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Research Article

Physiochemical and Functional Properties of (*Balanites Aeqyptiaca. Del*) Hydrolysates by Pepsin and Pancreatin Proteases

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Abstract:

Enzymatic hydrolysates from underutilized desert date could produce functional ingredient for desert region and population of the world. The current study investigated the physiochemical and functional properties of hydrolysates from Balanites aeqyptiaca using pancreatin, pepsin and combined pancreatin and pepsin enzymes. The hydrolysate obtained through the addition of the enzyme pancreatin, and pepsin combined gave (45.1%) yield. On the other hand, pancreatin hydrolysate gave maximum (45.9%) yield and pepsin hydrolysate was (37.4%) .The combined hydrolysate has maximum protein (80.58%),ash content (0.85%) mineral profile; Ca(0.12mg.100g),K(1.29mg/100g), Na(2.01mg/100g),Mg (1.09mg/100g),Fe(0.23mg/100g), Cu (0.09mg/100g) , Zn(0.26mg/100g) and excellent anti-nutrient alkaloid , oxalate ,phytate and saponin and total phenolic content of (0.084mgCat/g). Functional properties such as bulk density (1.02g/m) swelling index (3.45g/m) least gelation capacity (20.39%) of pancreatin hydrolysate and WAC (1.72g/g) and OAC (1.73g/g) from pepsin hydrolysate produced the best result than the combine hydrolysate. The maximum solubility (12%) at pH 9 was observed for all samples. The hydrolysate by combined enzymes had a high IVPD compared with pancreatin and pepsin hydrolysates. However, both showed a good general foaming and emulsifying properties. Foaming capacity of the pepsin hydrolysate samples increased with concentration at pH3 and pH7 while the foaming stability at pH7 and pH9 of combined hydrolysate was at 20 mg/ml and 40 mg/ml high comparable with pepsin and pancreatin, respectively. The emulsifying activity of combined hydrolysate was high at 10mg/ml and 50mg/ml at alkaline region. On the other hand, pepsin hydrolysate reveals excellent emulsifying activity (EAI) of 200 mg/m index (EAI) at pH 5. The hydrolysate obtained from pepsin decreased in emulsifying stability as concentration increased and pH shift to alkaline region. The hydrolysate by pepsin and pancreatin had better WHC, OHC and foaming properties due to their solubility however lower than the combined hydrolysate. The result indicates potential utilization of hydrolysate from Balanites aaqyptiaca. del for less protein rich cereal food formulations.

Key words: physical; chemical; functional; pepsin; pancreatin; hydrolysate

Introduction

The need to exploit more proteins and peptides from diverse natural resources are on the increase and with respect to climate change, it may double soon. A lot of forest and desert reserved plant source of food abound and needs to be exploited for human maximum beneficial use. Protease hydrolysis is an excellent way for solubilizing and exposing peptides conformation for cellular utilization of their essential amino acids or proteins in living cells. Hydrolysis of protein is adoptable in small and industrial scale, widely used in the food industry to make stable or semi stable products which could serve as finished or raw material in milk as replacer, beverage stabilizer and flavor enhancer. Plant food hydrolysates when derived by protease hydrolysis, tends to have better nutritional profile in terms of amino acids, peptides classes which could be used for human or animal feeds [30]. The greater benefit of using plant source food for hydrolysis is to modify and activates the functional properties of plants sourced free amino acid, reduce phytotoxins and generates a mixture of free amino acids, [2,3]. Derivable proteins or peptides from hydrolysis are crucial in their use as food ingredient [4] and as potential therapeutic food or drug incipient. Enzymatic hydrolysis of plant protein using proteases such as pepsin, pancreatin Alcalase, Flavoenzyme and Chymotrypsin have been opined [34]. Functional properties are related to structure -functions relationship such as amino acid composition, molecular weight, charge distribution [3]. It has been elaborated that hydrolysate stereochemistry helps interactions with other components like, ions, lipids, carbohydrates, and vitamins constituents, which are dependent on certain intrinsic physical parameters like pH which are in turn involved as modifiers at food preparation, processing, and storage [3,6,7]. Aduwa seed have been reported utilizable for human and feed use, the use of Aduwa leaves, fruits and seed have also been researched and reported with potential utilization at homes, industries and pharmaceutical applications as anti-diabetes remedy, anti-cancer, anti-helminthics as well as an antioxidant [2,8]. The objective of this work is to evaluate the properties of desert date enzymatically hydrolyzed with pepsin and pancreatin from toasted Aduwa seed to direct their application and use towards food and pharmaceutical products for human use.

2.Materials and Methods

2.1 Material

The raw material used was Aduwa seed. They were cracked and packaged from Gashua in yobe state, Northeast of Nigeria and there were transported to the Laboratory of college of Food Technology and Human Ecology, Food Science and Technology at Joseph Sarwan Tarka University, Makurdi. The seed were toasted and milled before defatted. The defatted meal was extracted by isoelectric precipitation into concentrates and then stored in an air tights container for further analysis. Samples were drawn and make into hydrolysate using pepsin and pancreatin at 4% w/v enzyme addition.

2.2 Preparation of Aduwa Concentrate (APC)

The Aduwa concentrate (AC) was prepared according to the method outlined by [9]. About 200 g weighed defatted Aduwa meal flour was dispersed in distilled water to final flour to water ratio of 1:10 dilution. Ratio. The mixture was stirred gently on a magnetic stirring thimble for 10 min until a suspension is form then the pH of the resultant slurry adjusted with 0.1 M HCl to pH 4. The precipitation process was allowed to proceed with gentle stirring for 2h at constant pH 4. During this phase, carbohydrates (oligosaccharides) and minerals are removed after centrifugation at 3,500 × g for 30 min using centrifuge. The collected precipitate (concentrate) was washed with distilled water to remove the residual minerals and soluble carbohydrates and then pH adjusted with 0.1 M NaOH to 7.0 for neutralization. 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.

2.3 Preparation of Aduwa protein hydrolysate using pancreatin enzyme

Aduwa meal protein hydrolysate (APH) was prepared using pancreatin enzyme optimum reaction conditions acting on the isolate. Pancreatin with pH 7.5 at 40 ºC using the method of [10] .A 1:20 w/v Aduwa seed protein concentrate was adjusted to pH 7.5 and incubated at 40 ºC following the addition of pancreatin at (4% w/w), based on protein content of Aduwa concentrate and then the mixture incubated at 45 °C following the addition of trypsin enzyme (4% w/w). 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 placing the mixtures in boiling water for 30 min to inactivate the enzymes for complete denaturation of enzyme and coagulation of undigested proteins. The mixture was allowed to cool to room temperature and later centrifuge to get the supernatant and freeze dried.

2.4 Preparation of Aduwa protein hydrolysate using pepsin enzyme

The Aduwa meal protein hydrolysate (AMPH) was prepared using pepsin enzymes in an optimum reaction condition (Pepsin with pH 2 at 37^{0}C), using the described method of [10]. A 1:20 w/v Aduwa protein concentrate slurry was adjusted to pH 2.0 and incubated at 37 ºC followed by addition of pepsin (4% w/w based on protein content of Aduwa protein isolate), The digestion was carried out for 4 h and the pH is maintained by adding 1 M NaOH or HCl where 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. The mixture was allowed to cool to room temperature and later centrifuged and supernatant collected and freeze dried.

2.5 Enzyme hydrolysis of APC for making combined Hydrolysate (APHpa+pe)

Enzymatic hydrolysis of APC was carried out using the method of Aluko and McIntosh (2004) with slight modification by [4]. The APC sample was dispersed in water (2%, w/v), and adjusted to pH 9.0 using 1 M NaOH solution for pancreatin while pH 2.0 was used for pepsin digestion. The dispersion was heated to 40ºC under continuous stirring on a hotplate. The enzymes (4% w/w) were added based on the protein content of the APC and incubated at constant temperature of 40ºC for 4 h. The reaction mixture was maintained at pH 9.0 using 1 M NaOH solution and after 4 h, the pH was adjusted to pH 2.0 with 1 M HCl for pepsin hydrolysis. At the end of the incubation period, the hydrolysates were transferred into a boiling water bath for 5 min to inactivate the enzymes. The hydrolysate was cooled to room temperature (22±2 ºC) using ice blocks and adjusted to pH 7.0 and finally freeze-dried.

