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Protein profile and functional properties of defatted flour, protein concentrate, and isolate of Al-Samh (*Mesembryanthemum forsskalei* Hochst) seeds

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Abstract

Protein profile and functional properties of the defatted flour, protein concentrate and isolate prepared from Al-Samh seeds were investigated. SDS-PAGE revealed the molecular weight distribution of samples protein was found in the range of ~15 and ~80 kDa. Both water and oil holding capacities were found to be significantly different among all samples. Protein isolate of Al-Samh seeds had the highest amounts of water and oil holding capacities 30 and 24.5 ml/100g, respectively. However, Al-Samh protein isolate showed a lower value of the emulsion stability compared to that of the defatted flour and protein concentrate Emulsion activity was found to be 41.4, 44.03 and 46.24% for the defatted flour, protein concentrate, and protein isolate of the seeds, respectively. The foaming capacity and capacity of Al-Samh seed products, revealed that the protein isolate had higher foam capacity and stability compared to that of protein concentrate and defatted flour. The maximum protein solubility of the defatted flour, protein concentrate, and protein isolate was noted at pH values from 7.0 to 12.0, while the minimum solubility values of the samples were obtained at pH levels between 4.0 and 6.0. Accordingly, it could be stated that Al-Samh protein has the potentiality to be a respectable source of protein elements in food processing.

Keywords: Al- Samh, Isolated protein, Concentrated protein, SDS-PAGE, Functional properties.

1. Introduction

In recent years, most of the researches are focused on new unexploited plant resources to feed the growing world population and food manufacturing purposes. In this sense, wild plants are considered as one of the main sources of macro and micronutrients for human consumption due to their tolerate to drought, heat and salinity. Al-Samh (*Mesembryanthemum forsskalei* Hochst) is considered as one of the interesting wild plant grown in the semi-arid zone of the Middle East region. In Saudi Arabia, this plant has been domesticated and grown in the northern parts particularly in Al-Jouf region in the spring season (Batanouny, 2001). Al-Samh seeds is being used for medical and nutritional purposes (Pandey et al., 1995). Traditionally, Al-Samh seeds are prepared by roasting, then ground to fine flours, and mixed with butter and dates, afterward, and baked and then consumed as a traditional food called Pakilla (Alruqaie and Al-Ghamidi, 2015). Many previous studies indicated that Al-Samh seeds are rich in nutrients as it has 22.25% protein, 5.6% fat, and 9.7% crude and 9.7% crude fiber. In addition, it contains appreciable amounts of minerals such as potassium, magnesium, sodium, and calcium (Al-Jassir et al.,1995; Mustafa et al., 1995).

Recently, Alderaywsh et al. (2019) investigated the effect of roasting, cooking and baking on the nutritional quality and *in vivo* biological value of Al-Samh. They concluded that processing particularly roasting enhanced the quality of the protein and the biological value of Al-Samh flour. Moreover, Al Qahtani and Al maiman (2011) studied the nutritional characteristics of Al-Samh seed products in term of defatted flour, protein concentrate and protein isolate. They found that Al-Samh seed products, particularly the protein isolate contained a sufficient amount of essential amino acids especially leucine and lysine. Furthermore, they stated that it had a high rate of the *in vitro* protein digestibility, which was found to be 80.1, 82 and 89.3% for the defatted flour, protein concentrate, and protein isolate, respectively.

Despite, there have been many studies carried out in Al-Samh seeds, the functional properties of Al-Samh seeds flour and protein isolates have not been studied thoroughly. Since the functional properties are considered to be fundamental physicochemical characteristics which may affect the behavior of food systems during processing and storage of food materials. Therefore, the objective of this study was to investigate the functional properties of defatted flour, protein concentrate and isolate of Al-Samh seeds for potential utilization of these seeds protein products as supplements and additives in the food industry.

2. Materials and methods

2.1. Materials

Al-Samh seeds were obtained from Al-Jouf (Dumat Al-Jandal) area, Saudi Arabia. The seeds were cleaned by using a 0.5mm sieve to remove impurities that magnify the seeds, and then another 0.35mm sieve was used to remove impurities that minimize the seeds (similar to the colour of the seeds). The grinding process was carried out by a laboratory miller and the fine flour was placed in sealed containers and kept in the refrigerator at 4 °C until used for analysis.

