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Aduwa (*Balanites eaqpytiaca*) FITR, amino acid profile and functional property of protein product by thermal pre-treatment

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ABSTRACT

Background: The work revealed the structure and functional properties of Balanites *aegyptiaca*. The meal was processed into a defatted meal and protein concentrate. The FTIR results indicate the presence of amino acid functional, bioactive, and carbonyl moieties, including CH, NC, CN, CO, CH_2, COC, and NH groups, with varying bands.

Objectives: Recent research efforts have focused on exploiting this underutilized Aduwa seed to make plant proteins that could compete against animal proteins.

Methods: Several methods were used to investigate Aduwa (*Balanites eaqpytiaca*) FITR, amino acid profile, and functional property of protein product by thermal pre-treatment. A detailed description of the methods used will be provided in the Methods and Materials section.

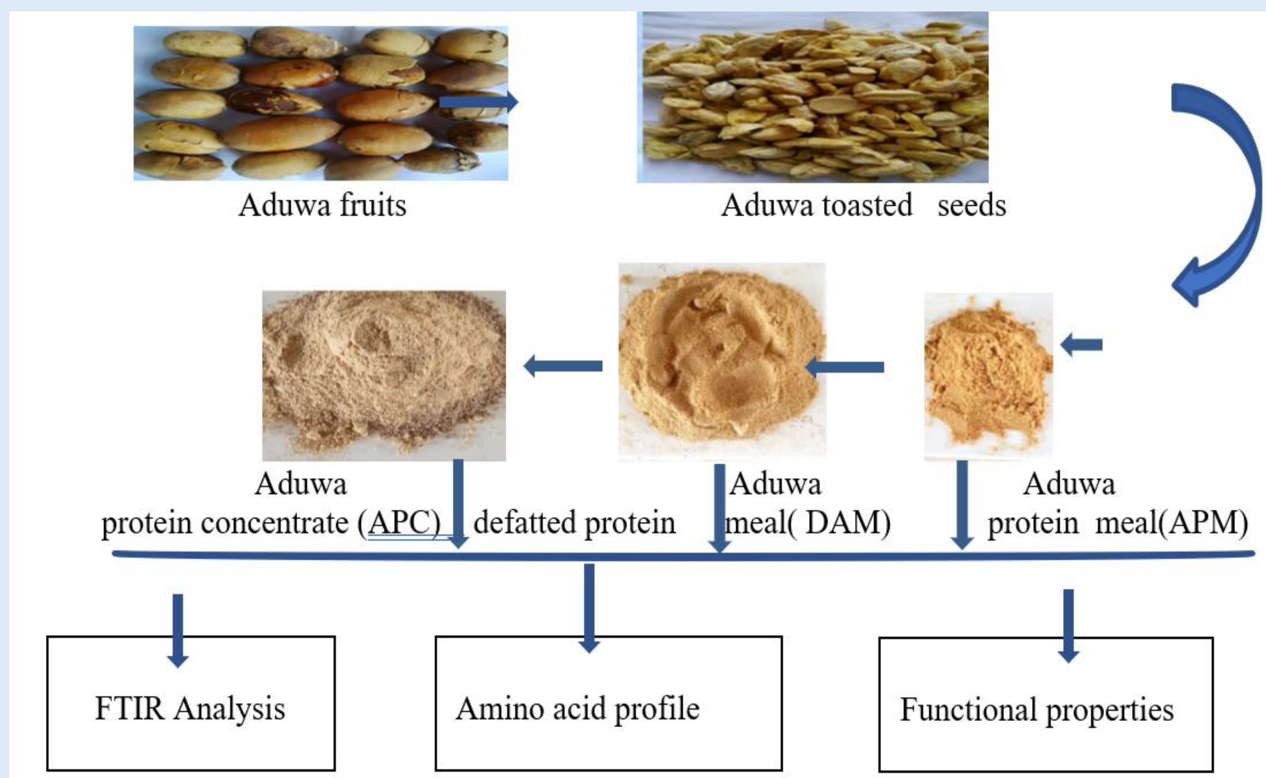
Results: *Aduwa* protein meal's (APM) amino acid profile shows a higher percent essential amino acid (48%), while protein concentrate (APC) had a greater concentration of hydrophobic amino acids (41%), aromatic amino acids (10.34%), sulfur-containing amino acids (3.15%), and positively charged amino acids (17.0%), these amino acid groups are good antioxidants that could prevent generation of reactive oxygen species. Aduwa solubility (PS) indicated that 61% of (APM) was soluble at pH 3. However, (DAM) and (APC) samples exhibited increased solubility at alkaline conditions starting from pH 7. The foaming properties demonstrated the foam capacity of APC was high (20 *mg/mL*) but decreased at pH 5,