2.6 Moisture content determination

Five grams (5g) of the hydrolysate sample was weighed accurately into pre-weighed clean dry dish provided with a removable lid. The uncovered dish was placed with lid open in a well-ventilated oven maintained at 103ºC for 3 hours. The dish was covered and transferred to a desiccator and cool for 30 minutes. The dish with the sample weighted again was placed in the oven for another 2 hours. The steps were repeated until decreases in mass was observed. The loss in weight was reported as the moisture content.

$$\%\,Moisture\ content\ = \frac{\mathrm{W1-W2}}{\mathrm{W1-W0}} \times 100$$

but

W_1 = First weight of crucible

W_2 = Weight of crucible and sample before drying

W_3 = Weight of crucible and sample after drying

2.7 Protein determination by Lowry Method

The [2] method was adopted. Here, one mg/ml of the soluble filtrate was pipetted with the addition of 3 ml of Lowry's reagent C was used and dissolved and make to the mark with distilled water in a 100 mL standard flask; Reagent X: (4 % $CuSO_4.5H_2O$) was dissolved and made-up to the mark with distilled water in 100 mL standard flask. The mixture was incubated at room temperature for 1 h. Also, 0.3 mL of diluted Folin Ciocateu phenol was added to the mixture and mixed vigorously using vortex mixer. The tubes were allowed to stand at room temperature for 45 min and the absorbance of the mixture was then measured at 600 nm using spectrophotometer. Bovine Serum Albumin (standard) was prepared in similar manner as the samples but at different concentration (1-100μg/mL). The standard curve obtained was used to find the protein concentration of the sample.

2.8 Ash content determination

Two grams (2g) of the hydrolysate sample was weighed into an empty porcelain crucible that was ignited and weighed. The hydrolysate sample was ignited over a hot plate in a fume cupboard to char. The crucible was thereafter placed in the muffle furnace maintained at a temperature of 600ºC for 6 hr. After ash, samples were then transferred directly to a desiccator and weighed immediately [13].

$$Ash(\%)\ = \frac{(Weight\ of\ the\ crucible\ and\ Ash)-(Weight\ of\ empty\ crucible)\times 100}{Weight\ of\ hydrolysate\ sample}$$

2.9 Crude fat determination

Crude fat determination was carried out using the method of [11]. Empty thimble was weight and recorded as W_1. Five (5) grams of oven dried hydrolysate sample was added and weighed as (W_2). Round bottom flask was used in the Soxhlet extraction with petroleum ether as extracting solvent. Soxhlet extractor was fixed with a reflux condenser to adjust the heat sources so that the solvent boils gently. The samples were put inside the thimble and inserted into the Soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 hours. After the barrel of the extractor became empty, the condenser and the thimble were removed. The thimble was taken to the oven at 100^{o}C for 1 hour and later cooled in the desiccator. The sample was then weighed as (W$_3$).

$$\% \, Fat = \frac{Weight\ loss\ of\ extracted\ fat)}{Original\ weight\ of\ hydrolysate\ sample} \times 100$$

2.10 *Crude fiber determination*

Crude fiber content of the sample was determined using the method described in [11]. Two (2) grams of sample was weighed and transferred into 250ml beaker. It was then boiled for 30 minutes with 100ml of 0.12M H_2SO_4 and filtered through a funnel. The filtrate was washed with boiling water until the washing is no longer acidic. The solution was boiled for another 30minutes with 100ml of 0.012MNaOH solution; filtered with hot water and methylated spirit three times. The residue was transferred into a crucible and dried in the oven at 103^{0}C for 1 h. The crucible with its content was cooled in a desiccator and then weighed (W$_1$). The residue was then taken into a furnace for ash at 600°C for 1 h. The ach sample was removed from the furnace and put into the desiccator to cool and later weighed (W2). The percentage crude fiber was calculated thus:

$$\% \, Crude\ fiber = \frac{W1 - W2}{Weight\ of\ sample} \times 100$$

Where:
W1 = Weight of crucible and residue
W2 = Weight of final ash sample

2.11 Carbohydrate content determination

Carbohydrate content determination was determined by difference[14]

$$\%Carbohydrate = 100\% - \%(Protein + Crude\ fat + Ash\ + Moisture + Crude\ fiber)$$

2.12 Determination of tannins

The modified vanillin – hydrochloric acid (MV – HCl) method of [15] was used. This entails the preparation of standard solution and stock solution.

The Preparation of Calibration curve was based on these various concentrations (0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of the catechin standard solution which were pipetted into clean dried test tubes in duplicate. To one set 5.0 ml of freshly prepared vanillin – HCl reagent prepared by mixing equal volume of 4% (w/v) vanillin/MeOH and 8% (v/v) HCl/ MeOH will be added and to the second set will be added 5.0 ml of 4% (v/v) HCl/methanol to serve as blank. The solutions will be left for 20 min before the absorbance will be taken at 500 nm. The absorbance of the blank will be subtracted from that of the standards. The difference will be used to plot a standard graph of absorbance against concentration. Sample procedure followed these steps*;* Aduwa samples (0.2 g) was extracted separately with 10 ml of 1.0% (v/v) HCl – MeOH. The extraction time is 1 hour with continuous shaking. The mixture was filtered and made up to 10 ml mark with extracting solvent. Filtrate (1.0 ml) was then reacted with 5.0 ml vanillin – HCl reagent and another with 5.0 ml of 4% (v/v) HCl –MeOH solution to serve as blank. The mixtures were be left to stand for 20 min before the absorbance was taken at 500 nm. Tannin was calculated using the formular.

$$\text{Tannin}=\frac{\text{Xmg/ml}\times 10\text{ml}}{0.2\text{ g}}=50\times\text{mg/g}$$

Where x - value obtained from standard catechin graph
Preparation of trypsin solution 0.5 mg/ml in 0.001 N HCl

2.13 Determination of oxalate

Oxalate was determined by the method of [16]. Four grams of the sample were weighed in triplicate into 250 ml conical flasks and was extracted with 190 ml distilled water and 10 ml 6M HCl. The suspension was placed in boiling water for 2 h, filtered and made up to 250 ml with water in a volumetric flask. To 50 ml aliquot, 10 ml 6M HCl was added and filtered, and the precipitates were washed with 10 ml of hot water. The filtrate and the wash water were be combined and titrated against concentrated NH_4OH until the salmon pink color of the methyl red indicator changed to faint yellow. The solution was heated to 90 °C and 10 ml 5 % (w/v) and $CaCl_2$ solution added to precipitate the oxalate overnight. The precipitates were washed free of calcium with distilled water and then washed into 100 ml conical flask with 10 ml hot 25% (v/v) H_2SO_4 and again with 15 ml distilled water. The final solution was heated to 90 °C and titrated against a standard 0.05M $KMnO_4$ until a faint purple solution persisted for 30 s. The oxalate was calculated as the sodium oxalate equivalent as shown in equation

1ml of 0.05M $KMnO_4$ = 2mg sodium oxalate equivalent/ g of sample

2.14 Determination of saponin

The spectrophotometric method [17] was used for saponin analysis. One gram of finely ground sample was weighed into 250 ml beaker and 100 ml of isobutyl alcohol added. The mixture was shaken on a Brunswick incubator shaker (USA) for 5 h to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman No 1 filter paper into a 250 ml beaker and 20 ml of 40% saturated solution of magnesium carbonate added and the mixture made up to 250 ml. The mixture that was obtained with saturated $MgCO_3$ was filtered through a Whatman No 1 filter paper to obtain a clear colorless solution. 1 ml of the colorless solution was pipetted into a 50 ml volumetric flask and 2 mL of 5% $FeCl_3$ solution added and made up to mark with distilled water. The mixture was allowed to stand for 30 min for blood red color to develop. Saponin stock was prepared and 0 – 10 ppm standard saponin solution were prepared from saponin stock solution. The standard solution was treated similarly with 5% of $FeCl_3$ solution as was done for 1 mL of sample above. The absorbance values of the sample as well as the standard solution was read after color development in spectrophotometer (722-2000 spectronic 20D, England) at a wavelength of 380 nm.

