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Structure and functional properties of (*Balanites aeqyptiaca del) Aduwa* protein meals and concentrate

Abstract

The study revealed structure and functional properties of *Aduwa* meals and concentrate. The SDS-PAGE show appearances of bands above >180 kDa in the non-reducing condition and the band was reduced to <140 kDa in the presence of β-mercaptoethanol. The FTIR contains the following functional groups; C-H, N-C, C=N, C=O, C-H_2, C-O-C, N-H and band intensities. The amino acid of *Aduwa* protein meal (APM) has greater amount of essential amino acid (47.84%) but the concentrate (APC) was high in hydrophobic amino acid (10.56%). The protein solubility (PS) show that 60.73% of the (APM) meal was soluble at pH 3.0 but APC was least soluble at pH 3.0. The foam properties showed that the foam capacity of the protein concentrate was high at 60 mg/ml, suggesting increase in the sample concentration enhanced by foam formation of the protein molecules from the concentrate. The emulsion properties revealed that sample concentration of 10 mg/ml is the threshold concentration for the samples to create enough interfacial tensions to stabilize the emulsion formed by the samples, at pH 9.0. The study concluded that *Aduwa* proteins from defatted meal and concentrate have potentials for expanded utilization in the food industries from the plausible molecular weight behaviour, functional properties, FTIR and amino acid profile.

Keywords: *Aduwa*, functional properties, polypeptide composition, Fourier transform infrared, ingredients

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Introduction

The importance of lesser-known seeds in the production of protein rich materials cannot be over-emphasized in the face of the recent need to feed the ever-increasing population of the world with more rich and nutritious foods. Similarly, the geometric increase in the cost of animal protein has created a scenario where the consumption of animal protein is beyond the reach of common man. As a result of this, research effort has been geared in recent times towards exploiting these under-utilized seeds to produce vegetable proteins that can compete favourably with animal proteins.[1,2] Many seeds and nuts, such as peanut,[3] Mustard seed,[4] Ackee apple[5] and Kariya seeds[6] have been used to produce protein rich bye-products, with demonstrated potentials to be used as ingredients to tackle protein malnutrition.

Defatted meal or flour is obtained from whole meal by simply removing lipids from the whole meal either by Soxhlet extraction method or using a screw press. While protein concentrate is produced by pH adjustment of the suspension of defatted flour in distilled water to iso-electric point and subsequent neutralization of the precipitated proteins, isolate is obtained by a combined process of solubilization, precipitation and neutralization.[7]

Dry desert date (*Balanites aeqyptiaca L.*) is one of the less exploited protein rich seed, that is abundantly found in the Northern part of Nigeria, hence, it is called *Aduwa* in the native language. The seed is consumed in unprocessed form in the Northern part of Nigeria and the leaves are used as part of ingredients in vegetable soup preparation.[8] The oil obtained from the seed has been traditionally used as cooking oil in the Northern part of.[9,10] Studies on the proximate composition of *Aduwa* seed showed that, about 23 % to 24.01 % crude protein content.[8]

The relatively high protein content of the seed suggests its potential to be used as source of protein in the food industry. For lesser-known protein seed like *Aduwa* to be effectively utilized in food system, it is important to understand the functional and structural properties of the proteins.[11] This is because an understanding of functional properties, such as the foaming and emulsion properties and solubility profiles would help to determine the specific food application of its proteins. The structural properties such as the behaviours proteins within the food matrices when in denatured or processed state and the amino acid profiles are important to establish the identity of a particular amino acids or combinations of amino acids that are responsible for a particular functional property.[12]

Therefore, an interaction between the structural and functional properties would determine the overall utilization of the *Aduwa* seed proteins as ingredients in food system. These properties also depend on some environmental factors of the proteins such as the ionic concentrations and buffering pH. These factors are important to ensure necessary manipulations of the protein to suit a particular food system.[13] To the best of our knowledge, information on the functional and structural properties of the *Aduwa* meals and protein concentrates are scanty in the literature, hence the objective of this study.

Materials and Methods

Materials and seed processing

Matured *B. aegyptiaca del* seeds were obtained from the popular Gashua market in Yobe State of Nigeria and authenticated at the Department of Agronomy, Federal University of Gashua, Yobe State, Nigeria. The seeds were toasted using dry heat at 70^{0}C for 30 minutes and allowed to cool. The toasted seeds were milled, and the oil was expressed using centrifugal semi-automated screw press according to the method described by Ogori et al.[8] to obtain the meal cake-(APM)

Preparation of defatted sample of Aduwa meal (DAM)

The process of removing fat from seed meal described by Gbadamosi et al.[14] was used to obtain Defatted *Aduwa* meal (DAM). 100 g of the meal cake was dissolved in 500 ml of acetone to make flour-solvent ratio of 1:5 w/v. The mixture was stirred over a magnetic

stirred for 4 h. The slurry was then filtered through a muslin cloth. The residue was re-extracted twice in a similar manner. The residue was then de-solventized by drying in a fume hood at room temperature and the dried flour was finally ground in a blender to obtain homogeneous flour and stored in an air-tight container as defatted sample of *Aduwa* meal.

Preparation of Aduwa protein concentrate (APC)

Aduwa protein concentrate (APC) was prepared by a method modified by method described by Gbadamosi et al.[15] A known weight (200 g) of the defatted flour were dispersed in distilled water (2-L) to give final flour to water ratio of 1:10. The dispersion were then gently stirred on a magnetic stirrer for 10 min to form a suspension, after which the pH of the resultant slurry was adjusted with 0.1 M HCl to pH 4. The precipitation process was allowed to proceed with gentle stirring for 2 h keeping the pH constant. Soluble carbohydrates (*oligosaccharides*) and minerals were removed by centrifugation at 3,500 × g for 30 min using a centrifuge. The precipitate (concentrate) was afterward washed with distilled water to remove the residual minerals and soluble carbohydrates and the pH was later adjusted with 0.1 M NaOH to 7.0 for neutralization and then centrifuged at 3,500 × g for 10 min again. The resultant precipitate (concentrate) was collected and dried in an oven at 45 ºC for 8 h and kept in air-tight container for further analysis.