particularly for the APC sample. This suggests that sample concentration improved foam of formations in APC, except pH 5, where it was close to its isoelectric point solubility, which might have resulted in high protein-protein interactions. Emulsification properties indicated that sample concentrations of 10 mg/mL to 15 mg/mL were enough for Balanites *aegyptiaca* (APM) and (APC) samples to create sufficient interfacial tension to migrate to the surface, while concentrations of 10 mg/mL and 20 mg/mL stabilized the emulsions formed by the samples.

Conclusion: This study demonstrated that Balanites *aegyptiaca* DAM and APC have the potential for use in the food formulating industry, given their functional and bioactive properties, FTIR data, and amino acid profiles. The research has shown that *Aduwa* protein meal and concentrate are bioactive ingredients that could be used and developed into novel foods.

Novelty: Balanites *aegyptiaca* seeds are underexploited sources of protein and oil in Nigeria. This study revealed various protein meals and their values, with potential uses as bioactive ingredients. The analysis of the *Aduwa-derived protein meal and concentrate shows* that Balanites *aegyptiaca* proteins could be functional ingredients in food design. This could improve the lives of low-income earners through the use of these widely underused plant-rich proteins, especially now that animal proteins are expensive. Balanites *aegyptiaca* protein meal (APM) and concentrates have important amino acid fractions that favor nutritional profile and protein solubility.

Keywords: *Aduwa*, meals, concentrate, Functional property, FITR, Ingredients,



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INTRODUCTION

The use of less-known oil seeds in preparing and producing bioactive-rich ingredients cannot be overemphasized in light of the increase in global demand for nutritious food from plant sources. Additionally, the rise in protein from animal sources has created a scenario in which household uses are beyond the average or poor individuals. Consequently, recent research efforts have focused on exploiting this underutilized Aduwa seed to make plant proteins that could compete against animal proteins [1] and [2]. Various plant seeds and nuts, such as peanuts [3], Mustard seeds [4], Ackee apples [5], and Kariya seeds [6], are being used to produce protein-rich by-products, and this has demonstrated these plant's sourced potential as ingredients to combat protein malnutrition and nutritional diseases. Seed from *balanites aegyptiaca* is one of the unused rich seeds in the northern part of country Nigeria, where it is known as Aduwa in the local Hausa language. The seeds are consumed in a raw or processed form, while the Leafe is used in vegetable soup [7]. Similarly, seed oil extracted from its seeds has traditionally been used as cooking oil in northern Nigeria [8] and [9]. Studies on the proximate composition of Aduwa raw seed have shown crude protein contents range of 23% - 24.01%, as reported by [10] and [7]. The relatively high protein content from Aduwa raw seed suggests a potential source of protein in food and allied industries. To effectively bring to the fore the utilization of less-known protein seeds such as Balanites aegyptiaca food application, it is crucial to understand the structural-functional properties of the proteins [11]. The knowing of functional properties, such as foam capacity, emulsion properties, and solubility profiles, can help determine the specific food applications. FTIR structural properties and amino acid profiles are important in identifying the functional groups, amino acids, and combinations of amino acids responsible for certain functional properties (FP) [12]. Therefore, the interactions between structure-function properties could help determine the overall utilization of *Aduwa* seed proteins as ingredients in food systems. However, structure-function properties are influenced by various factors, such as ionic concentrations and pH ranges, which are important for the necessary manipulation of the proteins to fit into specific food systems [13]. To the best of our knowledge, information on the structure-function properties (SFP) interplay of *Aduwa* meal and protein concentrate is scarce in the literature.