2.2. Methods

2.2.1. Defatted flour preparation

Al-Samh flour was defatted according to AOAC (1995). The hexane solvent was added to the sample in a ratio of 10:1 and then stirring with an electric motor overnight at room temperature. Then the solvent was removed using rotary evaporation and the sample was dried at room temperature and kept in a refrigerator at 4 ° C for the further analysis.

2.2.2. Preparation of protein concentrate

The protein concentrate of Al-Samh flour was prepared according to Muttil (1974). Water was added to the defatted flour (10:1) and then the pH was adjusted to 9 using 0.1N NaOH. The alkaline mixture was stirred for 2 h at room temperature. After filtration, 80% methanol was added and allowed to stand for 2 h at 4 ºC. The alcohol was removed and sample dried and kept in airtight containers in the refrigerator at 4 ° C until it was used for analysis.

2.2.3. Preparation of protein isolate

The protein isolate was prepared by mixing the defatted flour with water (1:10) and adjusting the pH to 9 using sodium hydroxide (0.1N). The mixture was then stirred for 2 h and centrifuged at 5000 × g for 20 min to obtain dissolved protein. The rest of the protein and the protein was precipitated at the isoelectric point of the Protein pH 4.57 using HCl following the same way of precipitation Wagner et al. (2000).

2.2.4. SDS–PAGE analysis of Al-Samh protein

The discontinuous SDS-PAGE was performed according to Lee et al. (2015). The sample of the total, concentrated and isolated protein of Al-Samh seeds was dissolved in the buffer (50 mM Tris-HCl buffer, pH 6.8, 14.4 mM ß-mercaptoethanol, 2.0 % SDS, 20.0 % (v/v) glycerol, and 0.01% (w/v) Bromophenol Blue) and then heated at boiling temperature for 5 min. After cooling, the samples were loaded in a volume of 10-20 µL and subjected to the electrophoresis with a constant current 30 mA per gel. The gel was stained with a staining solution Colloidal Coomassie G-250 and then de-stained with the solution (ethanol, orthophosphoric acid and H_2O) and then stored in a 5% glycerol solution for imaging

2.2.5. Water and oil holding capacities of Al-Samh

The water holding capacity (WHC) and the oil holding capacity (OHC) of of Al-Samh flour samples were estimated using the procedure described by Sudha, Baskaran, and Leelavathi (2007). Flour samples were suspended in refined sunflower oil (3 g sample in 30 mL). Suspensions were shaken for 30 min and then centrifuged at 3000 rpm for 15 min at 20 °C. The supernatant was decanted and the WHC was estimated as the amount of water retained per gram of the sample, whereas the OHC was expressed as the amount of oil bound per gram of dry matter of the sample.

2.2.6. Emulsification properties of Al-Samh

The emulsifying properties of Al-Samh samples were measured according to the method of Elkhalifa and Bernhardt (2010). About 2 g of the sample was agitated with 20 mL H_2O in a high-speed laboratory blender. Refined sunflower oil was added gradually (0.4 mL/s) during blending until phase separation occurred. The ratio of the emulsified layer to the total volume was used to express the emulsification activity (EA):
EA (%) = Height of emulsified layer/Height of total volume × 100.
The emulsion was centrifuged again, heated at 80ºC for 30 min, and then cooled to 15ºC. The emulsion stability (ES) was calculated as the percent of the total volume remaining emulsified after heating:
ES (%) = Height of the emulsified layer after heating/Height of total volume × 100.

2.2.7. Foaming properties of Al-Samh

The foaming capacity (FC) and foam stability (FS) of Al-Samh flour were measured according to the method of Wang et al. (2018). A mixture of a sorghum sample and H_2O (0.7 g in 100 mL) was whipped in a high-speed laboratory blender for 5 min and then decanted into a 250- mL measuring cylinder, and the foam volume was recorded. The FC was calculated according to the following equation:
FC (%) = (Volume after whipping – Volume before whipping)/Volume before whipping × 100.
The FS was calculated in terms of the drop in volume of the foam in the measuring cylinder after 60 min:
FS (%) = Foam volume after 60 min/Initial foam volume × 100.

