Food Production, Processing and Nutrition

RESEARCH Open Access

Characterization of okra seed flours, protein concentrate, protein isolate and enzymatic hydrolysates

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Abstract

The need to feed the increasing world population with high quality protein and the enormous post-harvest losses of okra fruits necessitated the research on okra seeds in this study. Whole flour (WF) of matured okra seed was processed to obtain defatted flour (DF), protein concentrate (PC) and protein isolate (PI), followed by hydrolysis of the protein isolate by three different proteases; pepsin (PHp), pancreatin (PHc) and trypsin (PH_T) to produce hydrolysates. The okra seed flours, proteins and the hydrolysates were analyzed for changes in the functional groups using Fourier, Transform Infrared (FTIR), amino acid composition, solubility profile and some functional properties. The FTIR results showed the presence of N-H stretching, C=O stretching, C=N stretching N-H bending and C-N stretching in the samples. Protein hydrolysate had higher essential amino acids (51.32–53.01%) than unhydrolysed samples (36.31–37.99%). PI and WF had the highest water absorption and swelling capacities respectively. The solubility profiles of the samples were minimal at pH 2–4 and then increased after the isoelectric point. The PC was more soluble than PI between pH 2–8. The foaming properties of the samples was least in the absence of salt but high in the presence of 0.5 M sodium chloride. The emulsion capacity of the samples was least in the presence of 1.0 M NaCl. The in-vitro protein digestibility results showed that okra seed protein hydrolysates were more digestible (83.26–86.08%) than unhydrolyzed proteins (36.48–80.90%). The results of the antioxidant properties showed that PHp and PH_T exhibited better radical scavenging and metal chelating activities respectively than the other samples. The study concluded that okra seed proteins and hydrolysates demonstrated potentials as ingredients in functional food preparation and this may be considered as a strategy to reducing the post-harvest losses of okra fruit and subsequently feeding the world with quality proteins.

Keywords: Okra seed, Functional properties, Antioxidant properties, Functional group

Introduction

The recognition of the importance of protein in human diet and the need to feed the growing population with protein rich food has increased research efforts on lesser-known protein rich seeds. Okra (*Abelmoschus esculentus*), is a vegetable that belongs to the family of *Malvaceae* (Naveed et al. 2009). The fresh fruit of okra can be consumed, together with the seed, but when it is matured and dry, the seeds are separated from the flesh, and replanted (Arapitsas, 2008).

Nigeria ranks among the largest producers of okra fruits in the world, with annual production of 15,000 tones/ha of land (Naveed et al. 2009). The increase in cultivation of okra fruits in Nigeria usually end up in yearly post-harvest losses, because the utilization is limited to preparation of house hold dishes. During production glut, any seed not used for re-plantation is discarded and this constitutes great post-harvest losses. On dry weight basis, the seed has 35% crude protein, 14% crude fat and 6% ash content (Gopalan et al. 2007). This level of protein in the seed suggests that okra is an important protein seed for human nutrition, that can be

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exploited to address the protein-energy malnutrition. The crude proteins in the dry seeds may be modified to produce high-value protein by-products, such as the defatted flours, protein concentrate and isolate. Many protein seeds, such as hemp seed (Malomo et al. 2015), mucuna beans (Lawal and Adebowale 2005), walnut (Gbadamosi et al. 2012) pumpkin seed (Muhamyankaka et al. 2013) and *kariya* seed (Famuwagun and Gbadamosi 2016) have been modified to produce value added protein products. However, the benefit of plant proteins may not be fully realized if the functional properties such as water and oil absorption capacity, protein solubility, emulsifying and foam properties etc. are not well understood. For example, protein that has high forming capacity can find application in baking industries while those with high oil absorption capacity would be useful in confectionary industries, because it will enhance flavor retention. Also, protein with high emulsifying and good gelation properties would be useful in food industries as food stabilizers (Gbadamosi et al. 2012; Famuwagun and Gbadamosi 2016; Adebowale et al. 2005). It is also important to understand changes in the molecular structure of proteins during value addition processes. Fourier transform infrared (FTIR) spectroscopy identifies chemical bonds by using an infrared spectrum that is absorbed by the material (Hinterstoisser et al. 2001). The change in the chemical structure of the okra seed protein products with respect to different value addition methods would be revealed using the infrared spectroscopy.

Protein hydrolysates are another group of protein by-product that are encrypted in the primary protein structure. Enzymatic hydrolysis is one of the cost-effective methods to release these peptides from their parent protein (Aluko 2015). Evidence has shown that bioactive proteins and peptides from various plant sources possess various physiological functions, such as antioxidant activities and these possible health benefit functions have been explored extensively (Alashi et al. 2014; Girgih et al. 2011; Arise et al. 2016). Previous studies on okra seed have focused on the nutritional composition of the whole seed and defatted flours (Manal et al. 2015) and effect of fermentation on the nutritional quality of okra seed flours (Adelakun et al. 2017). Therefore, the aim of this work was to evaluate the functional properties of various protein by-product from okra seed flour, examine the antioxidant and amino acid composition of different protein hydrolysates and investigate changes in the functional groups of the protein products with respect to value addition processes.

Material and methods

Materials

Matured okra seeds were obtained from the Teaching and Research Farms of Obafemi Awolowo University, Ile-Ife, Nigeria. Enzymes (pepsin, pancreatin and trypsin) and other reagents, such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) hydrate powder, Ferrozine, 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, Iron II sulfate heptahydrate, and potassium bromate (KBr) were of analytical grade and purchased from Sigma accredited agent in Osun State, Nigeria.

Methods

Production of okra seed flours

Mature okra seeds were sorted, cleaned and washed with clean water. The cleaned seeds were dried with hot air oven (Uniscope Laboratory Oven, England) at 50 ± 2 °C for 8 h. The dried seeds were milled using laboratory blender (VLC sapphire grinder, IS-4930, England) at speed 4_{max} and sieved using 615 μm laboratory sieve (test sieve, ISO0330-1, Endecotts Ltd., London, England) to obtain okra seed whole flour (WF). The chaff was removed and the sieved endosperm were defatted using n-hexane in a Soxhlet extraction apparatus. The WF was de-solventized by drying in a fume hood to remove the residual solvent and finely ground in a grinder (VLC sapphire grinder, IS-4930, England) set at speed four to obtain homogenous defatted flour (DF). The defatted flour obtained was stored in an air-tight plastic bottle and kept in a freezer until use.

Production of protein concentrate

Okra seed protein concentrate (PC) was prepared by a method modified by Gbadamosi et al. (2012). One hundred grams of defatted flour was dispersed in 1 L distilled water to give final flour to water ratio of 1:10. The dispersion was 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 1.0 M HCl to the point at which the protein was least soluble (pH 4; a value obtained from preliminary solubility results of the defatted flour) to precipitate the proteins. The precipitation process was allowed to proceed with gentle stirring for 4 h, keeping the pH constant. Soluble carbohydrates (oligosaccharides) and minerals was removed by centrifugation at 3500×g for 30 min using a centrifuge (Bosch, TDL-5, United Kingdom). The precipitate (concentrate) was afterward washed twice with distilled water to remove the residual minerals and soluble carbohydrates and the pH was adjusted with 1.0 M NaOH to 7.0 for neutralization and then centrifuged at 3500×g for 10 min. The resultant precipitate (concentrate) was collected and dried in an oven at 45 °C for 8 h (Uniscope SM9053 Laboratory Oven, Singerfriend, England) and kept for further analysis. The protein yield in the concentrate was calculated as the ratio protein in the dried material to the protein in the defatted flour.