MATERIAL AND METHODS

Material and *Aduwa* Seed Processing: Mature Aduwa seeds were obtained from the popular market in Yobe State, Nigeria, and authenticated at the Department of Plant and Soil Science, Federal University of Gashua, Yobe State. These seeds were toasted using dry oven heat at 70°C for 30 min and then allowed to cool. The toasted seeds were milled, and the oil from the seeds was expressed by the use of a centrifugal semi-automated screw press, following the method described by [7] to obtain the *Aduwa* protein (APM) meal and eventually molded by hand into irregular shapes.

Making of Defatted Sample of Balanites *aegyptiaca* DAM: The method outlined by[14a], was used to obtain defatted Balanites aegyptiaca meal (DAM). One hundred grams of meal cake was made into flour and then dissolved in 500 ml of acetone to make an *Aduwa* flour-solvent ratio of 1:5 (w/v). The sample mixture was stirred under a magnetic stirrer for four (4) hours. Slurries were then filtered through muslin cloth to collect the residue. The residue collected was re-extracted again in a similar manner. The final residue after the second process of defatting was dried under a fume hood at room temperature before the dry flour was grounded using a blender to obtain homogeneous flour, which was stored

in an airtight container as the defatted sample (DAM).

Making of *Balanites aegyptiacal* APC: The *Balanites aegyptiaca* (APC) was prepared using a method modified by [14b]. 200 g of defatted Aduwa meal was dissolved in 2 L of double distilled water to arrive at a final meal-to-water ratio of 1:10. The dispersion was gently stirred on a magnetic stirrer for 10 minutes to form a suspension, after which the pH of the resulting slurry was adjusted to pH 4 by use of 0.1 *M* HCl. The process was allowed by gentle stirring for 2 hours at a constant pH of 10 for Soluble carbohydrates (oligosaccharides) and minerals to be removed by centrifugation at 3,500 × g that lasted for 30 minutes. The concentrate was washed with double distilled water to remove residual minerals and soluble carbohydrates. The pH was subsequently adjusted to 7 for neutralization purposes by using 0.1 M NaOH, and finally, the mixture was centrifuged again at 3,500 × g for 10 minutes. The resulting APC was collected, freeze dried and stored in an airtight container for further analysis.

Methods of Analysis: FTIR (Fourier Transform Infra-Ray Analysis) of (*Balanites eaqpytiacal*) derived protein products. FTIR spectra of the sample were read by using FTIR (Bruker Germany Model 25) coupled PC-based software for operation and data processing. Ten (10 mg) of each Aduwa sample were made into pellets using KBr suitable for FTIR analysis. The infrared transmittance data were collected over a wavelength range of 3900 cm^{-1} to 4900 cm^{-1}. All *Aduwa* samples were analyzed in triplicate, with plain KBr pellets serving as blank. The spectral data from read out were compared with reference values to identify the functional moieties present in the samples.

The Amino Acid profile of (*Balanites eaqpytiacal)* derived protein products: Amino acid composition was determined following the method described by [14] using an S433 Amino Acid Analyzer (SYKAM, Eresing, Germany). Samples were freeze-dried and hydrolyzed for 24 hours at 110 °C with *6 M* HCl. After hydrolysis, the samples were stored in sodium citrate buffer at pH 2.2. For analysis, 50 µL of the hydrolysate was directly injected into the analyzer. Tryptophan was determined separately by hydrolyzing the sample with sodium hydroxide. Cysteine and methionine were quantified after performic acid oxidation prior to hydrolysis in *6 M* HCl, measured as cysteic acid and methionine sulfone, respectively [15] (Girgih et al., 2011).

Protein Solubility (PS) of (*Balanites eaqpytiacal*) derived protein products: The solubility of the sample was determined according to the method by [15]. 10 mg sample was dispersed in 1 mL of 0.1 *M* phosphate buffer solutions at various pH values of 3, 5, 7, and 9) to obtain a 0.1% (w/v) concentration. The resulting mixture was vortexed for 2 minutes and then centrifuged at 3,500 × g for 20 min. Protein content in the supernatant was determined using the modified Lowry method as described [16]. Total protein content was determined by dissolving the protein samples in 0.1 *M* NaOH solution. Protein solubility was expressed as the 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).

