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MORPHOLIGICAL AND MOLECULAR DOCKING STUDIES ON THE FORTIFICATION OF STARCH FROM MAIZE WITH ISOLATED AND CHARACTERIZED DEFATTED SOYBEAN GLOBULINS POWDER

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ABSTRACT

Fortification of indigenous infant weaning meal with protein from leguminous plants such as soybean can actually improve its nutritive value. This study investigates the stability and composition of soy-based formulations with maize starch and determines the nature of intermolecular interaction between the protein and the starch molecules. Dry protein powders were isolated from the legumes and the percentage crude protein was confirmed by proximate analysis. Scanning Electron Microscope (SEM) was used to visualize the surface morphology of the defatted soybean, starch, dry protein powders and protein–starch composite. Molecular docking was used to investigate the nature of the binding and interaction between the 7S and 11S globulin storage protein in soybean and the Cyclo-Amylose and amylopectin present in starch. The results reveal that the blending of maize starch with the dry protein powders yielded a uniformly mixed protein–starch gel in all proportions with the best binding pose of the amylopectin with the 11S and 7S globulins having binding affinity energies of -11.0 and -10.8 kcalmol^{-1} respectively, while for the amylose, the binding affinity energies are -3.8 and -3.7 kcalmol^{-1} for the 11S and 7S globulins respectively. These results indicate higher propensity of amylopectin in the formation of protein–starch complex.

Keywords: Legume, globulins, amylose, amylopectin, protein–starch, binding-affirnity

INTRODUCTION

Good nutrition is of great importance for survival, physical growth, mental development, health and well-being across the entire lifespan of man. Such a need is equally important at the early stages of fetal development, at birth and through infancy, childhood, adolescence, and into adulthood. Malnutrition has placed children at a great risk of dying from common infections, increase in the severity of these infections and also causes delays in recovery. Malnutrition is responsible for nearly half of the deaths in children under the age of five years. On the other hand, good feeding practices would result in an improved nutrition, optimum physical growth, reduced vulnerability to common childhood illnesses and better resistance to cope with them [1].

In many West African countries, exclusive breastfeeding is usually sufficient for children from birth till the ages of three or four months, but after this period exclusive breastfeeding may become grossly

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insufficient to support the nutritional needs of the growing infant. So, there is a need to introduce soft, easily swallow-able foods to supplement the infant's feeding early in life. In Nigeria, the common weaning food used by both the low-income and middle-income groups is pap, (locally called ogi, *akamu*, or *koko*) which can be made from maize (*Zea mays*), millet (*Pennisetum americanum*), or guinea corn (*Sorghum spp*) [2]. This food has low nutritive value and is characterized by low protein content. The protein content of maize is poor and is low in lysine and tryptophan, amino acids which are necessary for the growth of children.

Soybean rank first among all food crops for its protein content and second among all legumes for its oil content. It is a legume that contains no cholesterol and is low in saturated fat [3]. Soybeans contain approximately 40 % protein, 20 % oil and 35 % carbohydrates on dry basis Soybean is the only vegetable food that contains all eight essential amino acids [4].

The majority of soybean proteins are storage proteins (65 – 80 %) as opposed to functional and structural proteins. Soy storage proteins are basically composed of two types of proteins; glycinin (primarily 11S) and beta-conglycinin (primarily 7S). Soy storage proteins are mostly globulins and are deposited in protein bodies, which are spherical in shape with size range of 2 – 20 µm. Electron microscopy has shown that soybeans also have lipid-containing spherosomes ranging in size from 0.2 - 0.5 µm between protein bodies [5].

Glycinin (11S globulin) is classified as a legumin and makes up to 25 – 35 % of the total seed protein, characterized by molecular weights of 300 – 400 kDa and sedimentation coefficient of 11S ± 1S. It consists of one acidicand one basicpolypeptide linked by a single disulphide bond, except for the acidic polypeptide A4. The quaternary structure of glycinin is composed of 12 subunits, forming a dimer of two identical hexamers. Three of the hexamers are acidic in nature and are present at 40 – 50 % while the other three are basic components that represent 50 – 60 %. Glycinin is capable of forming disulfide polymers, associating to increase the molecular weight and dissociating into subunits [6]. Beta-conglycinin (7S globulin) is a trimeric glycoprotein which is associated via hydrophobic and hydrogen bonded interactions without the contribution of disulphide bonds. They comprise of three random combinations of subunits: α-, α′-, and β- and are present in soybean. In contrast to 11S globulin, the presence of tryptophan and Sulphur-containing amino acids of 7S globulin is very low. As opposed to 11S, 7S globulin is not able to form disulphide bonds and dimerizes at 0.1 ionic strength, giving rise to the 9S sedimenting form [7]. 11S fraction differs noticeably from the 7S fraction in physiochemical, nutritional and functional properties [8].

