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# *In vitro* protein digestibility, surface hydrophobicity and functional properties of *Aduwa (B.aegyptiaca* Del*)* membrane protein products

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**ABSTRACT**

Over the years, the demand for plant-based protein ingredients has continued to attract food processors globally. This study reports the protein recovery, in vitro protein digestibility (IVPD), Surface hydrophobicity **(So), and functional properties** of *Aduwa* meal using various extraction procedures. The meal was defatted to obtain a protein concentrate (APC) and was further processed with membrane molecular cut-off ultrafiltration to obtain membrane sodium hydroxide (*m*NaOH) and membrane sodium chloride (*m*NaCl) concentrates. APC samples had high yield in percent proteins, APC and *m*NaCl samples showed higher protein digestibility (86.46%,81.73%) and surface hydrophobicity (1023, 174.10%)) compared to *m*NaOH (71.73% and 66.69%) sample, however low solubility (20%–55%) was seen in APC compared to *m*NaOH (50%–60%) and *m*NaCl (70%–100%). The *m*NaCl sample formed better emulsions at pH 9.0 compared to the other samples, and its foaming capacity and stability were better at a 15 mg/mL concentration. The extraction of membrane sodium chloride concentrates from *Aduwa* protein is a promising functional ingredient with potential applications in the beverage, pharmaceutical, and food industries.

**Keywords:** Aduwa, protein concentrate, membrane concentrates, yield, digestibility, proximate, functional, properties,

## INTRODUCTION

The demand for plant-derived protein has increased tremendously compared to animal protein. The demand may be attributed to various micro and macroeconomic factors. Proteins from plant-based foods have been extensively studied and are considered valuable food ingredients due to their affordability and availability (Adrián et al., 2022). The desert date (*Balanites aegyptiaca Del*) is rich in minerals and bioactive nutrients (Muhammed et al., 2002). Recent research efforts have focused on exploiting plant materials to produce protein ingredients that can compete favorably (Nnamezie et al., 2021). Reports on plant seeds and nuts have demonstrated that protein-rich products can be used as ingredients to address nutritional deficiencies in food formulations (Wu et al., 2009). There is limited information on the Protein digestibility and functional properties of Aduwa (B.aegyptiaca Del) concentrate, alkaline, and salt-soluble extractions. Findings from this study could be valuable in enhancing the applications of the *Aduw*a protein ingredient in food and pharmaceutical formulations.

## MATERIALS AND METHODS

**Sample Collection and Preparation:** Mature *Aduwa* (*Balanites aegyptiaca*) cracked kernels were purchased from the local Bada market in the North East State of Yobe, Nigeria. Dried *Aduwa* seeds were toasted in an air oven at a temperature of 60–70°C for 25 minutes and then allowed to cool to 35 °C. Aduwa heated seed was milled and seed oil was removed by a centrifuge screw press to obtain Aduwa meal cake (APM). The APM was packaged in airtight containers and stored at 4°C.

**Preparation of Defatted *Aduwa* Meal:** Defatting APM was carried out at room temperature using acetone at a 10:1 meal weight to solvent volume ratio, with continuous stirring using a magnetic stirrer for 2 hours. Mixture was decanted and drained using a muslin cloth with a 90×40×45 mesh size. The residue was defatted again for another 2 hours. The final defatted Aduwa sample was aerified overnight (~12 hours) using an open plate in a fume cabinet or hood. The dried meal was then milled using a coffee grinder, labeled (DAPM) as defatted protein meal, and stored in an air-sealed container at 20 °C in a freezer.

**Preparation of Protein Concentrate:** The defatted *Aduwa* meal (DAM) was mixed with double-distilled water at a ratio of 1:20 (w/v). The mixture pH was adjusted to 10.0 by adding 1 M NaOH, and the mixture was stirred for 1 hour. Then, the mixtures were centrifuged (3100×g) at -4°C for thirty minutes. The supernatant was fetched and filtered through cheesecloth, and the pH was adjusted to 4.5 using 1 *M* HCl. After 30 minutes of stirring, the mixture was centrifuged again. The supernatant was washed with distilled water to remove non-protein biomaterial before centrifuging to obtain the final slurry precipitate. The precipitate mixture was dissolved in double-distilled water and adjusted to pH 7.0. Before freeze-drying, it was used as Aduwa protein concentrate (APC).

**Preparation of *Aduwa* Protein Concentrates by Membrane Ultrafiltration *Aduwa* Membrane Sodium Hydroxide (mNaOH) Concentrate:** The defatted Aduwa meal was mixed with double-distilled water at a one-to-twenty (w/v) ratio (50 g to 1000 mL) and adjusted to pH 10.0 using 1 M NaOH and stirred continuously for 1 hour. The mixture was then centrifuged at 31000×g at -4°C for 30 minutes. Then the supernatant was fetched and filtered through cheesecloth. The pH of the filtrate was adjusted to 5.5 using 1 M HCl, and the solution was digested with an enzyme mixture containing 1% cellulase and 1% alpha-amylase at 50°C for 1 hour. After digestion, the pH of the mixture was adjusted to 7.0, and the mixture was allowed to cool to 37°C, forming the Aduwa alkaline extract. This extract was filtered through a 5-kDa ultrafiltration membrane. Ultrafiltration continues with periodic dilution using double-distilled water until the permeate is clear. After the Aduwa permeate became

clear, retentate was collected and freeze-dried as membrane NaOH-soluble protein (mNaOH).