Foaming Capacity and Foaming Stability of *(Balanites eaqpytiacal*) derived protein products: The method of [17] was used. Suspensions of different sample concentrations were prepared in 100 mL of distilled water in separate beakers, and the pH of each protein solution was adjusted using either *1 M* HCl or *1 M* NaOH. The solution was then homogenized for 2 minutes using a blender (O'Qlink, China) set at high speed (5-max) and poured into a 250 mL measuring cylinder. Foaming capacity was calculated as the percentage increase in volume compared to the original volume of the protein solution in the measuring cylinder. The FC was expressed

as the percent foam volume remaining in the measuring cylinder after 30 30-minute quiet periods compared to the first volume.

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

Foaming stability or (FS) is the capacity to retain air for a period of time. The Foaming stability was calculated by measuring the foam volume after storage at room temperature for 30 minutes and expressed as a percentage of the first foam volume

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

Emulsifying Properties of (*Balanites eaqpytiacal)* derived protein products: The method described by [5] was used to measure the EAI of the sample. Different concentrations of 10 mg/mL, 15 mg/mL, and 25 mg/mL of Aduwa samples were prepared in double distilled water. The pH of each solution was separately adjusted to 3, 5, 7, and 9 using either 1 *M* HCl or 1 *M* NaOH. Each sample slurry was mixed with 20 *mL* of vegetable oil, and the mixtures were homogenized by using VLC, Saphire, England blender, set at high speed for 60 seconds.

Fifty microliters of emulsion aliquots were transferred from the bottom of the blender after homogenization and mixed with 5 mL of Sodium Dodecyl sulfate (SDS) 0.1% solution. The absorbance of the diluted emulsions was measured at 500 nm using a spectrophotometer specification (722-2000 Spectronic 20D, England) in a 1 cm path length cuvette. The first absorbance was recorded, after which turbidity and EAI were calculated using the f formula:

$$T = 2.303xA/I \quad \text{(iii)}$$

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

The emulsion activity index (EAI) was calculated as:

$$Emulsifying\ activity\ index(m^2/g) = \frac{2xT}{0.2xC} \quad \text{(iv)}$$

The letter T was turbidity, while the letter C was the weight of protein per unit volume of aqueous phases before the emulsion was formed (g/ml). The value 0.2 is the volumetric fraction of oil, and the value 2 is a constant. The emulsion stability index (ESI) was determined after emulsions were allowed to stay for 30 min and the absorbance of the mixture read at 500 nm and then calculated using the formula:

$$Emulsion\ stability\ index = \frac{EAIat10min}{EAIat0min}x100 \quad \text{(v)}$$

Analysis of Statistics: Three readings were subjected to (ANOVA) followed by Duncan's multiple range test to compare treatments for foaming and emulsification properties. Differences from reading were considered significant at $p < 0.05$ using the statistical package for the social sciences window program (SPSS V23) software.

RESULT AND DISCUSSION

Fourier transforms infrared (FTIR) of (balanitis eaqpytiacal) derived protein products: FTIR analysis was conducted to identify functional groups based on the energy that corresponds to changes in vibrational energy at -the infrared region between 4000–400 cm^{-1}. Appearances of absorption bands in these regions are used to determine the functional group present within the molecular samples [18]. As shown in Table 1, the absorption band of *Aduwa* APM, defatted DAM, and APC exceeded five bands, which indicated that all the *Aduwa* samples are complex in nature [5] and [19]. Comparing these results with other published work, the analysis revealed proteins and polyhydroxy substances of bands between 3443.05 and 3421.83 cm^{-1} wavelength, with low absorption bands observed in the DAM and APC. The defatted DAM and APM were characterized by CH_2 and CH moetic stretch, with bands of 2928.04 cm^{-1} and 2852.84 cm^{-1}respectively, indicating the presence of CH and methoxy ether compounds, which are protein and carbohydrates skeletons. However, these wavelength absorption ranges were low compared to those reported

at 2960 cm^{-1} and 2900 cm^{-1} by [20] and [21]. Functional groups such as CO, CO, CN, CH, and COH corresponded to aliphatic hydrocarbon, halogenated compounds, and carbohydrate moieties. Carbohydrate moieties were more abundant in APM and DAM but lower in APC. This pattern can be attributed to the sample extraction protocol during the protein concentrate production [22]. Similarly, the observed shift to low energy levels in Aduwa protein APM, DAM, and APC may result from the formation of detached amino acids during the extraction [23] and [1].

Table 1. FTIR peak assignment for aduwa meal, defatted flour, and protein concentrate.

