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Pasting properties of starches and physicofunctional properties of proteins from some underutilized legumes

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ABSTRACT

Neglected and underutilized legumes abound in storage proteins and starches but are not exploited to the fullest for industrial applications. Thus this study was to investigate the surface functional properties of the storage proteins and the pasting properties of their starches. The pasting properties of the starches were studied using Brabender viscoamylograph. Four factors; concentration of protein (0.01-0.08%), pH (7-10), source of protein (*V. subterranea*, *C. ensiformis* and *C. cajan*) and type of solvent for extraction (0.01M NaOH, 0.05M NaCl) were projected into quadratic model to study the two main surface responses of the isolated proteins; foaming properties and emulsion capacities. The *V. subterranea* starch had maximum paste viscosity (400 BU) and a breakdown viscosity of 21 BU compared *C. ensiformis* starch. The optimum surface functional properties was respectively achieved at protein concentration, pH, solvent and protein source of 0.08%, 8.5, NaOH and *V. subterranea*. The surface functional properties of foaming and emulsion capacities could be attributed to the storage protein of 80kDa present at 70% in the *V. subterranea* NaOH extract. The potential therefore exist for food industrial applications of *V. subterranea* as far as pasting and surface functional properties are concerned.

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KEYWORDS

Legume starch;
Pasting;
Surface active properties;
Neglected legumes;
storage proteins.

INTRODUCTION

In many developing countries and in the tropics beans and peas collectively known as pulses or legumes are largely important sources of proteins. In Ghana, consumers depend on a very limited number of crops like cassava, yam, sweet potato and other roots and tubers

as well as cereals as sources of dietary energy to meet the needs of staple diets. Starch forms a major ingredient in all staple foods and can be used in its purified and unmodified form by the food, beverage, paper and textile industries. More recently, chemical and/or enzymatic modifications of isolated root and tuber starches have been carried out to further increase its application and

utilization^[13]. Though legumes contain substantial amount of starch these are not exploited industrially^[19]. Recently, there have been calls for the improvements of such legumes leading to extraction of starch from other sources in addition to the traditional roots and tubers.

According to Kessler, et al.^[14], *V. subterranea* and *C. ensiformis* have international importance as a future source of food. Therefore such leguminous starches have the potential of contributing greatly to textural properties of various foods. They are therefore expected to perform such industrial applications as thickener, stabilizer, gelling agent, bulking agent, water retention agent and as adhesive^[23,24]. Also due to inadequate supplies of animal proteins, there has been a continual search for new protein sources for use as both functional food ingredients and nutritional supplement^[20]. This search has led to increasing global protein consumption and the quest for new sources of food proteins, particularly plant sources^[17]. Attention must therefore be given to these legumes which hold tremendous alternative sources of proteins and starches. Thus this study was designed to firstly, determine the percent protein concentration, pH, source of protein and the solvent for the extraction of proteins that could support maximum surface functional properties and secondly, to evaluate the pasting properties of the legume starches.

MATERIALS AND METHODS

Materials

All reagents of analytical grade, used were purchased from Sigma-Aldrich Inc., USA. Electrophoresis apparatus (Bio-Rad ECPS 3000/1500) was used to measure the molecular characteristics of the proteins.

Source and preparation of sample

C. ensiformis, *V. subterranea* and *C. cajan* were obtained from Plant Genetic Resource Research Institute of the Council for Scientific and Industrial Research (CSIR-PGRRI) at Bunso in Ghana. One kilogram of the legumes was solar dried for a maximum of four days to a moisture content of about 10%. The dried samples were milled using MPE roller mills (Model GP-140 Grinder) with pore size of 250 microns. Eighteen liters of hexane as solvent was used to defat the flour in a Solid-Liquid Extractor (model E1 VS, France). The

defatted meal was solar dried in solar tent dryer for two days to expel all the volatile solvent after which it was stored in plastic bags at -4°C.

Methods

The extraction of storage proteins and starch

The extraction of protein fractions from the flours was carried out using the method described by Gomez-Brenes, et al.^[11], after a minimum modification. Proteins were extracted from 50g flour by adding 200ml of 0.01M NaOH and 0.05M NaCl separately. The resulting slurry was then agitated on a G24 Environmental incubator shaker (New Brunswick,) at 150 rpm at room temperature for 2 hrs. After filtering to remove the insoluble polysaccharides, the filtrate was centrifuged (Towson and Mercer G24 Centrifuge) at 2500rpm for 15 minutes into a debris free supernatant containing the proteins which were later precipitated at the isoelectric point (pI = 4.10) with 0.5M HCl. The proteins were washed with 500ml distilled water and re-suspended in 100ml distilled and stored in the refrigerator at 4°C. The insoluble starch which remained as the residue together with the insoluble polysaccharides was washed thoroughly with distilled water and sieved with a nylon cloth membrane with 60µm pore size. The starch was degummed with 250 ml 45% hexane on the incubator shaker for 2 hrs and was solar dried for 72 hrs, weighed and packaged in plastic containers pending further analysis.