$$\text{saponin}=\frac{\text{absorbance of sample }\times\text{dil. factor }\times\text{ gradient of standard graph curve}}{\text{sample weight }\times 10{,}000.}$$

2.15 Determination of phytate

The phytate content of the samples was determined following the method described by [11]. Four gram of the grinded sample was weighed into a beaker and was soaked in 100 ml of 2% HCl for 5 h and then filtered. 25ml of the filtrate was taken into a conical flask and 5 ml of 0.3% potassium thiocyanate solution was added. The mixture was titrated with a standard solution of Iron (III) chloride ($FeCl_3$) until a brownish yellow color persisted for 5 min. The concentration of the $FeCl_3$ was 1.04%w/v and Mole ratio of Fe (iron)to phytate = 1:1 100 x weight of sample concentrations of phytate phosphorous = Titre value x 0.064

2.16 Determination of alkaloids

Alkaloids was quantitatively estimated using the method by[18] . Five grams of the sample was extracted in 200 ml of 10% acetic acid in ethanol in a 250 mL beaker. Samples was incubated for 4 h at room temperature, then filtered and the filtrate concentrated on a water bath to one-quarter of the original volume. The extract was then precipitated by the addition of drops of concentrated ammonium hydroxide and allowed to settle. The precipitate was washed with dilute ammonium hydroxide and then filtered. The residue which comprised of the alkaloid, was dried, and weighed. The alkaloid content was determined using the formula:
alkaloid (%) = final weight of sample/initial weight of extract × 100.

2.17 Bulk density

Five (5) gram of flour sample of the tiger nut was poured into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g /cm^3) was calculated as weight of flour (g) divided by volume of flour (cm3). [19].

$$\text{Bulk density} = \frac{Weight\ of\ sample\ (g)}{\text{Volume of sample(ml)}}$$

2.18 Foam capacity (FC) and Foaming stability

FC was determined according to the method described by [20] using slurries that was prepared at 20, 40, or 60 mg/mL (protein weight basis) dispersions in 50 mL graduated centrifuge tubes containing 0.1 M phosphate buffer, pH 3.0, 5.0, 7.0, and 9.0. Sample slurry was homogenized at 20000 × *g* for 1 min using a laboratory blender (Bl231japan). The capacity of the continuous phase to include air (FC) was determined as follows using the mean of measurements.

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

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

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

2.19 Water *Absorption capacity (WAC)*

The WAC was determined using the method of [20] with slight modifications. Protein sample (1 g) was dispersed in 10 mL distilled water in a 15 mL pre-weighed centrifuge tube. The dispersions were vortexed for 1 min, allowed to stand for 30 min and then centrifuged at 5000 × *g* for 25 min at room temperature. The supernatant was decanted, excess water in the upper phase drained for 15 min and tube containing the protein residue was weighed again to determine amount of water retained per gram of sample.

2.20 Oil *Absorption capacity (OAC)*

The OAC was determined using the method of [11] with slight modifications. Protein sample (1 g) was dispersed in 10 mL pure canola oil in a 15 mL pre-weighed centrifuge tube. The dispersions were vortexed for 1 min, allowed to stand for 30 min and then centrifuged at 7000 × *g* for 25 min at room temperature. The supernatant was decanted, excess oil in the upper phase drained for 15 min and tube containing the protein residue was weighed again to determine amount of water or oil retained per gram of sample.

2.21 Swelling index

Swelling index was determined according to the method by [21]. Two (2) grams of the flour sample was poured into a 50ml measuring cylinder and the volume it occupied was recorded. Already boiled water was added up to 50ml mark and the measuring cylinder was allowed to stand for 45mins after which the new volume of flour was recorded. The ratio of the initial volume to the final volume was taken as the swelling index.

$$\text{Swelling index} = \frac{change\ in\ volume\ of\ sample\ (ml)}{Original\ volume\ of\ sample(ml)}$$

2.22 Emulsifying properties.

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined using the method modified by [20]. Protein slurry of. 10, 25, or 50 mg/ml each were mixed with 30 ml of deionized water. This protein solution was mixed with 10 ml of sunflower vegetable oil, and the pH was adjusted to 3, 5, 7, and 9. The mixture was homogenized at a speed of 1,000 rpm for 1 min. Fifty microlites (50 mL) of the aliquot of the emulsion were transferred (using pipette) from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using spectrophotometer (UVIKON 930, BIO-TEK Kontron, Germany). This was calculated EAI and ESI using the method suggested by [11].

Emulsifying activity index $\text{EAI} = \frac{\text{2x2.303x Ao}}{\text{0.23xprotein weight g}}$

Emulsion stability index $\text{ESI (min)} = \frac{\text{A10 x Dt}}{\text{DA}}$

where A_0 is the absorbance at 0 min after homogenization; A10 is the absorbance at 10 min after homogenization; Dt = 10 min; and
DA = A0 _ A10.

2.23 Least gelation concentration (LGC)

LGC was determined according to the method of [20] with slight modification. I gram sample were suspended in water at different concentrations (2% to 20%, w/v, protein weight basis). The mixture was vortexed, placed in a water bath at 95 °C for 1 h, cooled rapidly under tap water and left in the refrigerator (4 °C) for 2-14 h. The sample concentration at which the gel did not slip when the tube was inverted was taken as the LGC.

2.24 Determination of protein Dispersibility

Dispersibility was determined by using the method described by [22]. 10g of the samples were weighted into a 100ml measuring cylinders and water added to make up to 100ml. The set- up was stirred vigorously and allowed to stand for three hours. The volumes of settled particles were taken and subtracted from 100.

% Dispersibility =100 -Volume of settled particle

2.25 Protein solubility (PS)

The method by [32] was used and modified as follows. An aqueous solution (1% w/v) of protein sample was stirred for 30 min. With either 0.5 M HCl or 0.5 M NaOH, each solution was adjusted to the desired values (pH 3.0-8.0). The solution was centrifuged at 10000xg for 20 min. Modified Lowry method was used to determine the protein content using bovine serum albumin (BSA) as the standard. Percentage PS was expressed as: (protein content of each sample / total protein content) x 100. All determinations were carried out in triplicates.

2.26 In-vitro protein digestibility (IVPD) of Aduwa hydrolysates

In-vitro protein digestibility of the samples was measured according to the method described by Chavan *et al.* (2001). Two hundred and fifty milligrams of the sample were suspended in 15 mL of 0.1 M HCl containing 1.5 mg pepsin, followed by gentle shaking for 1 h at room temperature. The resultant suspension was neutralized with 0.5 M NaOH and treated with 4.0 mg pancreatin in 7.5 mL of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 24 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 [13] as described earlier.

Protein digestibility was obtained using the equation.

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

$$\text{where, I} = \text{protein content of sample before digestion}$$
$$\text{F} = \text{protein content of sample after digestion}$$

2.27 Mineral analysis of Aduwa hydrolysates

The analyses for essential mineral elements were carried out by the atomic absorption spectrophotometric method described by **[24]** with modification. The sample (0.5g) was weighed into 75 ml digestion flask and 5 ml digestion mixture (10ml HNO_3 and 10ml HCl) added and digested at 150 °C until the solution becomes clear. It was cooled and 30 ml of distilled water added. The tube was vigorously stirred. The blank sample was prepared following the procedure describes earlier but with exception of the sample. A sample aliquot was then transferred to the Autoanalyzer (Technicon AAU model) for total mineral analysis at 420 nm. The left-over digest was used to determine the other elements (calcium, magnesium, and iron) on the Atomic Absorption Spectrophotometer (Perkin Elmer, model 402) while sodium and potassium were determined by flame photometry.

2.28 Data analysis

The result of three replicate experiments were analyzed for mean ± standard deviation. A one-way analysis of variance (ANOVA) and the least significance difference (LSD- turkey test) were carried out. Significance difference was accepted at $P \leq 0.05$.