Methods of Analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Gel electrophoresis was determined using the method by Aluko and McIntosh.[16] The samples were prepared for non-reduced sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by mixing the samples with Tris-HCl buffer solution (pH 8.0) containing 10% SDS and 0.01% Bromophenol blue. The sample was placed in boiling water for 5 min. and cooled to room temperature, then centrifuged at 16, 000 x g for 5 min. An aliquot (1 µL) of the supernatant was then loaded onto the 8-25% gradient gel. Reduced samples were prepared by adding 5% (v/v) 2-mercaptoethanol (ME) to an aliquot of the supernatant from 10% SDS samples, and 1 µL was then loaded onto the gel. Polypeptide separation and staining were carried out using the BIO base Separation and Development electrophoresis unit (BIO-base).

Fourier transform infrared (FTIR)

The FTIR spectra of the samples were recorded in FTIR instrument (Model/ Make: IFS 25, Bruker, Germany), with PC based software-controlled instrument operation and data processing. Ten milligrams (10 mg) of meal samples were made into pellets using KBr for FTIR analysis and a thin film was prepared by applying pressure. The data of infrared transmittance was collected over a wave number ranged from 4000 cm-1 to 500 cm- 1. All the samples were analysed in triplicates with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the samples.

Amino acid composition (AAC)

Amino acid composition was determined following the method described by Gbadamosi et al.[15] using S433 Amino Acid Analyzer (SYKAM, Eresing, Germany). Samples were freeze-dried and then hydrolysed for 24 h at 110 °C with 6 M HCl. After hydrolysis, the samples were freeze-stored in sodium citrate buffer at pH 2.2. When ready for analysis, a 50 µL of the hydrolysates was directly injected into the analyser. Tryptophan was determined separately by hydrolysis of the sample with sodium hydroxide. Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 M HCl, and measured as cysteic acid and methionine sulphone respectively.[17]

Protein solubility (PS)

The protein solubility of the samples was determined according to the method described by Malomo et al.[18]A 10 mg of sample were dispersed in 1 mL of 0.1 M phosphate buffer solutions at different pH values (3.0, 5.0, 7.0, 9.0) to obtain a 0.1% (w/v) concentration and the resulting mixture will be vortexed for 2 min and centrifuged at 3,500 × g for 20 min. Protein content of the supernatant were determined using the modified Lowry method as previously describe.[19] Total protein content was determined by dissolving the protein samples in 0.1 M NaOH solution. Protein solubilities were expressed as percentage ratio of supernatant protein content (samples dissolved in buffer solutions at different pH values) to the total protein content (samples dissolved in 0.1N NaOH).

Foam capacity and foam stability

Foam capacity and foam stability as influenced by pH (3.0, 5.0, 7.0, and 9.0) and sample concentration (20, 40 and 60 mg/ml) was determined by a modification of method described by Chavan et al.[20] A suspension of different sample concentrations was made in 100 ml of distilled water at different a beaker and the pH of the protein solution was adjusted separately to pH values adjusted with either 1 M HCl or 1 M NaOH.

The solution was then homogenized for 2 min using a blender (O'Qlink, China) set at high speed 5max then poured into 250 ml measuring cylinder. The percentage ratio of the volume increase to that of the original volume of protein solution in the measuring cylinder was calculated and expressed as foam capacity or whip ability. Foam stability was expressed as percentage of the volume of foam remaining in the measuring cylinder to that of the original volume after 30 min of quiescent period.

$$\text{Foam Capacity}(\text{FC}) = \frac{\text{Vol.after homogenization} - \text{Vol.before homogenization}}{\text{Vol.before homogenization}} \quad \text{(i)}$$

Foam Stability (FS): The ability to retain air for a certain period of time (foam stability, FS) was calculated by measuring the foam volume after storage at room temperature for 30 min and expressed as percentage of the original foam volume

$$\text{Foaming Stability}(\text{FS}) = \frac{\text{Volume after standing} - \text{Volume before whipping.ml}}{\text{Vol.before whipping.ml}} \quad \text{(ii)}$$

Emulsifying Properties

The effect of pH and sample concentration on emulsifying activity index (EAI) was determined by a modified turbidimetric method described by Famuwagun and Gbadamosi.[5] Different concentrations (10, 15 and 25 mg/ml) of the *Aduwa* samples were made in distilled water. The pH of the solution was adjusted separately to 3, 5, 7 and 9 with either 1 M HCl or 1 M NaOH. The sample slurry was each mixed with 20 ml of vegetable oil and the mixture was homogenized using a blender (VLC, Saphire, England) set at high speed for 60s. Fifty microlitres of the aliquot of the emulsion was transferred from the bottom of the blender after homogenization and mixed with 5 ml of 0.1 % sodium dodecyl sulphate (SDS) solution.

The absorbance of the diluted emulsions was measured at 500 nm using spectrophotometer (722-2000 Spectronic 20D, England) in 1 cm path length cuvette. The absorbance was read initially, after which turbidity and EAI was calculated using the following formula

$$T = \frac{2.303\,x\,A}{I} \quad \text{(iii)}$$

Where T = turbidity, A = absorbance at 500 nm and I = path length of cuvette (cm).

The emulsion activity index (EAI) was calculated as:

$$Emulsifying\ activity\ index\left(m^2 / g\right) = \frac{2\,x\,T}{0.2\,x\,C} \quad \text{(iv)}$$

Where T is the turbidity, C is the weight of protein per unit volume of aqueous phase

before the emulsion is formed (g/ml); 0.2 is the volumetric fraction of oil and 2 is a constant. The emulsion stability index (ESI) was determined after the emulsion is allowed to stay for 30 minutes and the absorbance of the mixture was read at 500 nm and calculated using the formular:

$$Emulsion\ stability\ index = \frac{EAI\ at\ 10\ min}{EAI\ at\ 0\ min} x100 \quad \text{(v)}$$

Statistical Analysis

Mead data from three readings were subjected to analysis of variance (ANOVA) followed by Duncan Multiple range test to compare treatment means for foaming and emulsifying properties. Differences were considered significant at $p<0.05$ using the statistical package for social sciences for window program (SPSS V23) software.

