbal *et al*., 2005). Research conducted by Michaelsen and Henrik (1998) has also proven that intake of cerealbased food which is bulky, with little energy, low nutrient density and high antinutrient content has contributed to the malnutrition in children. Besides, the accessibility of animal proteins is hindered due to their insufficient source accompanied by rising costs, there by stressing on the need to exploit another source to meet the recent protein requirements (Khattab *et al*., 2009). In order to meet the protein demands in developing countries, intensive research has been geared towards finding alternative cheaper sources of protein.

An alternative plant source that can replace animal protein is the seeds of leguminous plants, including bean seeds, in many circumstances (Chau *et al.*,1997; Iqbal *et al.,* 2006).

In the developed countries, plant proteins are now regarded either as useful ingredients or as biologically active components more than as essential nutrients (Marcello and Gius, 1997). There is therefore the need by food industry, consumers and health experts to manufacture foods made from legumes to improve overall nutritional status (Guillon and Champ, 1996). Siddhuraju and Becker (2003) reported that conventional legumes have been playing a major part as a food and foodstuff in most of these countries, but their production does not meet the demand of the growing population and animal feed industries (Siddhuraju and Becker, 2003). There is therefore the need to exploit on neglected underutilized legumes (Agbede and Aletor, 2005; Janardhanan *et al*., 2003).

A lot of wild legumes have been identified but their use is limited due to absence of dietary information (Viano *et al*., 1995; Vijayakumari *et al*., 1994). Although the amino acid profile of *Phaseolus lunatus*, *Vigna subterranean*, *mucuna pruriens*, *Cajanus cajan* and *Canavalia ensiformis* has been reported in several publications (Ezeagu *et al*., 2004), 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 in elastin in food, especially in composited underutilized legume flour.

Elastin is a protein essential for the formation of new blood vessels of all the organs in our bodies. Cancers and tumors can grow with the formation of these blood vessels, therefore

cancers must be deprived of elastin to minimize or inhibit growth. 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.

1.3 Justification of Work

To broaden the protein sources for human diet, there is the need to explore on viable wild legumes as alternative foods. 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 and its exploration may serve as an alternative source of cancer and diabetic patient. 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

Whole flour or composite of different legumes has attracted increasing research interest. Studying their elastin score is important to efficiently utilize the flours produced from these underutilized legumes and help consumers easily accept them. Due to inadequate supply of protein diets, malnutrition in children and lactating women is evident in developing countries, which can be compensated by wild legumes (Pelletier, 1994; Vadivel and Janardhanan, 2001a). Exploration of economically viable wild legumes as an alternative source of food may expand the protein sources for nutrition.

Elastin is the main component of elastic fibre, which provides resilience and elasticity to many tissues such as skin, lungs, ligaments and arterial walls. Major types of domains are found in tropoelastin. There is substantial evidence that the hydrophobic domains are necessary for the self-aggregation of tropoelastin via coacervation, which is thought to concentrate and align tropoelastin molecules for cross-linking (Vrhovski *et al*., 1997). Hydrophobic domains of tropoelastin are rich in amino acids such as glycine, proline, valine, and leucine, which are present in a variety of tandem repeat sequences. Elastin also confers elasticity, preventing dynamic tissue creep by stretching under load and recoiling to their original configurations after the load is released.

1.4 Objective

The objective was to use the mixture design to composite five NULs): Bambara groundnut *(Vigna subterranea)*, Velvet bean (*Mucuna pruriens*), Jack bean (*Canavalia ensiformis*), Pigeon pea (*Cajanus cajan*) and Lima beans (*Phaseolus lunatus)* to obtain the score of amino acids that could theoretically supply minimum amino acids to build the protein elastin, required for building blood vessels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Elastin Formation

Elastin is an essential part of various human tissues that depend on elasticity found in the form of fibres. They are found as membranes in the elastic ligaments, elastic blood vessels, and other compliant tissues such as lung, arteries and skin, (Rosenbloom *et al*., 1993; Uitto *et al*., 1991; Vrhovski and Weiss, 1998). Generally, the most mature, thicker elastin fibres are found deep in the dermis, where they function as an inter-penetrating elastin network (Kielty and Shuttleworth, 1997; Ushiki, 2002). The protein tropoelastin is the fundamental building component of all elastin. There is only one tropoelastin gene (ELN) in humans which mainly occurs before birth and in the first few years of age.

The constituent amino acids found in elastin are branched chain amino acids (valine, leucine and isoleucine), alanine, proline and glycine and the latter comprising almost one quarter of it. Szpak (2011) from research gave the abundance of these amino acids residues/1000 in elastin as; glycine 329 (32.9 %), proline 126 (12.6 %), alanine 109 (10.9 %), leucine 24 (2.4 %), valine 22 (2.2 %) and isoleucine 11 (1.1 %).

2.1.1 Branched chain amino acids

Valine was first isolated by Schutzenberger in 1879 (Belitz *et al.*, 2009). It is an essential amino acid and is present in meat and cereal proteins (5–7 %) and in egg and milk proteins (7–8 %). Elastin contains notably high concentrations of valine (15.6 %). It is needed for muscle metabolism tissue repair, and the maintenance of a proper nitrogen balance in the body. Valine is found in high concentrations in muscle tissue. It is one of the branched-chain amino

acids that are the energy source for muscle tissue. It is good for correcting the type of severe amino acid deficiencies that can be caused by drug addiction. An excessively high level of valine may lead to such symptoms as a crawling sensation in the skin and even hallucinations.

Leucine was isolated from wool and from muscle tissue by *Braconnot* in 1820 (Belitz *et al*., 2009). It is an essential amino acid and its content in most proteins is 7–10 %. Cereal proteins contain variable amounts of leucine which are 12.7% in corn and 6.9 % in wheat. Leucine works together with other branched-chain amino acids to protect muscle and act as fuel. They promote the healing of bones, skin, and muscle tissue, and are recommended for those recovering from surgery. Leucine also lowers elevated blood sugar levels, and aids in increasing growth hormone production. An excessively high intake of leucine may also contribute to pellagra, and may increase the amount of ammonia present in the body.

Isoleucine was first isolated from fibrin by Ehrlich in 1904 (Belitz *et al*., 2009). It is an essential amino acid. Meat and cereal proteins contain 4–5 % isoleucine; egg and milk proteins, 6–7 %. Isoleucine is needed for haemoglobin formation. It also stabilizes and regulates blood sugar and energy levels. It is metabolized in muscle tissue. It has been found to be deficient in people suffering from many different mental and physical disorders. A deficiency of isoleucine can lead to symptoms similar to those of hypoglycaemia

2.1.2 Unbranched chain amino acid

Proline was discovered in casein and egg albumen by Fischer in 1901 (Belitz *et al*., 2009). It is present in numerous proteins at 4–7 % and is abundant in wheat proteins (10.3 %), gelatin (12.8 %) and casein (12.3 %). It is obtained primarily from meat sources. It improves skin texture by aiding in the production of collagen and reducing the loss of collagen through the

aging process. It also helps in the healing of cartilage and the strengthening of joints, tendons, and heart muscle. It works with vitamin C to promote healthy connective tissue.

Glycine is found in high amounts in structural protein. Collagen contains 25–30 % glycine. It was first isolated from gelatin by Braconnot in 1820 (Belitz *et al*., 2009). It retards muscle degeneration by supplying additional creatine which utilized in the construction of DNA and RNA. Glycine is essential for the synthesis of nucleic acids, bile acids, and other nonessential amino acids in the body. It is used in many gastric antacid agents. Because high concentrations of glycine are found in the skin and connective tissues, it is useful for repairing damaged tissues and promoting healing. Glycine is necessary for central nervous system function and a healthy prostate. It functions as an inhibitory neurotransmitter and as such can help prevent epileptic seizures. It has been used in the treatment of manic (bipolar) depression, and can also be effective for hyperactivity. Having too much of this amino acid in the body can cause fatigue, but having the proper amount produces more energy. When necessary, it can be converted into the amino acid serine in the body.

Alanine was isolated from silk fibroin by Weyl in 1888 (Belitz *et al.*, 2009). Alanine is considered nonessential for humans. It is present in most proteins and is particularly enriched in silk fibroin (35 %). Gelatin and zein contain about 9 % alanine, while its content in other proteins is 2–7 %. It is found in appreciable levels in many foods including beef, lamb, milk products, corn meal, peas and potatoes. It aids in the metabolism of glucose, a simple carbohydrate that the body uses for energy. One form of alanine, beta-alanine, is a constituent of pantothenic acid and coenzyme A, a vital catalyst in the body. It plays a significant role in

several metabolic processes and in regulating blood sugar. It can therefore be used not only against acute low sugar shocks, but is also able to stimulate insulin excretion in the pancreas and in doing so significantly improve the metabolism of glucose over longer periods of time. Other functions of this proteinogenic amino acid are to support, the ability to perform physically and build muscle mass. And its supplements are therefore popular with athletes who are thus able to achieve increases in their performance. It can further contain the uncontrolled growth (cancer) of the pancreatic tissue and is thus used to treat both prostate cancer as well as its symptoms (Shiga *et al.*, 2008).

2.2 Amino Acids in Food Proteins

Food protein quality is a key nutritional issue because it varies from one food protein to another and it is important to consider in dietary protein requirements. The main determinant of food protein quality is the content and availability of essential amino acids which have been shown to play an important role in the growth, reproduction and maintenance of the human body (FAO/WHO/UNU, 2007). The FAO/WHO (1991) has recommended that the composition of amino acids in diets be taken into consideration to determine the chemical composition of diets and to be able to estimate the protein quality of the diets.

There are nine essential amino acids out of twenty amino acids and must be provided in our foods because it cannot be produced by the human body. The amino acids considered as essential for humans are phenylalanine, valine, threonine, tryptophan, methionine, Leucine, isoleucine, lysine, and histidine. Essential amino acids such as cysteine, tyrosine and arginine are vital for infants and growing children.

Animal protein is expensive and cannot be afforded by most urban and rural dwellers, therefore the need for regular and cheaper protein foods from other sources. The world‘s source of protein for man is 65 % of plant protein of which 45 – 50 % are from cereals and 10 – 15 % are from legumes or vegetables (Mahe *et al*., 1994).

Plant proteins are the alternative to complement the diet for improving nutritional status. Generally, plant proteins do not have all but rather lack one or more of the essential amino acids. Legumes for example, lack the essential amino acid, methionine whiles cereals lack the essential amino acid lysine (Amjah *et al*., 2003). There is second limiting amino acids which are tryptophan in legumes and threonine in cereals (Duranti, 2006) whilst vegetables and fruits have limited amount of phenylalanine and methionine (Macdonald *et al.*, 2003).