$$Protein\ yield\ (\%) = \frac{protein\ in\ the\ dried\ powder}{protein\ in\ the\ starting\ material} x\ 100 \quad (1)$$

Preparation of okra seed protein isolate

Okra seed protein isolate (PI) was prepared by a method described by Gbadamosi et al. (2011). One hundred grams of the okra seed defatted flour was dispersed in 1 L of distilled water to give final flour to liquid ratio of 1: 10. The suspension was gently stirred on a magnetic stirrer for 10 min. The pH of the resultant slurry was adjusted by drop-wise addition of 2 M HCl with constant stirring until the pH was adjusted to the point at which the protein was most soluble (pH 10.0) and the extraction was allowed to proceed with gentle stirring for 4 h keeping the pH constant to solubilize the proteins. The mixture was centrifuged (Harrier 15/80 MSE) at 3500×g for 10 min to remove the non-soluble materials (residue). The proteins were precipitated from the supernatant by adjusting the pH to the point at of least soluble (pH 4.0) and the soluble proteins was recovered by centrifugation (3500×g for 10 min). After separation of proteins by centrifugation, the precipitate was washed twice with distilled water to remove the excess salt formed during the pH adjustment. The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to freeze-drying using a freeze-dryer (Laboao LFD 10 A Vacuum freeze-dryer, Zhengzhou Labooo instrument equipment, Co Ltd., China). The freeze-dried protein was stored in air-tight plastic container at room temperature for further use. The protein yield in the isolate was calculated as the ratio protein in the freeze-dried material to the protein in the defatted flour.

$$Protein\ yield\ (\%) = \frac{protein\ in\ the\ freeze-dried\ powder}{protein\ in\ the\ starting\ material} x\ 100 \quad (2)$$

Preparation of okra seed protein hydrolysate

Okra seed protein hydrolysate was prepared using different enzymes such as pepsin ((> 250 units/mg), pancreatin (> 250 units/mg) and trypsin (> 250 units/mg) with different optimum reaction conditions) in different containers using the method of Omoni and Aluko (2006). A 5% okra seed protein isolate's slurry was adjusted to pH 2.0 and incubated at 37 °C followed by addition of pepsin (> 250 units/mg; 4% w/w, on the basis of protein content of okra seed protein isolate), another slurry was adjusted to pH 7.5 and incubated at 40 °C followed by the addition of pancreatin (> 250 units/mg; 4% w/w, on the basis of protein content of okra seed protein isolate) and lastly, another protein slurry in water was adjusted to pH 7.5 in another container and incubated at 45 °C followed by the addition of trypsin enzyme (> 250 units/mg; 4% w/w, on the basis of protein content of okra seed protein isolate). The digestion was carried out for 4 h and the pH was maintained by adding 1 M NaOH or HCl when necessary. The digestion was terminated by adjusting the pH to 4.0 and then place the mixtures in boiling water for 30 min to inactivate the enzymes which ensure complete denaturation of enzyme protein and coagulation of undigested proteins. The mixture was allowed to cool to room temperature and later centrifuged (3500×g for 30 min). The resulting supernatants was freeze-dried (Labooo LFD 10 A Vacuum freeze-dryer, Zhengzhou Laboao instrument equipment, Co Ltd., China) to produce the respective enzymatic hydrolysates. The protein yields in the hydrolysates were determined as the ratio protein in the freeze-dried material to the protein in the starting material.

$$Protein\ yield\ (\%) = \frac{protein\ in\ the\ freeze-dried\ powder}{protein\ in\ the\ starting\ material} x\ 100 \quad (3)$$

Determination of functional groups by Fourier transform infrared (FTIR)

The FTIR spectra of okra seed flours, protein isolate and hydrolysates 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 powdered 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 analyzed in triplicates with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the sample.

Determination of protein and fat contents

The protein and fat content of the samples (whole flour, defatted flour, protein concentrate, protein isolate and hydrolysates) were determined using the AOAC (2012) official methods.

Determination of protein solubility

The protein solubility was determined according to the method described by Malomo et al. (2015). A 10 mg of sample was dispersed in 1 mL of 0.1 M phosphate buffer solutions at different pH values (2.0, 4.0, 6.0, 8.0 and 10.0) to obtain a 0.1% (w/v) concentration and the resulting mixture were vortexed for 2 min and centrifuged at 3500×g for 20 min. Protein content of the supernatant was determined using the modified Lowry

method (Markwell et al. 1978). Total protein content was determined by dissolving the protein samples in 0.1 M NaOH solution. Protein solubility was 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 0.1 M NaOH).

Determination of functional properties

Water absorption capacity (WAC)

The WAC of the samples at room temperature and at elevated temperatures (50,60,70, 80, 90 and 100 °C) was determined following the of AOAC (2012) method. One gram (1 g) (w_1) of each sample was weighed into a centrifuge tube and the sample was weighed (W_2). Ten millilitres (10 mL) of distilled water was added to the sample in tube. The temperatures of the tube and its contents were maintained for 10 min (Julabo 565, England). The tubes were then centrifuged (BOSCH centrifuge, TLD-500, England at 3500×g for 20 min). The supernatant was decanted and the contents of the tube was drained at an angle of 45° angle for 10 min and weighed (W_3). The WAC was expressed as percentage of the volume of water absorbed by the sample

$$\textit{Water absorption capacity}\ (\%) = \left(\frac{W_3 - W_2}{W_1}\right) \times 100 \quad (4)$$

W_3 = weight of tube + sample after centrifuging and decanting W_2 = weight of tube + sample before water was added, W_1 = weight of sample.

Least gelation concentration (LGC)

The method of Adebiyi and Aluko (2011) was used for the determination of gelling concentration. Sample suspensions of 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20% (w/v) was prepared in 5 mL distilled water separately using test tubes. The tubes and their contents were heated in a boiling water bath for 1 h followed by rapid cooling under running cold tap water. The tubes were further cooled for 2 h at 4 °C. Least gelling concentration of the samples was determined as the concentration when the sample from the inverted test tube did not fall down or slip.

Effect pH and NaCl concentration on foaming properties

Foam capacity and foam stability as influenced by pH and salt concentration was determined by a modification of the method described by Chavan et al. (2001). Approximately 500 mg of protein sample was dispersed in 100 ml of distilled water at different NaCl concentration (0.5 and 1.0 M) inside a beaker and the pH of the protein solution was adjusted separately to pH 2, 4, 6, 8 and 10 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 5_{max} 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 whippability. 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.

$$\textit{Foaming capacity}\ (\%) = \frac{V_2 - V_1}{V_1} \times 100 \quad (5)$$

$$\textit{Foaming stability}\ (\%) = \frac{V_3 - V_1}{V_1} \times 100 \quad (6)$$

V_1 = volume before whipping (ml)
V_2 = volume after whipping (ml)
V_3 = volume after standing (ml)

Effect of pH and NaCl concentration on emulsifying properties

The effect of pH and salt concentration on emulsifying activity index (EAI) was determined by a modified turbidimetric method described by Famuwagun and Gbadamosi (2016). About 500 mg sample was dispersed in 80 mL of distilled water at different NaCl concentrations (0.5 and 1.0 M). The pH of the solution was adjusted separately to 2, 4, 6, 8 and 10 with either 1 M HCl or 1 M NaOH. The sample slurry was mixed with 20 ml of vegetable oil and the mixture was homogenized using a blender (VLC, Saphire, England) set at high speed for 60 s. 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

$$T = \frac{2.303\ x\ A}{I} \quad (7)$$

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

The emulsion activity index (EAI) will then be calculated as:

$$\textit{Emulsifying activity index}\ \left(m^2/\mathrm{g}\right) = \frac{2\ x\ T}{0.2\ x\ C} \quad (8)$$