Functional group	APM	DAM	APC	Suggested Nutrients	Literature range
O-H bond. N-C, N-H bond	3443.05	3443.05	3443.05	Protein and polyhydroxy compounds	[21]
CH_2 s bond	2928.04	2928.04	2924.18	Alkane compounds	[20]
C-H bond	2852.84	2852.84	2852.81	Methoxyl, methyl, and ether compounds	[21]
N-H bond	2361.96	2542.26	2360.96	Amino acid and compound	[20]
CN, X=C=Y	2065.83	2361.96	2065.83	Nitriles, Isothiocyanates	[20]
CO bounding	1867.16	2065.83	1867.16	Triglycerides and acid chlorides	[18]
C=O bounding	1732.13	1732.13	1732.13	Triglycerides and esters	[18]
O-H bending N, CO bounding	1635.64	1635.64	1635.69	Protein and amide	[18]
C-H_2 symmetric, asymmetric bending	145.63	1533.46	1456.3	Proteins and fat,	[21]
C-O, CO anti-symmetric bending	1384.94	1438.94	1384.94	Carboxylic acids and polypeptides	[21]
C-H2 wagging	1317.43	1384.94	1317.43	Carboxylic acids	[18]
P=O;C-N Stretch	1247.97	1317.43	1247.99	Nucleic acid	[18]
CN, C-O, C-C bounding	1145.75	1257.63	1145.75	Tertiary amin	[21]
CN, C-O, C-C bounding	1103.32	1147.68	1103.32	Primary amin	[18]
C=N Stretch	1062.81	1103.32	1062.81	Primary amin and Nucleic acid	[18]
CN, C-O stretch	1035.81	1055.1	1035.81	Primary amine and Carbohydrates	[18]
-C-O-C stretch	893.07	887.28	893.07	Aliphatic hydrocarbon and compounds	[20]
C-CL	781.2	781.2	781.2	Aliphaticchloro compound	[20]
C-H, C-Br	667.39	611.2	667.39	Aliphatic and bromo compounds	[20]
C-H, C-Br	617.24	518.87	617.24	Aliphatic and bromo compound	[20]
CX, CCl,CI	516.94	3443.05	516.94	Aliphatic substances	[18]

Legend; *Apm= Aduwa Meal, Dam= Defatted meal, Apc=Aduwa Concentrate*

Amino acid composition of (*Balanites eaqpytiacal*) derived protein products: Amino acids are building blocks of proteins and are important parameters for determining the quality of protein in food ingredients

[24]. Table 2 below shows the amino acid composition of *Aduwa protein* meal, defatted protein meal, and protein concentrate. The essential amino acid (EAA) content in these samples ranged from 37.35% - to 47.84%, with APM having a significant ($p>0.05$) high value followed by APC. This revealed that *Aduwa* protein meals (APM) and the extracted concentrate (APC) are good-quality sources of protein. Similarly, (AAA) aromatic amino acids, hydrophobic amino acids (HAA) and positively charged amino acids (PCAA) in the samples ranged from 5.24% - 10.56%, 39.96% - 40.95%, and 15.90% - 17.00%, respectively, and the APC sample was significantly ($p>0.05$) high even in values hence potential bioactive sample special as anti-oxidant and anti-hypertensive bioactive ingredients. A high concentration of hydrophobic amino acid (HAA) in the APC has implications for the structural and bioactive behaviors of the proteins [25]. The observation may be linked to many functional amino groups present, as indicated by the FTIR results.

The value for sulphur-amino acids ranged from 3.1% - 3.2%, with APM with the highest content implicated as a better antioxidant sample as well. The trend in sulphur amino acids may have influenced the antioxidant properties of studied samples. The tryptophan content ranged from 1.16% - 1.18%, which was higher compared to the 0.1% and 0.12% reported for okra seed flours and isolated okra proteins [1]. Similarly, histidine content ranged from 3.00% - 3.39%, and the APC sample had the highest value. However, the differences in amino acid content between the meal, defatted meal, and concentrate could be attributed to the additional varied techniques applied during the production of the APC concentrate [26].