SDS-PAGE Electrophoresis of proteins

The molecular weights of the NaOH and NaCl solvent protein extracts were determined using polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulphate (SDS). It was performed on 12% polyacrylamide gels according to the procedure described by Laemmli^[16] in a Mini-Protean II dual slab cell (Bio-Rad Laboratories, Richmond, CA). The sample buffer was briefly heated with 1% β-mercaptoethanol before loading into the wells. Standard protein markers containing seven proteins ranging from myosin (205 kDa) to lysozyme (18.5 kDa) was used for molecular weight determination. The separated bands of proteins were stained with Coomassie brilliant blue R-250. Molecular weights were determined by measuring the distances of migration in comparison with the standards.

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Image analysis of electrophoregram

The electrophoregram from the SDS-PAGE was scanned using HP Scanjet 2,400 at 1,200 dpi (Hewlett-Packard, Glasgow, Scotland, U.K.) and analyzed using the imageJ software according to the gel analyzer option as described by Ferreira and Rasband^[8]. Subsequently the percentage peaks of the separated bands against the logarithm of the molecular weights of the proteins (having calculated them previously from their Rf's) were run in a statistical tool^[25].

Experimental design for the surface functional properties

Presented in TABLE 1 are four factors; concentration of protein (A) (0.01-0.08%), pH (B) (7-10), source of protein (C) (*V. subterranea*, *C. ensiformis* and *C. cajan*) and type of solvent for extraction (D) (0.01M NaOH, 0.05M NaCl), which were varied in a response surface design into 27 experimental runs. Using Design Expert^[7] statistical tool, the D-optimal initial design was chosen to project a quadratic model to study the two main surface responses of the isolated proteins; foaming properties and emulsion capacities.

Foaming capacity and stability of proteins

Foam capacity and stability was determined by the Lin, et al.^[16], methods. Twenty five milliliters of protein solution obtained from each experimental run were suspended in 0.01M phosphate buffer. The resulting solution was whipped vigorously in a Stuart magnetic stirrer (SM4, Ireland) at 100 rpm at room temperature for 5 minutes. Volumes were recorded before and after whipping. The volume of the foam generated was expressed as foam capacity and the volume of the foam after a period of 120 minutes was expressed as foam stability. The percentage volume increase was calculated according to the following equation;

$$\% \text{ Volume} = (V_2 - V_1) / (V_1) \times 100$$

where V_2 = volume of protein solution after whipping; V_1 = volume of solution before whipping.

Emulsion capacity of proteins

The emulsion capacity was determined using the method described by Beuchat, et al.^[4]. Twenty five milliliters of protein solution suspended in phosphate buffer obtained from each of the experimental runs (TABLE

1) was agitated on Stuart magnetic stirrer (SM4, Ireland) at 150 rpm at room temperature as a refined vegetable oil (*frytol*) was added continuously from a burette until there was separation into two layers, when emulsion breakpoint was reached. Emulsification capacities were measured as volumes of oil added per gram of protein and these were determined in duplicates at 25°C.

Data analysis

The response data obtained was loaded into the Design Expert^[7] statistical tool and run to generate regression parameters which were studied. Statistical significance of the terms in the regression model was examined by analysis of variance (ANOVA) for each response.

Prediction regression-(pred R²) and adequate precision -(adeq precision) of the models selected were studied and used to judge the adequacy of the model that were generated. The p-values for the regression models as well as the interactions among the factors of extraction were tested against p<0.05. Optimization was performed according to the constraints presented in table 2 in order to predict the optimum conditions for maximum foaming and emulsion properties. In addition the conditions of concentration of meal slurry for extraction from flour, pH study of responses, solvent for the protein extraction and the source of protein.

Physicochemical composition of legume starches

The phenol-sulphuric acid or the anthrone method was used to determine the carbohydrate content of the legumes. The standard AOAC^[2] methods were used for the measurement of amylose. The Coffman and Garcia^[5] procedure was used to determine the least gelation concentration. Ogungbenle^[19] method was used to determine the swelling power/ solubility. The starch granule sizes measured by staining with 0.2g I2/2g KI and viewing under a light microscope using a Meiji microscope model MX4300H (Meiji Techno Co., Ltd., Saitama, Japan), equipped with a JVC color camera.