Protein–starch composites are becoming more common in food processing when looking for enriched foods. Several studies have been carried out with focus on combining vegetable or animal proteins with starchy matrixes to produce different composites [9,10, 11]. Starch is a polymeric carbohydrate which consists of large number of glucose units joined by glycosidic bonds. It is composed of two types of molecules: the linear and helical chain amylose and the branched chain amylopectin. Both consist of polymers of α-D-glucose units in the $^{4}C_{1}$conformation. Depending on the plant, starches generally contain 20 – 30 % amylose and 75 – 80 % amylopectin by weight. Amylose is a polysaccharide made up of α-D-glucose units, bonded to each other through α-(1-4) glycosidic bonds. Because of

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its tightly packed helical structure and the linear chains of amylose, it is more readily crystallized than amylopectin and is more resistant to digestion than other starch molecules. It is therefore, an important form of resistant starch [12]. Amylopectin is formed by the non- random α-1⟶6 branching of the amylose-type α-(1⟶4)-D-glucose structure. This branching is determined by enzymes that leave each chain with up to 30 glucose residues. Each amylopectin molecule usually contains between 1– 2 million residues with about 5 % of it forming the branch points [13].

Many interactions exist among complex biomolecules, and the dominant interaction depends on molecular characteristics. Both starch and protein are biomolecules found in food and significantly affect food texture and rheology. The nature and extent of protein–starch interactions can be further understood by employing computational modeling techniques. With the increasing availability of high-resolution X-ray crystallography and NMR structures of biomolecules, the study of interactions between biomolecules in detailed atomic levels is now becoming more and more possible. The combination of this information with computational and visualization tools, helped spawn the field of structure-based ligand design. Molecular docking is a common step in the design cycle such that possible binding geometries of a molecule (ligand) with a macromolecule (receptor) are studied. The computational goal of molecular docking is to rapidly assess millions of possible binding orientations between molecules by filtering out the majority that are predicted to be extremely unlikely to bind, and then prioritizing the remaining ones. This approach is, in fact, a successful strategy, and several publications have demonstrated impressive enrichment of active compounds [14-19].

In this study, we isolated pure dried protein powders (DPP) from soybean that will serve as a fortifier to local infant weaning meal (carbohydrates) in order to combat the prevailing health challenges in infants as induced by protein deficiency. The physio-chemical properties such as proximate analysis, amino acid profile, morphological studies, as well as the molecular docking of the storage protein in soybean bean on amylose and amylopectin in starch, were investigated.

MATERIALS AND METHODS

Apparatus and Reagents

Volumetric flasks, Measuring cylinders, standard conical flasks, Pipette, Burette, filter paper, funnel, Kjeldahl apparatus, Weighing balance (S. Mettler analytical balance), pH meter, Freeze drier (Lab Kits FD-12-MR), Soxhlet Fat Extractor (Cytiva Whatman), Amino Acid Analyzer (Applied Biosystems 120A), rotary evaporator (Buchi rotavapor R-124), freezer (Lab Kits FD-12-MR), SEM machine (VEGA3 TESCAN).

All chemical reagents and solvents were of analytical grade from BDH chemicals and were used without further purification.

Collection of Materials and Sample preparation

Soybean seeds and maize grains were purchased from a local market in Ilorin metropolis, Kwara State, Nigeria. The soybean seeds were cleaned and pulverized and then dried at room temperature. The maize grains were cleaned, washed and stepped in water for three days in a plastic container, after which it was pulverized into a slurry. The slurry was then sieved using a muslin cloth which separated the pomace from the filtrate. The filtrate was allowed to settle for few hours and then the upper layer

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was decanted leaving the starch. The starch was then freeze-dried using theLab Kits FD-12-MRfreeze drier.