3. Results and discussion

3.1 Percentage yield of Aduwa protein hydrolysates.

The percentage oil recovery and material yield are shown in Table 1. The percentage material yield of pancreatin hydrolysate APHpa (45.9%) and pepsin hydrolysate **APHpe ((**37.4%) and APHpan+pe, (45.1%) respectively showed that APHpa and APHpan+pe had better yield when compared to enzymatic pepsin hydrolysate samples **APHpe**. This observation could be due to peptide molecular sizes, peptides bonds that are been attacked and broken by enzymes during hydrolysis. The pancreatin hydrolysate had higher material yield and could be more economically viable to processors than pepsin enzymatic hydrolyzed peptides.

3.2 Proximate composition of Aduwa hydrolysates

Proximate composition of Aduwa hydrolysates is shown in Table 2**.** Moisture content in food matrixes is one of the most important components of food processing and preservation. The moisture content is of direct economic importance to consumer, processor, and transporters. It is very significance; however, moisture affect the stability and quality of foods. The moisture content of the hydrolysate sample by all protease showed no significant difference at (p>0.05) The moisture content of APHpe was (8.33%), APHpan (8.91%) and APHpan+pe (8.96%) respectively.

The protein content of Aduwa protein hydrolysate by pancreatin, pepsin and combined enzymes- pancreatin and pepsin significantly (p>0.05) differ as sample protein hydrolysate are being made with different and combined enzymes. Crude protein content of Aduwa protein hydrolysate by combined enzymes APHpan+pe (80.50%) is significantly higher than separate enzyme hydrolysate, APHpan was (73.82%) and APHpe was (79.31%). The variation could be attributed to enzyme nature, activities, and possible cleaving site these proteases could have cleavage. peptides protein is an essential component of the diet required for the survival of both humans and animals. Aduwa protein hydrolysate can serve as a source of bio nutrient fortification. These could also serve as source of specific protein fractions for animal feed making at this hydrolysate state. Fats are macronutrients, along with carbohydrates and protein. Fat is an important foodstuff for many forms of life and serves as both structural and metabolic functions. They are necessary part of the diet of both humans and animals and the most efficient form of energy storage. The crude fat content of the hydrolysate samples is significantly different at (p>0.05). The pancreatin Aduwa hydrolysate has significantly high fat content at (p>005) than APHpen (0.18%) and APHpan+pe (0.14%).[25], reported (9.63%) fat content on desert date kernel and this result was supported by [26]. This reported value by [25] is however high and far above hydrolyzed Aduwa samples. The low-fat content of the hydrolysates is an indication that it can be a good source material for food products required at low fat mix.

The ash content of the hydrolyzed sample analyzed was found to be significantly different at (p>0.05). *Aduwa* protein hydrolysate by combined enzyme APHpan+pe (0.85%) are significantly (p>0.05) higher than APHpe (0.76%), and APHpan (0.64%) respectively. Since ash is the index of mineral content, the combined hydrolysate meal has mineral contents or profile that could be physiologically important.

The Aduwa hydrolysates analyzed in this study contain no amount of crude fiber and significantly (p>0.05) not different in all the samples ; APHpep (0.00%) , APHpan (0.00%) and APHpan +pe (0.00%) .The low values in crude fiber content could be because of the different proteases used on the concentrate samples .Low crude fiber content in nuts could lead to constipation if excess of it is being consumed as crude fiber enhances bowel movements Crude fiber is known to expand the inside walls of the colon, easing the passage of waste, and this makes it quite effective against constipation[27] .

Carbohydrates, alongside fats and proteins, are one of the three macronutrients in our diet with their main function being to provide energy to the body. The carbohydrate content of the Aduwa hydrolysates samples was significantly different. Aduwa APHpan (16.45%) has higher energy values significantly different at (p>0.05) than APHpan+pe (9.46%) and APHpep (11.46%). The low carbohydrate content in the hydrolysate sample might be due to the use of enzymes for hydrolysis, implying that Aduwa hydrolysate are not excellent source of carbohydrate rather peptides.

3.3 Mineral composition Aduwa hydrolysates

The mineral profile of protein materials from Aduwa is shown in Table 3. Mineral calcium was significantly p>0.05 high in APH pa+pe (0.92mg/100g) APM compared to APHpa (0.85 mg/100g) and APHpe (0.64 mg/100g). Potassium content in APH pa+pe (0.92mg/100g) is significantly higher than APH pe (0.64 mg/100g) and APHpa (0.85 mg/100g). Similar trend was observed in mineral sodium, iron and zinc: Soduim (APHpa2.06 mg/100g), APHpe(1.18 mg/100g) and APH pa+pe(2.09 mg/100g), Iron (APHpa (0.15 mg/100g), APH pe(0.20 mg/100g) and APH pa+pe (0.23 mg/100g), zinc APHpa(0.24 mg/100g), APH pe(0.12 mg/100g) and APH pa+pe(0.26 mg/100g) ,manganese(APHpa (0.96 mg/100g) , APH pe (1.08 mg/100g)and APH pa+pe (1.09 mg/100g) . Mineral cupper in APHpa (0.105 mg/100g) was significantly different at (p>0.05) compared to APHpe (0.03 mg/100g) and APH pa+pe (0.09 mg/100g). The results show that Aduwa meal hydrolysate samples are rich in potassium, calcium, sodium, and magnesium, while other mineral such as copper which can helps the body form collagen, absorbs iron, plays a role in energy production and zinc plays a role in wound healing as well as treatment to diarrhea. The findings in this study agree with similar findings reported by [26] Supplementing these protein materials could curb child and adult Tetany oesteomalacia and related diseases from due to lack of calcium. Potassium and sodium are electrolytes needed for the body to function normally and help in maintaining the fluid and blood volume of the body. Iron is a mineral that serves several important functions, its main function being to carry oxygen throughout our body and making red blood cells [28]

3.4 Anti Nutrients composition of Aduwa hydrolysates

Anti-nutrient composition of (Balanites aeqyptiaca del) *aduwa* enzymatic hydrolysate are shown in Table 4. The presence of alkaloid disappeared and are absent in all enzymatic hydrolysate samples. These variations in the understudy might be due to the treatments employed. Alkaloid is an antimicrobial bio active characterized by bitterness 29(Ogori *et al* .2019) however, the alkaloid is reduced to zero in Aduwa hydrolysate samples. However toxic at a very high amount and may have physiological activities [25].

Total phenol content TPC are conjugated bioactive materials but varies depending on exposed sites [29]. The phenolic content in this study decreased significantly**; APHpa (0.007**mg GAE/g**) APHpe (0.055** mg GAE/g**) and APHpa+pe (0.084**mg GAE/g**) respectively. The** results obtained in this study were lower than the values reported by [29] for soaked and roasted Aduwa samples. These indicates that enzymatic processing of *Aduwa* seed influenced phenolic profile content. The saponin contents of APHpa **(0.002**mg/g**), APHpe (0.011** mg /g**) and APHpa+pe (0.00mg**/g**) respectively** under this study were significantly low and safe below lethal levels. Tannin content under this study reduced significantly as material samples were resolved enzymatically **(**APHpa (0.044 mgCAT/g)**,** APHpe (0.022 mgCAT/g), APHpa+pe (0.009 mgCAT/g)**.** The phytate values under this study were completely absent APHpa(0.00mg/g), APHpe (0.00mg/g)and APHpa+pe(0.00mg/g. The phytate value obtained from Aduwa hydrolysates are lower than the lethal dose reported in other studies while the toxic effect of these anti-nutrients may not occur when these hydrolysates are consumed because their levels are not enough to elicit toxicity. Oxalate is another anti -nutrient moiety that causes intestinal. However, Oxalates were absent in all enzymatic hydrolysate samples. The values between 3-5mg/g have been pegged by [31] to be a lethal level. There was a significant decrease in hydrolysates, and these were within safety benchmark by[31]. Implying that enzymatic cleavage by pancreatin and pepsin had reducing effects on oxalate anti-nutritional factors

3.5 Functional properties of Aduwa (Balanaites aeqyptiaca del) seed meal, deffated meal, protein concentrate, isolate and hydrolysate.