**Aduwa Membrane Sodium Chloride (mNaCl) Concentrate**: The defatted meal sample was mixed with a 0.1 *M* NaCl solution at a 5:100 (weight/volume) ratio for 1 hour, then centrifuged to obtain the supernatant. The supernatant was filtered using a 5-kDa membrane ultrafilter, with periodic dilution using double-distilled water until the permeate became clear. After the permeate became clear, the retentate was collected and freeze-dried as membrane NaCl-soluble (mNaCl) protein.

## MATERIALS AND METHODS

**Surface Hydrophobicity (So) of Aduwa Protein Samples**: Surface hydrophobicity (So) was determined using the method outlined by Karaca et al. (2011) with 1-anilino-8-naphthalenesulfonate (ANS). A 10 mg/mL stock solution of each Aduwa sample was prepared in 0.1 *M* sodium phosphate buffer (pH 7.0) and diluted to concentrations ranging from 0.005% to 0.025%. About twenty microliters of 0.8 *M* ANS solution, prepared in 0.1 *M* sodium phosphate buffer (pH 7.0), was added to each protein concentration. Fluorescence intensity (FI) was measured at excitation 390nm and emission wavelength 470 nm, respectively, using the JasCo FP-6300 spectrofluorometer. The FI of the Aduwa protein samples without ANS was subtracted from the respective samples containing ANS. The slope of the FI versus protein concentration plot, obtained by linear regression analysis, was used as an index of protein surface hydrophobicity (So).

**Solubility Profile of Aduwa Protein (PS)** PS or Protein solubility was determined according to the method outlined by Malomo et al. (2014). Aduwa samples (ten milligrams each) were dispersed in 5 mL of 0.1 M acetate buffer (pH 3.0 and 5.0), phosphate buffer (pH 7.0), and tris buffer (pH 9.0), respectively, based on the protein content of the Aduwa samples. The resulting mixture was thoroughly vortexed, then allowed to hydrate for 1 hour. This was followed by centrifugation at 3100×g for 30 minutes at 4 °C. The protein content of each supernatant was determined using the modified Lowry method (Markwell et al., 1978). The total protein content of the Aduwa sample was also determined using the same method after dissolving the samples in 0.1 M NaOH solution. Protein solubility (PS) was calculated as:

PS (%) =Protein content of supernatant at given pH×100Total protein content of samplePS (\%) = \frac{\text{Protein of supernatant at given pH} \times 100} {\text{Total protein content of sample}PS(%)=Total protein of sample Protein content of supernatant at given pH×100

**Intrinsic Fluorescence (FI) of Balanites aegyptiaca Del Products**: Intrinsic fluorescence measurement was carried out as described by Ajibola et al. (2016). Aduwa sample dispersions (*10 mg/mL*) were prepared in acetate (*0.1* M) buffer of pH 3.0 and 5.0, phosphate buffer of pH 7.0, and Tris-HCl buffer of pH 9.0, based on sample protein content. The sample was solubilized for 1 hour, then centrifuged at 2100×g (-4°C) for 30 minutes, and the supernatant was collected. A stock concentration of approximately 2 mg/mL for each aduwa sample was diluted to 0.002% (w/v) using the respective corresponding buffer. The emission spectrum was recorded at a temperature of 25.0 °C using a Jasco FP 6300 spectrofluorometer (Jasco Inc., Japan), with a 1 cm path-length microcuvette (150 µL). Aduwa protein samples were excited at 280 nm, and emission wavelengths were recorded between 300 nm and -400 nm. The emission spectra in the buffer blanks were subtracted from those of the respective sample spectra to get the correct fluorescence spectrum. The max. fluorescence intensity called (Fmax) and corresponding Fλmax was determined.

**Aduwa Protein Samples: Emulsion Formation and Stability.** The emulsion sample was prepared as

described by Chao et al. (2018) with slight modifications. Aduwa sample fraction in slurries form in 10, 15, or 20 mg/mL concentrations were each prepared at *0.1 M in* acetate pH 3 and 5 buffers, phosphate pH 7, and tris-HCl (pH 9) buffer solution respectively, and then addition of *1 mL* of canola pure oil, The emulsion was prepared by homogenizing the oil-water mixtures for one minute at 20000 rpm speed using a Polytron PT 10-35 homogenizer equipped with ague, 12-mm non-foaming shafts. A Malvern Instruments 2000 particle size analyser, in mesmerizer mode, was used to measure the mean oil droplet size (d3,2) of the Aduwa sample made emulsions, using distilled water as a dispersant under constant shearing. Each emulsion was added dropwise to approximately 100 mL of water in the small-volume wet sample dispersion unit attached to the instrument, until the required level of occlusion was achieved. The instrument was set to take measurements in triplicate automatically, and the emulsion of each sample was prepared in duplicates. The results obtained include oil droplet size measurements of the emulsions, which were used as indicators of their emulsifying ability.