Preparation of defatted Seed Powder

The legume was defatted following a literature methodology according to AOAC [20] with slight modifications as described below.
The pulverized Soybean (70 g) was weighed into an extraction thimble and extracted in a Cytiva Whatman Soxhlet Fat Extractorwith n-Hexane for 6 hours. This procedure was repeated using diethyl ether and ethanol to ensure total extraction of the oil contained in the seed powder. After the extraction, samples were removed and kept to dry at ambient temperature. The defatted sample was obtained as fine whitish-yellow granules.

Preparation of Dried Protein Powder (DPP)

Soybean dried protein powder (DPP) was prepared using the reported methodology of Mattil[21]. The dried protein powder (DPP) of the defatted Soybean was obtained by alkaline extraction process using sodium hydroxide (0.2 M NaOH) at room temperature by varying the pH from 6.8 – 10. After the alkaline solubilization of the proteins has occurred, the insoluble material (residue) which contains materials like carbohydrate, sugars and other minor components was removed by centrifugation. The protein was precipitated isoelectrically by adding hydrochloric acid to the supernatant at pH 4.5to produce the isolate. The precipitate was creamy white in colour. It was then centrifuged to recover the proteins and washed repeatedly with water to free it from acid tinge. It was then neutralized to pH 7 using sodium hydroxide. Finally, the protein isolate was freeze dried using a freeze drier to obtain the DPP.

Test for Protein

The DPP was tested for protein according to Monrose-Forde [22] method, using sodium hydroxide and copper sulphate solutions. Sodium hydroxide solution ($1cm^3$ 0.1M) and copper (II) sulphate solution ($1cm^3$ 0.1M) were added in drop-wise to the DPP already dissolved in distilled water. The mixture was shaken well and allowed to stand for 5 minutes.

Proximate Analysis

The moisture, crude protein, and ash content were determined using the relevant AOAC, [23] methods. Fat and fiber contents were also determined using AOCS, [24] methods. Non-fiber carbohydrate content was determined as described by Mundi and Aluko [25].

Amino acid Profile

The Amino Acid profile in the Soybean protein was determined using methods described by Benitez [26]. The protein isolate was dried to constant weight, hydrolysed, evaporated in a rotary evaporator and loaded into the Parathyroid hormone (PTH) Amino Acid Analyzer (Applied Biosystems 120A).

Nitrogen Determination

The method of Benitez [26] was adopted for the nitrogen determination. 150 mg of the DPP was weighed in a Whatman filter paper and put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10 ml) was then added. 0.5 mg of a catalyst mixture containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules was then added. The flask was put in Kjeldahl digestion apparatus for 3 hours until the liquid turned light green. The

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digested sample was cooled and diluted with distilled water to 100 ml in a standard volumetric flask. Aliquot (5 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of the distillate was collected. The distillate was then titrated with standard 0.01 N Hydrochloric acid to grey coloured end point.

Percentage Nitrogen =

$$\frac{(a-b)x\ 0.01\ x\ 14\ x\ V\ x\ 100}{W\ x\ C} \quad \text{---------- (1)}$$

Where: a = Titre value of the digested sample, b = Titre value of blank sample,
V = Volume after dilution (100 ml), W = Weight of dried sample (mg),
C = Volume of the sample used (5 ml), 14 = Nitrogen constant in mg.

Hydrolysis of the sample

DPP sample (20 mg) was weighed into a glass ampoule and 7 ml of 6N HCl was then added. Oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen flame and put in an oven pre-set at 105 ^{o}C ± 5 ^{o}C for 22 hours. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness using rotary evaporator (Buchi rotavapor R-124). The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which was then kept in the freezer.

Parathyroid Hormone (PTH) Analysis of the Hydrolysate Sample

The cartridge of the analyzer was loaded and dispensed with 60 µl tubes. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Preparation of the Composite

The components that were used are DPP and freeze-dried starch granules. The DPP (20 mg) and starch (40 mg) were weighed in ratio 1: 2 into a conical flask containing 50 ml of distilled water. It was then transferred to a mechanical shaker and the components were shaken together for 15 hours. The mixture was then freeze-dried to produce the powdered protein-starch blend.