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 10 min and the absorbance of the mixture was read at 500 nm and calculated using the formular:

$$Emulsion\ stability\ index = \frac{EAI\ at\ 10\ \ min}{EAI\ at\ 0\ \ min}\ x\ 100 \quad (9)$$

In-vitro protein digestibility (IVD)

In-vitro protein digestibility of the samples was measured according to the method described by Chavan et al. (2001). Two hundred fifty milligrams of the sample were suspended in 15 mL of 0.1 M HCl containing 1.5 mg pepsin (> 250 units/mg), followed by gentle shaking for 1 h at room temperature. The resultant suspension was adjusted to pH 7.0 with 0.5 M NaOH and treated with 4.0 mg pancreatin (> 250 units/mg) in 7.5 mL of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 2 h at room temperature. The mixture was then filtered using Whatman No 1 filter paper and the residue washed with distilled water, air-dried and used for protein determination using Lowry method (Markwell et al. 1978) as described earlier. Protein digestibility was obtained using the equation.

$$In\ vitro\ protein\ digestibility\ (\%) = \left(\frac{I - F}{I}\right) \times 100 \quad (10)$$

where,

I = *protein content of sample before digestion*
F = *protein content of sample after digestion*

Amino acid determination

Amino acid composition was determined following the method described by Gbadamosi et al. (2012) 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 (Girgih et al. 2011).

Determination of antioxidant properties

2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method

DPPH radical scavenging activities was determined by method described by Pownall et al. (2010). Samples were made to 1 mg/1 mL and the mixture was centrifuged to obtain the clear supernatant. One milliliter of the sample solution was mixed with 1 mL of 0.3 mM DPPH in methanol. The mixture was vortexed for 60 s and incubated in the dark for 30 min. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm against a DPPH control which contained only 1 ml of DPPH solution and 1 ml of methanol. Methanol was used as blank. The free radical scavenging ability was calculated using the equation below.

$$\%DPPH = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\ \ x\ \ 100 \quad (11)$$

Metal chelating ability

The metal (iron) chelating activity (MCA) of the samples was determined according to the modified method of Xie et al. (2008). Protein samples/standard were prepared to final concentrations of 1–5 mg protein/mL in distilled water. A 1 ml aliquot of the sample solution or blank (distilled water) was mixed with 50 μL of 2 mM $FeCl_2$ and 1.85 mL double distilled water in a reaction tube. This was followed by the addition of 100 μL of 5 mM Ferrozine. The mixture was vortexed thoroughly and incubated at room temperature for 10 min. After incubation, a 200 μL aliquot of the reaction mixture was transferred into clean tubes and absorbance values of both the blank (Ab) and samples (As) were measured at 562 nm using microplate reader. The metal chelating activity was calculated as follows;

$$Metal\ chelating\ activity\ (\%) = \frac{(Ab - As)}{Ab}\ x\ 100 \quad (12)$$

Where Ab is the absorbance of the blank and As is the sample absorbance

Ferric reducing antioxidant power (FRAP)

The FRAP of the protein isolates, hydrolysates and peptide fractions was determined using the modified method of Benzie and Strain (1999). FRAP working reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20 mM $FeCl_3$ in the ratio of 5:1:1, respectively to obtain a straw-colored solution, and the temperature of the mixture raised to 37 °C. Samples were dissolved in distilled water to a final concentration of 1 mg/mL in a clean tube and 200 μL of FRAP reagent was added and absorbance read at 593 nm. Iron II sulfate heptahydrate ($FeSO_4.7H_2O$) at 0.025–0.25 mM was used as standard. Iron reducing activity of the samples was determined from the standard curve and the results expressed as Fe^{2+} (mM).

Statistical analysis

Some of the analyses were performed in triplicates and the preparations of the samples (concentrate, isolate and hydrolysates) were done win six independent replicates. The results were subjected to analysis of variance using SPSS version 18.0. The statistical significance of differences ($p < 0.05$) between mean values were determined using the Duncan Multiple Range Test

Result and discussion

Functional groups by Fourier transform infrared

FTIR analysis uses the vibration of functional groups within macromolecules of materials and indicate the molecular structural changes of the molecules through a shift in wave numbers (Chen et al. 2013; Saguer et al. 2012). Table 1 showed the frequencies and band assignments for FTIR spectra (4000–500 cm^{-1}) derived from okra seed whole flour (WF), defatted flour (DF), protein concentrate (PC), protein isolate (PI), protein hydrolysates obtained by pepsin enzyme (PH_P), pancreatin enzyme (PH_C) and trypsin enzyme (PH_T) in comparison with published literature range. The absorption bands of all the samples were greater than 5, suggesting that the samples were complex materials. The spectra of whole flour (Table 1) showed a protein and polyhydroxyl absorption band at 3419.90 cm^{-1}. The band with the same energy level was observed in DF but lower absorption band (3417.98 cm^{-1}) was observed for PC (Table 1). The protein and the polyhydroxyl was characterized by intermolecular bonded O-H stretching and N-C stretching (Table 1). The spectrum band (3419.90 cm^{-1}) was shifted to a lower energy level (3417.98 & 3416.05 cm^{-1}) in the protein hydrolysates. A shift to lower energy level may be due to the formation of free amino acids, as a result of the hydrolysis of the N-C bonds during protein digestion. The 2917.46–2964.69 cm^{-1} corresponds to presence of alkane compound, which was due to asymmetric CH2 stretching. The spectra bands were found in enzymatic protein hydrolysates but not in the flours, protein concentrate and isolate. The 2892.36–2887.53 cm^{-1} bands correspond to methoxy methyl ether compound and these were due to O-H stretching and asymmetric CH2 stretching. The intensities were greater in DF, PC and PI than WF but the bands were not observed in the protein hydrolysates. The 2359.02–2362.88 cm^{-1} for amino acid component, which was due to N-H stretching was observed in all the samples. A characteristic band of triglycerides at 1745.64 cm^{-1}, resulting

Table 1 FTIR peak assignment for okra seed flours, protein isolate and enzymatic hydrolysates

Functional group	WF	DF	PC	PI	PH_P	PH_C	PH_T	Suggested nutrients	Literature range Saguer et al. (2012)
Intermolecular bonded O-H stretching; N-C stretching	3419.90	3419.90	3419.98	3419.98	3417.98	3417.98	3416.05	Protein and poly-hydroxyl compounds	3029–3639 (3334)
CH2 Stretching asymmetric	N.D.	N.D.	N.D.	N.D.	2962.76	2964.69	2917.46	Alkane compounds	2960
O-H stretching; CH2 stretching symmetric	2887.53	2892.36	2890.425	2892.36	N.D.	N.D.	N.D.	Methoxy methyl ether compound	2890
N-H stretching	2359.02	2359.02	2360.95	2360.95	2359.02	2359.02	2362.88	Amino acid component	2332–2359 (2344)
C=O stretching of esters	1745.64	1740.64	N.D.	N.D.	N.D.	N.D.	N.D.	Triglycerides	1763–1712 (1737.5)
C=O stretching, C=N stretching	N.D.	1637.62	1637.62	1647.62	N.D.	N.D.	1647.62	Protein amide I band	1620–1650 (1635)
N-H bending; C-N, stretching	1541.18	1541.18	1541.18	1541.18	1541.18	1541.18	1541.18	Protein amide II band	1481–1585 (1533)
CH2 bending asymmetric; CH3 bending asymmetric	1456.30	1456.3	1458.23	1456.3	N.D.	N.D.	N.D.	Protein and fats	1425–1477 (1451)
C-O stretching symmetric of COO- groups of carboxylates	1379.22	1384.94	1484.15	1384.94	1404.22	1402.3	1406.15	Carboxylic acid	1357–1423 (1390)
P=O stretching asymmetric; C-N stretching amine	1277.88	1277.88	1278.85	1279.81	1276.92	1286.57	1286.57	Nucleic acid	1191–1356 (1273.5)
C=N stretch (amide III),	N.D.	N.D.	1159.26	N.D.	1153.47	N.D.	N.D.	Tertiary amine	1210–1150 (1170)
C=N stretch (amide I),	1066.67	1076.32	1037.74	1065.701	1074.39	1078.24	1056.07	Primary amine	1090–1020 (1055)
C-Cl stretch,	719.47	N.D.	719.47	N.D.	N.D.	773.48	N.D.	Aliphatic Chloro-compound	800–700 (750)
C-Br stretch	643.28	643.28	643.28	643.28	623.03	613.38	609.53	Aliphatic bromo compounds	700–600 (750)