The Bulk density, WAC, OAC, LGC, and dispersibility of (Balanaites aeqyptiaca.del) *Aduwa* hydrolysates are shown in Table 5. AHpan (1.02%) samples had significantly better packaging properties than APHpep (0.12%) and APHpan +pe (0.11g/ml), hence good weight and space relationship. The ability of biomaterial to absorb moisture and swell to a given capacity is determined by hydrophilic or hydrophobic site exposure on their biomolecules. Swelling index from the Table 5 revealed that Aduwa APHpa 1.02 g/mL are significantly high than APHpe (0.12%) and APHpe+pa (0.11). This variation could be attributed to enzyme or the protease activities. The ability of protein material micelles to hold water molecules depends on the conformational position of the protein material, size, and shape. [32]. According to [33] this behavior is attributed to the hydrophilic and hydrophobic balance of the residual amino acid in the material. The WAC in APHpe (1.72 g/g) is significantly high than APHpan+pe (0.17g/g) and APHpa (1.58g/g. The variation observed could be attributed to the hydrolysis process. The OAC decreased (p>0.05) from APHpa+pe(1.02)g/g to ,APHpa (1.72g/g) and then 1.73g/g in APHpe . The *Aduwa* hydrolysate by pancreatin and pepsin had the least OAC and are significantly different when compared to hydrolysate by combined enzymes APHpa+pe. However, this value did not agree with the value obtained from peas, chicken peas and lentils concentrate at these range (1.10-2.3g/g), [34] and walnut protein concentrate (2.50 g/g) [35]. This may suggest that *Aduwa* hydrolysate samples has good nonpolar amino acids, greater surface area of macro molecules, charges, and hydrophobicity properties. The LGC in APHpe (14.36) % and, APHpa (14.32) % are lower than APHpa+pe. The least gelation concentration of protein material confers gel formation through aggregation of denatured protein molecules. Gelation concentration helps in food system to ascertain degree of thickening and gelling especially in pudding and sources [36]. The ability of the hydrolysate samples to disperse easily in solution increased significantly at p>0.05, APHpa+pe (74.01%) had the higher value when compared to in APHpe (52.84) % and, APHpa (62.12) %. The reconstitution ability of pepsin and pancreatin hydrolysate in aqueous medium were low compared to APHpa+pe. This observation maybe due to their bonding sit resulting in their high percentage dispersion in water solution.

Sample	Quantity	Mean	Stdev	% Yield
APHpa (Pancratin Hydrolysate yield)	Q1=200g Q2=92g, Q3=91.6g Yield= Q2/Q1 =0.46 Yield= Q3/Q1 =0.458	46.0 45.8	0.1	45.9
APHpe (Pepsin Hydrolysate yield)	Q1=200g Q2=75.2g, Q3=74.4g Yield= Q2/Q1 =0.376 Yield= Q3/Q1 =0.372	37.60 37.2	0.2	37.4
APHpa+pe(Combined Hydrolysate yield)	Q1=500g Q2=222g, Q3=231g Yield= Q2/Q1 =0.444 Yield= Q3/Q1 =0.462	44.40 46.2	0.9	45.1

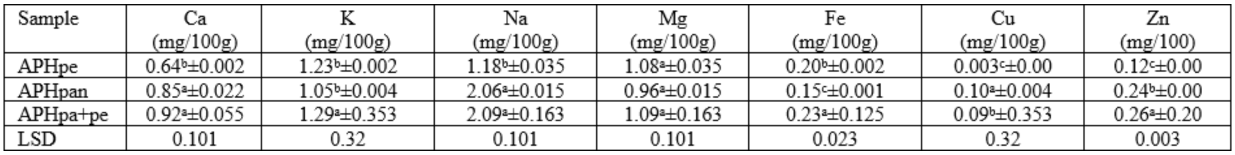
Key: APHpa= *Aduwa* protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysate by pepsin, APHpa+pe= *Aduwa* protein hydrolysate by pancreatin +pepsin combined

Table 1: Percentage Yield of *Aduwa* protein hydrolysates

Sample	Moisture Content (%)	Fat (%)	Crude Fiber (%)	Protein (%)	Ash content (%)	Carbohydrate (%)
APHpe	8.33^{b}±0.208	0.14^{c}±0.015	0.00^{a}±0.00	79.31^{b}±0.85	0.76^{b}±00.02	11.46^{b}±0.7
APHpan	8.91^{a}±0.051	0.18^{a}±0.012	0.00a±0.00	73.82^{c}±0.11	0.64^{c}±00.02	16.45^{a}±0.14
APHpa+pe	8.96^{a}±0.020	0.16^{b}±0.006	0.00^{a}±0.00	80.58^{a}±0.86	0.85^{a}±00.02	9.46^{c}±0.84
LSD	0.00	0.00	0.00	0.418	0.62	0.06

Mean value is from three determinations. Means followed by the same alphabetic on the column are not significantly different at p>0.05. Key. isolate APHpa= *Aduwa* protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysate by pepsin, APHpa+pe= *Aduwa* protein hydrolysate by pancreatin +pepsin combined

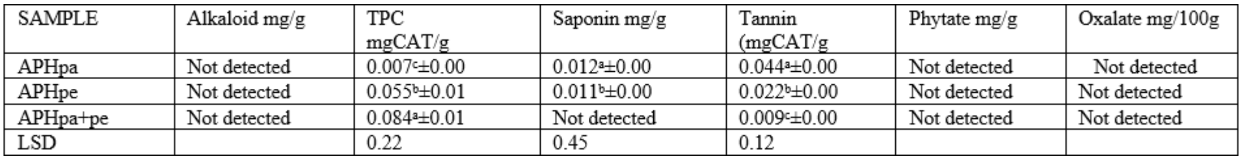
Table 2. Proximate Composition of Aduwa protein hydrolysates



Sample	Ca (mg/100g)	K (mg/100g)	Na (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Cu (mg/100g)	Zn (mg/100)
APHpe	0.64^{b}±0.002	1.23^{b}±0.002	1.18^{b}±0.035	1.08^{a}±0.035	0.20^{b}±0.002	0.003^{c}±0.00	0.12^{c}±0.00
APHpan	0.85^{a}±0.022	1.05^{b}±0.004	2.06^{a}±0.015	0.96^{a}±0.015	0.15^{c}±0.001	0.10^{a}±0.004	0.24^{b}±0.00
APHpa+pe	0.92^{a}±0.055	1.29^{a}±0.353	2.09^{a}±0.163	1.09^{a}±0.163	0.23^{a}±0.125	0.09^{b}±0.353	0.26^{a}±0.20
LSD	0.101	0.32	0.101	0.101	0.023	0.32	0.003

Mean values are readings from triplicate determinations; Means followed by the same alphabetic on the column are not significantly different at p>0.05 Key. AHPa= Aduwa protein Hydrolysates by pancreatin, AHpe= Aduwa protein hydrolysate by pepsin, AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Table 3: Mineral composition Aduwa hydrolysates



SAMPLE	Alkaloid mg/g	TPC mgCAT/g	Saponin mg/g	Tannin (mgCAT/g	Phytate mg/g	Oxalate mg/100g
APHpa	Not detected	0.007^{c}±0.00	0.012^{a}±0.00	0.044^{a}±0.00	Not detected	Not detected
APHpe	Not detected	0.055^{b}±0.01	0.011^{b}±0.00	0.022^{b}±0.00	Not detected	Not detected
APHpa+pe	Not detected	0.084^{a}±0.01	Not detected	0.009^{c}±0.00	Not detected	Not detected
LSD		0.22	0.45	0.12		

Mean values are readings from triplicate determinations; Means followed by the same alphabetic on the column are not significantly different at p>0.5

Key.APM=AHPa= Aduwa protein Hydrolystaes by pancreatin, AHpe= Aduwa protein hydrolysate by pepsin,AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Table 4. Anti -nutrients composition of Aduwa hydrolysates