After measuring the foam capacities, the emulsion in the tube was allowed to stand for 30 minutes without agitation, and the oil droplet size measurement was repeated to determine the stability of the respective emulsions. Emulsion stability was calculated as:

*= (Mean oil droplet size at 0 min) / (Mean oil droplet size after 30 min) × 100*

**Water-holding and oil-holding capacities of Aduwa Protein Samples:** The method outlined by Malomo et al. (2014) was adopted to determine the oil-holding capacities of Aduwa samples. An aqueous solution of Aduwa protein samples (40 mg/mL) was prepared in a 15 mL centrifuge tube containing phosphate buffer. For the oil holding capacity, a similar sample concentration was prepared using pure canola oil instead of buffers. The sample dispersion (water or oil) was vortexed thoroughly and allowed to stand for 30 minutes at room temperature. The mixtures were then centrifuged at 3100× g at 4°C for 30 minutes, and the supernatant, containing excess water or oil, was drained for 15 minutes. The centrifuge tubes were reweighed to determine the weight of water or oil retained per gram of Aduwa samples.

**Least Gelation Concentration (LGC) of Aduwa Protein Samples**: The Least gelation concentration (LGC) of Aduwa samples was evaluated by the method described by Malomo et al. (2014). Aqueous Aduwa solutions were prepared in 0.1 M phosphate buffer at concentrations ranging from 2% to 20% (w/v). The mixture was placed in test tubes, vortexed for 5 minutes, and then heated in a water bath at 95°C for 1 hour. The tubes were then cooled to room temperature and stored at 4°C for 14 hours. The LGC was determined as the minimum concentration at which the gel did not slip when the tube was turned downwards.

**In Vitro Protein Digestibility (IVPD) in Aduwa Protein Samples**: The in vitro digestibility of Aduwa protein was determined following the procedure described by Hsu et al. (1977). The Aduwa membrane sample was suspended in an aqueous solution containing double-distilled water and adjusted to pH 8.0 with 0.1 M NaOH, then stirred at 37°C. A 3 mL of enzyme solution (containing 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase) was taken from the enzyme solution in an ice bath and added to 30 mL of each protein suspension. The drop in pH of the mixture was recorded every 30 seconds over 10 minutes using a pH meter. The analysis was repeated to obtain triplicate results. The percent of protein digestibility of each Aduwa sample was calculated using the Hsu et al. (1977) regression equation:

**PD or Protein Digestibility (%) = 210.46 - 18.10 x X_f**: where (Xf), the final pH value of each sample after a 10-minute digestion.

**Statistical Analysis**: The data in the analysis were reported as the mean ± standard deviation from triplicate determinations. Statistical analysis, of one-way ANOVA and Duncan's multiple-range test of $p < 0.05$, was performed using SPSS 26.1 version.

## RESULTS AND DISCUSSION

**Protein Yield, Digestibility, Gelation Concentration, and Surface Hydrophobicity**: The protein yield and salt-induced gelation from mNaOH are significantly higher than those obtained with APC and mNaCl (Table 6). The better gelation concentration (LGC) and higher surface hydrophobicity (So) of APC imply that this sample has more exposed hydrophobic clusters compared to mNaOH and mNaCl. The high yield in mNaOH may be attributed to the solubilization of both storage and non-storage proteins (Table 6). Protein quality also depends on the amount available for absorption from the intestinal tract after digestion. Protein digestibility is an important quality measure, considering factors such as anti-nutrient moieties, protein sizes, and their folded or conformational structures. The results obtained from APC and mNaOH can be compared to previously reported values for quinoa protein isolate (78.4%), flaxseed (68%), and cowpea (73.5%) (Elsohaimy et al., 2015; Marambe *et al.,* 2013). The lower digestibility of the membrane salt-extracted Aduwa protein sample is consistent with its higher denaturation temperature, indicating a more native conformation compared to APC.

The least gelation concentration defines the amount of protein needed to form a gel that can be self-supportive. This ability depends on the extent of denaturation and the formation of protein networks. Gelation results showed that mNaCl had higher gelation capacities compared to APC and mNaOH for both normal and salt-induced gelation. The gelation concentration in this study for mNaCl was lower compared to the 10.75% reported for green lentil salt-extracted protein concentrate (Osemwota et al., 2021), 10% for defatted cashew nut protein (Adewole & Adebowale, 2007), and 12% for lupin protein concentrate (Lqari et al., 2002).