Amino acids are primarily made up of four key elements: carbon, nitrogen, oxygen and hydrogen. Each has an overall molecule characteristic _side chain‘ which contains traces of different elements. It is these _side chains‘ that distinguish between the different types of amino acids. They are building blocks of protein which have several different purposes in our lives such as acting as neurotransmitters in our brains where they carry information from one nerve cell to another, used in the manufacture of fertilizer, biodegradable plastics and certain drugs. Some proteins contain as few as three amino acids, while others can contain hundreds. Amino acids have different effects on cell. Some help them to grow and reproduce while others keep them healthy.

2.3 Production and Uses of Legumes

Legume seeds are important sources of protein, complex carbohydrate and dietary fibre

(Morrow, 1991). It also provides vitamin B and valuable mineral substances like potassium, calcium, magnesium, phosphorus and iron salts (Fachmann and Kraut, 2000). They are low in fat and have a variety of micronutrients and phytochemicals as well (Siddhuraju and Becker, 2007). Over the past years, the usage of proteins from plant seeds has improved greatly because of greater knowledge of their functional properties, processing and nutritive value. Soybean is one of the convectional legumes that have been consumed worldwide. Currently, soybean and its products are in higher demand because of their reported beneficial effects on nutrition and health and have gained competitive advantage over other legume seeds (Yin *et al.*, 2011).

Underutilized legumes such as *Canavalia ensiformis, Cajanus cajan*, (Bhat and Karim, 2009) 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 (Adsule *et al*., 1989). Underutilized legumes, such as cowpea (*Vigna unguiculata*) and horse gram (*Macrotyloma uniflorum*) have been documented as potential sources of protein and other nutrients (Sreerama *et al.*, 2012). Recently, there has been a tendency to use exotic species such as soybean and peanut for food and for this, Pandey and Srivastava (1990) 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 local legumes underutilized (unconventional) legumes in Ghana.

2.3.1 Cultivation and nutritional usage of underutilized legumes

***Canavalia ensiformis*:** *Canavalia ensiformis* (Jack bean) is one of the under exploited tropical dry beans. It is however, fairly widely cultivated in Africa, Asia, West Indies, Latin America and India. The nutritional characteristics and food potentials of jack bean (*Canavalia ensiformis*) have been reviewed. The bean is a good source of protein, 23 to 34 %, and carbohydrate 55 % (Akpapunam and Sefa-Dedeh, 1997) and accrording to Udedibie and Nkwocha (1990), the seed of jack bean contains about 300 g/kg crude protein and 600 g/kg carbohydrates. It is also a good source of Ca, Zn, P, Mg, Cu and Ni.

Jack bean protein is adequate in most essential amino acids with the exception of methionine and cystine which may be nutritionally limiting (Akpapunam and Sefa-Dedeh, 1997). Antinutritional and toxic factors including trypsin inhibitors, hemagglutinins, cyanogen glucosides, oligosaccharides and others are present in jack bean.

Properly processed jack bean could be used to prepare some of the popular dishes made from cowpea, peanut, pigeon pea and soybean. Industrial products such as protein concentrates and isolates, starch, flakes, grits and flours can be produced from the bean. Development of new highly nutritious food products based on whole or processed jack bean should increase production and expand use (Akpapunam and Sefa-Deden, 1997). *Canavalia ensiformis* ranks among the underutilized legumes that could ameliorate protein deficiency in human nutrition, particularly in developing countries.

Table 2.1: Amino acid composition of *Canavalia ensiformis*

Amino acid	***Canavalia ensiformis*** **(mg 100mg-1)** .
Glutamic acid	2.4-16
Aspartic	2.3-14
Serine	1.1-5.0
Threonine	1.0-5.0
Proline	0.8-4.3
Alanine	0.1-4.7
Glycine	0.9-4.3
Valine	1.1-5.3
Cystine	Trace-0.9
Methionine	Trace-1.2
Isoleucine	-
Leucine	2.5-16
Tyrosine	0.8-3.3
Phenylalanine	1.1-5.2
Tryptophan	0.3-1.2
Lysine	1.3-6.8
Histidine	0.6-3.2
Arginine	1.1-5.6

Source: Arora (1995).

***Mucuna pruriens*:** The seeds of *Mucuna pruriens* have been traditionally used in Ghana in food preparations as a thickener for soups and stews (Ahenkora *et al*., 1994). It is currently being promoted as an efficient cover crop in Ghana (Osei-Bonsu and Buckles, 1993). Due to the very limited quantities used as human food, seeds are left after harvesting. On the other hand, it is a rich source of protein (Ahenkora *et al*., 1994), containing more than 200 g per kg on dry matter basis. Four accessions of the under-utilized legume, velvet bean (*Mucuna pruriens*) were evaluated by Vadivel and Janardhanan (2000) from different locations of Western Ghats and South India. Proximate composition, mineral profiles, protein fractions, amino acid profiles of the seed protein, in vitro protein digestibility and certain antinutritional factors were determined.

They reported crude protein ranged from 20.2-29.3 %, crude lipid 6.3-7.4 %, total dietary fibre 8.7-10.5 %, ash 3.3-5.5 % and carbohydrates 49.9-61.2 %. Mineral profiles like sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc and manganese ranged from 43.1-150.1, 778.1-1846.0, 393.4-717.7, 174.9-387.6, 98.4-592.1, 10.8-15.0, 0.9-2.2, 5.0-10.9, 3.9-4.3 mg/100 seed flour, respectively. Moreover, data on seed protein fractions revealed that the globulins constituted the major bulk of the seed protein as in most legumes. The seed contains all essential amino acids except threonine, leucine and lysine in black-coloured seed coat accessions and phenylalanine and tyrosine in white-coloured seed coat accession compared with the FAO/WHO (1991) requirement pattern.

Table 2.2: Amino acid profile of *Mucuna pruriens*

Amino acid	***Mucuna pruriens* (Saduragiri) (g/100g)**
Glutamic acid	14.11
Aspartic	12.98
Serine	4.40
Threonine	3.78
Proline	2.80
Alanine	4.24
Glycine	5.95
Valine	3.90
Cystine	0.54
Methionine	1.24
Isoleucine	5.94
Leucine	7.24
Tyrosine	4.94
Phenylalanine	3.98
Tryptophan	0.88
Lysine	6.01
Histidine	4.44
Arginine	5.06

Source: Fathima *et al*. (2010).

Vigna subterranea*:* Bambara groundnut (*Vigna subterranea*) is a crop originating from Africa and it is eaten in almost all parts of Ghana. It also contains some appreciable amount of minerals like calcium (95.5-99 mg/100mg), iron (5.1-9 mg/100mg), potassium 1144714355 mg/100mg and sodium 2.9-10.6 mg/100mg (Karikari *et al*., 1997). It makes a complete food as it contains sufficient quantities of protein, carbohydrate and fat (Goli, 1997) and its gross energy exceeds that of other common pulses such as cowpea, lentils and pigeon pea (FAO, 1982). Bambara groundnut is reported by (Olomu, 1995) contains crude protein 20.60 %.Bambara groundnut is one of the legumes that are highly nutritious which contains 32 % of the total essential amino acid and 66.10 % non-essential amino acid (Minka and Bruneteau, 2000; Amartefio *et al*., 2006).

Table 2.3: Amino acid profile of raw *Vigna subterranea*

Amino acid	**Amount (N/16g)**
Glutamic acid	15.76
Aspartic	12.95
Serine	4.46
Threonine	3.11
Proline	3.60
Alanine	3.52
Glycine	3.01
Valine	4.51
Cystine	1.25
Methionine	1.04
Isoleucine	4.56
Leucine	7.76
Tyrosine	3.19
Phenylalanine	5.43
Tryptophan	NB
Lysine	6.01
Histidine	4.44
Arginine	5.06

ND = Not detected (Akande *et al*., 2009)

It also a good source of leucine, phenylalanine, histidine and valine in appreciable amount making it a completely balanced food when composited with cereals (Rowland, 1993). Lysine and Leucine are the predominant essential amino acids present in Bambara groundnut (Mune *et al*., 2011; Olomu, 1995; Adu-Dapaah and Sangwan, 2004; Aremu *et al.,* 2006). However, the seed is deficient in tryptophan (Akande *et al.,* 2009). The seed is regarded as a balanced food because when compared to most food legumes, it is rich in iron and the protein contains high lysine and methionine.

***Cajanus cajan*:** Pigeon pea (*Cajanus cajan*) is one of the oldest food crops. India alone contributes over 90 % of the world pigeon pea production. It is also a food crop in many other tropical countries and is commercially important in East Africa, the Caribbean and Latin America. It has low concentrations of fat, moderate amount of fibre, good amount of proteins and starch and a reasonably balanced range of all dietary essential minerals.

The protein content of commonly grown pigeon pea has been reported to range between 18–26 % (Swaminathan and Jain,1973). Pigeon pea flour has been found to be suitable as a protein source for supplementing baked products such as bread, cookies and *chapatties* due to its high level of protein, iron (Fe) and phosphorus (P) (Harinder *et al.*, 1999). It has therefore been recommended in school feeding programmes and vulnerable sections of the populations in developing nations (Damaris, 2007). The protein-rich seeds have also been incorporated into cassava flour to produce acceptable extruded products (Rampersad *et al.*, 2003). Pigeon pea is a rich source of carbohydrates, minerals and vitamins.

The seeds contain a range of 51.4–58.8 % carbohydrates (Faris and Singh, 1990), 1.2–8.1% crude fibre and 0.6–3.8% lipids (Sinha, 1977). Some potential uses of pigeon pea for human

consumption in Africa include the production of noodles (Singh *et al.*, 1989), *tempe* (Mugula and Lyimo, 2000) and other fermented products (Onofiok *et al.*, 1996). Elsewhere, pigeon pea is used as a flour additive to other foods in soups and with rice (Centre for New Crops and Plants Products, 2002).

Table 2.4: Amino acid profile of *Cajanus cajan*

Amino acid	**Amount (N/16g)**
Lysine	7.79
Histidine	3.66
Arginine	5.86
Aspartic acid	11.56
Threonine	3.12
Serine	3.59
Glutamic acid	9.23
Proline	3.17
Glycine	3.07
Alanine	3.79
Cystine	1.19
Valine	5.85
Methionine	1.19
Isoleucine	3.47
Leucine	6.78
Tyrosine	2.63
Phenylalanine	6.15
Tryptophan	ND

Source: Akande *et al*. (2010). **ND= Not detected**

Pigeon pea flour is an excellent component in the snack industry and has been recommended as an ingredient to increase the nutritional value of pasta without affecting its sensory properties (Torres *et al.*, 2007). Millet and pigeon pea biscuits are reportedly highly nutritious and provide a cheaper alternative to wheat imports in Nigeria (Eneche, 1999).

Pigeon pea leaves have been used to treat malaria (Aiyeloja and Bello, 2006) in Nigeria, while in Southern Africa; pigeon pea is currently one of the indigenous crops being promoted for potential medicinal use (Mander *et al.*, 1996).