WF Whole flour, *DF* Defatted flour, *PC* Protein concentrate, *PI* Protein isolate, PH_P Pepsin protein hydrolysate, PH_C Pancreatin protein hydrolysate, PH_T Trypsin protein hydrolysate, *ND* Not detected; Saguer et al. (2012)

from C=O stretching of esters was found in okra whole flour. A shift of the intensity of the band to lower energy level (1740.64 cm^{-1}) in the defatted flour suggests the reduction of some of the triglycerides during the defatted process. This band was absent in the PC, PI and the protein hydrolysates, suggesting little or no triglycerides in the samples except WF. The 1637.62 and 1541.18 cm^{-1} bands were due to N-H bending/C-N stretching and C=O stretching/C=N stretching for amide I and amide II respectively. The amide I bands of the protein was found in the defatted flour, protein concentrate and trypsin hydrolysate. The spectra bands 1379.22–1406.15 cm^{-1} of carboxylic acids were due to symmetric stretching C-O of COO- groups of carboxylates. The intensities were weaker in WF, DF, PC and PI (lower wave numbers) when compared to strong signals in the protein hydrolysates. The high signals in the energy levels of carboxylates may suggest increase in the negatively charged amino acids of the hydrolysates, which may have implications in the radical reducing activities of the hydrolysates (Armstrong et al. 2006 ; Baron et al., 2005).

Protein contents and protein yields

The protein content of protein isolate (PI: 90.24 ± 1.89%) was significantly ($p < 0.05$) higher when compared with protein concentrate (PC: 75.81 ± 1.19%), defatted flour (DF: 41.81 ± 0.83%) and whole flour (WF: 25.48 ± 0.76%). For PI and PC, the values were well compared with the 75.56 and 89.26% reported for walnut protein concentrate and isolate respectively (Xiaoying and Hua 2011). The higher protein contents of PI compared with PC may be attributable to the extra processing step (precipitation), which hitherto may have further purified protein isolate. The protein content of the PI was comparable with 89.26% reported for protein isolate produced from custard apple seed (Saguer et al. 2012) but higher than 67.87% reported for pumpkin seed protein isolate (Muhamyankaka et al. 2013). The results of the protein contents showed okra seed protein concentrate and isolate may be considered as good sources for protein enrichment.

Among the protein hydrolysates, protein hydrolysate by pepsin (PH_p) had higher protein content (93.95%) when compared with protein hydrolysate by trypsin enzyme (PH_T: 86.30 ± 0.58%) and protein hydrolysate by pancreatin enzyme (PHc: 78.92 ± 1.08%). The high protein content of PH_p compared with the starting material (protein isolate: 90.24%) suggest that the peptide bonds in the protein isolate were made more available for pepsin enzyme for hydrolysis during protein digestion to release more free amino acids (Horton et al. 2002). The protein hydrolysates have varied protein contents, though with the same starting material, suggesting the effects of specificities of the enzymes on the substrate. The protein contents of the hydrolysates compared well with 81.07 to 92.22% reported for pumpkin seed protein hydrolysates (Muhamyankaka et al. 2013). The protein yields of the isolate was 55.54 ± 1.43%, which was higher when compared with the 37.65 ± 2.65% obtained for the protein concentrate. Among the protein hydrolysates, PH_p had the highest protein yield (49.88 ± 0.48%), when compared with 15.86 ± 0.21 and 13.02 ± 0.12% reported for PHc and PH_T respectively. The pattern of the protein yields was in line with protein contents of the hydrolysates, where PH_p and PHc had the highest and lowest protein contents respectively. Previous study reported 55.05 to 77.6% for the protein yield of canola protein hydrolysates (Alashi et al. 2014). The differences may be related to variation in the source of proteins (okra vs canola).

Amino acid composition

The amino acid composition of okra seed okra seed flours, protein and hydrolysates is shown in Table 2. The PI had higher contents of threonine, valine, methionine, phenylalanine and lysine contents when compared with PC, DF and WF. The process of solubilization and precipitation may have enhanced the release of these amino acids during protein isolation. The WF had greater amounts of aspartic (11.80%) and glutamic (17.80%) when compared with DF, PC and PI. The enzymatic hydrolysis improved the contents of methionine, isoleucine, tyrosine, phenylalanine, histidine and tryptophan amino acids. The hydrolysates contained higher contents of essential amino acids (51.57–53.01%), aromatic amino acids (9.96–15.23%), hydrophobic amino acids (33.55–38.97%) and Sulphur amino acids (7.23–7.65%) than the protein isolate and okra seed flours. The post hydrolysis processes such as heat inactivation and ultrafiltration processes may ay be responsible for the high amino acid composition of the hydrolysates compared with the isolate and the flours. The results also showed that the hydrolysates had different amounts of amino acids, for instance, the essential amino acids in PH_P were 53.01%, PH_T had 51.32% and PHc contained 51.27%. In a similarly manner, 38.97% hydrophobic amino acid was obtained in PHc, while 33.55 and 35.85% was obtained in PH_P and PH_T respectively. The difference in the amino acids among the hydrolysates may be attributable to different specificities of the enzymes used during hydrolysis (Horton et al. 2002; Rajapakse et al. 2005). The contents of amino hydrophobic amino acids in this study compares well with 39.10–41.50% reported for canola protein hydrolysates (Alashi et al. 2014) and 36.13% reported for hemp seed protein hydrolysate (Girgih et al. 2011). The greater amounts of these amino acids in the hydrolysates may have implications in the structural, functional and amino acids contents of the hydrolysates.