Surface hydrophobicity (So) is an important indicator for predicting foaming and emulsifying characteristics, as well as other surface-related functional properties. The level of protein intermolecular interactions, which can result in protein conformational change, best defines So (Arogundade et al., 2007). Variations in So among different proteins, as shown in Table 6, may be attributed to their varied amino acid position and their exposed environments. The process of extracting Aduwa protein concentrates increased exposure of hydrophobic clusters in APC, which may have accounted for the high surface hydrophobicity compared to the milder membrane processes used to make mNaOH and mNaCl. This observation was also noted by Osemwota et al. (2021) during the isolation of green lentil protein. The extent of denaturation influenced by pH on the hydrophobicity of APC agreed with the results reported for African yam bean protein from ultrafiltration (Arogundade et al., 2016). Although mNaCl is also a membrane concentrate product, it has a higher solubility, because of the higher content of aromatic amino acids (See Table 4). Thus, methods of protein extraction, such as washing and re-washing, freeze-drying, and other unit operation during sample processing, influenced the hydrophilic and hydrophobic balance of the protein structures (Wang et al., 2000), significantly impacting the So behaviour of the protein materials from amino acid clusters either at the surface or interior of the sample. Similar observations were noted by Osemwota et al. (2021) during the processing of green lentil protein isolate.

**Table 1.** Aduwa yield of protein, digestibility, least gelation concentration (LGC), and Surface hydrophobicity of *Aduwa* protein concentrate (APC), membrane protein concentrates from NaOH (*m*NaOH) and NaCl (*m*NaCl) extracts

| Sample | Protein Yield (%) | Protein digestibility (IVPD)% | LGC (%) | 0.5% NaCl LGC (%) | Surface hydrophobicity |
|---|---|---|---|---|---|
| **APC** | $62.22^{b}$±1.24 | $86.46^{a}$±0.04 | $16.00^{b}$ | $14.00^{c}$ | $1023.8^{a}$±0.00 |
| ***m*NaOH** | $86.77^{a}$±6.23 | $71.43^{c}$±0.09 | $18.00^{a}$ | $16.00^{b}$ | $66.69^{c}$±0.00 |
| ***m*NaCl** | $43.48^{c}$±1.24 | $81.73^{b}$±0.19 | $0.00^{c}$ | $20.00^{a}$ | $174.10^{b}$±0.00 |

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

**Solubility:** The Aduwa protein concentrate was soluble at pH 3.0; however, the percentage of soluble proteins decreased progressively as the pH was adjusted from 3.0 to 5.0 (Fig. 4). Results show that approximately 70.0% of mNaCl, 50% of mNaOH, and 20% of APC were soluble when the pH was 3.0. The membrane Aduwa concentrates were soluble at pH 3.0. The protein concentrate was expected to exhibit better solubility at acidic pH; however, the low solubility of APC at pH 3.0, compared to alkaline and salt-extracted membrane concentrates, may be attributed to high protein complexation during isolation (Molina et al., 2001). The same pattern of results was reported for okra seed meal and protein isolate, showing poor solubility in the acidic pH region (Nnamezie et al., 2021). APC did not show any difference in solubility, even as the pH value increased from 3.0 to 9.

The mNaCl and APC had the lowest protein solubility (45% and 10%), respectively, at pH 4.0, their isoelectric point, while mNaOH had the lowest solubility (35%) at pH 5.0, before increasing to pH 9. The high solubilities of all the samples at pH nine observed agreed with the pattern of solubility results reported for walnut protein (Mao & Hua, 2012). The low protein solubility values of APC and membrane concentrate at pH 4 and 5, respectively, are consistent with the zwitterionic nature of plant proteins, which have a zero net charge or isoelectric point. Solubility decreases as the pH increases until the isoelectric point is attained and then increases again. Within the isoelectric point range, where loss of electrostatic repulsive forces created favourable conditions for protein network formation, leading to high bulk density and large diameter of aggregates, which could result in protein precipitation (Singh et al., 2005). The higher values and solubility patterns observed in mNaCl and mNaOH may be advantageous for use as a potential ingredient in the formulation of acidic drinks.

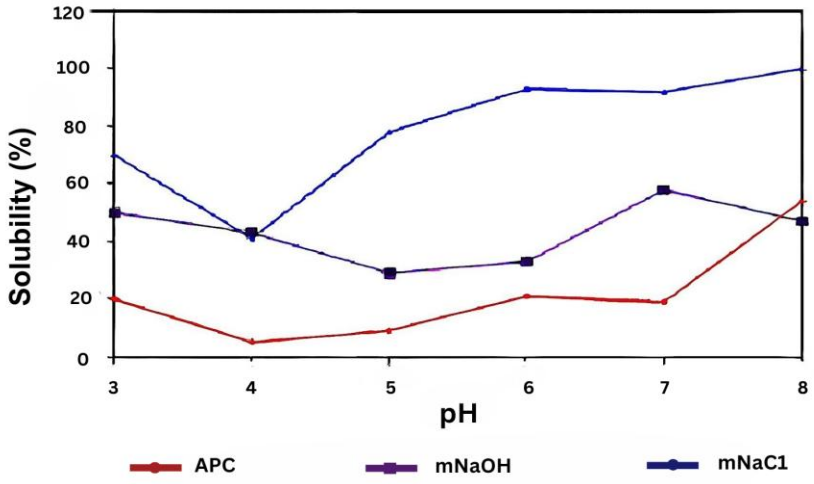


**Figure 1.** Solubility of Aduwa Protein Concentrate (APC), Membrane Protein Concentrates from NaOH (mNaOH), and NaCl (mNaCl) Extracts