***Phaseolus lunatus* (Lima bean):** It is a good source of protein and have been used as a source of substitute for other protein sources of plant like plant (soy) and animal milk protein in industrial preparation of commonly consumed foods such as ice cream or regionally consumed foods such as miso in Japan and kishk in Eastern Euro (Nestares *et al*., 2001).

Currently, the bean does not have a price in the international market and as well as in the recent four years. The other grains have experienced the decline of 10 or 15 % in the price and in the case of beans, it has been reduced by 60 %. However, the increase in the cost of production has been 70 % (Romero-Arenas *et al*., 2013).

Lima bean is one of the most widely cultivated pulse crops both in temperate and subtropical regions. It is adapted to highly leach infertile soils of the more humid regions. In recent time plant geneticists have improved the lima bean enormously and a number of early maturing, disease and pest resistant, non-toxic cultivars are widely available commercially (Elegbede, 1998). Lima bean is rich in niacin, thiamine and riboflavin (Sathe *et al*., 1984). They are said to contain high levels of potassium, phosphorus, calcium and iron (Ologhobo and Fetuga 1984; Osagie *et al*., 1996). *Phaseolus lunatus* (lima bean) is a New World legume that has been domesticated in areas corresponding to present day Peru and Mexico (Kaplan, 1965; Moraes *et al*., 2000) and is currently cultivated in many tropical regions of the World (Smartt, 1990).

Table 2.5: Amino acid of *Phaseolus lunatus*

Amino acids	Amount (g/100g protein)
Lysine	6.54
Histidine	2.27
Arginine	6.99
Aspartic acid	10.61
Threonine	4.01
Serine	3.06
Glutamic acid	15.87
Proline	3.21
Glycine	4.92
Alanine	3.05
Cystine	1.54
Valine	5.41
Methionine	1.58
Isoleucine	4.51
Leucine	7.04
Tyrosine	3.46
Phenylalanine	4.96

Source: ieanacho, 2010

2.4 Compositing of Legumes

Legume seeds represent the most abundant source of protein. The most common combination of plants product usually comes from cereals and legumes to overcome the essential amino acid deficiencies (Brody, 1999). Farzana and Khalil, 1999) reported that legumes contain adequate amount of lysine but are deficient in the sulphur containing amino acids which are methionine, cystine and cysteine.

Iqbal *et al.* (2005) conducted a research on chickpea, cowpea, lentil and green pea and analysed the protein and the amino acids of these legumes. It was reported that the four legumes were

rich in lysine, leucine and arginine and were deficient in sulphur containing amino acids and tryptophan. Composited food product such as wheat and non-wheat flour have assumed great relevance in developing countries including Ghana and has become important due to the rising demand and cost of importing wheat flour to countries where the climate is not favorable for growing wheat (Troester, 2013).

Compositing foods for product have become popular because of the economic and nutritional benefits. Different types of flours have been blended together to enhance specific quality attributes (Dendy, 1993). According to Liener (1981), the high protein content of soybean along with its relatively high tryptophan, lysine and minerals gives soybeans the potential to be used to supplement other flours. Kadam *et al.* (2012) reported that several researches have been made about the effect of the addition of legume flours on the functional properties of bread dough and final bread quality.

Kayitesi *et al*. (2012) reported that the use of marama flour in sorghum composite flours have improved the nutritional quality in porridge. It has contributed to better amino acid balance and increased protein content and energy value. It has also helped alleviate protein energy malnutrition in developing countries. Composite flour prepared from carob seeds and cereals contain potential ingredient used in foods for celiac people (Feillet and Roulland, 1998).

Compositing most of the neglected and underutilized legumes (NULS) may provide alternative source of plant protein to soybean. Composites NULS merit to be explored as likely sources of low cost protein for possible use as food or feed to meet the gap of protein and energy deficiency. It follows that people with low incomes are least likely to eat quality diets (Ruel *et al*., 2001) and the judicious combination of the NULs can progress nutritive intake,

creating the option for reintegration through variation. This composite represents an economic way to improve the protein value of legume-based foods.

2.5 Research Methodolgy

2.5.1 Protein compositing materials

Amino acid profile is a key aspect of protein quality. Protein sources include seafood, pulses, milk, meat, milk etc. A number of cereals and legumes have been composited to complement one another (Fernandez *et al*., 2002; Oguntona and Akinyele, 1995). Vegetable has also been used with other food groups to composite protein (Applewhite, 1990).

2.5.2 Protein compositing designs and Methods of analysis

Protein compositing designs: Response Surface Methodology (RSM) has been used to produce different composited foods. It is currently the most popular optimization technique in food science due to its comprehensive theory, reasonably high efficiency and simplicity.

It can be used in problems involving ingredients and processing conditions as variables (Arteaga *et al.,* 1994) and has been successfully applied to optimize food-processing operations (King and Zall, 1992; Madamba, 2002; Oomah and Mazza, 2001; Unal *et al.,* 2003). It is a statistical tool that uses statistical techniques for modeling and analyzing problems where a response of interest is being influenced by many factors to provide an optimized response (Triveni *et al*., 2001). It overcomes the weakness and limitations of the classical method (Liyana-Pathirana and Shahidi, 2005) by taking into account the possible interrelationship among the test variables while minimizing the number of experiments (Silva *et al.,* 2007).

One of the main objectives of Response Surface Methodology is the determination of the optimum settings of the control variables that result in a maximum (or a minimum) response over a certain region of interest, *R*. This requires having a _good' fitting model that provides an adequate representation of the mean response because such a model is to be utilized to determine the value of the optimum (Jinap *et al.*, 2007). Optimization techniques used in RSM depend on the nature of the fitted model (Khuri and Mukhopadhyay, 2010). Fabbrooq *et al.* (2012) used the RSM to optimize composite flour for the production and enhancement of the storability of leavened flat bread. Singh *et al.* (2004) also used RSM to optimize the ingredient level of sweet potato-based pasta products.

Defatting of raw material: Raw materials that have high levels of oil must be defatted before protein isolation. During protein extraction the oil is removed to prevent the formation of emulsion. Samples are acquired by defatting the raw materials to be fat -free with solvents such as hexane (Abbot *et al*., 1991; Kumagai *et al*., 2002) and petroleum ether (Sathe *et al*., 2002) or by mechanical press (Shrestha *et al*., 2002). Plant proteins such as legumes that contain high levels of fat should first be ground, defatted before protein extraction.

Protein and isolation of extraction of legumes: There have been different procedures in the extraction of proteins to produce a suitable mixture for use in food system. The first step in obtaining an enriched protein product is to extract the raw material with a suitable solvent which is usually an aqueous solvent. Other factors apart from the source of raw material that intend to influence the extraction include the nature of the raw material and type of protein (Yada, 2004).

Extractable protein determines the amount of protein that can be made available from a particular source for food and non-food application. One of the preliminary factors that determine whether or not a protein source could be embraced for commercial using is the protein extraction efficiency of such protein (Liu, 1997). Protein extraction and isolation can be effective in reducing many of the antinutritional problems, while creating a protein rich commodity with marketable nutritional and functional properties. Protein isolation is a very important step when incorporating proteins from oil-producing plants into food products. Protein isolates are the most refined protein product containing the greatest concentration of protein, but unlike protein flour and concentrates, contain no dietary fibre. They are very digestible and easily introduced into foods such as sports drinks and health beverages as well as infant formulas (Hoffman and Falvo, 2004).

Protein extraction usually involves the use of acid, alkaline and saline solution (Eromosele *et al*., 2008). Different types of protein contained in raw materials would favour certain treatment but the most common solvent seen in the literature is an aqueous alkaline solution and this has been used for Karkade (*Hibiscus sabdariffa*) (Abu-Tarboush, 1995), amaranth seeds (Ventureira *et al*., 2011), sesame seed (Bandyopadhyay and Ghosh, 2002). The use of alkali as a solvent for protein extraction is now well-liked due to its high degree of solubility that can be achieved. The fact that all these researchers made use of alkaline in their protein extraction, the pH of the solution is very important in order to obtain a higher protein yield. Higher pH values, especially pH 12 and above, (Deng *et al*., 1990) have been found to increase the formation of lysinoalanine. This has resulted in researchers preferring a lower pH alkaline solution to solubilize proteins.

Protein Recovery: Researchers have used various procedures in precipitating proteins from solutions. Protein extracted using alkaline solution is mostly precipitated with pH adjustment. Maximum protein precipitation has been reported at pH 4.0 (Klockeman *et al*., 1997), with only about 53 % of the protein precipitates (Chen and Rohani, 1992). Karkade seed flour protein had a pH range of 3.0 to 5.0 (Abu-Tarboush, 1995). This finding agrees with data given by other investigators. Hang *et al*. (1970) reported that several bean proteins, namely mung bean, pea bean and red kidney bean have a common point of minimum precipitation at pH 4.0.

The precipitate contains mostly the protein of interest and is collected by centrifugation (Kumagai *et al*., 2002). The protein isolate may be neutralized to pH 6.9-7.2 with dilute alkali (usually NaOH) to give a more soluble product called proteinate. The supernatant that remains after precipitated protein isolate has been removed contains soluble proteins that can be recovered by membrane processing. The supernatant is processed by ultrafiltration and diafiltration to remove low molecular weight substances, especially salts and peptides to produce a retentate, which is freeze dried as the soluble protein isolate (Xu *et al*., 2003).

Amino acid analysis: Amino acid analysis is used to quantitatively determine the amino acid composition of a protein. The protein sample is first hydrolyzed to release the amino acids. Amino acids are then separated using chromatographic techniques and quantified. Ionexchange chromatography, reversed-phase liquid chromatography and gas–liquid chromatography are three separation techniques used.

The analysis of amino acid in food samples is currently usually done using HPLC following pre-column derivatization using various reagents including O-phthalaldeyde

(OPA),ninhydrin,dansyl chloride, phenylisothiocyanate(PITC), dansyl chloride and 9fluorenylmethylchloroformate (FMOC-Cl). The use of these reagents have drawbacks, including long derivatisation times (PITC, dansyl chloride), need for removal of excess reagent after derivatization (PITC, FMOC-Cl), unstable derivatives, failure to react with secondary amines (OPA) and decreased derivatization efficiency in the presence of buffers and detergents (FMOC, PITC) (Liu *et al*., 1995; Sarwar and Botting, 1993).

In this studies 6-aminoquinolyl-N hydroxysuccinymidyl carbamate (AQC) was used because it has numerous advantages in comparison to the other possible derivatizing reagents (Cohen and Michaud, 1993). The reagent has been successfully used for determination of amino acids in foods (Bosch *et al*., 2005, 2006).