Sample	Bulk density (g/ml)	Swelling index (g/ml)	WAC(g/g)	OAC(g/g)	LGC (%)	Dispersibility (%)
APHpa	1.02^{a}±0.15	3.45^{a}±0.50	1.58^{b}±0.06	1.72^{b}±0.15	20.39^{a}±0.6	62.12^{b}±0.15
APHpe	0.12^{b}±0.15	0.22^{b}±0.21	1.72^{a}±0.05	1.73^{a}±0.50	14.36^{b}±0.62	52.84^{c}±0.14
APHpa+pe	0.11^{c}±0.10	0.15^{c}±0.10	0.17^{c}±0.12	1.02^{c}±0.02	14.32^{b}±0.48	74.01^{a}±0.42
LSD	0.000	0.0007	0.00017	0.0000	0.9000	1.1600

Mean values are triplicate determinations: Means followed by the same alphabetic on the column are not significantly different at p>0.05 Key. APHpa= *Aduwa* protein Hydrolysates by pancreatin, APHpe= Aduwa protein hydrolysate by pepsin,APHpa+pe= *Aduwa* protein hydrolysate by pancreatin +pepsin combined

Table 5. Functional properties of Aduwa hydrolysates

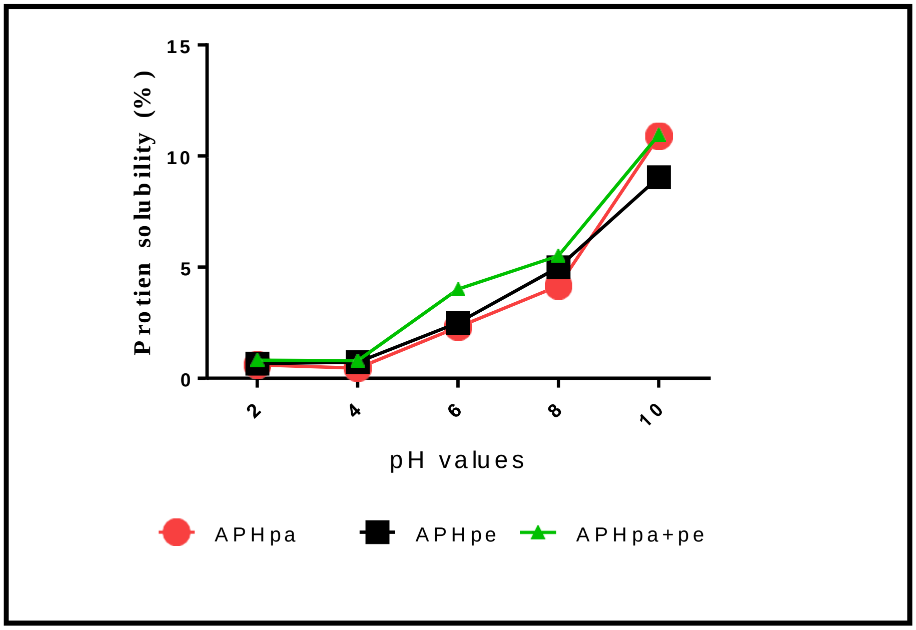
Samples	Protein content before digestion (%)	Protein content after digestion (%)	*In-vitro* protein digestibility (%)
APHpan	54	7.43	84.93 ^{b}± 0.10
APHpep	27	5.34	81.58 ^{c}± 0.20
APHpan+pep	61	6.95	89.53 ^{a}± 0.01
LSD	33.23		

Mean values are readings from triplicate determinations; Means followed by the same alphabetic on the column are not significantly different at p>0.05 Key. AHPa= Aduwa protein Hydrolystaes by pancratin, AHpe= Aduwa protein hydrolysate by pepsin, AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Table 6. Invitro protein digestibility of *Balanites aeqyptiaca.* del Aduwa hydrolysates

3.6 Protein solubility of Aduwa hydrolysate at different pH

Figure 1 shows the solubility profile of *Aduwa* hydrolysate with respect to different pH (3, 5, 7 and 9) values. The results showed that the samples were most soluble at pH 4.0. The percentage soluble peptides decreased progressively as the pH value was adjusted from 4 to 8. The results showed that protein hydrolysate by combined enzyme, pancreatin and pepsin were least soluble at the very acidic pH value (pH 2-4). Ordinarily, the protein hydrolysates were expected to show better solubility at the acidic pH, but the low solubility of the enzymatic Aduwa hydrolysates and at pH 2 when compared to the Aduwa protein meal may be attributed to high protein aggregation at the pH value, which reduced the solubility. Similar pattern of results was reported for okra seed meals and protein isolate [37]. Beyond pH 4.0, hydrolysate samples did show marked difference in the solubility, even as the pH value increased from 3-9. The protein hydrolysate had lowest protein solubility at pH 4.0 and thereafter increased progressively till pH 9.0, which agreed with the pattern of results reported for walnut protein [35]. The low values in solubility of the hydrolysates at pH 4.0 have helped to justify the iso-electric point of the hydrolysates. Usually, solubility decreases as the pH increases until it reaches the isoelectric point. The loss of electrostatic repulsive forces provides beneficial conditions for the formation of protein aggregates; high bulk density and large diameter of the aggregates results in precipitation of protein [38]. The difference between the pattern of solubility in protein hydrolysates may be due to the enzymatic hydrolysis. However, the low values and the pattern of solubility of the hydrolysate samples may be a disadvantage when considering its use as ingredients in acidic drinks.



Key.APM= API= aduwa proten isolate AHPa= Aduwa protein Hydrolysates by pancratin , AHpe= Aduwa protein hydrolysate by pepsin,AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Figure 1: Protein solubility of Aduwa protein isolate and hydrolysate at different pH

3.7 Invitro protein digestibility of Balanites aeqptiaca del Aduwa protein hydrolysates

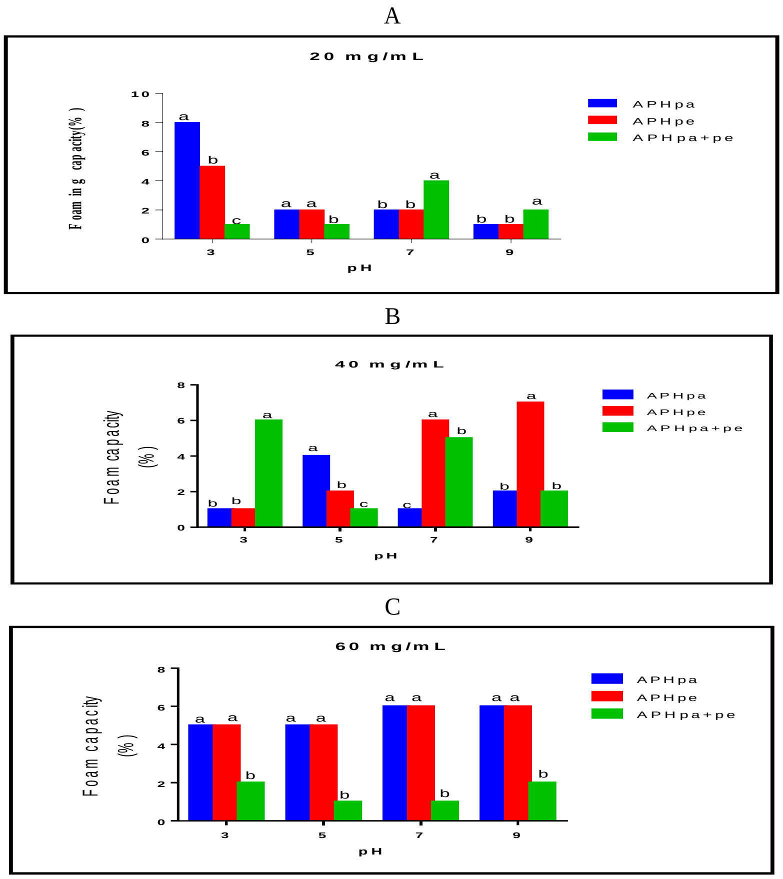
In-vitro protein digestibility of *Balanites aeqptiac*a del Aduwa hydrolysate is shown in Table 6. The invitro protein digestibly increased significantly in APHpa+pe (89.53%) compared to APHpan (84.93%), and APHpe (81.58%). The high value of protein digestibility observed in hydrolysate samples may be due to their peptide fractions release, The Increase in *in vitro* protein digestibility experienced in hydrolysate samples may be due to the reduction in the levels of antinutritional factors.