**Intrinsic Fluorescence:** The aromatic amino acids of the *Balanites aegyptiaca* Del extracted protein were activated at a wavelength of 280 nm, revealing fluorescence peaks (Fig. two). The maximum fluorescence (FI) took place at a wavelength < 350 nm for all *Aduwa* protein samples. Aromatic amino acids can emit fluorescence spectra at wavelengths of 350 nm (Trp), 303 nm (Tyr), and 260 nm (Phe), which are excitable in the UV region. These emission wavelengths reflected some conformational changes that could result from the folded or unfolded state of the samples, depending on the exposure of aromatic amino acids to a hydrophilic or ionic environment (Osemwota et al., 2021). The emission wavelength after excitation can also indicate functional properties such as protein solubility (Malomo et al., 2014). The results in Figure 2 suggest an unfolded nature of the polypeptides, particularly the Trp residues in APC, while the Try and Phe moieties revealed a folded nature at pH 7 and pH 9 with an extensive hydrophobic interior. In general, fluorescence peaks were observed only for the APC, while mNaOH and mNaCl showed no observable peaks. These results indicate a more hidden conformation and possible extensive hydrophobic or hydrophilic interiors of the mNaOH and mNaCl samples under the FI environment.

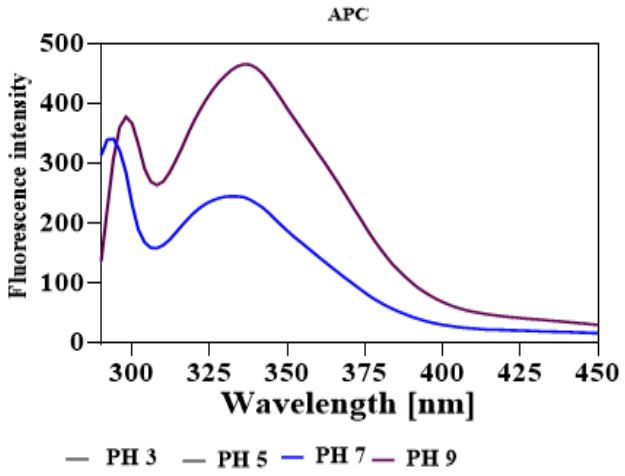


**Figure 2.** Intrinsic fluorescence (FI) intensity of isoelectric pH-precipitated *balani toward Tes aegyptiaca* Del seed protein concentrate (APC). No observable FI was observed for the mNaOH and mNaCl samples.

**Water (WAC) and Oil (OAC) Absorption Capacities: The** Abilities of a protein material to hold water molecules or surround itself with oil molecules depend on the conformational position, dimension, and configuration of the protein material (Chavan et al., 2001). The WAC of APC at pH 3.0 and pH 5.0 is significantly higher than that of mNaOH and mNaCl (Fig. 6A). mNaCl had the least WAC across the pH range tested. The variation observed could be attributed to the method of protein extraction. According to Malomo et al. (2014), this behavior was attributed to the balance of hydrophilic and hydrophobic properties of the residual amino acids in the material samples.

The OAC increased significantly ($p < 0.05$) from APC, mNaOH, and finally to mNaCl (Fig. 6B). APC had the least OAC, which was significantly ($p < 0.05$) different from the membrane concentrates. The OAC values obtained for the membrane concentrates are similar to those reported for B. aegyptiaca concentrate (Asmau & Essan, 2016), chickpeas, and lentils (1.10–2.3 g/g; Boye et al., 2010), and walnut protein concentrate (2.50 g/g) (Mao et al., 2012). This suggests that membrane salt-extracted

concentrates have good ratios of surface non-polar amino acid, greater surface area of macromolecules, and charges, as well as hydrophobicity properties. The mNaCl concentrate could be a useful ingredient for formulating meat and baked goods where oil retention is an important food quality attribute.

