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Hydrolysate Yield, Surface Hydrophobicity and Amino acid Properties of Desert Date (*B.aegyptiaca Del)* Protein Hydrolysates.

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Abstract

Desert date, called B.aegyptiaca Del commonly wild in desert regions of the world. The seed was processed into Aduwa Protein Concentrate (APC) and afterward hydrolyzed using two combined endogenous Pepsin-Pancreatin (PP) proteases and two different exogenous proteases (Alcalase and flavourzyme) to respective hydrolysates and analyzed using standard methods. The result of the analysis revealed that Alcalase hydrolysate had a higher protein yield (99%) compared to APC (62.22%), PP (83.23%) and flavourzymes (62.90%). The protein content and surface hydrophobicity of Alcalase hydrolysate were above the reference control and the other hydrolysates. The excellent surface-related properties exhibited by the Alcalase hydrolysate were below the APC reference control. The PP hydrolysate had higher content of hydrophobic amino acid and sulphur-containing amino acid (40.19%, 7.04%) compared to Alcalase (38.92%, 6.69%), Flavourzyme (37.43%,6.35%), and APC (39.97%, 6.95%) samples. The hydrolysates of PP and Alcalase have better amino acid residues and surface-related properties and could be employed as ingredients in the nutraceutical and functional food industries. Desert date called *B.aegyptiaca Del* commonly wild in desert regions of the world. The seed was processed into Aduwa Protein Concentrate (APC) and afterword hydrolyzed using two combined endogenous Pepsin-Pancreatin (PP) proteases and two different exogeneous proteases (Alcalase and flavourzyme) to respective hydrolysates and analyzed using standard methods. Result of analysis revealed that Alcalase hydrolysate had higher protein yield (99%) compared to APC (62.22%), PP (83.23%) and flavourzymes (62.90%). The protein content and surface hydrophobicity of Alcalase hydrolysate were above the reference control and the other hydrolysates. The excellent surface related properties exhibited by the Alcalase hydrolysate was below the APC reference control. The PP hydrolysate had higher content of hydrophobic amino acid and sulphur containing amino acid (40.19%, 7.04%) compared to Alcalase (38.92%, 6.69%), Flavourzyme (37.43% ,6.35%), and APC (39.97%, 6.95%) samples. The hydrolysates of PP and Alcalase has better amino acid residues and surface related properties and could be employed as ingredients in the nutraceutical and functional food industries.

Keywords; Desert date, concentrate, yield, enzymes, Hydrolysate.

Introduction

Nowadays, there is a wide interest in the effects of processing on the chemical composition and amino acid profile of seed nuts. Many bioactive compounds with nutraceutical potentials present in nut seed as peptides have captured researchers' interest worldwide (Malomo et al., 2020; Ogori et al., 2022 and Fanrui et al.,2020). Biologically active peptides and proteins are either naturally produced by enzymatic digestion, fermentation, germination, or enzymatic hydrolysis. Protein hydrolysis is an enzyme-aided digestion that can release shorter-chain compounds or peptides with lower molecular mass. Protein hydrolysates are physiologically active than intact proteins, because their intestinal absorption appears to be more effective due to the increased solubility of proteins Tang et al., 2010) and small surface area in relation to absorbing surface of the visceral lining. They are also considered as a potential dietary source of natural antioxidants with intact biological functions (Darmawan et al., 2010; Ogori et al., 2018). Balanites aegyptiaca, also known as Aduwa in the Hausa language from Northern Nigeria. The hydrolysis of Aduwa protein concentrate (APC) with pepsin and pancreatin proteases produced peptides with good phytochemical properties (Badu et al., 2020; Ogori et al.,2022). Balanites aegyptiaca belongs to the family of Zygophyllaceae and has been widely used as a medicinal herb, food and food material in households around the desert areas of the world (Mohammed et al., 2002; Obidah et al.,2010 and Badu et al., 2020). Numerous studies have reported higher levels of amino acids, protein, carbohydrates, minerals and medicinal phytochemicals and lower levels of anti-nutritive factors in Aduwa seed when toasted as compared to when cooked, boiled and raw (Datti et al., 2020 and Sabo et al., 2014). Reports of food protein-derived peptides acting as antihypertensive agents by inhibiting hypertension (Solis et al.,2018), renin activities (He et al., 2013; Girgih et al., 2014) and antioxidative properties (Ma et al., 2015) have been opined. Despite the physiological importance, the traditional folklore medicine believes that Aduwa seed has been used as a component in ancient medicines to treat diabetes, hypertension, anti-aging, wound healing, maybe because of its active peptide compounds (Chao et al.,2001; Admassu et al., 2013 and Okia et al., 2011). To the best of our knowledge, no work has been performed to evaluate yield, surface hydrophobicity and amino acid properties of protein hydrolysates by pepsin-pancreatin combined protease,