Determination of pasting properties of starch

The pasting properties were determined by the method described by Newport Scientific^[18]. The equipment was set to rotate the spindle at 75 rpm and to start pasting at 50°C. The temperature was set to in-

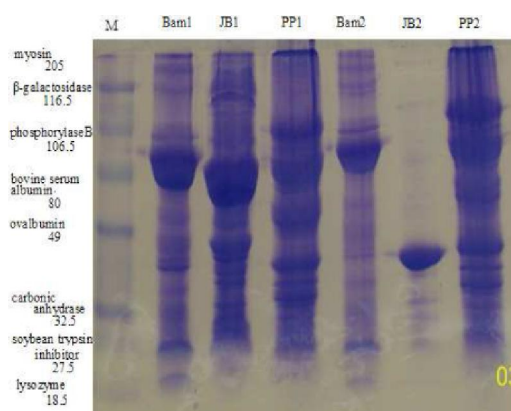


Plate 1 : The electrophoregram of marker proteins(M) and three legume proteins; *V. subterranea* (Bam), *C. ensiformis*,(JB) *C. cajan*(PP) which have been extracted by two solvents; 0.01M NaOH(1) and 0.05M NaCl(2)

crease at 1.5°C / min and programmed to hold the paste at 90°C for 15 minutes after which it was cooled at the same rate to 50°C for a further holding for 15 minutes. *C. ensiformis* and *V. subterranea* starches were pasted as described above using 38.1g (dry basis)/ 421.8 ml and 40.5g (dry basis)/ 419.5ml respectively. Pasting parameters such as beginning of gelatinization, maximum viscosity, start of holding, start of cooling, end of cooling, end of final holding, break down and set back were recorded in this determination.

RESULTS AND DISCUSSION

SDS-PAGE electrophoresis of proteins

SDS-PAGE of the six protein extracts gave multiple bands as presented by the electrophoregram (plate 1). *V. subterranea* which was extracted with 0.01M NaOH (Bam1) showed eleven bands (plate1, lane 2, Figure 1a) with a molecular weight range of 19-172 kDa, while the *V. subterranea* extracted with 0.05M NaCl (Bam2) showed ten bands (plate1, lane 3, Figure 2b) with a molecular weight similarly ranging from 19-172kDa. There was one very prominent peak of up to about 70% of a protein with a molecular weight 80kDa extracted with NaOH from the *V. subterranea* whereas the rest of the eleven proteins extracted constituted some 30% (Figure 1a). Similarly, a protein with molecular weight of about 90kDa constituted about 72% of the proteins extracted with NaCl from *V. subterranea* of which the rest make up some 28 % (Figure 1b).

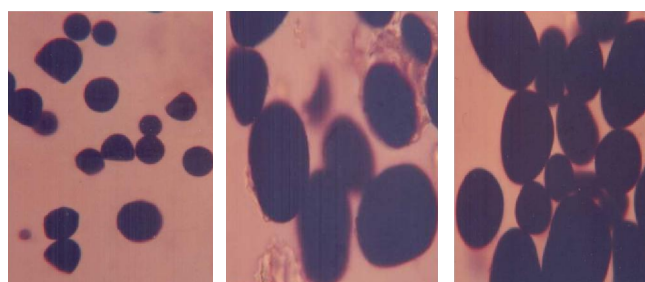


Plate 2 : Starch granule distribution showing small granules as round and polygonal shapes with large granules showing oval and elliptical shapes especially in the leguminous starches. The control starch is showing very small and many truncated shapes

The *C. ensiformis* extracted with 0.01M NaOH (JB1) showed nine bands (plate1, lane 4, Fig 2a) with molecular weight ranging from 27 to 173 kDa, while *C. ensiformis* extracted with 0.05M NaCl (JB2) showed four bands (plate1, lane 5, Figure 2b) with a rather low molecular weight range of 24-45 kDa. The effect of solvents was consistent in the extractions from *C. ensiformis*. For instance, in the NaOH extraction, there were nine bands of which five were pronounced due to a protein of molecular weight 75 kDa amounting to some 51% of the extract (Figure 2a) compared to a protein with a molecular weight 43.65 kDa with a strong peak of 88.92% in the four bands (Figure 2b) obtained from the NaCl extraction. It is suggested that only subunits of the parent albumins, globulins and possibly glutelins were extracted probably because of the low molecular weights shown^[1].

Also the *C. cajan* extracted with 0.01M NaOH (PP1) showed eight bands (plate1, lane 6, Figure 3a) and the 0.05M NaCl extract of *C. cajan* (PP2) had nine bands (plate1, lane 7, Figure 3b). Their molecular weights ranged between 24-174 kDa (Figure 3a) and 26-180 kDa (Figure 3b) respectively. The NaCl extraction of *C. cajan* gave nine bands of which six band were above 10% band area (Figure 3b). There was significant presence of peak strength of which the highest was 17.56% due to a protein of molecular weight of 179.52 kDa. The situation was similar for the NaOH extraction of *C. cajan* though this solvent was able to extract only eight proteins of which a protein with molecular weight 58.94 kDa had 21.99% band area (Fig-