In general, a protein sample is hydrolyzed in constant boiling 6*N* HCl for 24 h to release amino acids prior to chromatography. Accurate quantification of some amino acids is difficult because they react differently during hydrolysis. Consequently, special hydrolysis procedures must be used to prevent errors. Tryptophan is completely destroyed by acid hydrolysis. Methionine, cysteine, threonine, and serine are progressively destroyed during hydrolysis; thus, the duration of hydrolysis will influence results. Asparagine and glutamine are quantitatively converted to aspartic and glutamic acid, respectively, and cannot be measured. Isoleucine and valine are hydrolyzed more slowly in 6*N* HCl than other amino acids, while tyrosine may be oxidized.

In general, losses of threonine and serine can be estimated by hydrolysis of samples for three periods of time (i.e., 24, 48, and 72 h) followed by amino acid analysis. Compensation for amino acid destruction may be made by calculation to zero time assuming first-order kinetics.

Valine and isoleucine are often estimated from 72 h hydrolysate. Cysteine and cystine can be converted to the more stable compound, cysteic acid, by hydrolysis in performic acid and then hydrolyzed in 6 M HCl and chromatographed. Tryptophan can be separated chromatographically after a basic hydrolysis or analyzed using a method other than amino acid analysis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 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 neglected and underutilised legumes Farm at *Anwomaso* site of KNUST. Hexane was bought from Air Products Nederland BV (Utrecht, Nederland). Amino acid standards (2.5 mol/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.

3.2 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 GP140 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.

3.3 Preparation of Defatted Flour

Cold extraction method was used to defat each of the legume flour in separate containers.

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

3.4 Experimental Design

3.4.1 Mixture design

Response Surface D-optimal, Mixture Design (Design Expert, 2007) was used in this study to composite the five different legume flours according to the ratios as shown in Table 3.1. *Vigna subterranea* ranged from a low of 15 % to a high of 55 %. *Cajanus cajan* also ranged from a low of 10 % to a high of 50 % while *Phaseolus lunatus* ranged from a low of 20 % to a high of 60 %. *Canavalia ensiformis* ranged from a low of 10 % to a high of 50 % and *Mucuna pruriens* had the minimum low of 5 % and a high of 45 %. In all, there were a total of 45 runs (Table 3.2) based on the cubic design model in order to increase the flexibility of the design.

Table 3.1: 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 3.2: Experimental runs for compositing five legumes as designed by the D-

Optimal Mixture Design of Response Surface Methodology

Run	A:BamGrnt %	B:CajCaj %	PhaL %	Can Ensif %	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

3.5 Preparation of the Composited Flour

The five legume flours were weighed according to the ratios for each run of the composite as shown in Table 3.2. The composited samples were homogenized to obtain a uniform mixture. The composites were then sealed in a tight container and stored at room temperature until when needed.

3.6 Extraction of Protein

Protein was extracted based on a method described by Gomez-Brenes *et al.* (1983). 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 ThermoFisher Scientific, USA).

3.7 Determination of Amino acid Content

3.7.1 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.

3.7.2 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 of (0.02 mol/L Na_2HPO_4 + 0.02 mol/L NaH_2PO_4) and a mixture B of (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 derivatise the mixture. This test tube was then placed on a water bath of 60ºC 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 then measured and the respective amino acid quantified from the standard curve previously determined.

3.8 Statistical Method

3.8.1 Mixture design

The amino acid response data collected were loaded and run in the Design Expert (2007). 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 model p values as well as then p values of the legumes that interacted to produce effective amino acid score. Also the regressions in the data collected such as coefficients of regression- (R^2), adjusted regression- (adjR^2), prediction regression-(pred R^2), and other output such as the adequate precision - (adeq precision) were studied. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were plotted and performance of the factors and responds made.

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 importanly, the solution to the _trade off' set in the criteria section was ranked as the possible set of legumes ratios according to their _desireability' points (0 – 1, with 1 maximum). This gave the most likely theoritical 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 were re-run with the selected legumes.

3.8.2 Fuzzy design

To better explain the roles of the legume protein ratios composited in the flour with respect to the final AASE response scores, fuzzy linguistic for these responses was defined for the legumes as: *low, 'medium'* and *'high'*. The rest of the procedure for the fuzzy modeling are as explained below. When the FIS editor was opened, the inputs which were proteins

(*Vigna subterranea* (A), *Cajanus cajan* (B), *Phaseolus lunatus* (C), *Canavalia emsiformis* (D), *Mucuna pruriens* (E)) were set as the inputs of the interrractive composites; BC, BE, CE, ACD, BCD and BCE. The membership functions were then set to quantify the protein content of each interactive composite as low, medium, high for all the proteins in the interractive composites.

The output; AASE and its membership function ranging from low, medium, high were also set for all the innteractive composites. The rules were then set for each of the interractive composites. Each protein quantity was in three categories; *low, medium, high* in the composite protein flour that gave the AASE. In setting a typical rule the procedure goes through as ―*If antecedent, then precedent*‖ as illustrated below.

If

Vigna subterranea protein ***is medium***

and

Cajanus cajan protein ***is low***,

and

Phaseolus lunatus protein ***is low***

and

Canavalia emsiformis protein ***is low***

and

Mucuna pruriens protein ***is high***, then AASE ***is what****?*

By going through all the runs for the interractive composites; BC, BE, CE, ACD, BCD and BCD, the fuzzy design gave responses for the rules that were set. The low, medium and high data of the interractive legumes were input into the fuzzy model.

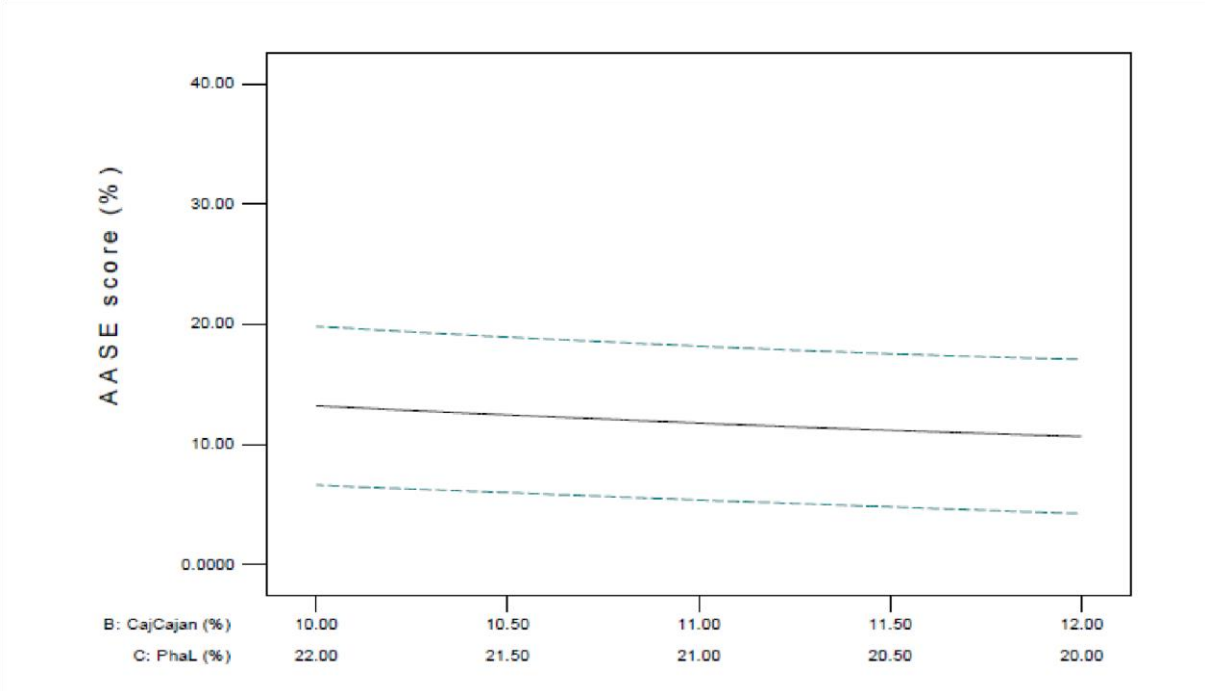

Figure 3.1: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual Cajanus cajan (10.00-12.00 %) and Phaseolus lunatus (20.00 – 22.00 %) at optimum percentage mixture of Vigna subterranea (15.00 %), Mucuna pruriens (33.50 %) and Canavalia ensiformis (19.50 %) respectively

Cajanus cajan ranged from 10.00 - 12.52 % and fuzzy linguistics set this as *low* (10.00 – 10.85 %), *medium* (10.86 – 11.7 %) and *high* (11.8 - 12.50 %). The range for *Phaseolus lunatus* protein was from 20.00 - 22.52 % and fuzzy linguistics set this as *low* (20.00 - 20.84 %), *medium* (20.85 – 21.70 %) and *high* (21.71 -22.52 %). The AASE range from 5.00 – 21.00 %

for the interractive composite BC; was set as *low* (5.00 – 10.00 %), *medium* (11.00 -6.00 %) and a *high* (17.00 -21.00 %). For the interractive composite BC (*Cajanus cajan* and *Mucuna pruriens*) proteins, Table 3.3 presented the final linguistic protein quantities and the final AASE for setting the rules.

nguistic protein quanities as *low, medium and high* in the

Table 3.3 Showing the li interractive composite; BC and their respective AASE responses

B: CajCajan	*C: PhaL*	*AASE score*
Low	Low	High
Low	Low	Medium
Low	Low	Medium
Low	Low	Medium
Low	Low	High
Low	Low	High
Medium	Medium	Medium
Low	Low	Medium
Low	Low	Medium
Low	Low	Low
Medium	Medium	Medium
Low	Low	High
Low	Low	Medium
Low	High	Low
Low	Low	Medium
Low	Low	Low

nguistic protein quanities as *low, medium and high* in the

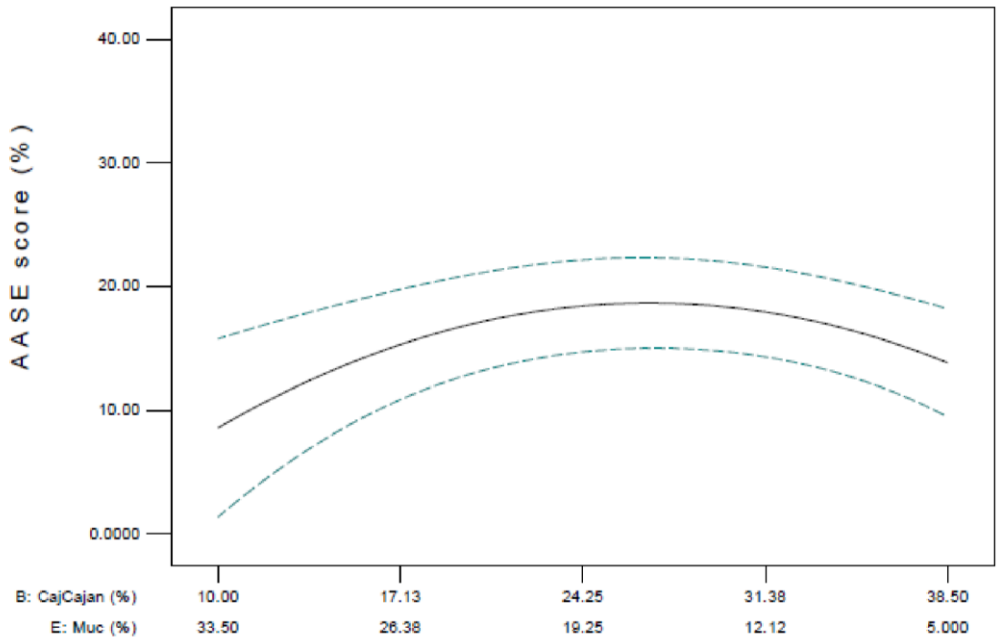

Figure 3.2: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual Cajanus cajan (10.00-38.50 %) and Mucuna pruriens (5.00- 33.50 %) at optimum percentage mixture of Vigna subterranea (15.00 %), Phaseolus lunatus (20.00 %) and Canavalia ensiformis (19.50 %) respectively

Cajanus cajan protein ranged from 10.00 – 38.50 % and the fuzzy linguistics set this as low (10.00 – 10.85 %), medium (19.60 – 29.00 %), high (29.10 – 38.50 %) and the range (5.00 – 33.50 %) for *Mucuna pruriens* protein as low (5.00 – 14.5 %), medium (14.6 – 24.5 %) and high (24.6 -33.50 %). The AASE ranged from 1.00 -15.00 % with a low (1.00 -5.00 %), medium (6.00 – 10.00 %) and a high (11.00 – 15.00 %).