Surface Characterization

The surface characterization was performed by Scanning Electron Microscopy (SEM). The technique was used to determine the microstructure, shape, size and surface morphology of all the prepared samples. The SEM machine (VEGA3 TESCAN) was used to investigate the morphologies of defatted soybean protein isolate, dry protein powder, starch (from maize) and the protein – starch composites. Samples were attached to the aluminum stubs and then examined using an accelerating voltage of 3.0 kV

Molecular Docking of the storage proteins in Soybean and Starch

The molecular modeling tool, AutoDock Vina [27] was used for this docking experiment. It was used to determine the possible binding geometries of the molecules i.e. the protein molecules and the starch macromolecules and also to determine the most favourable binding interactions between the molecules. The X-ray structures of the globulins from Soybean; 7S, 11S and the starch amylose were downloaded from the RSCB protein data bank (http://www.rcsb.org/pdb) with pdb codes

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3AUP [28] 1OD5 [29] and 1C58 [30] respectively. The structure of starch amylopectin was downloaded from the repository of PubChem [31] (with PubChem CID 439207). The structure files were prepared for the simulations by editing them appropriately to remove unwanted water and hetero atoms. The blind docking of the two starch molecules to each of the soybeans globulins receptors as targets were performed in AutoDock Vina [27] to form the protein – starch complex. The docking experiment is used to determine the magnitude of the binding affinity and the nature of the protein – starch interactions in accordance with the AutoDock4 docking protocol [32]. The LGA stochastic search method [33] was implemented with the semi-empirical free energy force field scoring function used to represent the potential energy surface of the ligand – protein interaction. A simulation box was defined around the binding site of each globulin receptor by considering a distance of 3.0 Å away from the outermost atoms in the *x*-, *y*-, and *z*-directions.

RESULTS AND DISCUSSION

Test for Protein

There was a change in the colour of the DPP sample from blue to deep violet after the addition of sodium hydroxide and copper sulphate solutions indicating the presence of protein. The colour change was visualized against a white background.

Proximate analysis of Soybean

The proximate composition of the Soybean seed is collected in Table 1. The result shows that Soybean seeds are rich in nutrients especially protein, crude fat and carbohydrate. The moisture content and crude fiber content falls in the range of the WHO/FAO recommended value of 10 % and 3 % respectively [34]. The low moisture content for soybean implies that they can be stored for a very long time since moisture which is an important medium for multiplication of microorganisms is very low in the flour samples. The fat content is important in a diet, as it promotes fat soluble vitamin absorption in diet. Most legumes contain 1.5 % crude fat. Soybean crude fat (28.2 %) is very high compared to most legumes because it is an oilseed. Thishigh crude fat content suggests that soybean may be a viable source of oil. The high carbohydrate content 48.89 % of the sample suggests that the flour sample could be used in managing protein-energy malnutrition. Since there is sufficient amount of carbohydrate to derive energy, protein is spared and used for its primary function of building the body and repairing worn out tissues rather than it being a source of energy. Soybean seed has been reported to have high crude protein with value of 34.55 % [35, 36]. The high protein content suggests that Soybean seed could be used in the management of protein deficiency cases such as kwashiorkor and can be used in improving the palatability of foods in which they are incorporated. Therefore, the high percentage crude protein serves as the basis for the isolation of dried protein powder needed for this experiment from the Soybean seed.

Amino acid Profile

The amino acid profile of soybean seeds is presented in Table 2 and Fig. 1. The amino acid profile of the protein isolate showed that the acidic amino acids, glutamic and aspartic acid had the highest concentrations with values in the range of 20.63 - 10.32 g/100 g while cystine had the least concentration with a value of 1.58 g/100 g. The results showed that the total amino acids contained in the protein isolate are eighteen, including all of the essential amino acids (EAA) and Sulphur-containing amino acids.