3.8 Foaming capacity of Aduwa hydrolysates at differentconcentration and pH

Figures 1a, b and c 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 the sample concentration of 20 mg/ml, the APHpe has high foaming capacity at pH3.0 and pH 5.0 respectively while the least foaming capacity was obtained at APHpa+pe and APHpan. The foaming capacity of Aduwa pancreatin hydrolysate APHpa and *Aduwa* pepsin hydrolysate APHpe decreased progressively as the pH of the solution increased from 3-9 at 20 mg/mL. The pattern was different in combined enzyme hydrolysate whereby the foaming capacity of the samples were relatively stable as the pH of the samples increased towards the basic region. The pattern of the results on enzymatic hydrolysate samples is in line with the increase in the net charge of the samples at the neutral and basic region, with the potentials to increase the net charge which eventually resulted in increase in 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 the high pH values. Similar pattern of results was observed in the foaming capacities of fenugreek seeds, bambara seed and walnut isolated proteins [35] . As the sample concentration was increased from 20 to 60 mg/ml, an increase in the foaming capacity of the APHpe was observed, basically in pH values 3, 7 and 9 but the foam formation at pH 5.0 remain substantially stable. For the APHpa and APHpe, an apparent increase in the foam capacity of the samples were observed up-to 60 mg/ml but decreased in values afterwards. A possible explanation for this pattern may that of protein crowding in APHpa result in from protein protein interactions. Although, an increase in the protein concentration is necessary to generate adequate foams; increase beyond 40 mg/ml may lead to generation of excess protein micelles that reduced the capacity to generate foams in the pancreatin and pepsin hydrolysate [39]

3.9 Foaming stability of Aduwa hydrolysates at different concentration and pH

Foaming stability is the ability of foam to keep its shape and volume over a specified period. 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* enzymatic hydrolysate with respect to variations in sample concentration (20, 40 and 60 mg/mL) and pH (3, 5, 7 and 9) values is shown in Figures 2 a,b and c.



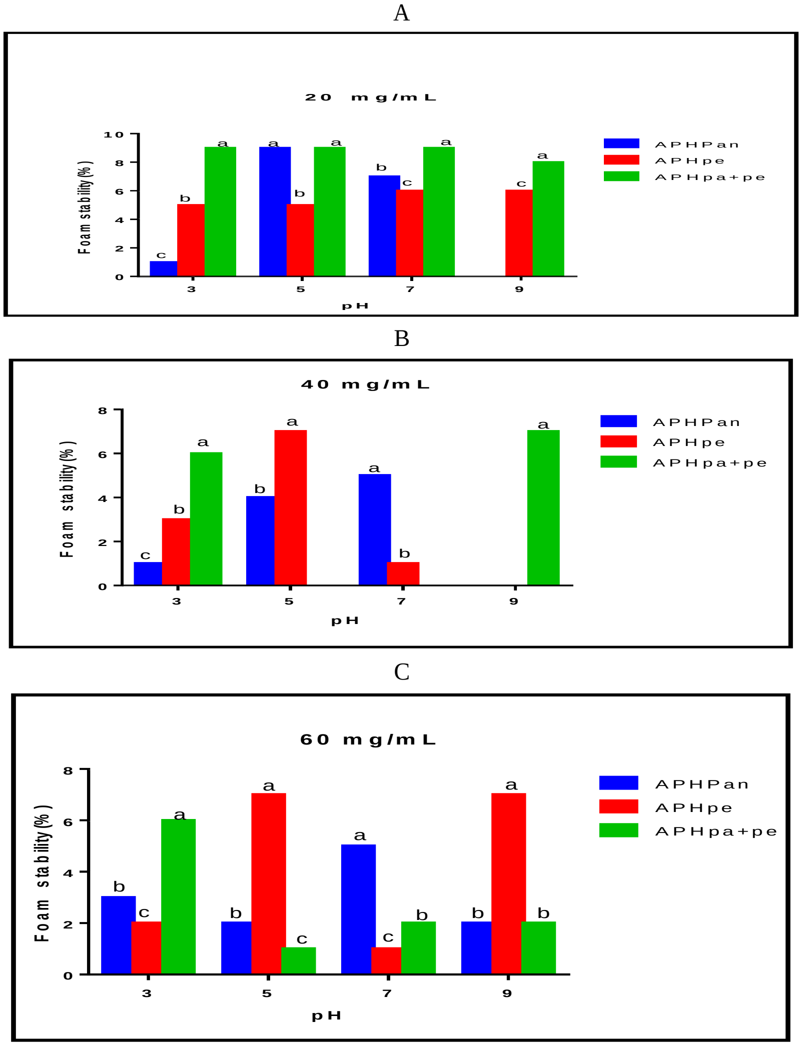
Key. AHPa= Aduwa protein Hydrolysates by pancreatin, AHpe= Aduwa protein hydrolysate by pepsin,AHpa+pe= Aduwa protein hydrolysate by pancreatin +pepsin combined

Figure 2. a,b and c :Foaming capacity of aduwa hydrolysates at different concentration and pH
Means are readings from triplicate determinations. Means followed mean followed by the same alphabetic on the bars are not significantly different at p>0.05

At 20 mg/mL sample concentration, the foam became increasing stable at the acidic pH (3 and 5) but increased progressively as the pH moved towards the basic region (7 and 9) for enzymatic hydrolysate samples. The results also revealed that values obtained for the foam stability were high in the APHpa+pe, APHpe and AHpa sample at 20 mg/mL sample concentration. This observation or pattern in enzymatic hydrolysate samples may be attributed to the formation of stable molecular layers in the air-water interface that could have enhanced greater impartation of texture, stability, and more elasticity of foams. Similar pattern of results was reported for rapeseed by [40] . As the 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 of 3 and 5 and this may suggest production of adequate charge densities at these pH values by the protein molecules which had made charges available to participate in the formation of strong interfacial membrane [41].At another observation, the result also showed that the foam stability was higher at sample concentration of 60 mg/ml, at a high pH values which also may suggest that the increase in the sample concentration is desirable in such that more protein molecules are produced to enhance the intermolecular cohesiveness of the foams formed [33]. The samples exhibited different pattern of foam stability with respect to the pH and varied sample concentration, which may be related to differences in the structural properties of the samples, especially the surface hydrophobicity in hydrolysate samples.

3.10 Emulsifying activity of aduwa hydrolysates at different concentration and pH.

Figures 3a, b and c show the emulsion activity of Aduwa protein enzymatic hydrolysate as functions of variations in pH (3,5 7 and 9) and concentrations of sample at 10, 15 and 50 mg/mL. At 10 mg/mL, the emulsion capacity of the APHpa, decreased from 3-9mL when compared to APHpe and APHpa+pe. The result also showed that the emulsion capacities of the enzymatic hydrolysate samples were low at high concentration of (25mg/mL and 50 mg/mL), respectively, this may be attributed to the release of excess protein molecule which may have resulted in protein overcrowding or interaction and disruption in the interfacial properties [41].