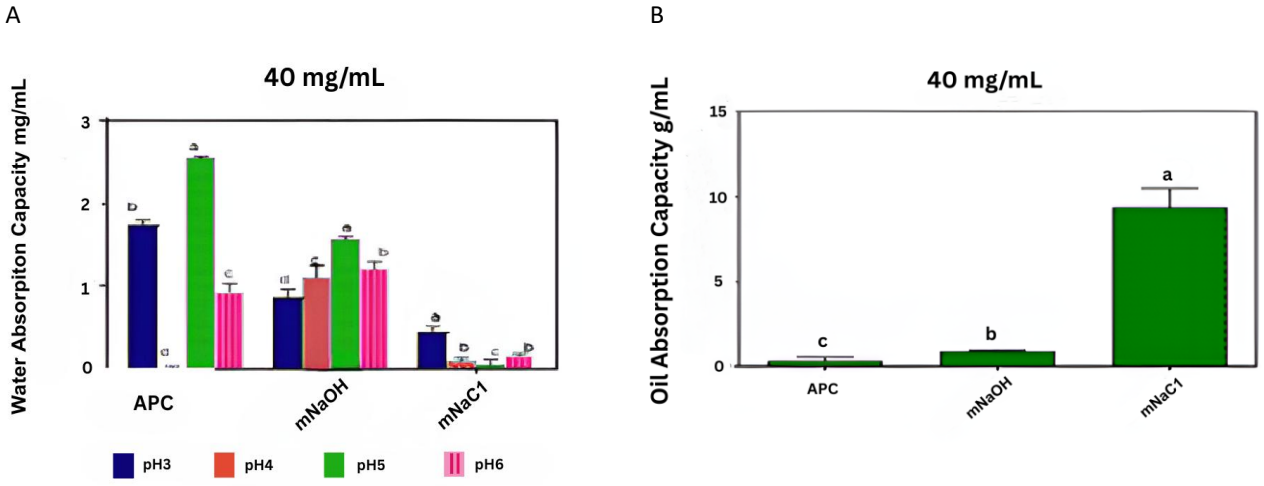


**Figure 3.** A. Water Absorption Capacity and (B) Oil Absorption Capacity of Aduwa protein concentrate (APC), and membrane protein concentrates from NaOH (*m*NaOH) and NaCl (*m*NaCl) extracts. Values obtained are the means and standard deviation of triplicate determinations. Different superscript characters (a, b, and c) indicate significant differences at $p < 0.05$ level on bars

## Foam Properties

**Foam capacity:** FC, or foaming capacity, and foaming stability (FS) of Aduwa membrane concentrates or materials within given pH values. Sample concentrations are shown in Fig. 7. FC and FS depend on the interfacial film formed by polypeptide chains. The interfacial film incorporated an air bubble in suspension, thus reducing the rate of phase merging (Ma et al., 2011). The FC of the Aduwa protein samples increased with concentration as the pH of the medium transitioned from acidic to alkaline (Fig. 7A) except in the mNaCl sample at 20mg/g concentration. APC has a high FC that increases with concentration and pH compared to the membrane concentrates. According to Alonso-Miravalles et al. (2019), the degree of processing can affect FC, as observed in isolated lentil protein, which exhibits higher solubility compared to ultrafiltered samples across the studied pH range. The higher foam capacity in APC towards alkaline pH could result from the enhanced ability to encapsulate air due to the extent of possible unfolded conformation and higher Surface Area. The foam capacity values for mNaOH and mNaCl samples decreased at the acidic region (pH 7.0 and pH 7.0), respectively, for mNaOH and mNaCl, and then increased, attaining the highest foam capacity at pH 9 across the (10 mg/g and 15mg/g) concentrations. The foam capacities reported in this study agreed with those of Osemwota et al. (2021) for green lentil protein isolate and ultrafiltered samples, as well as yam bean protein isolates (Arogundade et al., 2016) and hemp seed proteins (Malomo et al., 2014). A similar pattern was observed for the foam capacity from bambara seed, and walnut isolate proteins (Nasri et al. (2007); Lawal et al. (2006) and Mao and Hua (2012)). The high FC of the samples, especially APC and mNaOH, towards basic environments and at high concentrations, is attributed to increased solubility and net charge, which reduced the formation of large, unstable foam particles (Ijarotimi et al., 2018).

**Foam Stability:** The ability of a foam to maintain its shape and volume over time is described as foam stability (FS). These are important because food ingredients with good

foaming stability can find applications in the confectionery and beverage industries. The FS values in APC, mNaOH, and mNaCl with respect to variations in pH environment and concentration are shown in Fig. 7B. The 10 mg/mL and 15 mg/mL concentrations, the foams were more stable across the pH medium; however, stability decreased with the pH move towards the basic region (5and 7) in mNaCl sample and became stable at pH 9. The results also showed that foam stability values were higher in APC and mNaOH compared to mNaCl, especially at a 15 mg/mL concentration. The observed foam behavior may be attributed to the formation of stable protein molecular layers at the air-water interface in APC and mNaOH samples, which could enhance the foam texture, stability, and elasticity, thereby reducing foam drainage in these samples. The 20 mg/mL concentration did not result in significant protein crowding, and there was no migration of protein to the surface by mNaCl at this concentration, even in the alkaline environment at the air-water interphase, which would create reduced surface tension to encapsulate air. The same trend was observed for rapeseed flours and proteins, where defatted flour exhibited a higher FS than the protein concentrates (Mahajan et al., 2002). Aduwa protein samples exhibited different patterns of FS with respect to pH and varied sample concentration, which may relate to differences in structural properties, as also indicated from the solubility profile. The least foaming stability was observed at 20%, but the improved FS was at 100%, indicating that foams produced by Aduwa membrane protein samples were stable at 10-15 mg/mL concentrations. Foam stability in this work at pH 3.0 and 9.0 (30–100%) is higher than the 6–45% value reported by Alonso-Miravalles et al. (2019).