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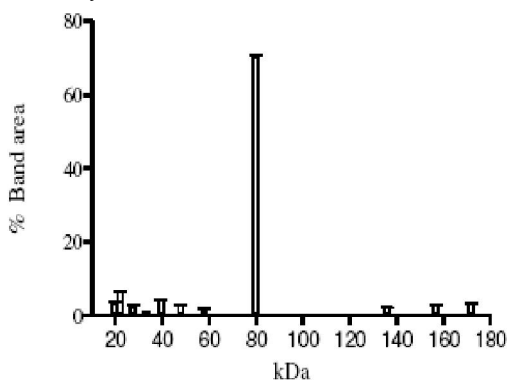


Figure 1a : Percentage band area of the molecular weights of storage proteins as extracted from *V. subterranea* using 0.01M NaOH solvent (Bam1)

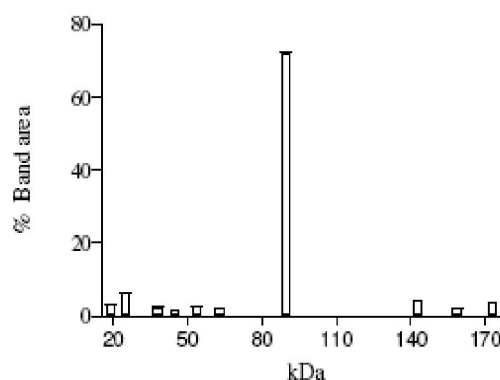


Figure 1b : Percentage band area of the molecular weights of storage proteins as extracted from *V. subterranea* using 0.05M NaCl solvent (Bam2)

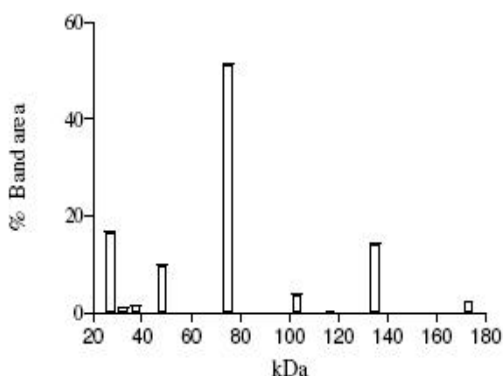


Figure 2a : Percentage band area of the molecular weights of storage proteins as extracted from *C. ensiformis* using 0.01M NaOH solvent (JB1)

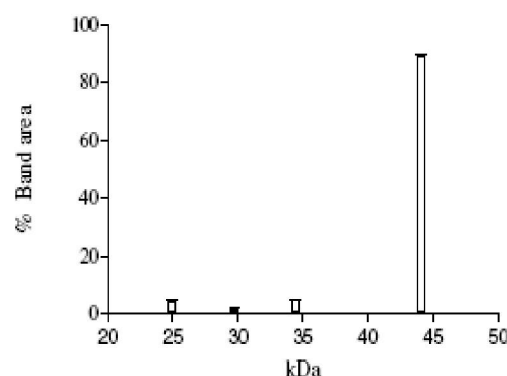


Figure 2b : Percentage band area of the molecular weights of storage proteins as extracted from *C. ensiformis* using 0.05M NaCl solvent (JB2)

ure 3b), not able to extract equal number of the storage proteins as in the case of the NaCl extraction.

It was observed that the solvent used for the extraction in *C. cajan* had a tremendous effect on the storage proteins extract since different molecular weights of proteins were recorded for the two solvents used. The *C. cajan* extraction did not provide a single most pronounced peak as compared to the extractions in the *V. subterranea* and *C. ensiformis* suggesting that the storage proteins in *C. cajan* were many different types. The low molecular weights of all the bands might also be due to dissociation of the protein subunits into smaller ones during heating in the presence of β -mercaptoethanol which encourage disulphide linkage reductions^[6].

Fitting the data collected

To explain the behavior of the treatment responses against the factors, plots such as the model designs were obtained. A summary of the statistics of model fitted

indicated the R^2 adjusted R^2 and predicted R^2 . These were indicators of how well the factors and responses models fitted the data. For goodness of fit, low standard deviations and R^2 near 1 were desired^[7].

Foaming capacity and stability of proteins

Analysis of the data revealed a linear model (Figure 5) for foaming at the zeroth minute which was significant ($p < 0.05$) for protein concentration, the legume source of protein and the type of solvent used for the extraction of the storage proteins. However, there were no interactions between the solvent used to extract the storage proteins and the source of the proteins. The regression parameters of R^2 (0.84), adj R^2 (0.77), pred R^2 (0.61) and adeq precision of 10.13, point to the very features of fitted models that could be used to navigate the design space and make predictions. Subsequently the equation (1) described the foaming response at the zeroth minute Y_0 and the dependent factors as;

$$Y_0 = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_7 X_7 + \beta_8 X_8 \quad (1)$$