2.2.8. Protein solubility of Al-Samh

A protein solubility determination for each sample was performed according to the method described by Elkhalifa and Bernhardt (2010). Defatted sorghum flour (1 g) was suspended in water (50 mL, pH 6). The suspension was shaken for 1 h at room temperature and then centrifuged at 3000 rpm for 20 min (Hettich Universal 30 F - Germany). The soluble protein content in the supernatant was determined using the Kjeldahl method (AOAC 2005).

$$\text{Protein solubility } \% = \frac{\text{soluble protein}}{\text{total protein}} \times 100$$

2.3. Statistical analysis

All analyses measurements were done in triplicate (*n* = 3). The data were statistically evaluated by analysis of variance. Differences at the 5% level of significance were compared using least significant difference test (LSD). Principle component of Analysis (PCA) cluster analysis was performed using GEE-biplot software, version 8.1.

3. Results and discussion

3.1. SDS page of Al-Samh flour, protein isolate, and protein concentrate

SDS-PAGE of the defatted flour, protein concentrate and protein isolate of Al-Samh seeds were used to compare the subunit profile of proteins (Fig. 1). Lane 1 standard molecular weight marker whereas Lanes 2, 3 and 4 are defatted flour protein, protein concentrate and protein isolate, respectively. The electrophoretic patterns of defatted flour, protein concentrate, and protein isolate were comparable showing most of the poly-peptides existing in the samples. Samples revealed similar protein profile with many numbers of protein bands. The molecular weight distribution of protein was ranged between approximately 12 and 80 kDa, however, the predominant polypeptides of the defatted flour, protein concentrate and protein isolate of Al-Samh seed were found in the range of 12 to 40 kDa.

Based on these findings Al-Samh seed proteins is considered to be a rich in globulin, glutelin and albumin fractions according to González- Pérez & Arellano (2009) who reported that low molecular weight (< 18) is related to these fractions.

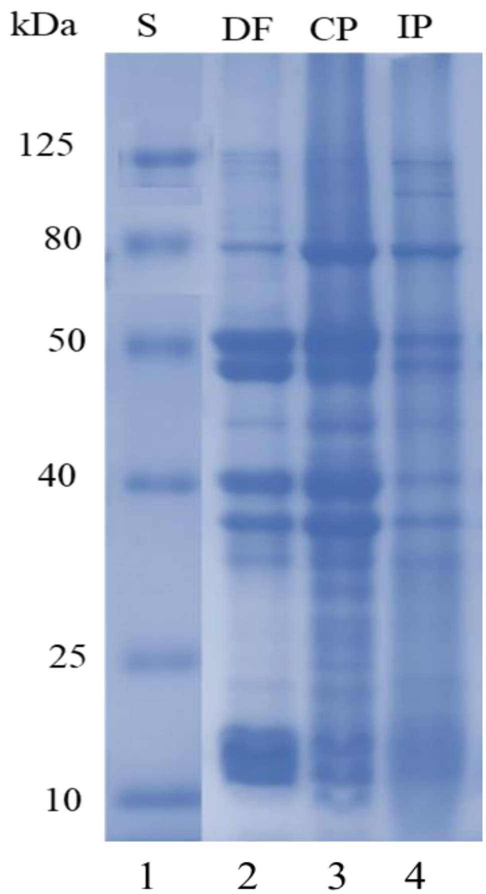


Fig. 1 SDS-PAGE pattern of Al-Samh protein samples. Lane 1: Molecular weight standards Lane 2: Defatted flour; Lane 3: protein concentrate; Lane 4: protein isolate.

3.2. Water holding capacity (WHC) and oil holding capacity (OHC) of Al-Samh flour, protein isolate, and protein concentrate

Figure 2 (a & b) present the WHC and OHC of defatted flour, protein concentrate, and protein isolate of Al-Samh seeds at their natural pH. As shown in figure 2a, the WHC was varied significantly ($P < 0.05$) among the samples. Protein isolate showed significantly higher WHC than protein concentrate and defatted flour. There was also significant difference in WHC between protein concentrate (27,5) and defatted flour (25.7). .Similarly, the OHC (fig 2b) of Al-Samh samples showed significant ($P < 0.05$) variation particularly between the defatted flour (23.6 ml/100g) and protein concentrate (25.7ml/100g).

WHC and OHC are essential in food manufacturing since they are required to improve the texture hardness and crispiness in many food products such as cake batters and sausages (Aletor et al., 2002). Our results showed that Al-samh, particularly the protein isolate, has high values of WHC and OHC. This might be due to excessive capability of protein isolate to swell, dissociate and unfold exposing additional binding sites, whereas, on the defatted flour and concentrated protein, others components such as carbohydrates and non-protein constituents weaken it (Kinsella, 1979).