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Table 1: Proximate analysis of soybean seed

Composition	***Soybean Seed (%)***
Crude Protein	34.54
Moisture Content	4.4
Crude Fat/Lipid	28.2
Ash	1.5
Carbohydrate	48.89
Fiber	0.5

Table 2: Amino acids profile of Protein Isolate from Soybean

Amino Acid	**Soybean Concentration (g/100 g protein)**	**% of Amino acid**	**Amino Acid**	**Soybean Concentration(g/ 100 g protein)**	**% of Amino acid**
Leucine	9.42	8.9	Tyrosine	2.41	2.3
Lysine	6.43	6.1	Histidine	2.81	2.7
Isoleucine	5.77	5.5	Cystine	1.58	1.5
Phenylalanine	5.15	4.9	Alanine	6.00	5.7
Tryptophan	1.90	1.8	Glutamic acid	20.63	19.5
Valine	5.62	5.3	Glycine	3.86	3.7
Methionine	1.65	1.6	Threonine	3.56	3.4
Proline	4.47	4.2	Serine	7.25	6.9
Arginine	6.72	6.4	Aspartic acid	10.32	9.8

The percentage of the total EAA; histidine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine and lysine are 40.08 %. The percentage of the total non-essential amino acids (NEAA) present in the samples is 59.91 %. The ratio of the EAA to NEAA present in the protein isolate was 0.67:1. The values obtained for percentage of total EAA and ratio of EAA to NEAA are comparable to the reference values of 40 % and 0.6 respectively, recommended by FAO/WHO [37]. Also, percentage composition of savoury amino acids (glutamic acid) is 19.55 %. The percentage of the sweet amino acids (glycine and alanine) was high with a value of 9.36 %. These values obtained in this study meet the recommended FAO/WHO [37]standards. In summary, Soybean is a complete protein source with a well-balanced amino acid profile and has relatively high levels of EAA. It is a good source for the curtailment of amino acid deficiencies present in the diet of many developing countries. Soybean protein can serve as dietary supplements to indigenous foods which are characterized with poor protein and EAA in order to combat malnutrition and nutritional disorder, especially in infants during the weaning stages of life.

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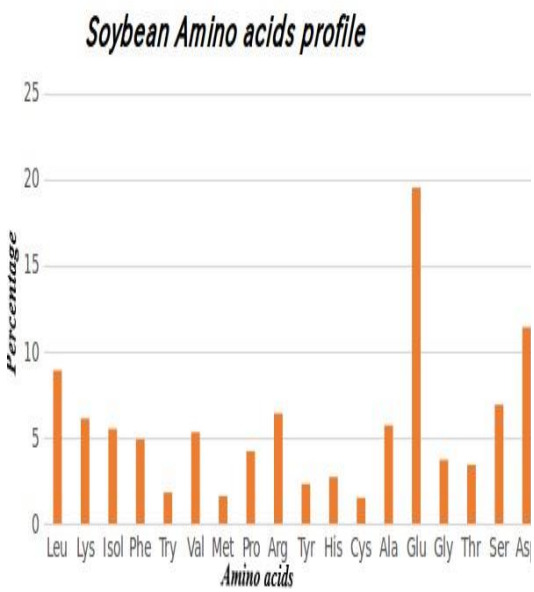

Fig 1: Amino acids profile for Soybean

Protein – Starch Composite Experiment

In the protein – starch composite experiment, the mixture of the DPP and the dry starch granules were shaken together for 15 hours on a mechanical shaker, the composite came out as a cloudy substance with no separation of the liquid from the solid. Hence the experiment showed that there was a successful binding between the protein and the starch meant to be fortified in this study. More so, if the dry protein is used based on recommended daily intake, it would effectively help any individual's protein supplementation. This further validates this work that is carried out for an affordable and reliable nutritious feeding. The composite which was later freeze dried came out as a fine uniform in ordered powder.

Microstructure and Morphology

The Fig. 2(a-b) shows that the defatted Soybean protein (Fig. 2a) comprises of rough but uniform granules of particles separated by air vacuoles that formed different clusters while the morphology of the dry protein powder (Fig. 2b) shows fine arrangement of smaller sized particles and this may facilitate faster hydrolysis of protein which in turn can improve the digestibility of the protein. It also showed the presence of some fragments and visible cracks but with a smoother morphology indicating a more homogenous structure. The incomplete coalescence of these small particles on the surface is probably the cause of the roughness on the surface of the protein and this is similar to the SEM result obtained by Wolf and Baker [38] for soybean flour.

Fig. 2(c-d) show that the starch granules has various particle sizes and shapes ranging from spherical, oval and irregular polygons which are well compacted together while the protein – starch composite has smaller particle sizes probably as a result of fragmentation of the granules while the larger particle sizes were as a result of the aggregation of composite granules.