alcalase and flavourzymes prepared from Aduwa protein concentrate (APC). From another study, it was found that the formations of bioactive compounds for physiochemical activity were obtained by only endogenous (pepsin and pancreatin) separate hydrolyzing enzymes on Aduwa protein concentrates, Ogori et. al.(2022). However, not with endogenous combined (pepsin-pancreatin) enzyme and exogenous (Alcalase and Flavoenzymes) hydrolyzing enzymes comparatively. Therefore, this study was to determine the effect of B. aegyptiaca Del protein concentrate and hydrolysates using exogenous and endogenous hydrolyzing enzymes on the production of bioactive peptides that will have better physiochemical properties for food formulation, thereby encouraging the tree propagation by farmers. Drive the tree's importance as a fruit seed that could also combat protein energy malnutrition.

Materials and methods

Source of raw materials.

The mature seeds of B. aegyptiaca (Aduwa) used for this study were bought from Gashua market in Yobe State of Nigeria, and were immediately transported to the Department of Biological Science for lot identification and then to the biochemistry laboratory of the Federal University Gashua. The sample seeds were sorted, toasted, Milled and packaged to the Department of Food and Human Nutritional Sciences, University of Manitoba, Canada, for physiochemical analysis. See Fig.1

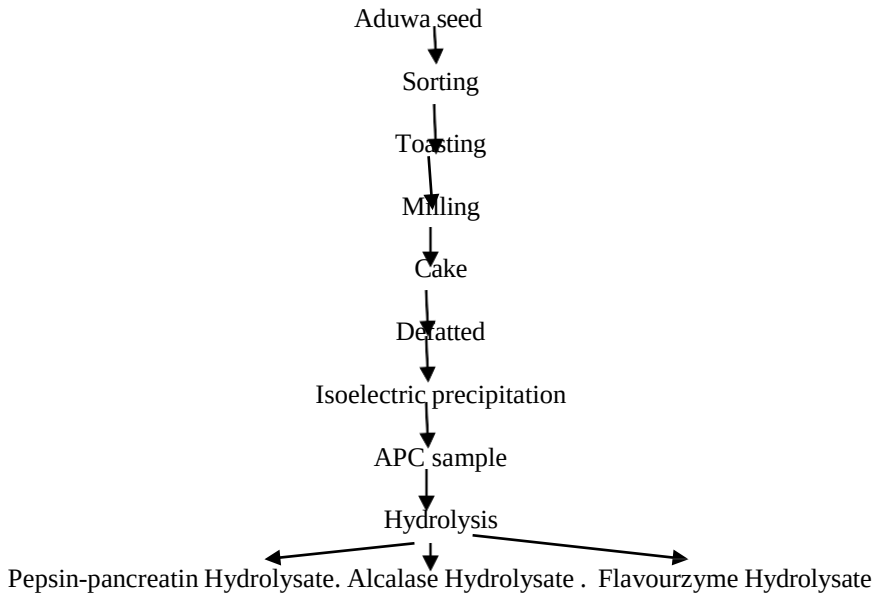


Figure 1:Process design of making Hydrolysate from Aduwa

Isoelectric precipitation method of B.aegyptiaca Del in producing concentrate.