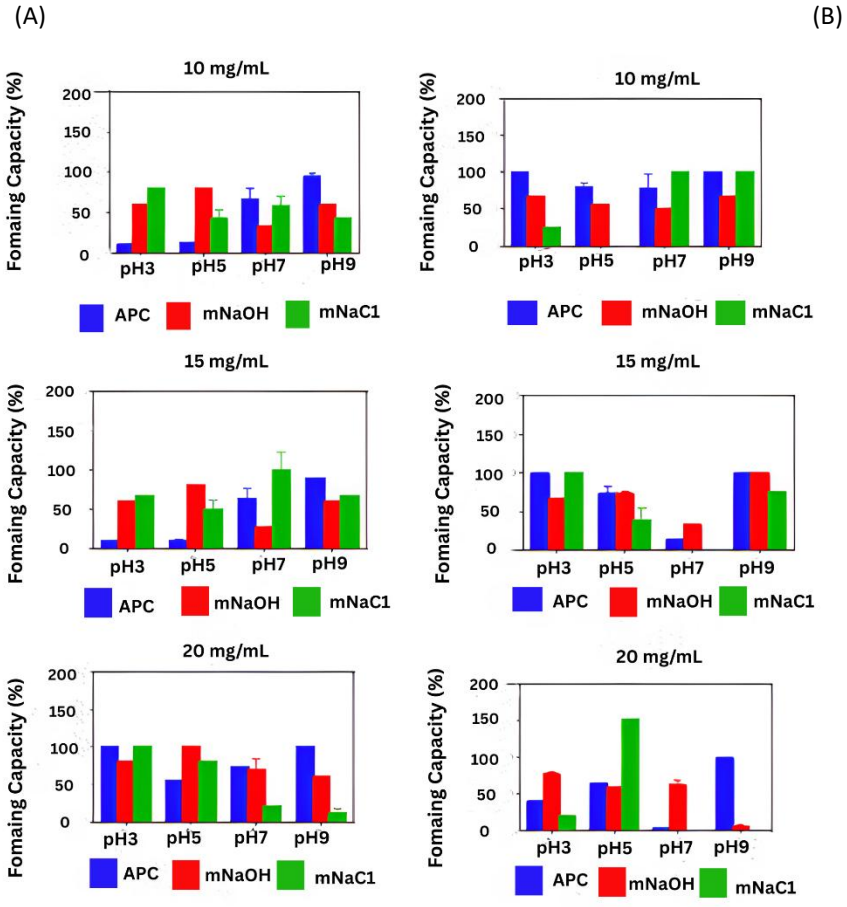


**Figure 4.** Foam capacity (A), Foam stability (B) of Aduwa protein concentrate (APC), and membrane protein concentrates from NaOH (*m*NaOH) and NaCl (*m*NaCl) extracts

**Emulsions:** Figure 8A shows the pH effects on Aduwa protein concentration regarding (A) the oil droplet size (d3,2) and (B) emulsion stability formed from Aduwa protein products. During the emulsification process, protein micelles can migrate to the oil-water interphase for absorption from the thick, viscous films surrounding the oil droplets. This results from hydrophobic and hydrophilic groups orienting towards oil and aqueous phases, respectively (Johnson et al.,2015). The lower the oil droplet size, the better the emulsifying ability of the samples. Surface area and droplet size of the oil in emulsions form in APC and membrane concentrate samples remain unchanged across various concentrations and pH ranges, reflecting a reduced effect of pH on the Aduwa protein's interaction with oil droplets. APC forms emulsions with larger oil droplet sizes at buffering pH 3 and 5 compared to emulsions formed by mNaOH and mNaCl. The sample APC oil droplet size emulsion decreased with increased concentration and pH of the medium. The lower oil-droplet size observed at pH 5.0 compared to pH 3.0 could be attributed to the lower net charge near the isoelectric point, thereby increasing the ability to form protein-protein interactions necessary for encapsulating oil droplets. All Aduwa sample emulsions have a small oil droplet size at pH 9.0, which is consistent with increased solubility, making more proteins available for interactions with the oil droplets. The pH change has a greater effect on Aduwa protein emulsion formation than protein concentration, indicating a strong influence of the environment and consistent with previous observations for isolated lentil (Osemwota et al., 2021) and pea (Shevkani et al., 2015) proteins.