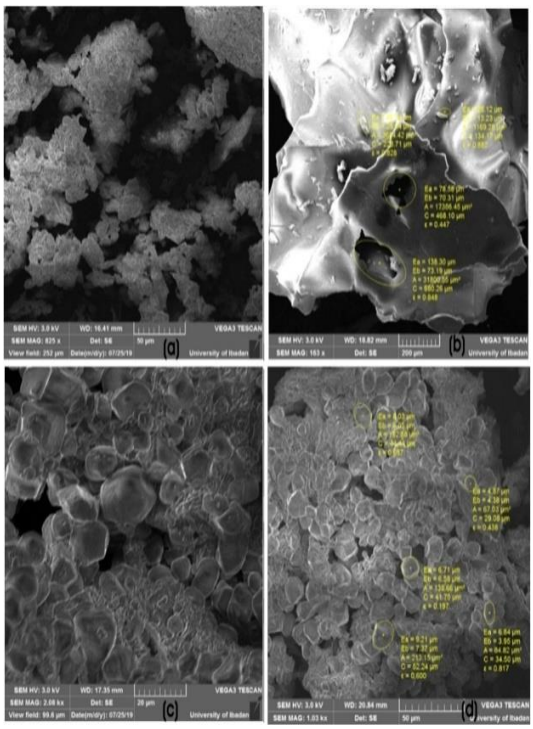

Fig. 2: The scanning electron micrographs of (a) defatted soybean protein (b) dry protein powder (c) starch (d) protein – starch composite at x50 µm magnification.

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The presence or addition of the protein slightly enhanced the cohesion of the composite as the blend appeared better bonded than the morphology of the slightly loosen-bonded starch. Significant differences in shape and size between the starch only and the protein – starch composite also show that there was a complete mixing of the protein and the starch.

Molecular docking of 7S and 11S globulins with Cyclo-amylose and Amylopectin

Molecular docking predicts the nature of the structural and intermolecular interactions present in complex formed between the 7S and 11S globulins protein and the Cycloamylose-26 and amylopectin starch molecules. The molecular models for the docking experiments are shown in Fig. 3(a-d), while the result of the binding affinities and molecular interactions are presented in Table 3. The binding sites interactions of the protein – starch composites are shown in Fig. 4(a-d). The two starch molecules successfully bind to the globulins protein to form a stable protein – starch complex. The amylopectin has higher propensity to bind to the 7S and 11S globulins than the cycloamylose as evident from its lower binding affinity energy of -10.8 kcalmol^{-1} and -11.0 kcalmol^{-1} for the best pose complex with 7S and 11S globulins respectively when compared cycloamylose complex which has the binding affinity values of -3.7 kcalmol^{-1} and -3.8 kcalmol^{-1} for the with the 7S and 11S globulins respectively. The interaction of amylopectin with the binding pocket residues of the 7S and 11S globulins is mainly through Hydrogen bonds and Van der Waals interactions while for the cycloamylose, the Van der waal interactions is more prominent. This also accounts for the greater stability of amylopectin – globulins complex. Therefore, in the formation of the protein – starch complex, the amylopectin component of the starch binds preferentially with relative ease to the globulins of the soybean than the amylase complex.

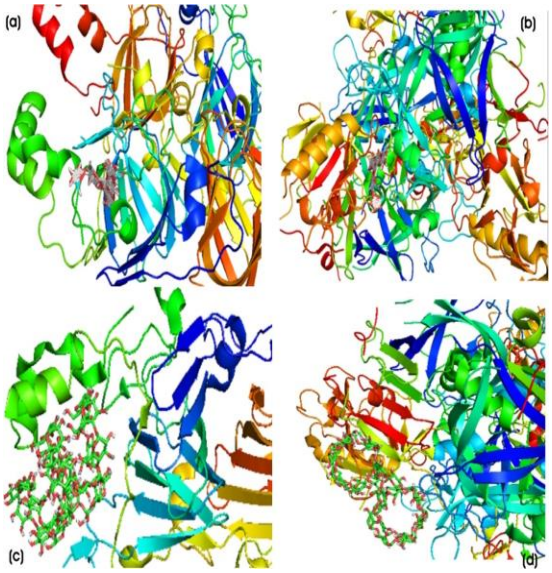

Fig. 3: Molecular model of the docked globulin – starch complexes (a). 7S – amylopectin (b). 7S – amylose (c). 11S – amylopectin (d). 11S – amylase