Results and Discussion

SDS- PAGE

The non-reducing and reducing SDS-PAGE electrophoresis gels of the *Aduwa* protein meal (APM), defatted meal (DAM) and protein concentrate (APC) are shown in Figure 1a, b respectively. In the absence of β-mercaptoethanol (non-reducing conditions), all the samples showed distinct bands above 180 kDa. However, the >180kDa in the (APM) and DAM were more visible, when compared with the concentrate (APC). The high molecular weight observed in these samples may be attributed to high hydrophobic character, which prevent the >180 kDa band to enter the gel.[2] The meal and the defatted flour also showed bands with molecular weight of 140 kDa, which was not visible in the protein concentrate. The low staining intensity in the protein concentrate, especially with the non-visibility of 10 kDa band could be attributable to the low solubility of the proteins in the electrophoresis buffer, which limits the interaction of the proteins with the gel. This pattern of results were observed in the dried alfalfa leaf proteins.

Under the reducing condition, there was disappearance of the >180 kDa bands in all the samples, which may suggest that the high molecular band contains some disulphide bonds.[21] There was also appearance of 45 kDa band in all the samples, which may have resulted from the reduction of the 140 kDa band. Also, there was appearance of highly stained and diffused small molecular bands between 10-25 kDa in all the samples. These highly diffused small polypeptide bands were common to all the samples. The appearance may be because of the reduction of the diffused bands in the non-reducing conditions of the samples. Smaller peptides have been attributed to better physiological performance such as in the management of chronic nutritional disease.[17,18]

Fourier transform infrared (FTIR)

FTIR analysis uses transition energy, corresponding to changes in vibrational energy state for many functional groups located in the mid-FTIR region (4000-400 cm^{1}) and hence the appearance of absorption band in this region can be used to determine whether specific functional groups existed within the molecule.[22] As shown in Table 1 and Figure 1. Based on the classification of simple and complex compounds, the absorption bands for the *Aduwa* protein meal (APM), defatted meal (ADM) and protein concentrate (APC) were more than five bands, suggesting that the samples were all complex in nature.[1,23]

Table 1 FTIR peak assignment for Aduwa meal, Defatted flour and protein concentrate

Functional group	APM	DAM	APC	Suggested Nutrients	Literature range Saguer et al. (2012); Mohamed et al. (2016); Zhang et al. (2017)
O-H stretching. N-C,N-H stretching	3443.05	3443.05	3443.05	Protein, poly hydroxyl compounds	3029-3334
CH_2 stretching	2928.04	2928.04	2924.18	Alkane compounds	2960
C-H Stretching	2852.84	2852.84	2852.81	Methoxyl, methyl, ether compounds	2890
N-H Stretching	2361.96	2542.26	2360.96	Amino acid compounds	23332-2359; 2344
C=N, X=C=Y	2065.83	2361.96	2065.83	Nitriles, Isothiocyantes	2270-1940
C=O stretching	1867.16	2065.83	1867.16	Triglycerides,acide chlorides	1810-1800
C=O Stretching	1732.13	1732.13	1732.13	Triglycerides, esters	1763-1712
O-H bending=N ,C=O Stretching	1635.64	1635.64	1635.69	Protein and amide II band	1620-1650
$C-H_2$ symmetric, asymetric bending	145.63	1533.46	1456.3	Proteins and fat,	1453, 1481-1585
C-O, C=O anti-symmetric bending	1384.94	1438.94	1384.94	Carboxylic acids, polypeptides	1357-1423
$C-H_2$ wagging	1317.43	1384.94	1317.43	Carboxylic acids	1357-1423
P=O;C-N Stretching	1247.97	1317.43	1247.99	Nucleic acid	1191-1356
C=N, C-O, C-C stretching	1145.75	1257.63	1145.75	Tertiary amines	1210-1150
C=N, C-O, C-C Stretching	1103.32	1147.68	1103.32	Primary amines	1090-1020
C=N Stretching	1062.81	1103.32	1062.81	Primary amines, Nucleic acid	1090-1020
C=N, C-O stretching	1035.81	1055.1	1035.81	Primary amines, Carbohydrates	1090-1020
-C-O-C stretching	893.07	887.28	893.07	Aliphatic hydrocarbon compounds	800-700
C-CL	781.2	781.2	781.2	Aliphatic chloro compound	700-600
C-H, C-Br	667.39	611.2	667.39	Aliphatic bromo compound	700-600
C-H, C-Br	617.24	518.87	617.24	Aliphatic bromo compound	700-600
C-X, C-Cl,C-I	516.94	3443.05	516.94	Aliphatic compound	

Keys: APM, Aduwa protein meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

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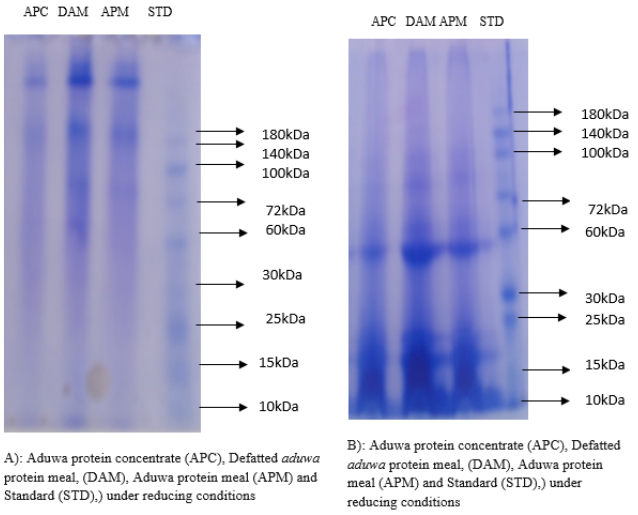
In comparison with published works in the literature, the result show protein and poly-hydroxyl compounds at bands between (3443.05 to 3421.83) cm (see Figure 1) with lower absorption band in protein meal APM, DAM and the protein concentrate APC. The defatted meal DAM and the proteins meal APM were also characterized by CH_2 stretching and C-H stretching, with absorption bands of 2928.04 and 2852.84 /cm respectively. This corresponds to the presence of alkane compounds and methoxy ether compounds. But the absorption range was lower, when compared to the range (2960 and 2890/cm) reported by Saguer et al.[24] and Zhang et al.[25]



A): Aduwa protein concentrate (APC), Defatted *aduwa* protein meal, (DAM), Aduwa protein meal (APM) and Standard (STD),) under reducing conditions

B): Aduwa protein concentrate (APC), Defatted *aduwa* protein meal, (DAM), Aduwa protein meal (APM) and Standard (STD),) under reducing conditions

Figure 1 (A) Aduwa protein concentrate (APC), Defatted *Aduwa* protein meal, (DAM) ,*Aduwa* protein meal (APM) and Standard (STD), under non-reducing conditions.