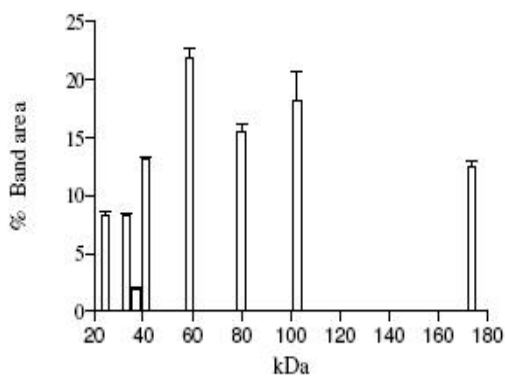


Figure 3a : Percentage band area of the logarithm storage proteins as extracted from *C. cajan* using 0.01M NaOH solvent (PP1)

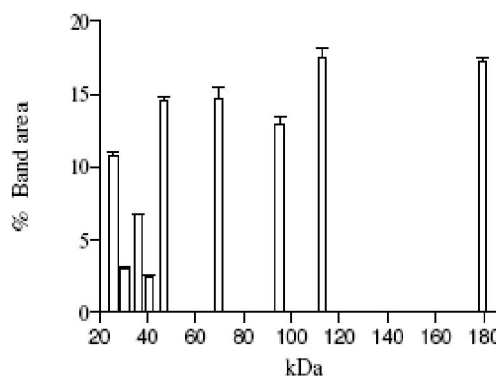


Figure 3b : Percentage band area of the logarithm storage proteins as extracted from *C. cajan* using 0.05M NaCl solvent (PP)

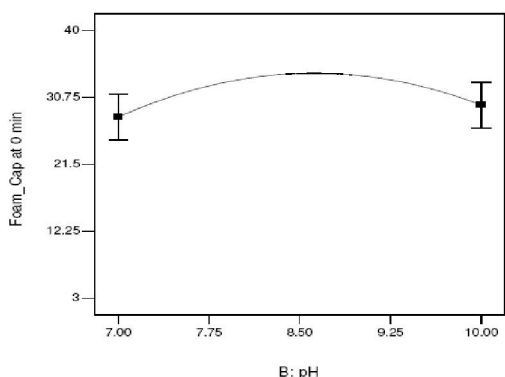


Figure 4: The relationship between foam capacity at the 0 minute and the pH at which foam was formed. A concentration of 0.08 % of *V. subterranea* proteins extracted with NaOH was used

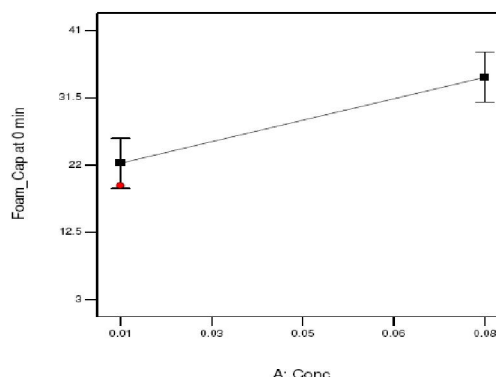


Figure 5 : The relationship between foam capacity at the 0 minute and the concentration of *V. subterranea* proteins extracted in NaOH at pH 8.5

The protein slurries of concentrations between 0.01M and 0.08M produced significant foaming that ranged between 22 to 35.1%. There was no significant interaction between foaming capacity and the dependent factors such as concentration of protein, type of solvent for extraction and type of protein.

However, pH had an influence on the foaming capacity at the zeroth minute for a protein concentration of 0.08% for particularly *V. subterranea* which were extracted with NaOH (Figure 4). At a pH of 7 (Figure 4), the foaming capacity rose from 28% and reached a peak of about 32% at pH 8.5, after which it declined to about 29 %, at pH 10. On the otherhand, the foam capacity response (Figure 6) at 120 minutes Y_{120} gradually rose to a maximum at pH 8.5 and declined to below 10% at pH 7. The model of the foaming capacity at 120 minutes is generally described by the regression model (2).

$$Y_{120} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_{3[1]} + \beta_4 X_{3[2]} + \beta_5 X_4 + \beta_6 X_1 X_2 + \beta_7 X_1 X_{3[1]} + \beta_8 X_1 X_{3[2]} + \beta_9 X_1 X_4 + \beta_{10} X_2 X_{3[1]} + \beta_{11} X_2 X_{3[2]} + \beta_{12} X_2 X_4 + \beta_{13} X_{3[1]} X_4 + \beta_{14} X_{3[2]} X_4 + \beta_{15} X_1^2 + \beta_{16} X_2^2 \quad (2)$$