Table 2 Amino acid profiles of okra seed samples

	WF	DF	PC	PI	PH_T	PH_P	PH_C
Aspartic/asparagine	11.80	12.28	10.67	9.67	8.37	8.07	8.04
Threonine	3.44	3.35	1.67	7.80	6.94	6.70	7.80
Serine	4.64	4.71	6.62	6.59	6.14	5.92	6.82
Glutamic/glutamine	17.38	16.46	12.86	9.64	7.43	7.17	8.03
Proline	6.35	6.02	7.80	7.44	6.08	5.87	6.22
Glycine	5.87	6.94	8.28	7.81	7.23	6.98	7.82
Alanine	4.52	5.00	7.52	7.34	6.64	6.41	7.45
Cysteine	0.10	0.34	0.74	0.72	0.58	0.56	0.61
Valine	5.73	5.58	7.19	7.39	6.77	6.54	6.24
Methionine	0.85	0.92	1.40	3.08	7.07	6.83	6.62
Isoleucine	3.53	3.59	2.15	1.83	3.76	3.63	4.21
Leucine	7.32	7.91	6.33	5.42	6.41	6.19	5.84
Tyrosine[a]	2.54	2.52	1.69	1.68	3.70	4.73	5.41
Phenylalanine[a]	4.94	4.66	6.64	6.70	6.17	7.11	8.43
Histidine	4.09	3.83	2.10	2.51	4.40	4.25	2.12
Lysine	5.45	5.19	7.03	7.08	6.00	5.80	3.21
Arginine	11.34	10.58	9.20	7.19	6.23	6.02	3.75
Tryptophan	0.10	0.10	0.11	0.10	0.08	1.24	1.39
EAA	37.99	37.67	36.31	43.59	51.32	53.01	51.27
AAA	7.58	7.28	8.44	8.48	9.96	13.08	15.23
HAA	28.69	29.37	31.62	30.46	33.55	35.85	38.97
PCAA	20.88	19.61	18.34	16.79	16.63	16.06	9.08
NCAA	29.18	28.74	23.53	19.31	15.80	15.25	16.06
SCAA	0.95	1.26	2.15	3.80	7.65	7.38	7.23
BCAA	16.59	17.09	15.67	14.63	16.95	16.36	16.29

Combined total of hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine and cysteine; aromatic amino acids (AAA) = phenylalanine, tryptophan and tyrosine; positively charged amino acids (PCAA) = arginine, histidine, lysine; negatively charged amino acids (NCAA) = aspartic + asparagine, glutamic + glutamine, threonine, serine; essential amino acids (EAA) = histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine and [a]Phenylalanine and Tyrosine; Sulphur containing amino acids (SCAA) = methionine and cysteine; total branched chain amino acids (BCAA) = leucine, isoleucine, valine

Solubility profiles

As shown in Fig. 1, okra samples were more soluble in the basic region (pH > 7) than in the acidic region (pH < 7). At pH 2.0, 45.17, 13.92, 11.76 and 15.03% of PC, PI, WF and DF were soluble. The results showed that PC and DF were more soluble than PI at very acidic pH. At pH 10.0, 33.92, 36.03, 62.48 and 67.89% of WF, DF, PC and PI were soluble, suggesting PI was more soluble at the very basic region than WF, DF and PC of okra seed samples. The solubility curve of the samples was not perfect U-shape, unlike peanut proteins (Yu et al. 2007) and for cashew nut proteins (Ogunwolu et al. 2009). The solubility profiles of the samples were minimal at pH 2–4 and then increased afterwards. This pattern may be due to the loss in electrostatic repulsion that provided conducive environment for

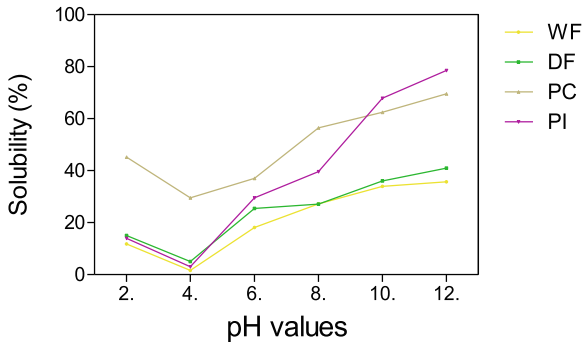

Fig. 1 Protein solubility of okra seed whole flour, defatted flour, protein concentrate and isolate

the formation of protein aggregates, that reduced of the samples in the acidic environment (Molina et al. 2001). The low solubility of WF and DF across the pH values compared with PC and PI may be presence of more non protein materials (in the form of fibrous materials) in the WF and DF which are not easily solubilized in the solution (Clemente, 2000). The low solubility of PI when compared with PC may be due to high aggregation of the okra PI, resulting from different drying processes (Oven drying for PC vs Freeze drying for PI). Low protein aggregation at pH 12 may have led to high solubility of PI at pH 12 compared to PC. The solubility profile of proteins is important in determining the functionality of such proteins (Molina Ortiz and Wagner 2002).

Functional properties

Water absorption and swelling capacity

At room temperature (RT), the water absorption capacities of PI (148%) were higher than the values reported for PC (92%), DF (75.33%) and WF (98%). The high-water absorption capacity of PI when compared to other samples may be attributed to high protein content, which may have influenced the structural conformation and composition of the protein molecules to absorb more water towards itself. For the PI, the value was lower than 221.43% reported for Bambara groundnut protein isolate (Eltayeb et al. 2011) and 333% for walnut protein isolate (Gbadamosi et al. 2011). At elevated temperatures (50–100 C), there was gradual increase in the water absorption capacities of the samples (Fig. 2) as the temperature increased. The increase may be attributed to the exposure of the water binding sites of the hydrophilic part of the proteins as the temperature increased. it may also be due to the unfolding of the proteins. However, the PI was highest up-to 100 C while DF and WF were higher than PC at elevated temperatures. The high-water absorption capacities observed in DF and WF when compared to PC may suggest rapid protein unfolding as the temperature increased and even the

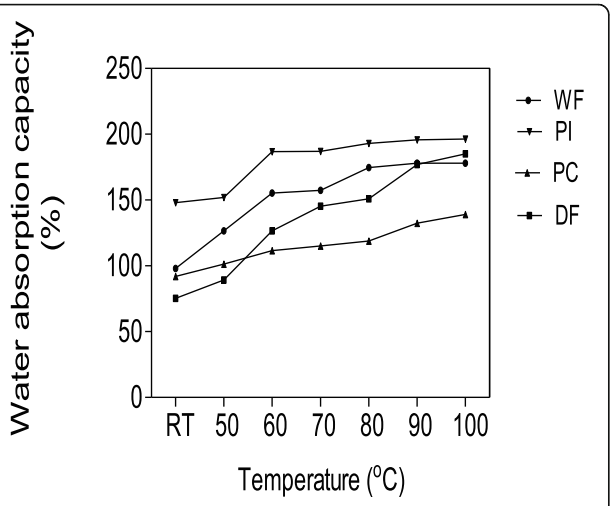

Fig. 2 Effect of temperature on water absorption capacity whole flour, defatted flour, protein concentrate and isolate

presence of high amounts of non-protein materials such as fibre (Ogunwolu et al., 2009; Sikorski et al., 2002)

Influence of pH and sodium chloride (NaCl) concentrations on foaming properties

The influence of pH (2–10) and salt concentrations (0.50 and 1.0 M) on the foaming properties of the samples is shown in Fig. 3a-c. The values ranged between 4 and 29%, 4 and 54%, 4 and 40% in the absence of salt, in the presence of 0.5 M and 1.0 M sodium chloride concentrations, respectively. The results showed low foam formation in the absence of salt but highest in the presence 0.5 M salt solution. The ability of the samples to form foams was lowered when the salt concentration was increased to 1.0 M. The pattern of results may suggest that the threshold salt concentrations of the samples is 0.5 M, since low amounts of foam was formed at 1.0 M salt concentration. A possible explanation for this trend may be that of the concentration of ions in the solution. Though the presence of ionic strength generated by the addition of salt is necessary for foam formations, as indicated in the presence of 0.5 M concentration, excess amounts may lead to overcrowded ions in the solution and hence, reduce the ability of the samples to entrap air to form foams (Xiaoying and Hua 2011; Yu et al. 2007). The foaming capacities of the samples were lower in the acidic region (pH < 7.0) than in the basic region (pH > 7.0). The lowest foaming capacity of the sample was obtained at pH 4.0 (isoelectric pH points) and the values increased towards the basic regions. The results were similar to the solubility profiles of the samples whereby the samples were least soluble at the isoelectric pH values and mostly soluble at the basic region. The trend was similar to the results of walnut proteins (Xiaoying and Hua 2011), mucuna protein products (Adebowale et al. 2005) and Bambara groundnut flours (Eltayeb et al. 2011). At all the pH and salt concentrations, PC had the highest values compared to PI, DF and WF, and this may be attributable to high solubility of PC. The high forming capacities of the samples at the basic region 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 (Ijarotimi et al. 2018).