APM, Aduwa protein meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

The absorption bands for the functional groups, such as the C-O, C=O, C=N, C-H and C-OH which correspond to aliphatic hydrocarbon with halogens compounds and carbohydrates were higher in the APM, DAM but lower in APC. This pattern of results could be attributed to the protein solubilization and precipitation processes during the protein concentrate extraction protocols.[26] In a similar manner, the shifts to lower energy level in *Aduwa* protein meal, defatted meal and protein concentrate might have resulted from free amino acids aggregation during processing (Figure 2).[27,1]

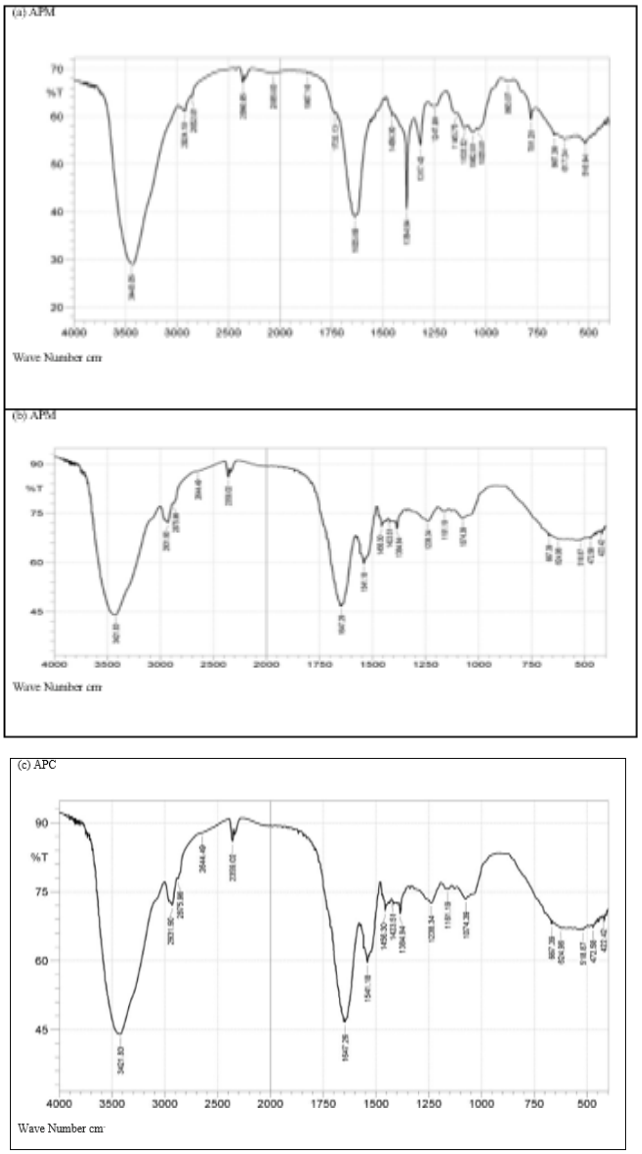


Figure 2 FTIR peak graphs for *Aduwa* Meal, defatted *Aduwa* meal and *Aduwa* protein concentrate

Amino acid Profile

Amino acid has been defined as the building blocks of proteins and it is an important parameter to determine the quality of protein in food ingredient. Table 2 show the amino acid composition of *Aduwa* meal, defatted meal and *Aduwa* protein concentrate. The essential amino acid (EAA) ranged between 37.35 and 47.84 %, and APM having the highest value, followed by the APC and this suggest that *Aduwa* protein meals APM and APC are good quality source of protein. In a similar manner, the aromatic amino acids, hydrophobic amino acids, positively charged amino acid of the samples ranged between 5.24 and 10.56 %, 39.96 to 40.95 % and 15.90 to 17.00 % respectively, and APC had higher amounts. The high concentrations of the hydrophobic amino acids in the APC may have implications on the structural behaviours of the proteins[28] such as solubility, foaming and emulsifying properties.

The values obtained for the sulphur containing amino acid ranged between 3.10 to 3.22 %, and APM has the highest value. The trend of the sulphur amino acid in the samples may dictate the antioxidant properties and anti-inflammatory property of the samples. The tryptophan content of the samples ranged between 1.16 and 1.18 %, and the values were higher than FAO recommend value 0.1 to 0.12 % reported for okra seeds flour and isolated protein Nnamezie et al.[1] The histidine contributes to anti -oxidant property from ability to donate electron .The content in the samples ranged between 3.00 to 3.39 %, above FAO referral value. The APC sample has the highest value. The differences in the amino acid contents of the meal and defatted meal when compared with those of protein concentrate may be attributed to the processing protocols applied when making APC.[29]

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Table 2 Amino acids composition of *Aduwa* meal, defatted meal and protein concentrate

	APM	DAM	APC		FAO
Leucine	12.01	8.60	8.47	6.6	
Lysine	8.41	5.32	5.35	5.8	
Isoleucine	5.62	4.50	4.55	2.8	
Phenylalanine	2.50	5.15	5.06		
Trytophan	1.78	1.18	1.16		1.1
Valine	7.49	5.02	5.06	3.5	
Methionine	2.21	1.58	1.62	2.5	
Proline	1.59	4.01	4.10		
Arginine	3.75	8.07	8.26		
Tyrosine	0.96	4.09	4.12	6.5	
Histidine	3.75	3.00	3.39	1.9	
Cystine	1.01	1.52	1.53		
Alanine	4.80	5.02	5.27		
Glutamic acid	8.65	18.22	18.08		
Glycine	8.21	4.11	4.26		
Threonine	5.86	4.81	3.86	1.4	
Serine	7.49	4.82	4.93		
Aspartic acid	13.93	10.99	10.93		
AAA	5.24	10.42	10.34		
BCAA	25.12	18.12	18.08		
HAA	39.96	40.67	40.95		
PCAA	15.90	16.39	17.00		
NCAA	35.93	38.84	37.80		
SCAA	3.22	3.10	3.15		
EAA	47.84	37.98	37.35		