In this model, it was observed that protein concentration has pronounced effect on foaming capacity and stability. These responses increased with increasing concentration of storage proteins as reported for mungbean protein isolate^[10]. The regression parameters of R^2 (0.94), adj R^2 (0.85), pred R^2 (0.35) and adeq precision of 11.68 were very good features for the model obtained. Therefore the model could be used to navigate the design space in order to explain the foam capacity and can thus be used to make predictions of foaming response. The foam capacity was optimized at around pH 8.5 and at a storage protein of 0.08% giving credence to the fact that at pH 8.5 the greatest nitrogen and hence protein solubility occurred. This suggest that at this pH protein polymers might have been polarized making them diffuse to the air- water interface rapidly to encapsulate air and subsequently form foam^[23,24]. pH increase from 7 to 8.5 may have led to the diffusion of gas from smaller bubbles to larger bubbles as reported by Rajeev and Djelveh^[22]. This could have

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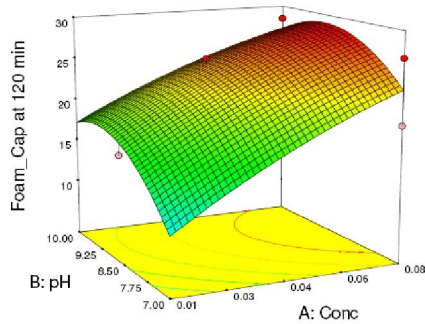


Figure 6 : Response surface plot of the relationship between foam capacity after 120 minutes and the pH and concentration of *V. subterranea* proteins extracted in NaOH solvent

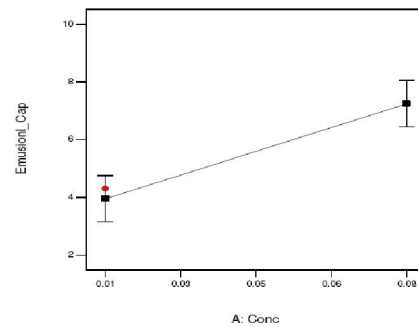
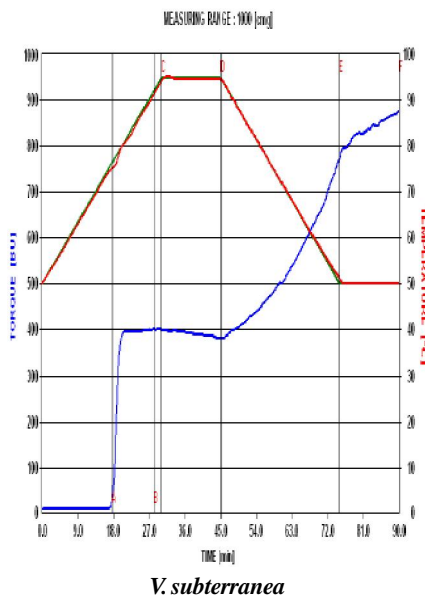
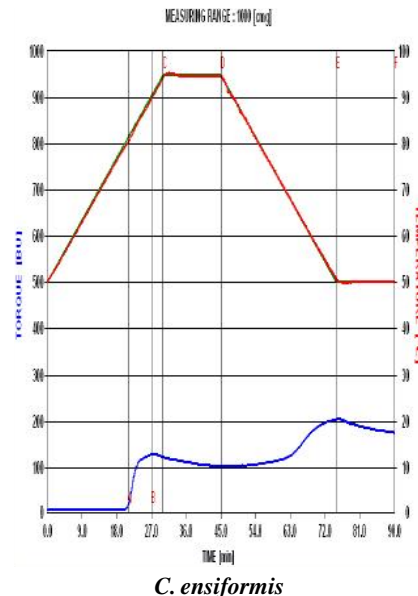


Figure 7 : The relationship between emulsion capacity after 120 minutes and the concentration of *V. subterranea* proteins solution of pH 8.5 extracted in NaOH solvent



V. subterranea



C. ensiformis

Figure 8 : Pasting characteristics of *V. subterranea* and *C. ensiformis* showing the beginning of gelatinization (A), maximum viscosity (B), start of holding period (C), start of cooling period (D), end of final holding period (E) and end of cooling period (F)

led to the disappearance of smaller bubbles into larger bubbles, a destabilization phenomenon called Ostwald ripening. This destabilization phenomenon seems not to be favourable at pH close to 7, probably due to poor lamellae formation and maintenance of foam integrity. According to Rajeev and Djelveh^[22], at an optimum pH which in this case is 8.5, the surface properties of proteins dominate as the surface tension drops to its lowest value. However, increasing the pH above 8.5 increases the surface tension therefore causing the disintegration of the foam.