The method of Osemwota et al. (2021) was adopted. The defatted Aduwa meal was mixed with double-distilled water at a 1:20 (w/v) ratio for solubilization at pH 10 using 1 M NaOH. The mixture was allowed to be stirred for 1h before centrifugation at 31000× g for 30 min. The supernatant was collected, filtered with cheesecloth, and adjusted to pH 4.5 with 1 M HCl, stirred for 30 min, and then centrifuged again. The collected precipitate was washed with running water to remove non-protein materials. The mixture was centrifuged again to obtain the final mix when double-distilled water was added and adjusted to pH 7.0. The slurry was freeze-dried as Aduwa protein concentrate (APC) .Production of B.aegyptiaca Del protein hydrolysate using pepsin and pancreatin combined enzyme

The method by Girgih et al. (2011) was used. A 40g isolate sample of 1:2 w/v slurry was adjusted to 37 °C incubation temperature and then given a pH 2, followed by the addition of

pepsin enzyme 3.04% w/w based on the molecular protein content of APC. The digestion was allowed for 2h at a constant pH of 2 with I M HCl. Pancreatin digestion starts by giving an incubation temperature of 40 °C, adjusted to pH 7.5. Pancreatin enzyme 4% w/w was added. The digestion was allowed for 2 hrs. at a constant pH of 7.5 with I M NaOH. The final digested mixture was terminated by adjusting the pH to 4.5, and then the mixture was placed in a boiling water bath at 95°C for 15 min to completely in actives the enzymes by denaturization. The mixture was cooled to room temperature and later centrifuged, and the supernatant was collected and freeze-dried. Production of B.aegyptiaca Del protein hydrolysate using Alcalase enzyme. The method of Aluko and Moni (2003) was adopted. A 40g isolate sample of 1:2 w/v slurry was given an incubation temperature of 50 °C, adjusted to pH 9 using 2M NaOH. Alcalase enzyme 3.04% w/w, based on the molecular protein content of APC, was added. The digestion was allowed to stir gently for 4 h at a constant pH of 9 with 2M NaOH. The digestion was terminated after 4 h by adjusting the pH to 4, and then the mixture was placed in a boiling water bath at 95°C for 15 min to completely inactives the enzyme pepsin by denaturization. The mixture was cooled to room temperature, centrifuged, and the supernatant was collected and freeze-dried.

Production of B.aegyptiaca Del protein hydrolysate using Flavourzyme

The method of Aluko and Moni (2003) was adopted. A 30g isolate sample of 1:2 w/v slurry was given, and an incubation temperature of 50 °C was adjusted to pH 6.5 using 2M NaOH. Flavoenzyme enzyme 3.04 % w/w, based on the molecular protein content of APC, was added. The digestion was allowed to stir gently for 4 h at a constant pH of 9 with 2M NaOH. The digestion was terminated after 4 h by adjusting the pH to 4.5 using 2 M HCl, and then the mixture was placed in a boiling water bath at 95°C for 15 min to completely in actives the enzyme pepsin by denaturization. The mixture was cooled to room temperature and later centrifuged, and the supernatant was collected and freeze-dried.

Materials and methods

Protein determination by the Lowry method

The Lowry et al. (1951) method was adopted. One mg/ml of the soluble filtrate of B. aegyptiaca Del concentrate and hydrolysates was pipetted with the addition of 3 ml of Lowry’s reagent C was dissolved and made to

the mark with distilled water in a 100 mL standard flask; Reagent D: (4 % CuSO4.5H2O) 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 Ciocalteu phenol was added to the mixture and mixed vigorously using a 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 a spectrophotometer 2000Sp (model S2000/S4000 Skyam Germany. Bovine Serum Albumin (standard) was prepared similarly to the samples but at different concentrations (1-100µg/mL). The standard curve obtained was used to find the protein concentration of the sample.