As shown in Fig. 3d-f, the values obtained for the foam stabilities ranged between 1.0 and 20.0%, 2.0 and 47% and 3.0 and 34% for samples in the absence of salt, in the presence of 0.50 and 1.0 M salt concentrations respectively. PC exhibited greater foam stability than PI, whereas WF was least stable. Foam formed in the presence of 0.5 M salt was most stable, while foam formation in the absence of salt was least stable. The results showed addition of sodium chloride up-to 0.5 M improved the stability of the foams, this is evidence in the low stabilities of foam formed in the presence of 1.0 M when compared to those foams formed in the presence of 0.5 M. Excess ionic formations at 1.0 M sodium chloride concentration may have led to overcrowding of foams thereby reducing the stability. The low foam stability of the samples at 1.0 M salt concentration may be attributed to salting out effects, which may have resulted from the competition between the proteins and the excess salt concentration (Xiaoying and Hua 2011). Malomo and Aluko (2015) reported that stability of foams are the results of intermolecular cohesiveness and the extent of elasticity of the proteins in solutions. The amounts of ions formed in the presence of 0.5 M sodium chloride concentrations may be enough to strengthen the intermolecular cohesiveness of the foam formed, which encouraged its stability. The PC and WF had the highest and lowest foam stabilities foams and this agreed with the trend of foam formation. The lowest foam stability was obtained at pH 4 while the highest was at pH 12 and this agreed with the solubility profiles earlier reported in this work. Earlier published work reported 60–80% species of *mucuna* flours (Adebowale et al. 2005), 11–67% for Bambara groundnut flours (Eltayeb et al. 2011) and 5 to 62% for walnut flours (Xiaoying and Hua 2011). The application of foaming agents in foods depends in greater parts on how long foam can be maintained. Many oil seeds have recently been discovered to exhibit good foaming properties and have recently found increasing use as aerating agents in whipped toppings, frozen desserts food and sponge cakes.

Influence of pH and sodium chloride (NaCl) concentration on the emulsifying properties

The emulsion capacities of the samples ranged between 43.07 and 102.39 m^2/g, 43.43 and 94.52 m^2/g and 59.46

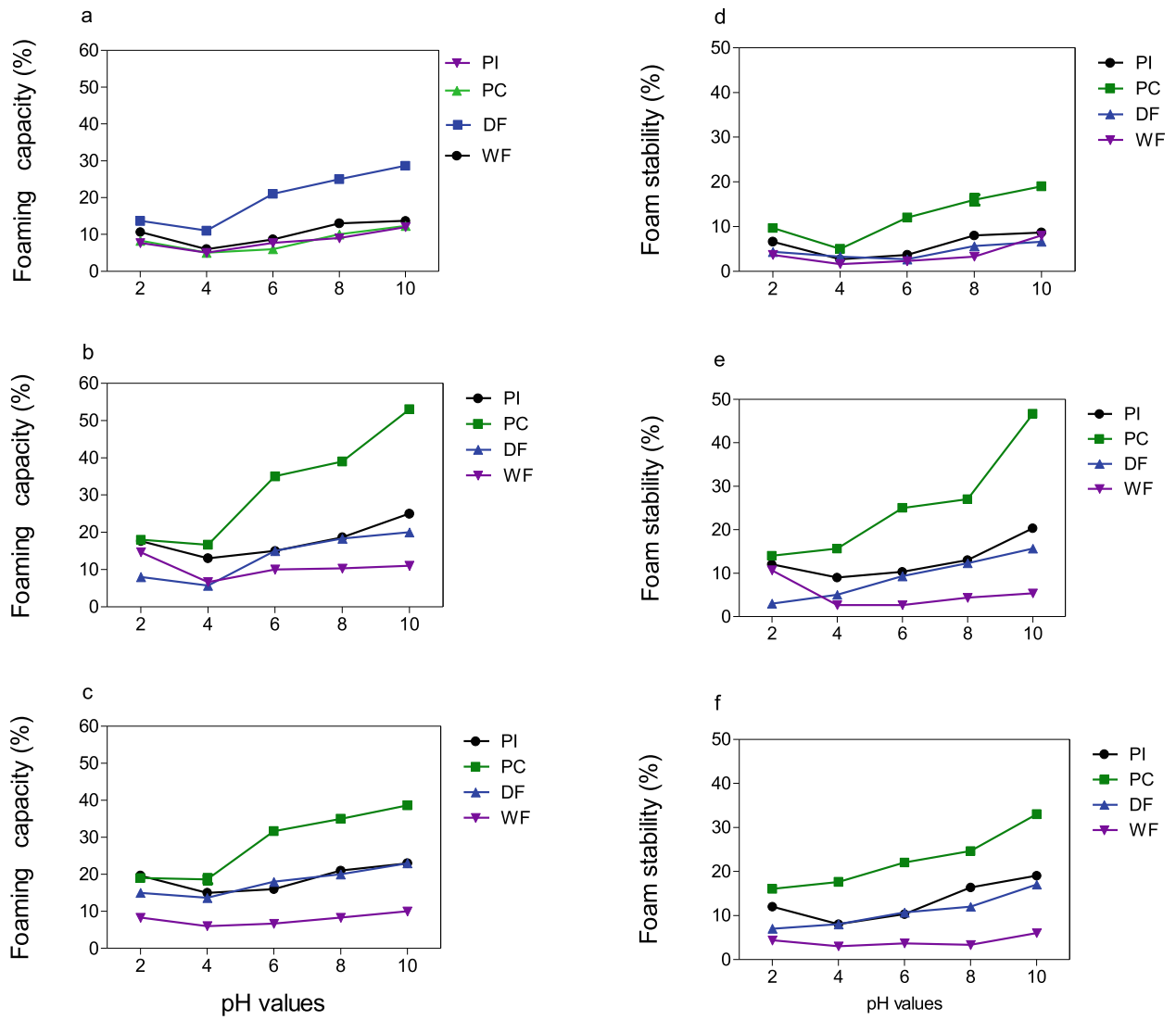

Fig. 3 Influence of NaCl concentrations and pH on the foaming capacity of okra seed samples at **a** no salt **b** 0.5 M NaCl concentration **c** 1.0 M NaCl concentration; and foaming stability at **d** no salt **e** 0.5 M NaCl concentration **f** 1.0 M NaCl concentration

and 90.23 m^2/g for samples in the absence of salt and in the presence of 0.5 M and 1.0 M solutions (Fig. 4a-c). At pH 10, the PC had the highest emulsion capacity while WF had the least emulsion capacity. The result showed minimum emulsion capacity at pH 4.0 while the maximum emulsion was obtained at pH 10.0, suggesting better emulsions formation in the basic regions than acidic. The formation of low change densities at the iso-electric point may have reflected in the low emulsions at pH 4.0. The results also showed greater emulsion formations of the samples in the presence of 0.5 M and 1.0 M Sodium chloride, suggesting that the ions created by the presence of salt enhanced the formation of emulsions. Ijarotimi et al. (2018) reported that that high emulsion formation of Wonderful cola seed flour and proteins depends on the alkalinity/acidity and ionic concentrations of the solutions. Previous works reported 71 to 120 m^2/g and 54 to 80 m^2/g for the emulsion capacities of Bambara nut proteins and varieties of *mucuna* flours (Eltayeb et al. 2011; Adebowale et al. 2005).