Keys: APM, Aduwa protein meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

Aromatic amino acid (AAA) = phenylalanine, tryptophan and tyrosine

Branched chain amino acids (BCAA) = leucine, isoleucine, valine

Hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine and cysteine

Positively charged amino acids (PCAA) = arginine, histidine, lysine

Negatively charged amino acids (NCAA) = aspartic, glutamic, threonine, serine

Sulphur containing amino acids (SCAA) = methionine, cysteine

Essential amino acids (EAA) = histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine

Solubility profiles

Figure 3 show the solubility profile of *Aduwa* protein meals APM defatted meal DAM and *Aduwa* concentrate APC with respect to different pH (3, 5, 7 and 9) values. The result revealed that the samples were soluble at pH 3.0. The percentage soluble proteins decreased progressively as the pH values were adjusted from pH 3 to pH5. About 60.73 % of the protein meal APM was soluble at pH 3.0, and the protein concentrate was least soluble at the very acidic pH (pH 3.0). Ordinarily, the protein concentrate is expected to show better solubility at the acidic pH, but the low solubility of the concentrate APC at pH 3 when compared to the protein meal APM may be attributed to high protein aggregation at the pH value, which reduced the solubility.[30] Similar result pattern was reported for okra seed meals and protein isolate,[1] where the protein isolate showed poor solubility at acidic pH region. Beyond pH 3.0, the protein meal APM did not show any marked difference in the solubility, even as the pH value increased from pH3-pH9. The protein concentrate APC defatted meal DAM has the lowest protein solubility (21.50 and 20.40 %) at pH 5.0 and thereafter increased progressively to pH 9.0, which agreed with the pattern of results reported for walnut protein.[28]

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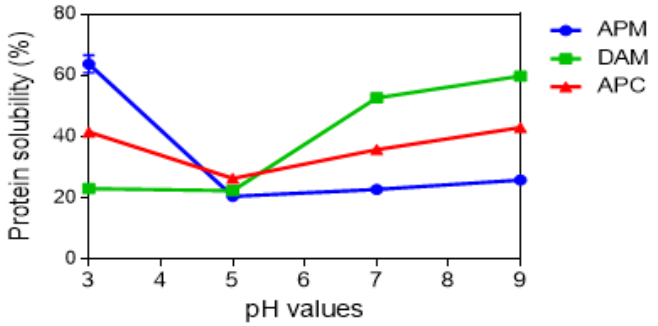

Figure 3 Protein solubility profiles of Aduwa protein meal, defatted meal and protein concentrate.

APM, Aduwa protein Meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

The low value of the concentrate protein APC at pH 5.0 have justify the iso-electric point of the *Aduwa* concentrate sample. Usually, solubility decreases as the pH increases until it reaches the isoelectric point and then increase again. The loss of electrostatic repulsive forces provides beneficial conditions for the formation of protein aggregates; high bulk density and large diameter of the aggregates had resulted in precipitation of protein.[31] The difference between the pattern of solubility of *Aduwa* protein meal and *Aduwa* protein concentrate may be due to the iso-electric precipitation during the concentrate protein process. At the basic region, especially pH 9.0, the amounts of soluble proteins for the samples were in the following order: 62%, 21.50% and 40.9 % for protein meal APM, defatted meal DAM and protein concentrate APC, respectively. The results showed that the protein meal was more soluble than the DAM and APC at the acidic region. But a reversed trend was observed at the basic region. The low values and the pattern of solubility of the protein concentrate may be a disadvantage when considering its use as ingredients in acidic drinks.

Foaming properties

Foaming capacity

Figure 4 show the influence of pH (3, 5, 7 and 9) and sample concentration (20. 40 and 60 mg/ml) on the foaming capacity of the samples. At sample concentration of 20 mg/ml, the APM have the highest foaming capacity when pH was 5.0, while the least was obtained at pH 3.0, which was not significantly different ($p > 0.05$) from the value obtained at pH 7.0. The foaming capacity of the defatted meal DAM decreased progressively as the pH of the solution increased from 3-9 at 20 mg/ml concentration. The pattern was different in protein concentrate APC whereby the foaming capacity of the samples increased as the pH of the samples increased, towards the basic region. The pattern of the results is in line with the increase in the net charge of the samples at the neutral and basic regions. At the neutral and basic regions there was observed increase in the net charges which eventually resulted in increase in APC protein-protein repulsion and a corresponding increase in the protein flexibility. When proteins become flexible, the tendency to accommodate more air bubbles increase and hence, an increase in the foaming capacity at this pH values. Similar pattern of results was observed in the foaming capacities of fenugreek seeds, Bambara seed and walnut isolated proteins.[32,33,28]

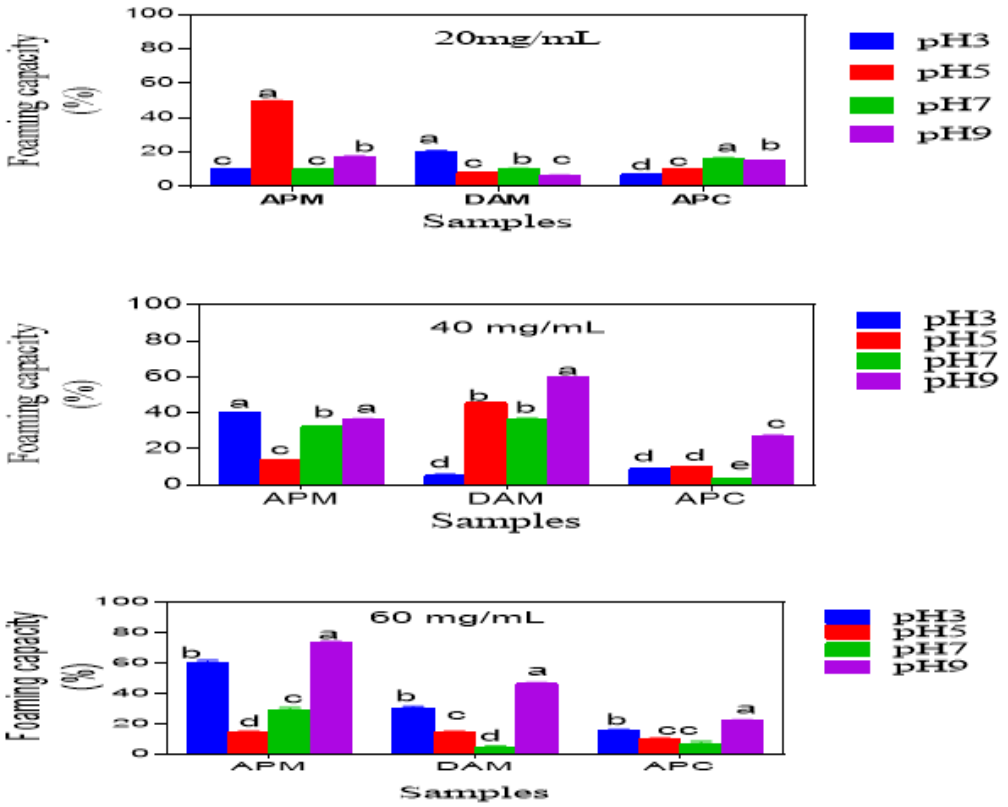

Figure 4 Effect of pH and sample concentrations on foaming capacity of Aduwa meal, Defatted meal, protein concentrate and isolate.