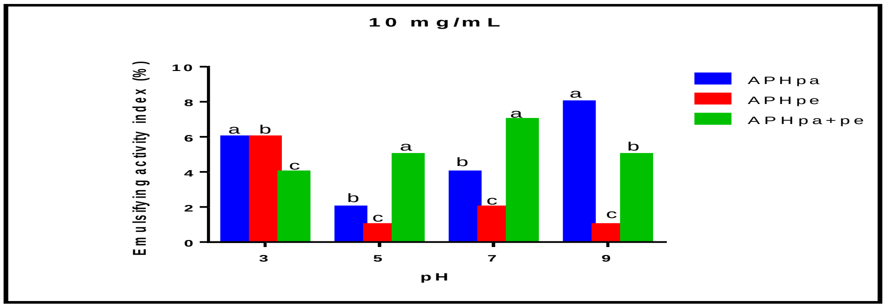
Key. AHPa= Aduwa Hydrolystaes by pancratin AHpe= Aduwa hydrolysate by pepsin,AHpa+pe= Aduwa hydroysate by pancreatin +pepsin combined

Figure 3: a,b and c :Foaming Stability of aduwa hydrolysates at different concentration and pH.Means are readings from triplicates determinations. Means followed mean followed by the same alphabet on the bars are not significantly different at p>0.05

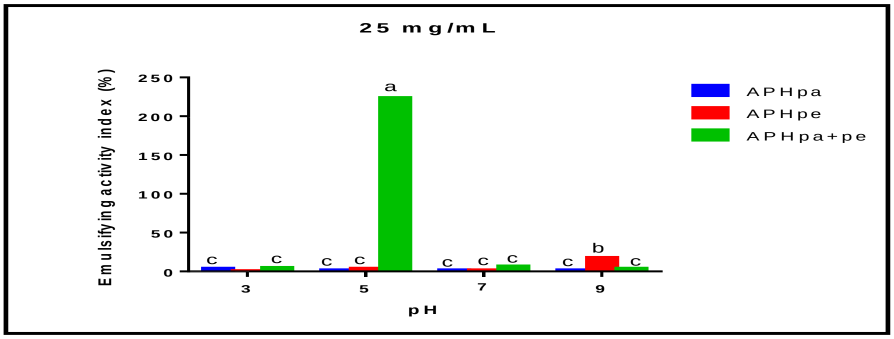
3.11 Emulsifying stability of Aduwa protein meal, concentrate, isolates and hydrolysates at different concentration and pH

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 [33]. Figures 4a, b and c show the emulsion stability of enzymatic hydrolysate as function of varied pH (3,5 7 and 9) and sample concentrations 10, 25 and 50 mg/mL respectively.

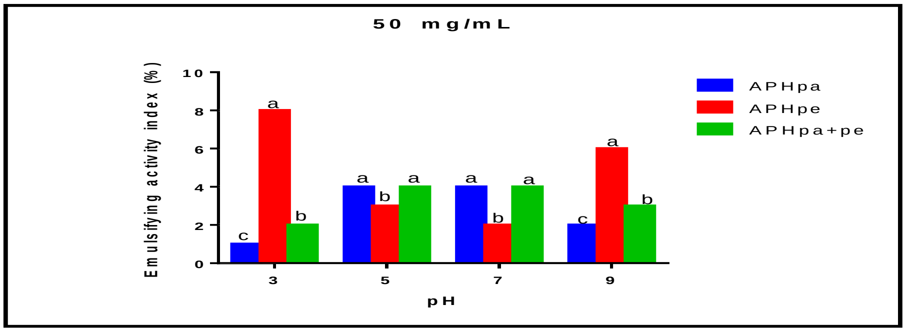
A



B



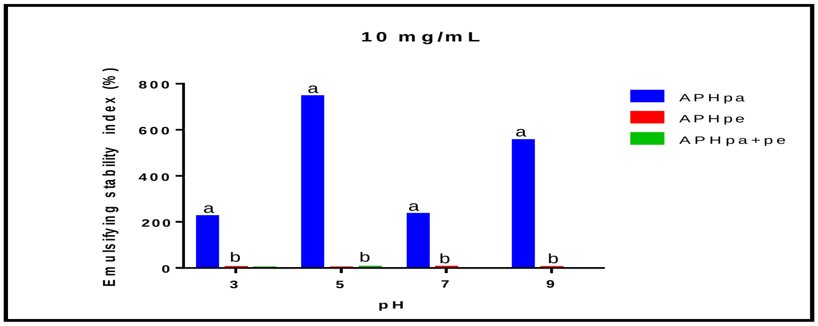
C



Key.AHPa= Aduwa protein Hydrolysates by pancreatin, AHpe= Aduwa protein hydrolysate by pepsin,AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Figure 4 a-b: Emulsifying activity of Aduwa protein hydrolysates at different concentration and pH**.** Means are readings from duplicate determination. Means followed mean followed by the same alphabet on the bars are not significantly different at p>0.05

A



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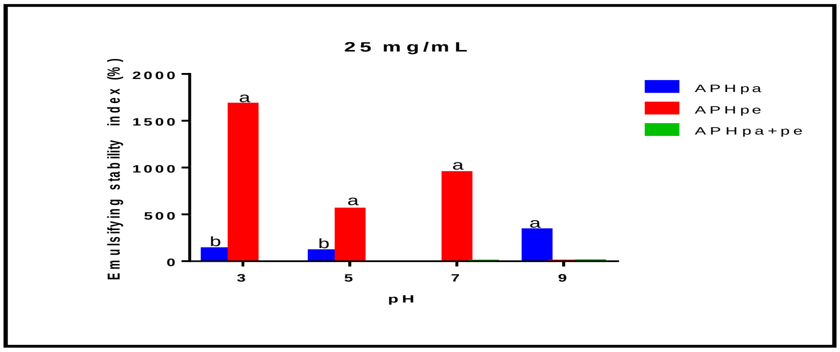

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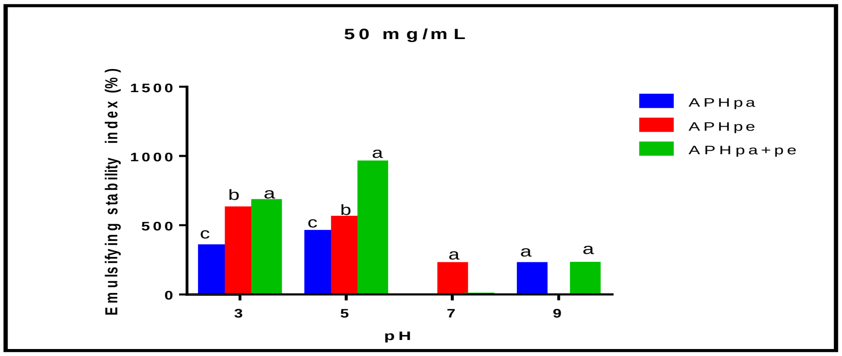

Key.APM= AHPa= Aduwa protein Hydrolystaes by pancratin ,AHpe= Aduwa protein hydrolysate by pepsin,AHpa+pe= Aduwa protein hydroysate by pancreatin +pepsin combined

Figure 5.a-b: Emulsifying stability of Aduwa protein meal, concentrate, isolates and hydrolysates at different concentration and pH. Means are readings from triplicate determinations. Means followed mean by the same alphabet on the bars are not significantly different at p>0.05

The emulsion stabilities of hydrolysate APHpa was high at 10 mg/ml, but the highest emulsion stability was obtained at pH 5 and pH9. But the emulsion formed at pH3, for APHpe was strong at 25 mg/mL sample concentration. However, at sample concentration of 50 mg/mL, pH 5.0 exhibited stronger APHpa+pe emulsion activities than at pH 3. The pattern of the emulsion stabilities in this study for the samples showed that sample concentration of 10 mg/mL and 50mg/mL are the threshold concentration for samples to create enough interfacial tensions to stabilize the emulsion formed by these hydrolysate samples.

4. Conclusion

The proximate, mineral, and phytochemical propertied of the combined hydrolysate had better advantages over the pancreatin and pepsin hydrolysate samples. The solubility of enzymatically hydrolysate for both single and combine hydrolysate samples compounds from Aduwa at alkaline pH, showing average solubility score. WAC, OAC and LGC was improved only in the pepsin hydrolysate compound. The hydrolysate digest obtained pancreatin and pepsin show a significant difference relative to combined hydrolysate. Emulsifying properties were not improved, as proteases were combined. The crude protein, content IVPD and zero anti nutrient levels obtained from combined enzymatically hydrolysate digests shows that when included as ingredients in other food products, they will improve nutritional quality, as they carry relevant amounts of peptides.

Conflict of Interest

The authors hereby declared no conflict of-interest

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