The values obtained for the emulsion stability of the samples were between 34.91 and 97.66%, 18.90 and 97.17% and 44.74 and 92.18% for samples with and without Sodium chloride as shown in Fig. 4d-f. The results showed progressive increase in the emulsion stability at pH > 4.0. The results showed greater emulsion stabilities at pH > 7.0 when compared to pH < 7.0, and this may suggest greater exposure of hydrophobic sides of proteins to charged ions. This pattern was in line with earlier published works on hemp and Wonderful cola seeds proteins (Malomo et al. 2015; Ijarotimi et al. 2018). In a similar manner, a low emulsion stability especially near

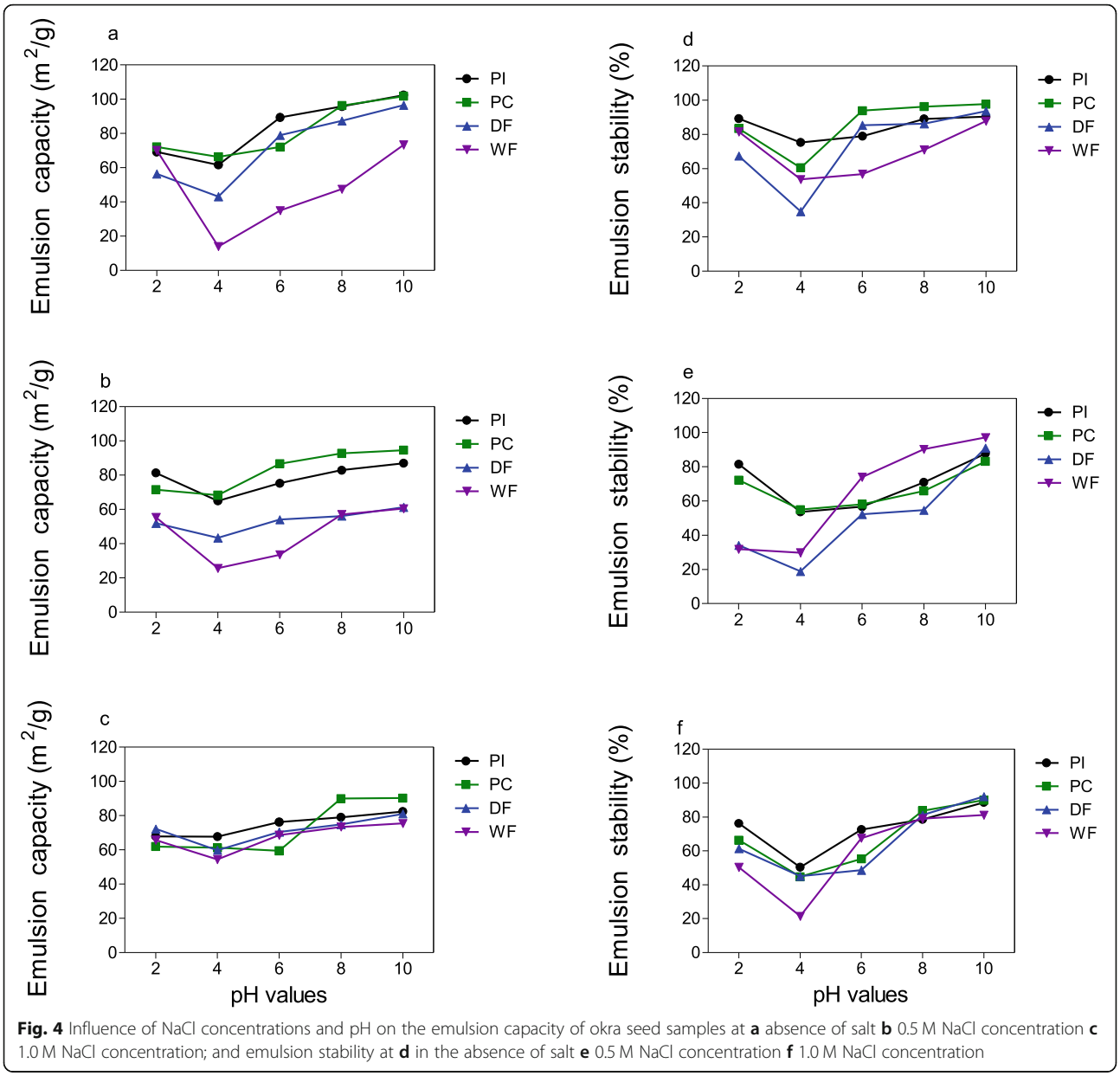

Fig. 4 Influence of NaCl concentrations and pH on the emulsion capacity of okra seed samples at **a** absence of salt **b** 0.5 M NaCl concentration **c** 1.0 M NaCl concentration; and emulsion stability at **d** in the absence of salt **e** 0.5 M NaCl concentration **f** 1.0 M NaCl concentration

iso-electric pH points may be attributed to increased interactions between the emulsified droplets, due to reduction in net charge on the proteins by the presence of chloride ions (Famuwagun et al. 2020). Between pH values of 4–10, the emulsion stabilities were lower when compared to samples in the absence of salts. The low emulsion stabilities in the presence of 1.0 M salt concentration may suggest reduction in the interfacial energy due to the presence of high ionic concentrations. However, when the emulsion stability was increased when the salt concentration was reduced to 0.5 M and this suggest presence of high interfacial energy due to reduced supply of ions. Previous works reported 11.30 to 13.50% for the emulsion stabilities of groundnut flours and protein isolate by Fekria et al. (2012) and 90–97% for ackee apple arils flours (Famuwagun and Gbadamosi 2016). High emulsifying properties of food additives are desirable properties for plant proteins to be used as ingredients in the production of mayonnaise and for the stabilization of emulsions in soups and cakes.

In-vitro protein digestibility

IVD is a measure of quality of protein in protein rich materials. Table 3 shows that okra seed protein hydrolysates had better digestibility (83.26–86.08%) compared to okra seed flours and protein isolate (36.48–80.90%). The higher protein digestibility of protein hydrolysates compared to the unhydrolysed samples may be

Table 3 In vitro protein digestibility and antioxidant properties of okra seed flours, protein isolate and hydrolysates

	In vitro protein digestibility (%)	DPPH radical scavenging activities (%)	Metal chelating activities (%)	Ferric reducing antioxidant properties ($mMFe^{2+}$)
WF	36.48 ± 1.45^{g}	60.06 ± 0.12^{g}	9.40 ± 0.56^{h}	0.06 ± 0.01^{c}
DF	44.77 ± 0.85^{f}	68.65 ± 0.76^{f}	12.45 ± 0.66^{g}	0.06 ± 0.01^{c}
PC	74.79 ± 0.96^{e}	74.56 ± 1.13^{e}	15.55 ± 0.95^{f}	0.07 ± 0.02^{c}
PI	80.90 ± 1.11^{d}	85.38 ± 2.05^{b}	29.05 ± 0.77^{e}	0.07 ± 0.02^{c}
PHp	85.39 ± 0.55^{b}	91.47 ± 0.85^{a}	81.00 ± 1.05^{c}	0.11 ± 0.01^{b}
PHc	83.26 ± 1.45^{c}	$85.50 \pm .073^{b}$	78.50 ± 1.11^{d}	0.14 ± 0.02^{b}
PH_T	86.06 ± 1.54^{a}	84.89 ± 0.55^{c}	90.30 ± 0.89^{b}	0.12 ± 0.02^{b}
Ascorbic acid	–	79.42 ± 0.69^{d}	95.03 ± 1.32^{a}	0.43 ± 0.07^{a}