APM, Aduwa protein Meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

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As the sample concentration was increased from 20 to 60 mg/ml, an increase in the foaming capacity of the APM was observed at pH values 3, 7 and 9 but the foam formation at pH 5.0 decreased substantially. For the *Aduwa* defatted meal DAM and *Aduwa* protein concentrate, APC an apparent increase in the foam capacity of the samples were observed up-to 40 mg/ml, but decreased in values afterwards. A possible explanation for this pattern may that of protein crowding. Although, an increase in the protein concentration is necessary to generate adequate foams, increasing beyond 40 mg/ml may lead to generation of excess protein micelles that reduced the capacity to generate foams in the *Aduwa* defatted meal DAM and *Aduwa* protein concentrate.

The high forming capacities of the samples especially of the *Aduwa* protein meal, defatted meal and APC samples towards the basic region and at high sample concentration may be attributed to the formation of large charges at these pH values which encouraged the formation of interfacial membranes. The formation of large interfacial membranes within the protein molecules may encourage greater solubility that would lead to high foams.[34]

Foaming stability

Foaming stability is the ability of foam to keep its shape and volume over a specified period of time. This is very important because food material with good foaming stability could find applications in beverages, coffee and baking industries. The foaming stability of *Aduwa* meal APM, defatted meal DAM and protein concentrate APC with respect to variations in sample concentration (20, 40 and 60 mg/ml) and pH (3, 5, 7 and 9) values is shown in Figure 5. At 20 mg/ml sample concentration, the foam was more stable at the acidic pH (3 and 5) but decreased progressively as the pH moved towards the basic region (7 and 9) for protein meal APM and defatted meal DAM samples. The results also showed that values obtained for the foam stability were greater in the protein meal and defatted meal when compared to the values obtained for the protein concentrate at 20 mg/ml sample concentration.

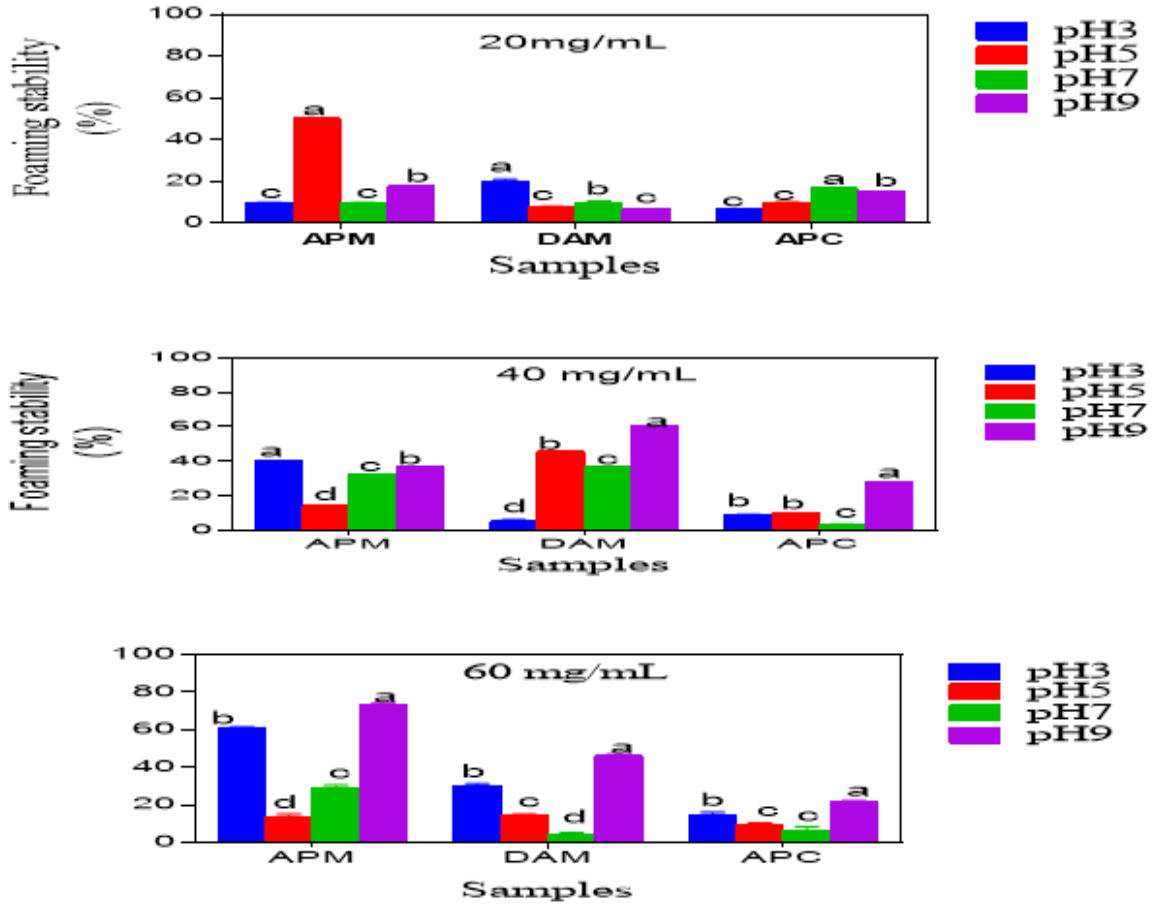

Figure 5 Effect of pH and sample concentrations on foaming stability of Aduwa meal, defatted meal, protein concentrate and isolate.