Table 2. Amino acids profile of *Aduwa* Meal, Defatted *Aduwa* meal, and Protein Concentrate

	APM	DAM	APC
Leucine	12.01a	8.60b	8.47b
Lysine	8.41a	5.32b	5.35b
Isoleucine	5.62a	4.50b	4.55b
Phenylalanine	2.50c	5.15a	5.06b
Trytophan	1.78a	1.18b	1.16c
Valine	7.49a	5.02b	5.06b
Methionine	2.21a	1.58b	1.62b
Proline	1.59b	4.01a	4.10a
Arginine	3.75b	8.07a	8.26a
Tyrosine	0.96c	4.09b	4.12a
Histidine	3.75a	3.00c	3.39b
Cystine	1.01b	1.52a	1.53a
Alanine	4.80c	5.02b	5.27a

	APM	DAM	APC
Glutamic acid	8.65a	18.22b	18.08c
Glycine	8.21a	4.11c	4.26b
Threonine	5.86a	4.81b	3.86c
Serine	7.49a	4.82c	4.93b
Aspartic acid	13.93a	10.99b	10.93b
AAA	5.24c	10.42a	10.34b
BCAA	25.12a	18.12b	18.08b
HAA	39.96b	40.67a	40.95a
PCAA	15.90c	16.39b	17.00a
NCAA	35.93c	38.84a	37.80b
SCAA	3.22a	3.10b	3.15c
EAA	47.84a	37.98b	37.35c7

Legend: Apm= Aduwa Meal, Dam= Defatted meal, Apc=Aduwa Concentrate, Aromatic amino acid (AAA) = phenylalanine, tryptophan and tyrosine, Branched-chain amino acids (BCAA) = leucine, isoleucine, valine, Hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine, and cysteine, Positively charged amino acids (PCAA) = arginine, histidine, lysine, Negatively charged amino acids (NCAA) = aspartic, glutamic, threonine, serine, Sulphur containing amino acids (SCAA) = methionine, cysteine, Essential amino acids (EAA) = histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine;

Solubility profiles of (*Balanites eaqpytiacal*) derived protein products: Our study shows the solubility profile of Aduwa protein meals (APM) and concentrate (APC) at different pH values. The result indicated that all samples were soluble at pH 3.0, with the percent solubility of the proteins decreasing progressively as the pH was adjusted from 3 to 5. Approximately 61.00% of Aduwa meals were soluble at pH 3, but the protein concentrate demonstrated the lowest solubility at the same pH. Typically, one would expect the protein concentrate to exhibit better solubility in acidic conditions; however, the observed low solubility of APC when it was pH 3 compared to APM may be a result of the increase in protein aggregates at this acidic pH, which may have reduced solubility [27], suggesting that APM sample is better functional ingredient (FI) to formulating beverage drink compare to APC due to the APC low solubility at acidic region. A similar result was reported for okra seed meal and protein isolate when the okra isolate showed poor solubility in the acidic pH region [1]. Beyond pH 3, APM did not show significant changes in solubility, even as the pH increased from 3 - 9. The defatted meal (DAM) and protein concentrate (APC) had low solubility values between 22% and 20.4% at pH 5, but solubility was observed to increase progressively to pH 9. This observation aligned with results reported for walnut protein, where increased negative charge in an alkaline environment was able to enhance solubility [28]. The low solubility in APC when pH was 5 suggests its proximity to the isoelectric point. Generally, solubility decreases as pH approaches the isoelectric point and then increases in alkaline conditions. Losses of electrostatic repulsion

when the pH was 5 have facilitated protein concentration and increased protein-protein interaction. High bulk density and larger aggregate diameters can lead to protein precipitation [29]. The differences in solubility patterns between APM and APC may be due to isoelectric precipitation in the concentrate and the amount of protein available, influenced by thermal pretreatment and denaturation. In the basic region, soluble protein levels were as follows: 61.5% for APM, 22% for DAM, and 41% for APC. However, APM was soluble compared to DAM and APC in acidic conditions. However reverse trend was observed in basic conditions. However, the poor solubility of the protein concentrate (APC) could pose disadvantages for APC sample utilization as an ingredient in making functional beverages (FB).