For the interractive composite BE *Cajanus cajan* and *Mucuna pruriens* proteins, Table 3.4 presented the final linguistic protein quantities and the final AASE for setting the rules.

Table 3.4: Showing the li interractive composite; BE and their respective AASE responses

B:CajCajan	E:Muc	AASE score
Medium	High	High
Low	Low	High
High	Medium	High
High	Low	medium
High	Low	High
Low	High	High
Low	High	High
Medium	Low	High
Medium	Medium	High
Medium	Medium	Medium
Low	High	High
Low	Medium	Medium
Low	Low	High
Low	Medium	High
Medium	Low	Medium
Low	Low	High
Low	Low	High
Low	Low	High
Medium	Medium	Medium
Medium	Low	Medium
Low	Low	High
Medium	Low	High
Low	Low	High
Medium	Low	High
Low	Medium	Medium
Low	Low	High
Low	Low	High
Low	High	Low
Low	Low	High
Low	Medium	High
Low	Low	High
Low	Low	High
High	Low	Medium
Medium	Medium	Medium
Low	Low	Medium
High	Low	Medium
Low	Medium	High
Medium	Low	High

nguistic protein quanities as *low, medium and high* in the

Low	Low	High

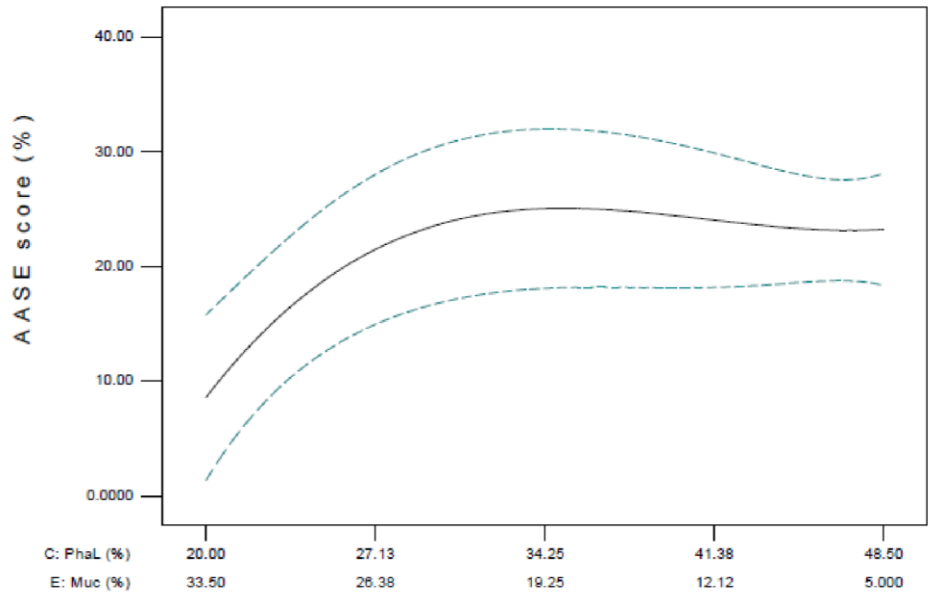

Figure 3.3: Two component mix plot of amino acid score in elastin (AASE) and its relation with actual Phaseolus lunatus (20.00- 48.50 %) and Mucuna pruriens (5.00- 33.50 %) at optimum percentage mixture of Vigna subterranea (15.00 %), Cajanus cajan (10.00 %) and Canavalia ensiformis (19.50 %) respectively

Phaseolus lunatus protein ranged from 20.00 – 48.50 % and the fuzzy linguistics was set as low (20.00 – 29.00 %), medium (30.00 – 39.00 %), high (40.00 – 49.00 %). The *Mucuna pruriens* protein ranged from 5.00 – 33.50 % and the fuzzy linguistics was set as low (5.00 – 14.00 %), medium (15.00 – 24.00 %) and high (25.00 – 34.00 %). The AASE ranged from 2.00 – 31.00 % and was set as low(2.00 – 11.00 %), medium (12.00 – 21.00 %) and high (22.00 – 31.00 %).

For the interractive composite CE (*Phaseolus lunatus* and *Mucuna pruriens*) proteins, Table 3.5 presented the final linguistic protein quantities and the final AASE for setting the rules.

Table 3.5 Showing the li interractive composite; CE and their respective AASE responses

C: PhaL	E: Muc	AASE score
Medium	Low	medium
High	High	high
Low	Medium	high
Low	Low	medium
Medium	Low	high
Low	Low	medium
Low	Low	low
Low	Low	high
Medium	Medium	medium
Low	Medium	medium
Low	High	high
Low	Medium	medium
Low	Low	high
Low	Medium	high
Low	Low	medium
Low	Low	high
Low	Low	high
Low	Low	high
Medium	Medium	medium
Medium	Low	medium
High	Low	high
High	Medium	high
Low	Low	high
Medium	Low	high
Low	Medium	medium
Medium	Low	high
High	Low	high
Low	High	low
Low	Low	high
Medium	Medium	high
Low	Low	high
Low	Low	high

nguistic protein quanities as *low, medium and high* in the

Low	Low	high
Low	Medium	medium
Medium	Low	medium
Low	Low	medium
Medium	High	high

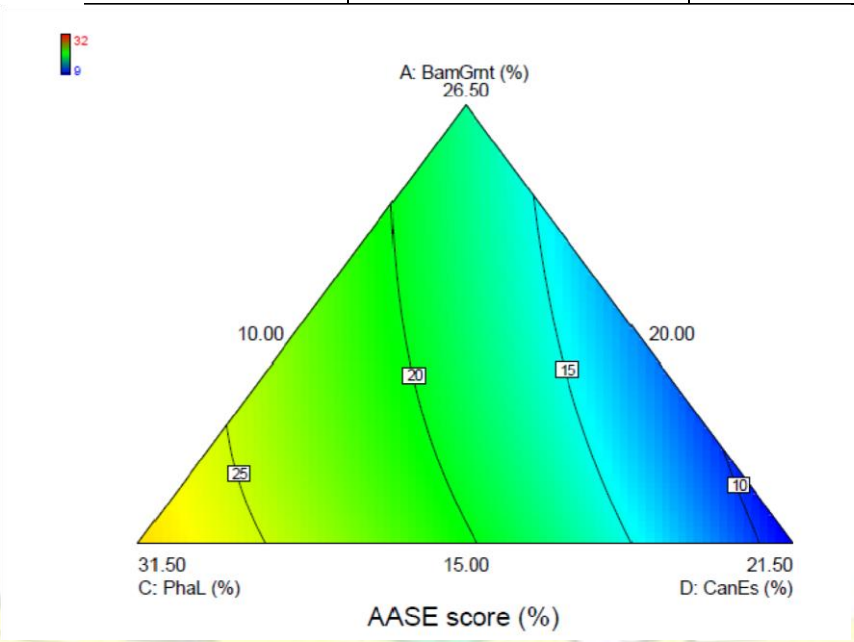

Figure 3.4: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual Cajanus cajan (10.00-21.65 %), Phaseolus lunatus (20.00-31.65 %) and Canavalia ensiformis (10.00-21.65 %) at optimum percentage mixture of Vigna subterranea (15.00 %), and Mucuna pruriens (33.35 %)

Vigna subterranea protein ranged from15.00 - 26.50 % and the fuzzy linguistics was set as low (15.00 - 18.88 %), medium (18.89 – 22.76 %) and high (22.77 – 26.65 %). *Phaseolus lunatus* protein ranged from 20.00 – 31.50 % and this was set as low (20.00 – 23.89 %), medium (23.89 – 22.76 %) and high (22.77 – 31.65 %). *Canavalia ensiformis* protein ranged from 10.00 – 21.50 % and this was set as low (10.00 -13.98 %), medium (13.99 – 17.96 %), and high (17.97 – 21.50). The AASE ranged from 8.57 – 31.96 % with a low (8.57 – 16.37 %), medium (16.38 – 24.70 %) and a high (24.80 – 31.97%).