Surface hydrophobicity of B.aegyptiaca Del protein concentrate and hydrolysate

Surface hydrophobicity of B.aegyptiaca Del protein concentrate and hydrolysate as outlined by Karaca et al. (2011), using ANS(1-anilino-8-naphthalenesulfonate) (ANS) as a litmus. The (10 mg/mL) stock solution of protein concentrate and hydrolysates was prepared (based on protein weight) dispersed in a 0.1 M sodium phosphate buffer (pH 7.0) for 1h, and then centrifuged at 10,000× g for 10 min. The collected supernatants were each diluted to final concentrations of 50, 100, 150, 200, and 250 µL/mL. For each sample, a 20 µL aliquot of 0.8 M ANS solution prepared in 0.1 M sodium phosphate buffer at pH 7.0 was added. Thereafter, the FI of each mixture was measured at excitation and emission wavelengths of 390 nm and 470 nm, respectively, using a spectrofluorometer FP-6300 (Jasco). Fluorescence values (Fv) for the mixtures without ANS were subtracted from those of the respective protein solutions containing ANS. The slope (S0) of the plot of Fv versus protein concentration was calculated by linear regression analysis and used as an index of the protein surface hydrophobicity.

Amino Acid Composition

Amino acid profile of the protein concentrate and hydrolysate was analyzed using an HPLC system with a pico-tag column after being digested with 6 M HCl for 24 h Bidingmeyer et al., 1984). The sulphur-containing amino acids (cysteine and methionine), as well as tryptophan, were then determined after performic acid oxidation and alkaline hydrolysis, respectively, Landry and Delhaje (1992).

Results and Discussion

Hydrolysate yield, protein content and S0 of protease hydrolysate from pepsin and pancreatin, Alcalase and Flavourzyme enzymes. The percentage material yield from APC, endogenous and exogenous hydrolyzing enzymes are shown in Table 1. The results showed that Alcalase had significant differences at a $p < 0.05$ probability level, higher yield than the concentrate of APC (62.22%), flavourzyme (62.90%), and the least was in combined enzyme peptin-pancreatin (22.40%). Hydrolysate from Alcalase had a better peptide yield compared to the other samples. This observation could be due to the wider specificity of Alcalase cleavage ability Fanrui et al., 2022) compared to pepsin-pancreatin, which could cleave N-terminal and C-terminal peptide bonds. Crude protein content of Alcalase hydrolysate (91.29%) is significantly higher than Flavourzyme (84.30%), pepsin-pancreatin (83.23%) and APC (74.05%). This high protein variation between hydrolysates and APC could be attributed to the degree of bond breakage or cleaving during processing. The extent of protein interaction results in a peptide bond shift, also defined as So. These differences in So observed in different samples in Table 1 below could be attributed to their peptide bonds and exposed bonding sites. The high So in APC (1023.8) compared to Alcalase hydrolysate (580.00), flavourenzyme (154.0) and the least from PP (151.00) may be due to process denaturation caused by isoelectric precipitation compared to the bond cleaving by the endogenous and exogenous hydrolyzing enzymes. The hydrolysate by Alcalase, flavourzyme had higher protein and material yield and could require less process economy than PP and APC.

Percentage of amino acid composition of B.aegyptiaca Del hydrolysate from pepsin-pancreatin, Alcalase and Flavourzyme proteases The amino acid profile of the protein concentrate sample and the hydrolysates is shown in Table 2. Hydrolysis did not substantially alter the constituents of amino acids. No huge differential changes were observed in the amino acid composition of the hydrolysates when compared to the concentrate APC, except, isoleucine and histidine content in the hydrolysates. In addition, the content of phenylalanine, tyrosine was also considerably higher in the hydrolysate samples when compared to APC. The contents of these flavor amino acids, aspartic acid/asparagine and glutamic acid/glutamine were high compared to the contents of APC which confirms their abundance in seed storage proteins Kazir et al.