Foam properties of (*Balanites eaqpytiacal*) derived protein products

Foaming capacity: Our study illustrates the influence of pH at 3, 5, 7, and 9, and sample concentration at 10, 15, and 20) *mg/mL* on the foaming capacity of the Aduwa-derived samples. At a sample concentration of 10 mg/ml, the *Aduwa* protein meal (APM) exhibited significantly ($p>0.05$) the highest foaming capacity at pH when it was 5, while the poorest was observed at pH 3, which was not significantly different ($p > 0.05$) from the value obtained when the pH was 7, foaming capacity of the defatted meal (DAM) decreased progressively as the pH increased from 3 - 9 with increase in sample concentration. In contrast, the protein concentrate (APC) displayed an increase in foaming capacity as the pH shifted towards the basic region. This trend aligned with an increase in the net charge of the samples in the neutral and basic pH ranges, leading to enhanced protein-protein repulsion and increased protein flexibility. As proteins become more flexible, their ability to accommodate air bubbles improves, which could result in greater foaming capacity at higher pH levels, hence making APC a better mayonnaise and cake functional ingredient (FI). Similar patterns have been reported in the foaming capacities of fenugreek seeds, Bambara seeds, and walnut protein isolates [30-31, 21].

As the sample concentration increased from 20 to 60 *mg/ml,* APM demonstrated a significant ($p>0.05$) increase in foaming capacity at acidic and basic pH values. However, foaming formation at pH 5 decreased substantially. For both the defatted meal (DAM) and protein concentrate (APC) samples, the initial increase in foaming capacity was observed up to 40 *mg/ml,* followed by a decrease. This pattern may be explained from the point where protein crowding started, while the increase in protein concentration is necessary to generate adequate foam. It was observed that concentrations beyond 40 *mg/ml* may lead to the formation of excess protein micelles, reducing the ability to generate foams in DAM and APC [32] and [33].

The high foaming capacity of samples, particularly APM, DAM, and APC, in the basic regions and at higher sample concentrations may be attributed to the formation of large charges at these pH values, which encouraged the development of interfacial membranes. The formation of these larger interfacial membranes within the protein molecules may enhance solubility, leading to increased foam formation [34]. These samples are better functional ingredients (FI) at higher concentrations and, therefore, are encouraged by small-scale food processing entrepreneurs to use them as functional ingredients to add values in terms of health and improve mechanistic properties of the food matrixes [35]

Foaming stability: Foaming stability refers to the ability of foam to maintain its shape and volume over time, which is crucial for applications in beverages, coffee, and the baking industries. Our study shows sample ability, *Aduwa* meal (APM), defatted meal (DAM), and Aduwa concentrate (APC) in relation to sample concentration (20, 40, and 60) *mg/mL* and pH levels 3, 5, 7, and 9 stability foams At sample concentration of 20 *mg/mL,* foam stability was significantly ($p>0.05$) high when pH

range was within acidic but decreased progressively as the pH was shifted toward the base regions in APM and DAM samples. Notably, foam stability values for APM and DAM were greater for APC in 20 *mg/mL,* concentration. This pattern may be attributed to the formation of stable molecular layers at the air-water interface, which may have enhanced the texture, stability, and elasticity of the foams. Similar findings have been reported for rapeseed flours and proteins. Here, (DAM) and (APC) samples exhibited significant and greater foam stabilities than APC, as observed by [36] as a behavioral principle. With sample concentration increase from 20 *mg/mL* to 60 *mg/mL*, it was observed that foam stability improved when the pH was 7 and 9 compared to when the pH was 3 and 5. This suggests that the protein molecules generate adequate charge densities at these pH values, facilitating the formation of strong interfacial membranes [34]. Additionally, the foam stability was observed to be high at a sample concentration of 60 mg/mL and at elevated pH levels, indicating that sample concentrations are beneficial, as they provide more protein molecules to enhance the intermolecular cohesiveness of the foams formed [37]. The samples demonstrated varying patterns of foam stability depending on pH and sample concentration, which can be linked to differences in their structural-functional properties, particularly in terms of surface dispersibility and stability of functional molecules (FM) before ingestion.