For the interractive composite ACD, (*Vigna subterranea, Phaseolus lunatus and Canavalia ensiformis*) proteins, Table 3.6 presented the final linguistic protein quantities and the final AASE for setting the rules.

n ***low, medium and high***

Table 3.6: Showing the li guistic protein quanities as in the interractive composite; ACD and their respective AASE responses A:BamGrnt C:PhaL D:CanEs AASE score

Low	Low	Low	High
High	Low	Low	Low
Low	High	Low	High
Low	Low	Low	Medium
High	High	Low	Medium
Low	Low	High	Medium
High	Low	Low	High
High	Low	High	Medium
Low	High	Low	Low
Low	High	High	Low
High	High	Low	High
Medium	Medium	Medium	Medium
Low	Low	Low	Medium
Low	Low	High	Low

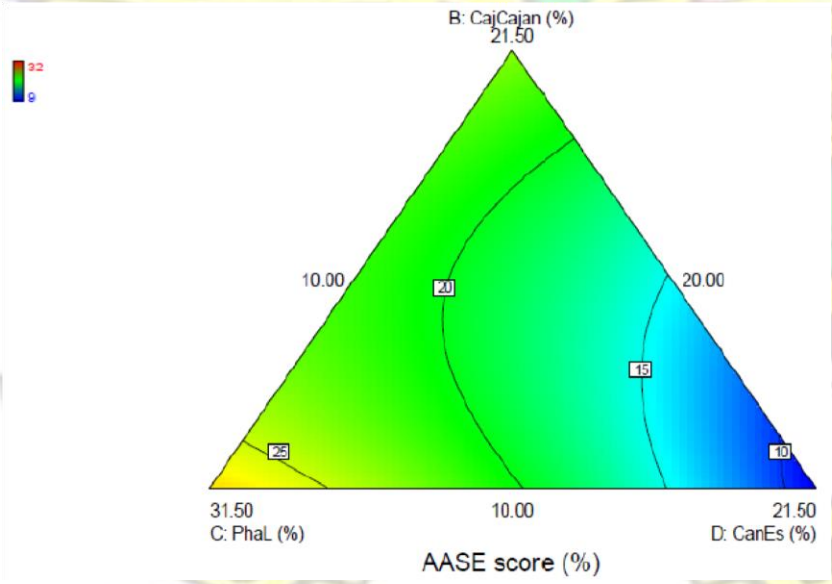

Figure 3.5: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual Cajanus cajan (10.00-21.50 %), Phaseolus lunatus (20.00-31.50 %) and Canavalia ensiformis (10.00-21.65 %) at optimum percentage mixture of Vigna subterranea (15.00 %), and Mucuna pruriens (33.35 %)

Cajanus cajan protein ranged from 10.00 – 22.00 % and the fuzzy linguistics was set as low (10.00 – 13.99 %), medium (14.00 – 17.99 %) and high (18.00 – 22.00 %). *Phaseolus lunatus* protein ranged 20.00 – 32.00 % and this was set as low(20.00 – 24.00 %), medium (24.10 – 28.00 %) and high (28.10 – 32.00 %). *Canavalia ensiformis* protein ranged from 10.00 – 22.00 % and this was set as low (10.00 – 14,00 %), medium (14,10 – 18.00 %), and high (18.10 – 22.00 %). The AASE ranged from 8.57 – 31.96 %) and this was set as low (8.57 – 16.37 %), medium (16.38 – 24.70 %) and high (24.80 – 31.97 %).

For the interractive composite BCD (*Cajanus cajan, Phaseolus lunatus and Canavalia ensiformis*) proteins, Table 3.7 presented the final linguistic protein quantities and the final AASE for setting the rules.

Table 3.7: Showing the linguistic protein quanities as *low, medium and high* in the interractive composite; BCD and their respective AASE responses B:CajCajan C:PhaL D:CanEs AASE score

High	Low	Low	High
Low	High	Low	Medium
High	Low	High	Medium
Low	Low	Low	High
Low	Low	High	Medium
High	Low	High	Low
Low	Low	Low	Medium
Medium	Medium	Medium	Medium
High	High	Low	Low
High	High	High	Low
Low	Low	Low	Medium
High	High	Low	Medium
Low	Low	Low	Medium
Low	High	Low	Medium
Low	Low	High	Low
Low	High	High	Low
High	Low	Low	Medium
Low	High	Low	High

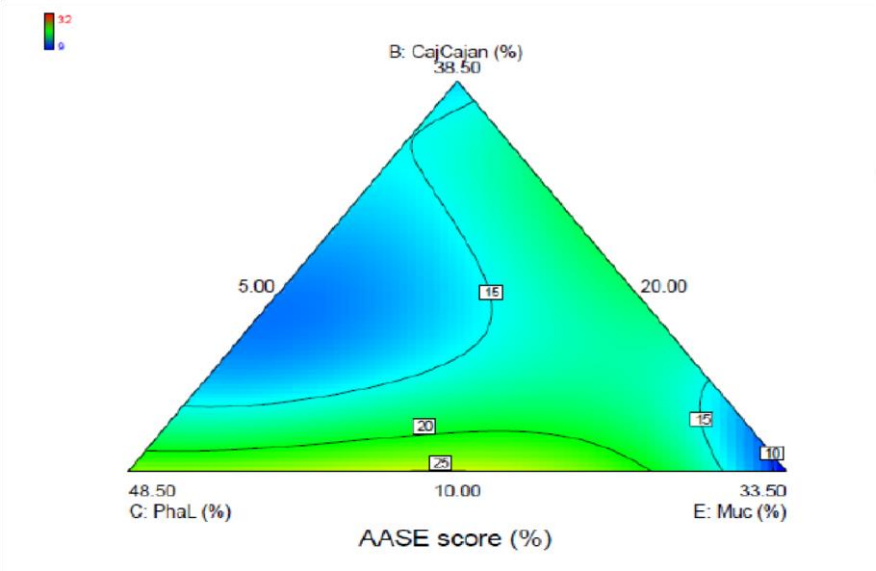

Figure 3.6: Three component mix plot of amino acid score in elastin (AASE) and its relation with actual Cajanus cajan (10.00-38.50%), Phaseolus lunatus (20.00-48.50%) and Mucuna pruriens (5.00-35.50%) at optimum percentage mixture of Vigna subterranea (15.00%), and Canavalia ensiformis (21.50%)

Cajanus cajan protein ranged from 10.00 – 38.50 % and the fuzzy linguistics was set as low (10.00-10.95 %), medium (19.6 -29.00 %) and high (29.10 – 38,50 %). *Phaseolus lunatus* protein ranged from 20.00 – 48.50 % and this was set as low(20.00 – 29.50 %), medium (29.60 – 39.00 %) and high (39.1 – 48,50 %). *Mucuna pruriens* protein ranged from 5.00 – 33.50 % and this was set as low (5.00 – 14.50 %), medium (14.60 – 24.50 %) and high (24.60 – 33.50 %). The AASE ranged from 8.57 – 31.96 % and this was set as low (8.57 – 16.37 %), medium (16.38 – 24.70 %) and high (24.80 – 31.97 %).

For the interractive composite BCE, (*Vigna subterranea, Phaseolus lunatus and Mucuna pruriens*) proteins, Table 3.8 presented the final linguistic protein quantities and the final AASE for setting the rules and their membership functions defined by their AASE scores. The data obtained was loaded into Fuzzy linguistic programming software (Matlab, 2013).

The rules were then set and the plots for interactions were obtained.

Table 3.8 Showing the linguistic protein quanities as *low, medium and high* in the interractive composite; BCE and their respective AASE responses B:CajCajan C:PhaL E:Muc AASE score

High	Low	Medium	High
High	Low	Low	Low
High	Medium	Low	High
Medium	Low	Low	Medium
Low	Medium	Medium	Medium
Medium	Low	Medium	Medium
Low	Low	High	High
Low	Low	Medium	Medium
Low	Low	Low	Medium
Low	Low	Medium	Medium
Medium	Low	Low	Low
Low	Low	Low	High
Low	Low	Low	Medium
High	Low	Low	Low

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Data Analysis

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 (Gao *et al.*, 2006). The data for dependent variable, which is AASE and the independent

variables; *Cajanus cajan, Canavalia ensiformis, Phaseolus lunatus, Mucuna pruriens* and *Vigna subterranea* were run in the Design Expert (2007) package to obtain a regression model that predicted the response within the given data set.

Table 4.1: Sequential model sum of squares table showing the suggesteted 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		

In RSM, it is always necessary to examine the fitted model to ensure that it provides an adequate approximation to the true system and verifies that none of the least squares regression assumptions is violated (Jinap *et al.*, 2007). The collected data was ran by fitting the summary and the sequential model sum of squares gave the following details. 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.

Table 4.2: Lack of fit test table showing the suggesteted 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		

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

The model summary statistics was studied from Table 4.3 which focused on the model maximizing the "*adjusted r-squared*" and the "*predicted r-squared*". It is aimed at selecting model with "*r-squared*‖ maximized and approaching 1. From the table, it suggested the cubic regression which had r-square of 0.086 which was approximately to1 over the other models the model. From Table 4.3 it is clearly seen that there are progressions of the sum of squares from the linear regressions and maximizing with the cubic regressions.

Table 4.3: Model summary statistics table showing the suggesteted model (*) of the highest order polynomial with the maximized r-squared value for the amino acids in elsatin score

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted 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		

Table 4.4: Analysis of variance table showing the significance(*) of the suggesteted model as well as the significance of the factors and their interractions in the regressional model that has been obtained for the amino acids score in eastin

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

The values of "P > F" less than 0.0500 indicate model terms are significant. In this case BC, BE, ACD, BCD, and BCE are significant model terms. 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 4.5: Showing the Adjusted R-Squared and Predicted R-Squared values

Std. Dev.	3.45
Mean	18.89
C.V. %	18.28
PRESS	2824.15

R-Squared	0.76
Adj R-Squared	0.56
Pred R-Squared	-1.48
Adeq Precision	9.23

A negative "pred R-Squared" of –1.48 implies that the overall mean is a better predictor of the response with an adjusted value of 0.56. Adequate precision which measures the noise ratio of 9.23 indicates an adequate signal.

4.2 Optimization of Legume Quantities by Response Surface Methodology to Obtain Mnimum AASE

In order to obtain the optimum quantities for the various legumes, constraints were set for all the legumes (Table 4.6). The AASE was also targeted at 8.57 in order to make available elastin to other organs of the body.

Table 4.6: 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 4.7: 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	

The model suggested all the following mixtures are shown in Table 4.10 but selected number one as the best mixture.

4.3 Mixture Design

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

The AASE ranges from 0 to 40.00 %, B also ranges from 10.00 % to 12.00 % and that of C ranges from 20.00 % to 22.00 % from Figure 3.1. 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 Score. 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 %

4.3.2 Interaction between B (*Cajanus cajan*) and E(*Mucuna puriens*)

There was a strong interaction between B and E. Figure 3.2 shows a quadratic curve which goes through two minimum ends and an upper middle part. As *Canavalia ensiforms, Vigna subterranea* and *Phaseolus lunatus* are kept at optimum percentages 21.50 %, 15.00 % and 20.00 % respectively,the amount of *Cajanus cajan* ranged from 10.00 - 38.50 % while *Mucuna puriens* 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 puriens* 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 puriens* 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.

4.3.3 Interaction between C (*Phaseolus lunatus*) and E (*Mucuna puriens*)

From Figure 3.3, as *Canavalia ensiforms, Vigna subterranea* and *Cajanus cajan* are kept at optimum percentages 21.50 %, 15.00 % and 10 % respectively, the amount of *Phaseolus lunatus* ranged from 20.00 - 48.50% while *Mucuna puriens* ranged from 5.00 - 33.50 %. AASE was at a minimum when the quantity of *Phaseolus lunatus* was 20.00 % and that of *Mucuna puriens* was 35.50 %. The amount of AASE increased when the amount of *Phaseolus lunatus* proteins increased from 20- 48.5 %. AASE was at maximum when quantities of *Mucuna pruriens* and *Phaseolus lunatus* proteins were 19.25 % and 34.25 % respectively.