Emulsion capacity of proteins

Analysis of the data revealed a linear relationship (Figure 7) model for emulsion capacity that is significant ($p < 0.05$) for protein concentration, source and

hence the type of protein and the type of solvent used for the extraction. There were interactions between the solvent used to extract the storage proteins and the concentration as well as the source of protein and the type of solvent used for the extraction of the storage proteins. As far as pH is concerned its relation between emulsion capacity rises through a maximum at 8.5 before decreasing just as in the case of foaming. The regression parameters; R^2 (0.36), adj R^2 (0.34), pred R^2 (0.27), and adeq precision (6.85) of the fitted model were just adequate and as such could be used to navigate the design space and make predictions. Subsequently the emulsion capacity of *V. subterranea* extracted with NaOH was modeled as:

$$Y_1 = \beta_0 + \beta_1 X_1$$

Differences in polypeptide profiles have been shown

TABLE 1 : Twenty seven experimental runs for four modification factors; concentration of protein (A) (0.01-0.08%), pH (B) (7-10), source of protein(C) (*V. subterranea*, *C. ensiformis* and *C. cajan*) and type of solvent for extraction(D) (0.01M NaOH, 0.05M NaCl) in a response surface D-optimal initial design projecting a quadratic model. The response factors were foam capacity at 0 and 120 min as well as emulsion capacity

Run	Factors				Responses		
	A:Conc %	B:pH	C:Proteins	D:SolExtr	Foam Cap at 0 min %	Foam Cap at 120 min %	Emulsion Cap %
1	0.080	10.00	CanV	NaCl	12.20	8.40	4.10
2	0.010	7.00	CanV	NaOH	3.80	2.80	3.20
3	0.045	10.00	CanV	NaOH	18.10	11.90	7.70
4	0.080	8.50	CanV	NaOH	32.20	22.50	7.10
5	0.045	7.00	CanV	NaCl	15.00	8.10	7.20
6	0.010	8.50	CanV	NaCl	7.50	4.70	5.80
7	0.045	7.75	CanV	NaOH	24.40	16.90	7.90
8	0.045	9.25	CanV	NaCl	11.90	9.40	3.90
9	0.045	7.00	CanV	NaCl	11.30	5.00	8.60
10	0.080	7.00	CajCan	NaCl	20.00	15.00	5.90
11	0.045	7.00	CajCan	NaOH	16.30	11.60	6.80
12	0.010	10.00	CajCan	NaOH	10.60	8.10	2.90
13	0.080	10.00	CajCan	NaOH	18.80	15.60	7.80
14	0.045	10.00	CajCan	NaCl	15.60	9.40	8.50
15	0.010	7.00	CajCan	NaCl	7.50	6.30	3.50
16	0.045	7.75	CajCan	NaCl	18.10	12.80	6.00
17	0.010	10.00	CajCan	NaOH	9.40	6.90	2.00
18	0.080	7.00	CajCan	NaCl	18.80	13.80	10.0
19	0.010	10.00	VigSub	NaCl	8.80	6.90	2.80
20	0.063	8.50	VigSub	NaCl	16.60	11.60	5.20
21	0.080	10.00	VigSub	NaOH	34.70	28.40	7.40
22	0.080	7.00	VigSub	NaOH	22.50	19.30	5.90
23	0.010	8.50	VigSub	NaOH	19.10	15.90	4.30
24	0.028	7.75	VigSub	NaCl	15.90	13.10	4.30
25	0.045	9.25	VigSub	NaOH	31.30	25.00	2.60
26	0.010	10.00	VigSub	NaCl	8.80	8.10	3.40
27	0.080	7.00	VigSub	NaOH	28.80	26.90	6.40

to have direct effects on the functional properties of protein samples. Phillips^[21] explained that the concentration dependent emulsifying capacity as based on adsorption kinetics. When protein concentration was low such as in this case at 0.01%, the rate of adsorption would be diffusion controlled, but at high protein concentration, there was activation barrier to adsorption.

Therefore, the ability of the protein molecules to create space in the existing film and to penetrate and rearrange on the surface was rate determining. Generally, emulsion activity was optimized at pH 8.5 for *V. subterranea* proteins which storage proteins have been extracted using NaOH compared to the other protein sources from *C. cajan* and *C. ensiformis*. The fact that *V. subterranea* proteins showed potential in the surface functional properties could be attributed to those proteins of molecular weight ranging from 80 kDa storage protein in the NaOH extraction which was present at 70% to 90 kDa in the NaCl extraction which was also present at 72%. The other legume protein sources delivered only low potential in surface functional properties probably because they do not have the storage proteins of the right polypeptide constituents or kDa to affect the appropriate functions. These proteins could give other food functional properties that still need investigation.

Physicochemical properties and composition of the legume starches

The yield of starch isolated from the defatted Bambara groundnut flour was 43.01% of which the quantities of proteins were 0.59%. Fat was present at 0.42%. The ash and fibre contents were respectively 0.45% and 0.50%. Within experimental or variation limits, Piyarat (2008) also recorded Bambara groundnut starch yield of 45.57% containing 0.61% protein, 0.44% fat, 0.47% ash and 0.60% fiber content. The *C. ensiformis* starch had the highest amylose content of 37.10% as compared to *V. subterranea* starch which had 21.44% and this was indeed far and over the value reported in the cassava starch used in this work (23.40%). After the degumming of the starches, the anthrone starch purity determination gave *V. subterranea* as being pure up to 39.95% compared to the *C. ensiformis* which was 62.6% purer. Failure to achieve close to 100% purity might be due to the presence of starch granule proteins or even polar pigments which are reported to be strongly bonded to starch granules during starch synthesis^[3].