Emulsion Properties of (*Balanites eaqpytiacal*) derived protein products

Emulsion Capacity: Our study shows the emulsifying capacity of *Aduwa* protein meal (APM), defatted *Aduwa* meal (DAM), and *Aduwa* protein concentrate (APC) across different pH levels and sample concentrations. These results from the emulsion study using an Aduwa-derived sample underscore the importance of both pH and sample concentration in determining the emulsion capacity of Aduwa-derived protein sources, highlighting their potential applications in food formulations where stable emulsions are required. At a sample concentration of 10 *mg/mL,* both the *Aduwa* meal and protein concentrate exhibited significant ($p<0.05$) poor emulsion capacity when pH was at 3.0. In contrast, higher emulsion capacities were recorded at pH levels ranging from 5 - 9. This increase may be attributed to the formation of more charge densities around 5-9 pH values, which enhanced the ability of sample protein to interact with oil and form stable emulsions [38]. At the highest concentration of 20 *mg/mL,* the emulsion capacity of the *Aduwa* meal peaked at pH 7.0, while the defatted *Aduwa* meal showed optimal emulsion capacity when pH was 5 and 7, respectively. For the protein concentrate sample (APC), the highest emulsion capacity was observed when the acid-based balance was 7.0, reflecting the ability of this functional ingredient (FI) to be used or applied in various food formulations judging from its high emulsion capacity at neutral pH. Interestingly, the overall emulsifying capacities of the samples were slightly low when the concentration was high, likely due to protein overcrowding. Protein overcrowding can disrupt interfacial properties, leading to less effective emulsification capacitation [34].

Emulsifying Stability: The emulsion stability of *Aduwa* protein meal (APM) and defatted *Aduwa* meal (DAM) displayed a similar trend of concentration, with the highest emulsion stability, which was observed with pH 5 and 9. The pattern of emulsion stability for these samples closely mirrored that of the protein concentrate (APC) stability stabilized at pH 7.0, but when the sample concentration was increased to 15 mg/ml, at pH 9.0 emulsion stability significantly ($p<0.05$) diminished, whereas the emulsion formed at pH 7.0 remained notably robust, particularly for APM and APC samples. At a concentration of fifty (50 mg/ml) and pH 5.0, the emulsifying stability of APM and APC was significantly ($P>0.05$) stronger, but DAM and APC achieved the highest and most significant ($p>0.05|$) stability at pH 9.0. Emulsion stability is crucial as it reflects the protein's

ability to interact with and stabilize immiscible phases, thereby preventing phase separation [36]. These findings suggested that 10 mg/ml serves as a maximum for creating sufficient interfacial tension to stabilize the emulsion formed by these samples and that could support bioactive molecules during the food use of these samples [39]. This has emphasized the importance of both pH and sample concentration in optimizing the functional properties of Aduwa-derived proteins for potential food applications

CONCLUSION

Bioactive ingredients (BI) are often encrypted and offer useful and healthy properties that are beyond basic nutrition, such as antioxidative effects, disease prevention, and disease management with the potential to lower disease risk [35]. Functional food is characterized by bioactive material derived via a careful and selective extraction process that has both physical attributes and physiological mechanistic attributes on food and food systems. FTIR results highlighted various functional groups in the samples, suggesting that differences in processing and protein modifications had played a significant role. Amino acid analysis indicated that Aduwa protein meal (APM) had a higher EAA (essential amino acid) while *Aduwa* protein APC was rich in HAA (hydrophobic amino acid); this influenced the structural-functional characteristics of the APC sample. The sample solvent-solvent interaction profile indicated that these proteins may not be ideal for use in acidic beverages or drinks. Foaming capacity tests showed that APC had a high foaming capacity at basic pH levels, particularly at pH 7, likely due to increased charge density promoting the formation of extensive interfacial membranes. Emulsion properties shifted with pH on *the Aduwa sample change in concentration; this has established that a* concentration of 10 mg/ml was optimal for achieving emulsion with good stability. Overall, the findings suggest that *Aduwa* seed meal DAM and concentrates APC have huge potential for use as ingredients in food product development from their functional and bioactive behaviour, especially of DAM and APC samples. The result of this work is an opening or avenue for Aduwa-derived protein products to be incorporated into various culinary applications and will improve the functional attributes and antioxidant properties of the food product.

Abbreviation	Meaning
APM	Aduwa protein Meal
DAM	Defatted Aduwa meal
APC	Aduwa protein Concentrate
FTIR	Fourier transform infrared
AAA	Aromatic amino acid
HAA	Hydrophobic amino acid
SCAA	Sulphur containing amino acid
EAA	Essential amino acid
FP	Functional property
FB	Functional beverage
FI	Functional ingredient
FM	Functional molecules
SFP	Structure functional properties
BI	Bioactive ingredient

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