(2019). The content of aromatic amino acids ranged between 16.38% -15.24%. Hydrolysis, however, resulted in increased contents of hydrophobic amino acids from APC (39.97%) to pepsin-pancretin (40.99%) compared to Alcalase (38.92%) and Flavourzyme (37.43%) hydrolysates, respectively. Isoelectric pH-precipitated B.aegyptiaca Del seed protein concentrate amino (50.47%) produced high contents of essential acids compared to pepsin-pancreatin hydrolysate (49.62%) and flavourzyme hydrolysate (49.83%), but Alcalase Hydrolysate had higher EAA (50.11%) content. The observed difference in EAA content may be due to the nature of the enzymes employed during hydrolysis. The high values of glutamate, asparagine, and arganin amino acid fractions in hydrolysate samples, where glutamate and asparagine donate electrons (strong antioxidants) and arganin acts as a good NO-precursor (Jakubczyk and Baraniak, 2014), were found to be higher in the hydrolysate samples compared to the APC sample. Study had revealed similar levels of glutamate and arginine that have been previously reported for cashew fractions (Liu et al., 2018). B.aegyptiaca Del hydrolysates is a rich source of hydrophobic amino acids, Aromatic amino acids, Essential Aromatic acids and sulphur-containing amino acids, and these could contribute to antioxidant potentials (Magana et al., 2015) of the ingredient produced. The quality of essential amino acids in Aduwa protein concentrates and their hydrolysates, compared similarly with the contents of amino acids as recommended by FAO (Tidjani et al., 2011)

Conclusion

This study has revealed the effect of different endogenous and exogenous protease enzymes in relation to peptides yield, hydrolysate surface-related properties and amino acid residues. The conversion of APC into Alcalase hydrolysate revealed better encrypted peptides and excellent physiochemical properties of the Alcalase hydrolysate, which may be attributed to Alcalase specificity. Alcalase and pepsin-pancreatin hydrolysates showed excellent essential amino acid profiles as well as good antioxidant properties. Due to the rich bioactives in desert date fruit, its tree afforestation should increase among other desertification mitigations and economic benefits. B.aegyptiaca Del seed hydrolysates and tree plants are a potential source of bioactive peptides and an economic desert mitigant.

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Table 1: Hydrolysate yield, protein content and S_0 of protease hydrolysate from pepsin- pancreatin, Alcalase and Flavoenzyme enzymes**.**

Sample	Hydrolysate yield (%)	Protein content (%)	Surface hydrophobicity
APC	62.22^{b}±1.24	74.05c±1.06	1023.8^{a}±0.00
PP	22.40^{c}± 0.0	83.23^{b}±1.95	151.00^{c}±0.00
Alcalase	99.00^{a}±0.01	91.29^{a} ±5.28	$580{\cdot}00^{b}$±0.00
Flavourzyme	62.90^{b}±2.99	84.30^{b} ±4.88	$154\ .00^{c}$±0.00

Key: APC= Aduwa protein concentrate , PP= pepsin-pancreatin hydrolysate

Table2. Percentage amino acid composition of *B.aegyptiaca Del* hydrolysate from pepsin- pancreatin, Alcalase and Flavoenzyme proteases