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Table 3: The binding affinity and molecular interactions of the complex between 7S and 11S globulins with the cycloamylose and amylopectin

		Binding affinity (kcal/mol)	Residues	Interaction type
1	7S globulin – Cycloamylose	- 3.7	Lys-58 Phe-64 Tyr-60 Ser-85 Leu-217 Phe-219 Lys-322 Asp-324 Arg-379	Van der waals Van der waals Van der waals Van der waals Van der waals Van der waals Van der waals Van der waals Van der waals
2	7S globulin – Amylopectin	-10.8	Ile-102 Phe-145 Ser-144 Thr-268 Val-272 Met-263 Pro-355 Thr-360	Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond
3	11S globulin – Amylose	-3.8	Gln-108 Ser-110 His-111 Trp-191 Phe-217 Lys-226 Leu-227 Arg-234 Val-240 Glu-241	Van der waals Hydrogen bond Van der waals Van der waals Van der waals Van der waals Van der waals Hydrogen bond Van der waals Hydrogen bond
4	11S globulin – Amylopectin	-11.0	Asn-159 Arg-161 Glu-172 Thr-176 Gly-202 Ser-203 Val-204	Van der waals Van der waals Van der waals Hydrogen bond Hydrogen bond Van der waals Van der waals

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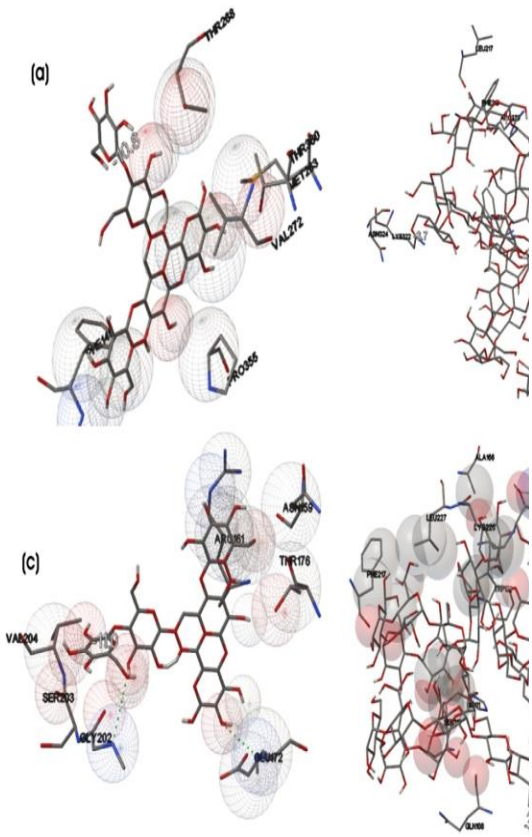

Fig. 4: Binding sites/pockets interaction of
(a). 7S interaction with amylopectin
(b). 7S interaction with amylose
(c). 11S Interaction with amylopectin
(d). 11S interaction with amylose

CONCLUSION

Soybean is a good source of protein as it has good nitrogen solubility, foaming and emulsifying properties, which in turn can improve the digestibility of the protein. Soybeans contain approximately 40 % protein. With this high protein content and it is assumed that this legume supports the isolation of dry protein powders. Dry protein powder isolated from Soybean can be used in fortifying indigenous infant meals of low protein quality.

The moisture content and fiber falls with the recommended value by WHO/FAO of 10%. The Proteins isolated is less sensitive to heat and other stresses when dried, it is chemically more stable and have a longer shelf life. The preliminary results indicated that Soybean seeds have a great potential for narrowing the amino acid deficit prevalent in the diet of many developing countries. The protein-starch blend experiment is a successful method of fortifying infant meal because the corresponding protein occupied the relatively loose starch granules based on SEM observation. Locally produced fortified meals are affordable for the rural parents in comparison to the fortified products produced by companies in the food sector of a country due to high or inconsistent cost of raw proteins, production and labour costs. So, fortifications take only a small fraction of one's income and since it increases human productivity and reduces a nation's healthcare expenditure fortifications are worth its cost implications.

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