4.3.4 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. The quantities of *Vigna subterranea, Phaseolus lunatus* and *Canavalia ensiformis* were varied while that of *Cajanus cajan* and *Mucuan puriens* were kept at optimum percentages of 10.00 % and 35.50 % respectively as shown in Figure 3.4. The range of the AASE was from 8.57 to 32.00 %. The amount of *Vigna subterranea* ranged from 15 - 26.50 %, *Canavalia ensiformis* at 10 - 21.50 % and *Phaseolus lunatus* at 20 -31.50 %.

Table 4.8: Predicted values ACD (*Vigna subterranea, Phaseolus lunatus* and *Canavalia*

***ensiformis*)**

Predicted values for for AASE (%)	Predicted values for *Vigna subterranea* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values for Predicted values *Canavalia ensiformis* (%)
10.0	16.02	20.35	20.13
15.0	19.48	21.78	15.24
20.0	19.70	24.42	12.38
25.0	16.88	28.87	10.75

4.3.5 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 3.5, 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 %.

Table 4.9: Predicted values for BCD (*Cajanus cajan*, *Phaseolus lunatus* and *Canavalia ensiformis*)

Predicted values for for AASE (%)	Predicted values for *Cajanus cajan* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values for Predicted values *Canavalia ensiformis* (%)
10.0	10.53	20.39	20.58
15.0	12.08	22.08	16.96
20.0	15.37	24.30	11.83
25.0	10.08	29.34	12.05

In Figure 3.5, *Mucuna pruriens* and *Vigna subterranea* were kept at minimum whiles that of *Cajanus cajan*, *Phaseolus lunatus* and *Canavalia ensiformis* were varied. 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.

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

From Figure 3.6, 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 %

Table 4.10: Predicted values for BCE (*Cajanus cajan*, *Phaseolus lunatus* and *Mucuna pruriens*)

Predicted values for for AASE (%)	Predicted values for *Cajanus cajan* (%)	Predicted values for *Phaseolus lunatus* (%)	Predicted values *Mucuna pruriens* (%)
10.0	10.59	20.35	32.57
15.0	13.52	21.92	28.07
20.0	12.45	34.94	16.10
25.0	10.06	36.41	17.32

4.4 Fuzzy Design

4.4.1 Interaction between *Cajanus cajan* and *Phaseolus lunatus*

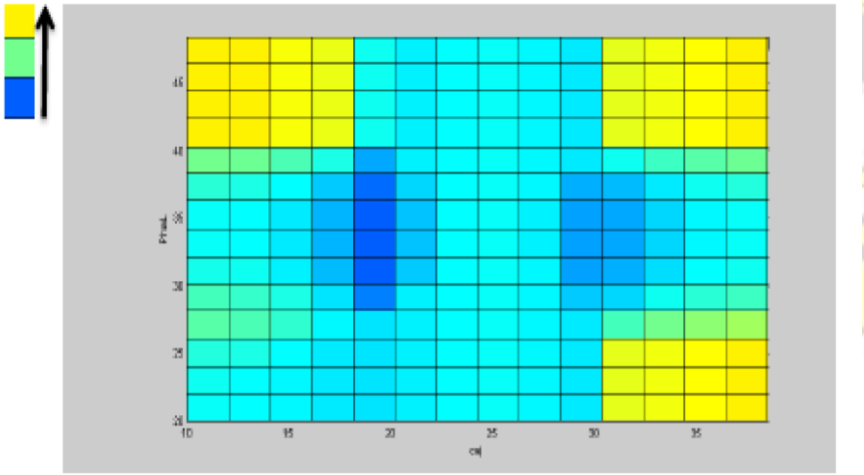

Figure 4.1: Plot showing the AASE resulting from the interactions between Cajanus cajan and Phaseolus lunatus proteins

AASE was at a minimum when *Cajanus cajan* was in the ranges 16.00 - 22.00% and 28.00-

32.00% and *Phaseolus lunatus* was also in the range 27.00 – 40.00%.

4.4.2 Interaction between *Cajanus cajan* and *Mucuna pruriens*

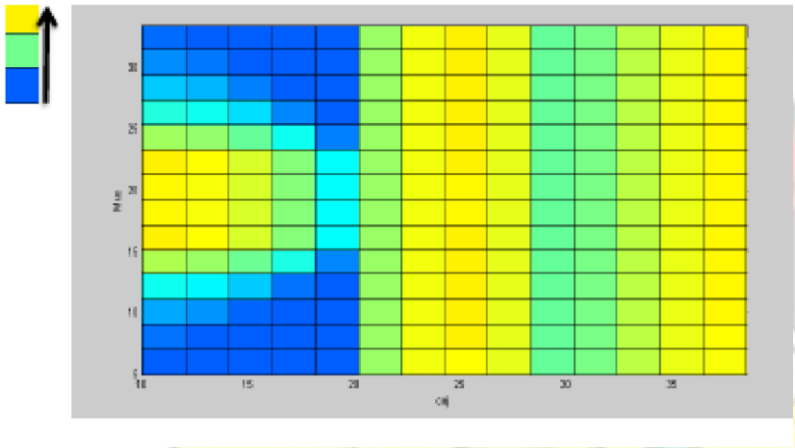

Figure 4.2: Plot showing the AASE resulting from the interactions between Cajanus cajan and Mucuna pruriens proteins

AASE was at a minimum when *Cajanus cajan* was in the ranges 10.00 – 20.00 % and

Mucuna pruriens was also in the range 5.00 – 15 % and 23.00 – 30 %.

4.4.3 Interaction between *Phaseolus lunatus* and *Mucuna pruriens*

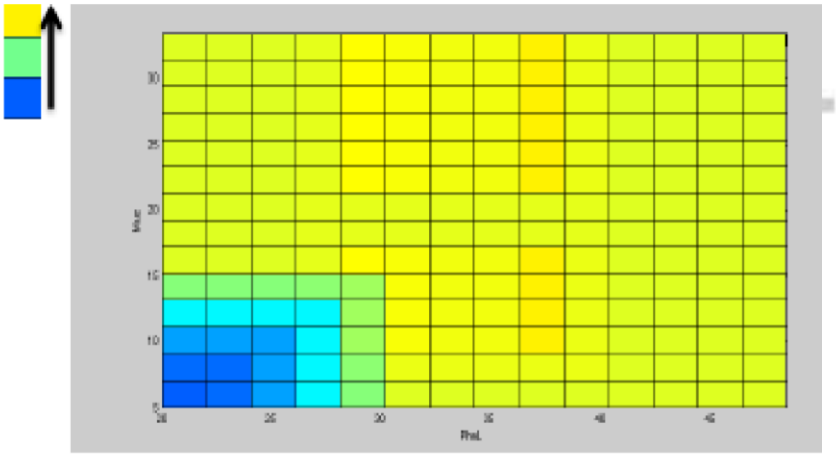

Figure 4.3 : Plot showing the AASE resulting from the interactions between Phaseolus lunatus and Mucuna pruriens proteins

AASE was at a minimum when *Phaseolus lunatus*, *Cajanus cajan* was in the ranges 20.00 – 25.00 % and *Mucuna pruriens* was also in the range 5.00 – 10.00 %.

Table 4.11: Predicted responses given by the model

Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low	95% CI high	99% Population TI low	99% Population TI high
AASE	8.57	8.57	score	-3.45	3.48	1.36	15.78	-9.21	26.36

Table 4.11 gave the predicted responses was almost closed after the selected mixture was input into model, the following were given for the actual response (Table 4.11).

4.5 Discussion

4.5.1 Mixture design

Interaction between BC (*Cajanus cajan* and *Phaseolus lunatus*): It has been reported by Bracalello *et al*. (2011) that the constituent amino acids found in elastin are valine, leucine, isoleucine, proline and glycine. Glycine constitute about one third (35 %) of elastin (Vrhovski and Weiss). According to Iheanacho (2010) *Phaseolus lunatus* contains these amino acids that makes elastin; glycine (4.92 g/100 g), proline (3.21 g/100 g), leucine (7.04 g/100 g) and valine (5.41 g/100 g) and (4.51 g/100 g) of isoluecine. From figure 3.1 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 *Phaseolus lunatus* is increased, there was a correspondence increase in the glycine which in tend increased the AASE.

Cajanus cajan contains glycine of amount of 3.07 g/100g, 3.17 g/100g of proline and isoleucine (3.47 g/100g) but higher quantities of leucine (6.78 g/100g), valine (5.58 g/100g) (Akande *et al.*, 2010). 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 %), alanine (10.9 %) are comparably higher than leucine (2.4 %), valine (2.2 %), and isoleucine(1.1%) (Szpak, 2011) and therefore have impact on the increase of the AASE.

Interaction between BE (*Cajanus cajanus* and *Mucuna pruriens*): From Figure 3.2, when *Cajanus cajan* was 38.50% and *Mucuna pruriens* was 5.00%, the AASE observed was higher (close to 10%) than at initial stage when *Cajanus cajan* was 10.00% and *Mucuna pruriens* was 33.50 %. As *Cajanus cajan* increased, the amount of AASE also increased whiles there was a decrease in the AASE as *Mucuna pruriens* increased. It was expected that AASE would increase as the quantity of *Mucuna pruriens* was increasing but the converse was observed. The constitutents of amino acids in elastin in *Mucuna pruriens* are as follows; ; glycine (5.92 g/100 g), proline (2.80 g/100 g), leucine (7.24 g/100 g) and valine (3.90 g/100 g) and (5.94 g/100 g) of isoleucine (Fathima *et al*., 2011). With the expection 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 (Szpak, 2011). The amount of proline in *Cajanus cajan* is 3.17 N/16g (Akande *et al*., 2010) which was higher than that of *Mucuna pruriens* with an amount of 2.80 g/100g (Fathima *et al*., 2010). 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* has contibuted to the increase in the AASE. Moreso, the aggregate of other amino acids in the legumes could also infleunce 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 cajanb* (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.

Interaction between CE (*Phaseolus lunatus* and *Mucuna pruriens*): From Figure 3.3, when the amount of *Phaseolus lunatus* was 20.00 % and *Mucuna pruriens* was at 33.50 %, AASE was at a minimum but when the amount of *phaseolus lunatus* was at 48.00 % and *Mucuna pruriens* was at 5.00 %, AASE was at maximum. The amino acid content of *Phaseolus lunatus* reported by Iheanacho (2010), for glycine was 4.92 g/100 g, and that of proline (3.2 g/100g), alanine (3.05 g/100g), leucine (7.04 g/100g), valine (5.41 g/100g), and isoleucine (4.5 g/100g). The amino acid content for *Mucuna pruriens* reported by Fathima *et al*., (2010), for glycine was 5.95 g/100g, proline (2.80 g/100g), alanine (4.24 g/100g), leucine (7.24 g/100g), valine (3.90 g/100g), and isoleucine (5.94 g/100g).