Values are mean ± standard deviation of triplicate determinations. Values with the same superscript along same column are not significantly ($p > 0.05$) different from each other

WF Whole flour, *DF* Defatted flour, *PC* Protein concentrate, *PI* Protein isolate, PH_P Pepsin protein hydrolysate, PH_C Pancreatin protein hydrolysate, PH_T Trypsin protein hydrolysate

attributed to the exposure of more protein active sites to the digestive enzymes. By virtue of the small molecular sizes of the hydrolysates as a result of enzymatic action, it may have been better digested by the digesting enzymes (pepsin and pancreatin) when compared to the unhydrolysed proteins. The protein digestibility of PI was significantly ($p < 0.05$) higher when compared to WF, DF and PC. The presence of non-protein materials such as the fibre in the whole and defatted flours, may be exposed to the active site of digesting enzymes, resulting to low digestibility. Similarly, the variation observed in the protein digestibility's of the protein hydrolysates may be attributed to differences in the specificities of enzymes used to produce hydrolysates (Ijarotimi et al. 2018). For the PI, the value (80.90%) compares with 78.34% for quinoa protein isolate (Elsohaimy et al. 2015) and 84% reported for other varieties of quinoa protein isolate (Repo-Carrasco-Valencia and Serna 2011) but lower when compared to 84–89% for hemp seed protein isolate (Malomo et al. 2015)

Antioxidant properties

2, 2-diphenyl-1-hydrazine (DPPH) radical scavenging activity

DPPH is a stable free radical that is scavenged by an antioxidant substance at absorbance of 517 nm in methanol. The method of scavenging DPPH free radical is based on the reduction of the absorbance of methanolic DPPH solution at 517 nm in the presence of a proton-donating substance (Xie et al. 2008). The values obtained for the DPPH radical scavenging activities (DRSA) of okra seed samples is shown in Table 3. Protein hydrolysates exhibited greater DRSA (84.89–91.47%) than the unhydrolyzed proteins (60.65–74.56%) and also higher than the standard ascorbic acid (79.42%). The high DRSA of the protein hydrolysates indicated that hydrolysis enhanced the proton donating ability of the hydrolysates. The results were in line with the amino acid patterns of the samples, whereby the hydrolysates composed greater amounts threonine, serine, methionine, isoleucine. Tyrosine, phenylalanine and histidine than the unhydrolysed samples. Previous works by Ajibola et al. (2011) and Famuwagun et al. (2020b) suggested that the release of free amino acids during enzymatic hydrolysis enhance greater radical scavenging activities of protein peptides. Among the protein hydrolysates, PHp exhibited greater (91.47%) radical scavenging ability when compared with PHc and PH_T. The high DPPH free radical scavenging activity of the okra seed hydrolysates, especially the PHp suggest their potential usefulness as ingredients in the management of free radical mediated disorders (Graham et al., 2017; Manley et al., 2002).

Metal chelating activities and ferric reducing antioxidant power

The active participation of ferrous ion in Haber-Weiss reaction, whose products, such as superoxides result in the formation hazardous hydroxyl radicals has been well documented (Xie et al. 2008). Therefore, chelation of ferrous ion (Fe^{2+}) will decrease the amount that will participate in the reaction thereby reducing the formation of lethal hydroxyl radicals. As shown in Table 3, the standard ascorbic acid exhibited better chelating activities (95.03%) than the protein hydrolysates (78.50–90.30%). The protein hydrolysates however demonstrated better chelating activities than the unhydrolyzed okra proteins and flours (9.40–29.05%), and this trend agreed with the results of some of the amino acids as indicated in Table 2. For instance, trypsin hydrolysate with the highest contents of histidine and methionine had better chelating activities (90.30%) when compared to other protein hydrolysates. These amino acids, especially the histidine have been reported to possess strong ability to chelate metals because of the special imidazole ring (Nam et al. 2008). Previous studies reported 61–78% and

70–72% for metal chelating activities of Bambara and hempseeds protein hydrolysates (Arise et al. 2016; Girgih et al. 2011).

The results of ferric reducing antioxidant power (FRAP) showed that the protein hydrolysates had significantly ($p < 0.05$) greater reducing abilities (0.11–0.14 mMol Fe^{2+}) when compared to the unhydrolysed proteins (0.06–0.07 mMol Fe $^{2+}$). The low molecular weights of the hydrolysates as a result of the enzymatic hydrolysis may be attributable to the high FRAP observed in the protein hydrolysates. Among the protein hydrolysates, PHc exhibited greater reducing ability than the other protein hydrolysates. The pattern is supported by its high contents of phenylalanine, aromatic and hydrophobic groups of amino acids. Earlier published works reported 0.108–0.139 mMol and 0.10 mMol Fe $^{2+}$ for FRAP contents of pepsin, trypsin and alcalase hydrolysates obtained from Bambara groundnut proteins (Arise et al. 2016) and for hempseed (Girgih et al. 2011) respectively.

Conclusion

The study reported the production and the potential functionality of okra seed flours, protein products and enzymatic hydrolysates. The results of FTIR showed that protein, fats and amino acids were responsible for the major spectra bands of the samples. The protein hydrolysates contained greater amounts of essential amino acids and hydrophobic amino acids than the seed flours and protein isolate. The higher water absorption of the samples at elevated temperature were linked with increase in the kinetic of the sample solution that enhanced the exposure of the water binding sites of the hydrophilic site of the proteins. The samples had better solubility in the basic than the acidic region. The high foaming ability of the samples in the basic region may be attributed to high solubility of samples in the region. The protein hydrolysates exhibited greater in-vitro protein digestibility and antioxidant properties, which were attributable to the effect of enzymatic hydrolysis. The results concluded that okra seed flours, concentrate, isolate and its resulting hydrolysates demonstrated potentials that would could enhance their utilization in food fortification and in the management of oxidation-mediated reactions.

Acknowledgements

The authors appreciate the Department of Food Science and Technology, OAU, Ile-Ife for the use of the facilities to carry out the experiments

Authors' contributions

GBADAMOSI Saka Olasunkanmi (GSO) conceived the idea, supervised and corrected the final draft. NNAMEZIE Anastasia Amaka (NAA) performed the analysis, interpreted the data and wrote the first draft of the manuscript. FAMUWAGUN Akinsola Albert (FAA) assisted in data interpretation and corrected the draft manuscript. The author(s) read and approved the final manuscript.

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Funding

Authors did not receive any form of funding from any organization

Availability of data and materials

The data and the materials are included in the manuscript and available for public use on request.

Declarations

Ethics approval and consent to participate

This study did not involve the use of animal and therefore no ethical form was obtained.

Consent for publication

All the authors have consented to publish the work in *Food Production, Processing and Nutrition*

Competing interests

The authors declare that there is no competing interest in the submission and the publication of this work. All the authors read and approved the manuscript for publication.

Received: 7 August 2020 Accepted: 15 March 2021
Published online: 10 May 2021

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