Like *Canna sp* starches the legumes starches had mixed population of large, medium and small. The mean size was however; different from that of the *Canna sp* starches which had mean granule diameter of 47.4 μm ^[12].

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TABLE 2 : The factors and responses goals of the constraints used to perform optimization

Name	Goal	Lower limit	Upper limit
Conc.	is in range	0.01	0.08
pH	is in range	7.00	10.00
Proteins	is equal to CanV	CanV	VigSub
SolExtr	is in range	NaOH	NaCl
Foam Cap at 0 min	maximize	3.80	34.70
Foam Cap at 120 min	maximize	2.80	28.40
Emulsion Cap	maximize	2.00	10.00

The particle size distributions of different sized granules separated from the legumes are presented in plate 2. *V. subterranea* showed mostly large elliptical and rather smaller spherical granules morphology and had sizes ranging from 15.78 μm (small) to 46.03 μm (large). It appeared elliptical in shape when large whereas the medium and small granules appeared spherical. *C. ensiformis* on the other hand showed mostly elliptical granules morphology and had rather larger granules sizes ranging from 15.78 μm (small) to 47.34 μm (large). The control cassava starch presented many round and truncated granules and had the smallest of the small sized granules (7.89 μm) as well as the smallest of the large sized granules (21.04 μm).

The results were in agreement with literature that root tuber starches small granules present round and polygonal shapes, whereas the large granules had oval and elliptical shapes^[12]. It also confirmed that most legume starches were oval in shape, but tend to have centric hilums. *C. ensiformis* starch had the lowest swelling power (22.71%) and solubility (0.0018%) compared to the *V. subterranea* of values 29.20 % and 0.0032% respectively. The cassava starch had comparable swelling power (23.90%) to that of *C. ensiformis* but had the highest solubility (0.062%) compared to all the legume starches. *V. subterranea* starch least gelled at 3.5% v/v whilst *C. ensiformis* starch least gelled at 2.5% v/w suggesting that smaller quantities of total solids is required by the *C. ensiformis* to gel compared to the *V. subterranea*.

Pasting properties of starches

The *V. subterranea* starch which had amylose content of 21.44% gelatinized at 75.1°C compared to *C. ensiformis* (37.10%) which gelatinized at 80.5°C probably because of the higher amylose content of the *C.*

ensiformis which might have presented greater crystallinity arising from hydrogen bonding hence requiring higher amount of heat for gelatinization. The *V. subterranea* starch had a higher pasting torque at 23 BU in the beginning of gelatinization as compared to the 18BU shown by *C. ensiformis* starch. The *V. subterranea* starch had preferable pasting characteristics because the maximum paste viscosity (400 BU) was higher and decreasing only slightly at start of holding (398 BU) and cooling (379 BU) as presented in figure 8. The final holding and end of cooling had torques of 772 BU and 876 BU respectively and producing only a small breakdown of 21 BU compared to *C. ensiformis* starch which rather had a lower maximum paste viscosity of 128 BU and start of holding and cooling of respectively 122 BU and 101 BU. The final holding and end of cooling were equally low hence producing a greater breakdown of 27 BU.

Even though a large setback of *V. subterranea* starch was observed (393 BU), the *C. ensiformis* starch had end of cooling paste which was still higher (876 BU) compared to that of *C. ensiformis* (174 BU). Many studies report the direct influence of granule sizes of various starch bases on the structure on their thermal properties like gelatinization and pasting behavior. However, it is not clear in this research how the granule sizes of the starch granules might have influenced the pasting behavior of the legume starches but it appears smaller granule size as in cassava starch as well as their truncated shapes might affect the pasting properties.

CONCLUSION

The concentration to be used to achieve optimum surface functional properties for all the three legume proteins was 0.08% at an optimum pH of 8.5. Of the two solvents, NaOH was the better solvent for the extraction of the proteins and *V. subterranea* storage proteins had the best surface functional properties of foaming and emulsion capacities which could due to the storage protein of 80kDa present at 70% in the NaOH extract. The *V. subterranea* starch had preferable pasting characteristics because the maximum paste viscosity (400 BU) was higher and decreasing only slightly at start of holding (398 BU) and cooling (379 BU). The final holding and end of cooling had torques of 772 BU and 876

BU respectively and showing only a small breakdown of 21 BU compared to *C. ensiformis* starch. Food processors should make conscious effort to upscale some of this underutilized legume for industrial applications in our quest to ensure sustainable food security.

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