APM, Aduwa protein Meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

The pattern may be attributed to the formation of stable molecular layers in the air-water interface that enhanced greater impartation of texture, stability and more elasticity of foams. Similar pattern of results was reported for rapeseed flours and proteins where defatted flour exhibited greater foam stability than protein concentrate.[35] It was observed that as sample concentration increased from 20-60 mg/ml, the foam stability increased at the pH values 7 and 9, when compared with the acidic regions (3 and 5) and this may suggest production of adequate charge densities at these pH values by the protein molecules from the *Aduwa* samples that would have made charges available to participate in the formation of strong interfacial membrane.[34]

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The results also showed that the foam stability was higher at sample concentration of 60 mg/ml a pH values which also may suggest that the increase in the sample concentration is desirable in such that more protein molecules were produced to enhance the intermolecular cohesiveness of the foams formed.[18]The samples exhibited different pattern of foam stability with respect to the pH and variations in sample concentration,[36-39] which may have translated to differencials in the structural properties of the samples, especially the surface dispersibility property.

Emulsion Properties

Emulsion capacity

Figure 6 show the emulsion capacity of *Aduwa* protein meal APM, defatted meal DAM and protein concentrate APC as functions of variations in pH (3,5 7 and 9) and sample concentrations (10, 15 and 50 mg/ml). At 10 mg/ml, the emulsion capacity of the protein meal and protein concentrate were poor at pH 3.0 compared to high emulsion capacity recorded at pH values between 5-9, and this could be attributed to the formation of high number of charge density at the high pH value to interface with the oil to form emulsion.[18] There was no significant difference ($p>0.05$) in the emulsion capacities of protein meal between pH7 and pH9, defatted meal DAM between pH7 and pH 9 and protein concentrate APC between pH5 and pH7. At concentration of (50 mg/ml), the emulsion capacities of the protein meal was high at pH 7.0, defatted meal was at pH 5 and 7, and protein concentrate APC was at pH5 and pH 7.

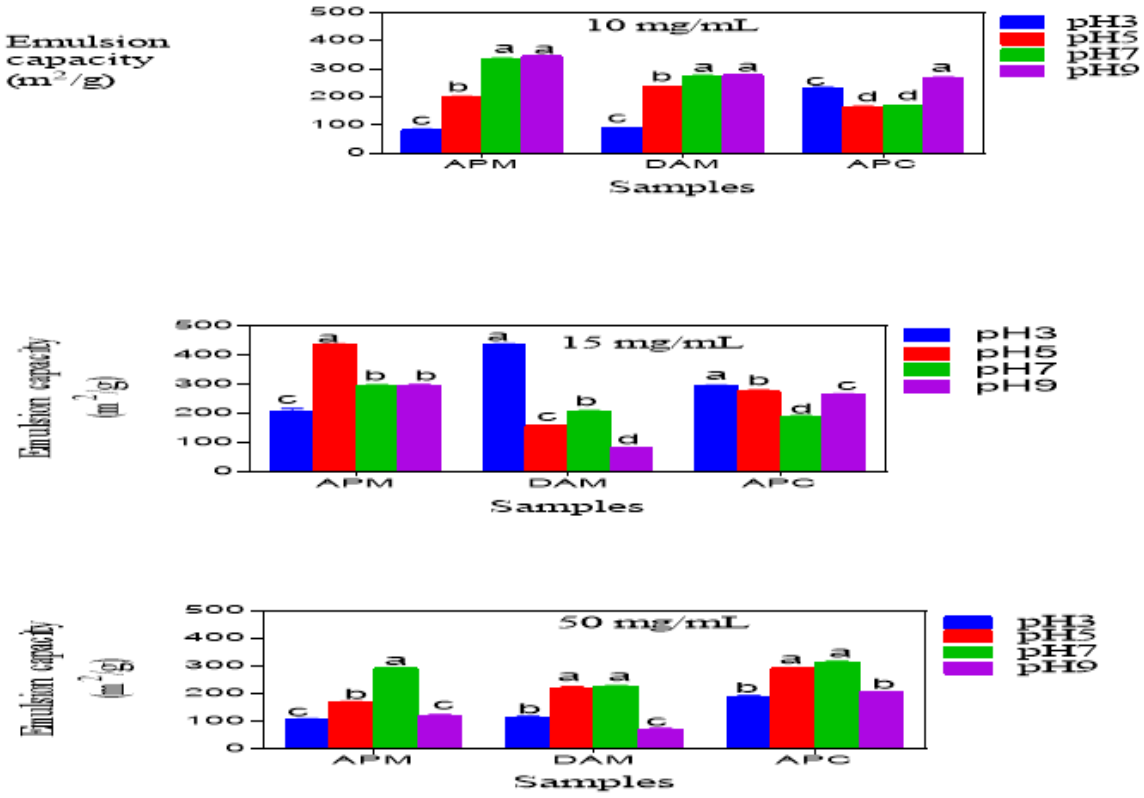

Figure 6 Effect of pH and sample concentrations on emulsion capacity of Aduwa meal, defatted meal, protein concentrate and isolate.

APM, Aduwa protein meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate

But the highest emulsion capacity was obtained at pH 7.0 for protein concentrate.[40-43] This also show that the emulsion capacities of the samples were lower at the highest sample concentration of (50 mg/ml).These variation may be attributed to the release of excess protein molecule which may have resulted in protein overcrowding and disruption in the interfacial properties.[34]

Emulsion stability

The emulsion stabilities of the protein meal APM and the defatted meal DAM followed similar pattern at 10 mg/ml and the highest emulsion stability was obtained at pH values of 5 and 9 (Figure 7). The result also showed that the emulsion stabilities of the whole meal AM, defatted meal DAM followed the pattern of protein concentrate APC as previously discussed, where the values undulated from pH 3-7 and peaked at pH 7.0. When the sample concentration was increased to 15 mg/ml, the emulsion formed at pH 9.0 became poor, and emulsion formed at pH 7.0 were stronger, especially for protein meal and protein concentrate APC. Emulsion formed at pH 5, for defatted meal DAM and APC was also strong at 15 mg/ml sample concentration.[44-46] At sample concentration of 50 mg/ml, pH 5.0 exhibited strong emulsion stability for protein meal APM and protein concentrate APC while the strongest emulsion stability was observed at pH 9.0 for defatted meal DAM and protein APC.[47,48] The potential of any protein to interact and bring together two immiscible phases such as oil and water and prevent phase coalescence is measured by emulsion stability.[18] The pattern of the emulsion stability in this study for the samples show that sample concentration of 10 mg/ml is the threshold concentration for samples to create enough interfacial tensions to stabilize the emulsion formed by the samples.