AA	APC	PP	Alca	Flav	FAO
Asx	4.95 ± 0.06c	5.06 ± 0.01^{b}	5.06 ± 0.13^{b}	5.27 ± 0.03^{b}	
Glx	10.96 ± 0.13a	10.79 ± 0.02^{a}	11.56 ± 0.39^{a}	11.65 ± 0.06^{a}	
Histidine	1.25 ± 0.00e	1.23 ± 0.02^{d}	1.32 ± 0.06^{c}	1.35 ± 0.00^{d}	1.9
Serine	4.95 ± 0.06c	2.03 ± 0.07^{c}	1.85 ± 0.08^{c}	1.36 ± 0.07^{d}	
Arginine	1.88 ± 0.11d	3.90 ± 0.02^{b}	3.79 ± 0.09^{b}	3.74 ± 0.02^{c}	
Glycine	3.80 ± 0.05c	13.91 ± 0.09^{a}	14.41 ± 0.52^{a}	15.14 ± 0.00^{a}	
Threonine	14.15 ± 0.16a	20.48 ± 0.11^{a}	20.71 ± 0.73^{a}	21.76 ± 0.09^{a}	1.4
Alanine	20.60 ± 0.21a	2.96 ± 0.02^{c}	2.74 ± 0.09^{c}	2.82 ± 0.00^{d}	
Proline	2.89 ± 0.04c	4.025 ± 0.16^{b}	3.50 ± 0.03^{b}	3.04 ± 0.02^{c}	
Cysteine	3.43 ± 0.04c	4.08 ± 0.02^{b}	3.95 ± 0.14^{b}	4.14 ± 0.07^{c}	
Lysine	4.15 ± 0.07c	2.37 ± 0.03^{c}	2.34 ± 0.14^{c}	2.25 ± 0.03^{d}	5.8
Tyrosine	2.39 ± 0.00d	3.59 ± 0.02^{b}	2.98 ± 0.11^{c}	2.95 ± 0.00^{d}	6.5
Methionine	3.28 ± 0.04c	2.95 ± 0.06^{c}	2.74 ± 0.17^{c}	2.40 ± 0.08^{d}	2.5
Valine	2.79 ± 0.05d	1.30 ± 0.02^{d}	1.38 ± 0.70^{c}	1.19 ± 0.04^{e}	3.5
Isoleucine	1.57 ± 0.01e	4.92 ± 0.03^{b}	4.89 ± 0.15^{b}	4.59 ± 0.00^{c}	2.8
Leucine	4.93 ± 0.07c	3.80 ± 0.00^{c}	3.80 ± 0.15^{b}	3.97 ± 0.02^{c}	6.6
Phenylalanine	3.85 ± 0.07c	6.67 ± 0.05^{b}	6.71 ± 0.23^{b}	6.18 ± 0.00^{b}	
Tryptophan	6.85 ± 0.09b	5.87 ± 0.06^{b}	6.19 ± 0.29^{b}	6.10 ± 0.02^{b}	1.1
AAA	16.34 ± 1.90b	16.13 ± 1.59^{b}	15.88 ± 2.02^{a}	15.24 ± 1.83^{a}	
EAA	50.47 ± 5.95a	49.62 ± 5.93^{a}	50.11 ± 6.00^{a}	49.83 ± 6.36^{a}	
BCAA	10.35 ± 1.71b	10.03 ± 1.85^{b}	10.08 ± 1.79^{b}	9.76 ± 1.81^{b}	
HAA	39.97 ± 1.61a	40.19 ± 1.53^{a}	38.92 ± 1.63^{a}	37.43 ± 1.18^{a}	
PCAA	3.65 ± 0.80d	3.60 ± 0.80^{d}	3.66 ± 0.71^{c}	3.61 ± 0.63^{c}	
SCAA	6.95 ± 0.95c	7.04 ± 0.079^{c}	6.69 ± 0.85^{b}	6.55 ± 1.23^{b}	

Values obtained are the mean and standard deviation of triplicates determinations. Different superscript characters (a, b and c) indicate significant differences at $p < 0.05$ level within a row.

Keys: APC=Aduwa protein concentrate (PP)= pepsin-pancreatin hydrolysate (Alca) =Alcalase hydrolysate. Fav= flavouzyme hydrolysate

AA=Amino acid

AAA=Aromatic amino acids = phenylalanine, tryptophan, and tyrosine

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

BCAA= Branched-chain amino acids = leucine, isoleucine, valine

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

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

SCAA=Sulfur-containing amino acids = methionine, cysteine