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 constitutent amino acids in 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/100g) and *Mucuna pruriens* (2.80 g/100g) 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.

Interaction between ACD (*Vigna subterranea* and *Phaseolus lunatus Canavalia ensiformis):* From the predicted values in (Table 3.4), AASE increased with an increase in the quantity of *Vigna subterranea* and *Phaseolus lunatus*. However, an increase in the quantity of *Canavalia ensiformis* did not increase the AASE. Increased quantity of *Vigna subterranea* and *Phaseolus lunatus* increased the AASE because of higher content of amino acids in *Vigna subterranea* reported in Table 2.3 by Akande *et al*. (2009) and *Phaseolus lunatus* in Table 2.5

reported by Iheanacho (2010). Their amino acid contents were comparably higher than that of *Canavalia ensiformis* and was able to increase the AASE.

The amino acid content for *Canavalia ensiformis* reported by Arora (1995) is as follows; 0.9 – 4.3 mg/100mg of glycine, 0.8- 4.3 mg/100mg of proline, 2.5 – 16 mg/ 100mg of leucine, 1.1 – 5.3 mg/100mg of valine, 0.1 – 4.7 mg/100 mg alanine. There was a decrease in the AASE as the quantity of canavalia increased, due to lower amount of relevant amino acids that were present. Thus their amino acids; glycine, proline, valine and leucine in *Canavalia ensiformis* were in their minimum ranges.

Interaction between BCD (*Cajanus cajan, Phaseolus lunatus* and *Canavalia ensiformis*):

Amino acid score in elastin(AASE) increased with an increase in the quantity of *Cajanus cajan* and *Phaseolus lunatus* in Table 3.5. The quantity of *Cajanus cajan* and *Phaseolus lunatus* increased as AASE increased as a result of their higher content of glycine, leucine, proline and valine (Akande *et al.,* 2010; Iheanacho, 2010). An increase in the quantity of *Canavalia ensiformis* did not increase AASE, due to lower amount of relevant amino acids that were present.

Interaction between BCE (*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 due to higher values of glycine (5.95 %) and alanine (4.24 %) (Fathima *et al.*, 2010). The amount of proline in *Mucuna prurien* was 2.80 g/100g (Fathima *et al*, 2010) which was lower as

compared to the amounts in *Cajanus cajan* (3.17 N/16g) and *Phaseolus lunatus* (3.21 g/100g). The higher values of proline in *Cajanus cajan* and Phaseolus lunatus have contributed to the increase of AASE.

4.5.2 Fuzzy design

Interaction between BC (*Cajanus cajan* and *Phaseolus lunatus*): Unlike the mixture design that could not give the exact quantity of legumes to be taken for the interaction, fuzzy design gave those ranges. From Figure 4.1, AASE was very low when *Cajanus cajan* was in the range 16 -22 % and 30 – 32 % and *Phaseolus lunatus* was also in the range 27 – 40 %.

Interaction between BE(*Cajanus cajan* and *Mucuna pruriens*): From Figure 4.2, AASE was at a minimum when *Cajanus cajan* was in the ranges 10.00 – 20 % and *Mucuna pruriens* was also in the range 5.00 – 15 % and 23.00 – 30 %.

Interaction between CE (*Phaseolus lunatus* and *Mucuna pruriens*): From Figure 4.3, AASE was at a minimum when *Phaseolus lunatus* was in the ranges 20.00 – 25.00 % and *Mucuna pruriens* was also in the range 5.00 – 10 %.

The optimized legume composite gave an AASE value (8.25 %) closer to the predicted value of 8.60 % as shown in Table 4.12. This implies that taking the legumes in their correct proportion in the composite as predicted by the mixture design will give an AASE value which will be almost the same as the predicted value.

Table 4.12: Actual response obtained using predicted optimum condition by RSM

Predicted	99% CI for Mean	Population of

Response	Mean	Median	Observed	Std Dev	SE Mean	95% CI low	95% CI high	95% TI low	95% TI high
AASE score	8.25	8.20	-3.00	3.33	1.25	14.00	-8.49	25.64	

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

There was a strong interaction between *Cajanus cajan* and *Mucuna pruriens* such that with almost equal quantities of *Cajanus cajan* and *Mucuna pruriens*, AASE value was at its maximum value. Increasing the quantity of *Canavalia ensiformis* in either *Vigna subterranean, Phaseolus lunatus* and *Canavalia ensiformis* interaction or *Cajanus cajan, Phaseolus lunatus* and *Canavalia ensiformis* interaction resulted in decreases in the AASE values. Increasing the quantity of *Mucuna pruriens* in either *Cajanus cajan* and *Mucuna pruriens* interaction or *Cajanus cajan, Phaseolus lunatus* and *Mucuna pruriens* interaction also resulted in a decrease in the AASE value. 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 %.

5.2 Recommendations

Studies should be conducted to determine the causes of the decrease in the AASE as the quantity of *Canavalia ensiformis* (D) and *Mucuna pruriens* (E) are increased. The functional properties of the composited legumes should be studied to determine their potential as food for cancer and diabetic patients. Diets made up of such composites should be evaluated to study the impact of the AASE scores.

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APPENDICES

Appendix A: Preparation of the Standard Solution

Dilute solutions (0.025, 0.05, 0.073, 0.10 and 0.12 moles/L) of the standard Amino Acids were then prepared by pipetting 20, 40, 60, 80 and 100 µL of the stock solution into a 15 ml centrifuge tube and diluted with 2.0 ml 0.2 M NaHCO3. 1 mL of 1% DNFB (a 1 mL portion of DNFB was dissolved in 100 mL of methanol). These test tubes were then placed on a water bath (60^{o}C) for 40 minutes. All derivatization reactions were stopped by addition of 0.5 mL of 1 mol / L HCL. The resulting derivative was then filtered. All the samples were treated in the same manner as described above for standard. That is, 1 ml of each sample were placed in a 15 ml centrifuge tube, 2 ml of NaHCO3 added and the resulting mixture derivatized by adding 1 ml of the 1 % DNFB(1-fluoro-2,4-dinitrobenzene).

Appendix B1: Experimental runs suggested by D-optimal of RSM with their response value of AASE

Run	Component 1 A:BamGrnt	Component 2 B:CajCajan	Component 3 C:PhaL	Component 4 D:CanEs	Component 5 E:Muc	Response 1 AASE score
	%	%	%	%	%	%
1	**15.00**	**10.00**	**60.00**	**10.00**	**5.00**	**26.50**
2	15.00	36.67	20.00	10.00	18.33	25.00
3	28.33	36.67	20.00	10.00	5.00	11.09
4	15.00	36.67	33.33	10.00	5.00	28.81
5	15.00	50.00	20.00	10.00	5.00	15.94
6	15.00	50.00	20.00	10.00	5.00	6.99
7	15.00	23.33	20.00	36.67	5.00	19.93
8	28.33	10.00	33.33	10.00	18.33	14.60
9	15.00	23.33	20.00	23.33	18.33	15.31
10	28.33	10.00	20.00	10.00	31.67	21.02
11	15.00	10.00	20.00	36.67	18.33	13.61
12	15.00	10.00	20.00	50.00	5.00	17.57
13	28.33	10.00	20.00	23.33	18.33	16.31
14	28.33	23.33	20.00	23.33	5.00	12.32
15	28.33	10.00	20.00	36.67	5.00	22.19
16	55.00	10.00	20.00	10.00	5.00	18.74
17	39.00	14.00	24.00	14.00	9.00	16.54
18	15.00	23.33	33.33	10.00	18.33	12.44
19	15.00	23.33	33.33	23.33	5.00	10.42
20	19.00	14.00	44.00	14.00	9.00	16.44
21	23.00	18.00	28.00	18.00	13.00	ND
22	55.00	10.00	20.00	10.00	5.00	16.00
23	28.33	23.33	33.33	10.00	5.00	19.28
24	41.67	10.00	20.00	10.00	18.33	15.47
25	41.67	10.00	33.33	10.00	5.00	17.38
26	15.00	10.00	46.67	23.33	5.00	16.92
27	15.00	10.00	20.00	23.33	31.67	8.80
28	19.00	14.00	24.00	34.00	9.00	17.00
29	15.00	10.00	33.33	23.33	18.33	22.26
30	15.00	10.00	20.00	50.00	5.00	21.28
31	41.67	10.00	20.00	23.33	5.00	17.55
32	28.33	10.00	46.67	10.00	5.00	ND

33	28.33	23.33	20.00	10.00	18.33	15.44
34	28.33	10.00	33.33	23.33	5.00	9.29
35	19.00	34.00	24.00	14.00	9.00	15.43
36	15.00	10.00	46.67	10.00	18.33	17.54
37	41.67	23.33	20.00	10.00	5.00	19.03
38	15.00	10.00	60.00	10.00	5.00	18.31
39	15.00	10.00	20.00	10.00	45.00	14.73
40	15.00	10.00	33.33	10.00	31.67	25.42
41	15.00	10.00	33.33	36.67	5.00	18.01
42	15.00	23.33	46.67	10.00	5.00	17.24
43	15.00	23.33	20.00	10.00	31.67	23.68
44	15.00	1.000	20.00	10.00	45.00	11.65
45	15.00	36.67	20.00	23.33	5.00	15.1205

Appendix B2: Table showing the goal and their importance of the factors

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	minimize	8	10	1	1	5

Appendix B3: Table showing constraints for the various legumes

Component	Name	Level	Low Level	High Level
A	BamGrnt	28.40	15	55
B	CajCajan	10.00	10	50
C	PhaL	34.42	20	60
D	CanEs	22.18	10	50
E	Muc	5.00	5	45

Total level = 100.00

Response	*Predicted mean*	*median*	*Std Dev*	*SE mean*
AASE score	9.64	9.64	3.87	3.72

Appendix C: ANOVA table for preliminary tests to processing factors

Design Summary File Version 9.0.3.1 **Study Type** Mixture **Runs** 45

Design Type D-optimal Point Exchange **Blocks** No Blocks

Design Model Cubic

Component	Name	Units	Type	Minimum	Maximum	Coded	Values	Mean	Std. Dev.
A	BamGrnt	%	Mixture	15.000	55.000	0.000=15.000	1.000=55.000	23.089	11.247
B	CajCajan	%	Mixture	10.000	50.000	0.000=10.000	1.000=50.000	18.089	11.247
C	PhaL	%	Mixture	20.000	60.000	0.000=20.000	1.000=60.000	28.089	11.247
D	CanEs	%	Mixture	10.000	50.000	0.000=10.000	1.000=50.000	18.089	11.247
E	Muc	%	Mixture	5.000	45.000	0.000=5.000	1.000=45.000	12.644	10.996
				Total =	100.00	**L_Pseudo Coding**			

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
R1	AASE score	%	43	Polynomial	8.573	31.9555	18.8903	5.20295	3.72746	None	RCubic