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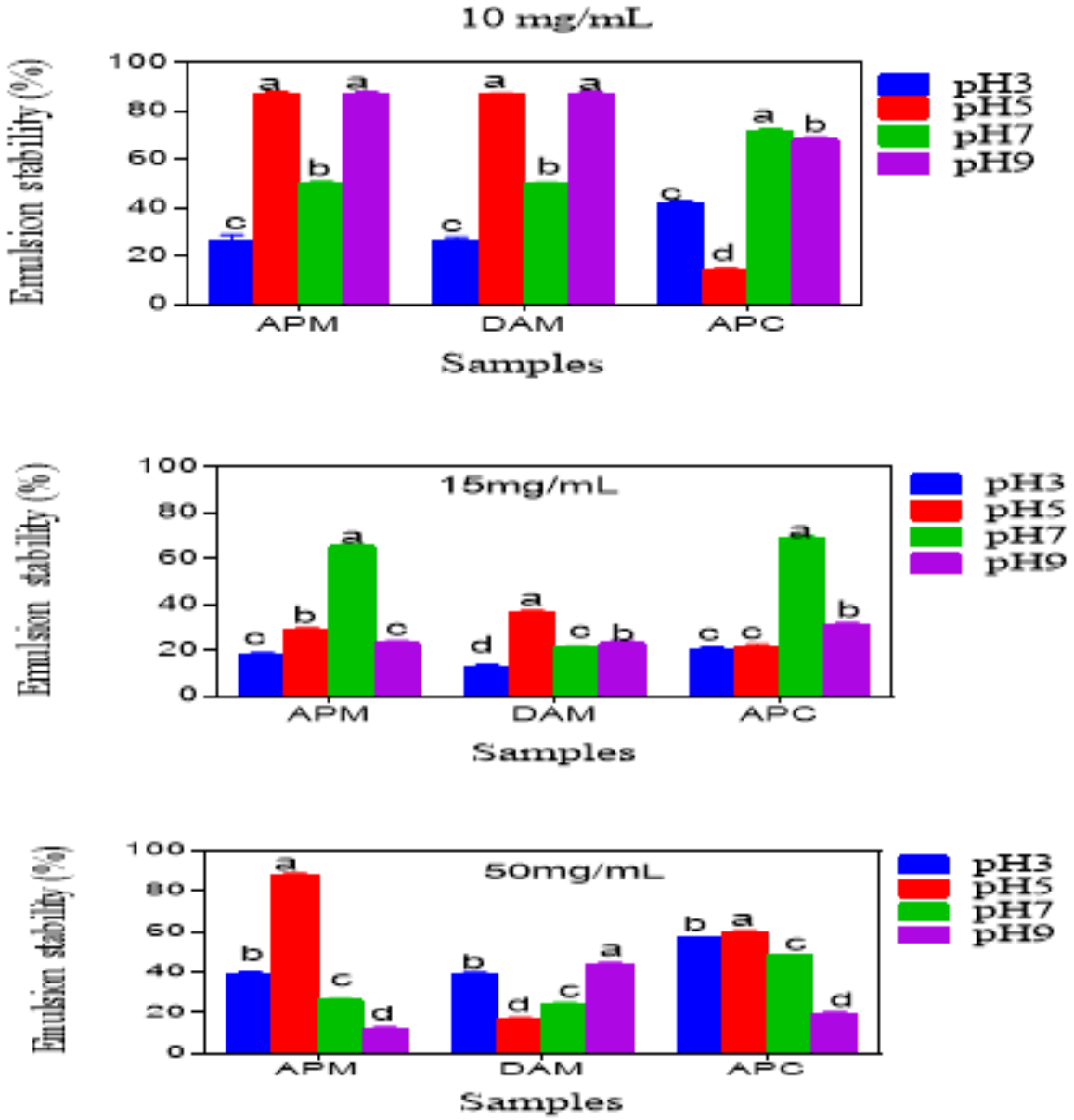

Figure 7 Effect of pH and sample concentrations on emulsion stabilities of Aduwa Meal, defatted meal, protein concentrate and isolate.

APM, Aduwa protein meal; DAM, defatted Aduwa meal; APC, Aduwa protein concentrate; API, Aduwa protein isolate

Conclusion

This study unveils the polypeptides composition, Fourier Transform Infrared (FTIR) and functional properties of *Aduwa* protein meals and concentrate. The result of SDS- PAGE showed emergence of new bands under reducing condition, suggesting the presence of sulfhydryl bonds and sulphur containing amino acid in the samples. The FTIR result revealed different functional groups which may have resulted from different processing and protein modification processes. The amino acid results showed that APM contained greater amounts of essential amino acid while protein concentrate APC was rich in hydrophobic amino acid, which may have implications on the structural characteristics of the concentrate. The pattern of solubility profiles of APM and DAM also suggest that they may not be suitable as ingredient in acidic drinks. The results of foaming capacity showed that the protein concentrate APC exhibited high foaming capacity at the basic region even as the concentration of the sample increase, and this was attributed to the formation of large aggregation of charges at pH 7 which encouraged the formation of greater interfacial membranes. The emulsion properties revealed variation in the emulsion capacity and stabilities of the samples with respect to pH and sample concentration. But the sample concentration of 10 mg/ml was established as the threshold to produce emulsion of good stability. *Aduwa* seed meal and protein concentrate demonstrated huge potentials that could expand its utilization as ingredient in the formulation of food products.

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Conflicts of interest

Conflict of interest Ogori akama friday declares that he has no conflict of interest. Girgih TA and Abu Joseph Oneh was of the technical partner. Eke ojotu micheal supported in review and final draft.

Author Contributions

Ogori Akama Friday, Data curation, investigation, writing of first original draft Data curation, Review, and editing. Eke Mike Ojotu, Project administration, Supervision, and review. Girgih TA, Investigation, supervision third original drafting and editing. Abu Joseph Oneh, Data curation, second original Draft, editing and review

Ethics statement

This article does not contain any studies with human or animal subjects.

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