**Emulsion Stability:** The ability of bioactive protein materials to form a stable emulsion, where two immiscible phases (typically oil and water) are combined without phase separation, describes the emulsion stability of protein materials (Malomo & Aluko, 2015). Change in oil-droplet size measured at the end of a 30-minute interval defines emulsion stability (ES). Figure 8B shows that emulsion stability did not vary much with differences in pH and protein concentration. At *10 mg/mL* and *20 mg/mL* concentrations, APC, mNaCl, and mNaOH samples demonstrate large emulsion stability at pH 3.0 and pH 9.0, while a reversed trend was observed at pH 5 at 15 mg/mL concentration in mNaOH. The results suggest that the presence of reduced net charge and high hydrophobic and hydrophilic material balance at pH 3.0 and 9.0 in APC, mNaCl, and mNaOH samples may have contributed to increased ES due to strong electrostatic repulsions, compared to pH 5.0 found in mNaOH and mNaCl sample at *15 mg/mL* and *10 mg/mL* concentrations. The trend of ES in this study showed that a 20 mg/mL concentration of Aduwa samples provided enough protein, as evidenced by values close to 100% for all samples, except for the emulsion stabilized by APC at pH 5.0.

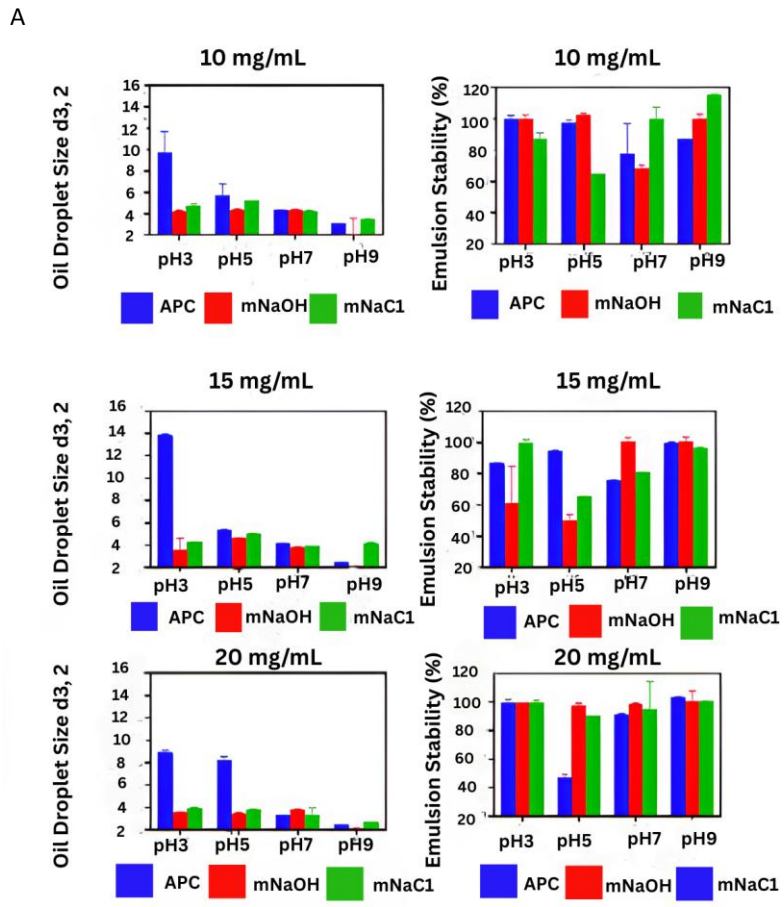


**Figure 5.** Effects of of pH and concentration on the (A) Oil droplet size ($d_{3,2}$) and (B) Stability of emulsions formed by Aduwa protein concentrate (APC) , membrane protein concentrates from alkaline (mNaOH) and salt (mNaCl) extracts.

## CONCLUSION

The study demonstrates how protein extraction methods impacted the physicochemical and functional properties of Aduwa meal-derived protein materials. The protein yield, protein digestibility, surface hydrophobicity, and functional properties of APC, mNaOH, and mNaCl samples differ. The APC and mNaCl samples have better in vitro protein digestibility, gelation concentration, and surface-related properties (So) compared to the mNaOH sample. The pH medium had a substantial effect on the physicochemical properties of *Aduwa* proteins, reflecting the effects of *Aduwa* protein/environment interactions on their properties. Sample mNaCl and next by APC sample exhibited better functional properties than mNaOH, which has provided insights into Aduwa seed ingredient protein choice that could be used to formulate a variety of food products.

**Conflict of interest: The** authors declare that they have no conflict of interest.

**Author contributions:** The authors confirm contribution to the paper as follows: formal analysis: Ogori A.F; validation: Ogori A.F; investigation: Ogori A.F, Girgih AT; draft manuscript preparation: Ogori A.F; visualization: Girgih AT; conceptualization: Girgih A.T; methodology: Ogori A.F; writing - review & editing: Ogori A.F and Girgih A.T; funding acquisition: Ogori A.F; supervision: Girgih A.F. All authors reviewed the results and approved the final version of the manuscript.

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