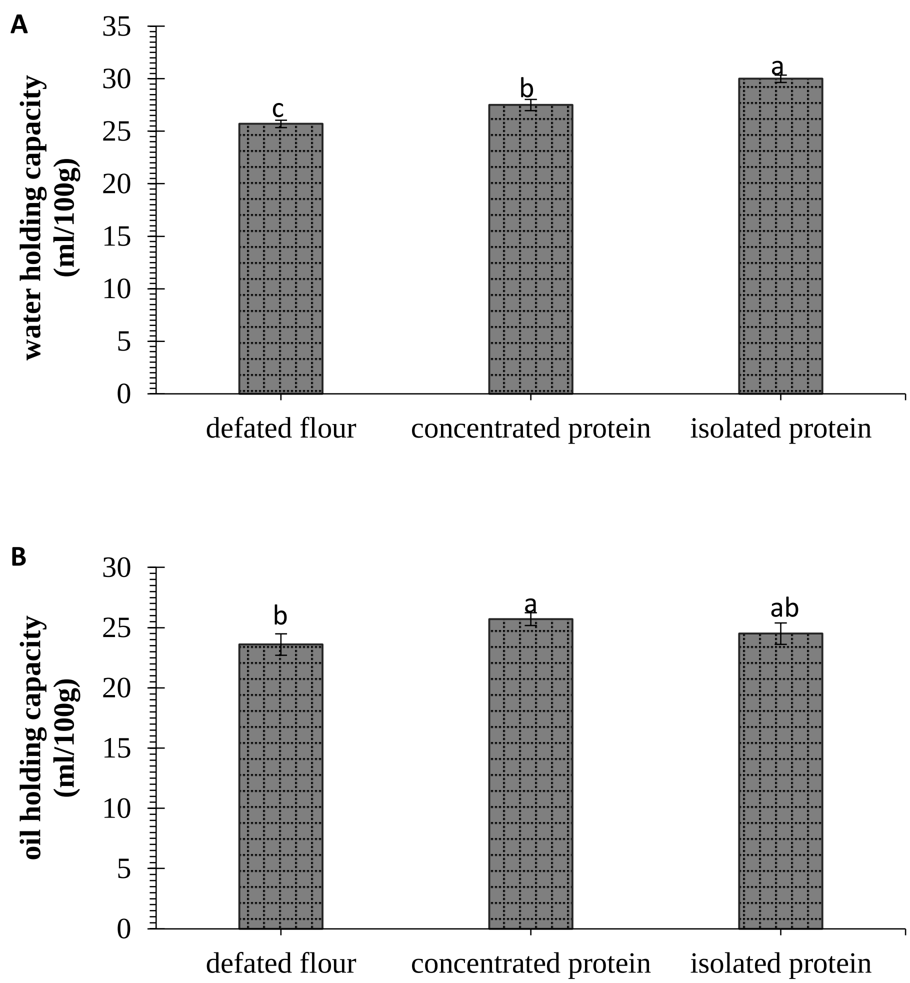


Fig. 2 Water holding capacity (A) and oil holding capacity (B) of Al-Samh seed protein. Values are means (±SD) of triplicate samples. Values without superscript are not significantly different (P < 0.05) as assessed by LSD. Values followed by the same letter are not significantly different (P < 0.05) as assessed by LSD.

Many other studies also revealed that isolated protein exhibit rich WHC and OHC compared to that of protein concentrate and defatted flour. Accordingly, the protein isolate of Al-Samh seed has the potential to be used as and natural ingredients in many food industries such as meat, bread, and cakes industries.

3.3. Emulsion properties of Al-Samh flour, protein concentrate, and protein isolate

Emulsification is an important function in different food industry such as ice cream, butter, cooked cheese, mayonnaise and sausage. Proteins play a key role in stabilizing these colloidal adhesions (Kinsella, 1976). The emulsion activity and emulsion stability of defatted flour, concentrated protein and isolated protein of Al-Samh seed are depicted in Figure 3 (A & B). As shown in figure 3A, the emulsion activity was found to be 41.4, 46.1 and 43.6% for the defatted flour, concentrated protein, and isolated protein of Al-Samh seeds, respectively. Also, it was observed that increment of protein concentration of Al-Samh seeds significantly ($P < 0.05$) increased its emulsion activity. In contrast, the emulsion stability of the protein concentrates and protein isolate (41.7, 37.2) were significantly lower than those of e defatted flour (36.7%) (fig 3B).

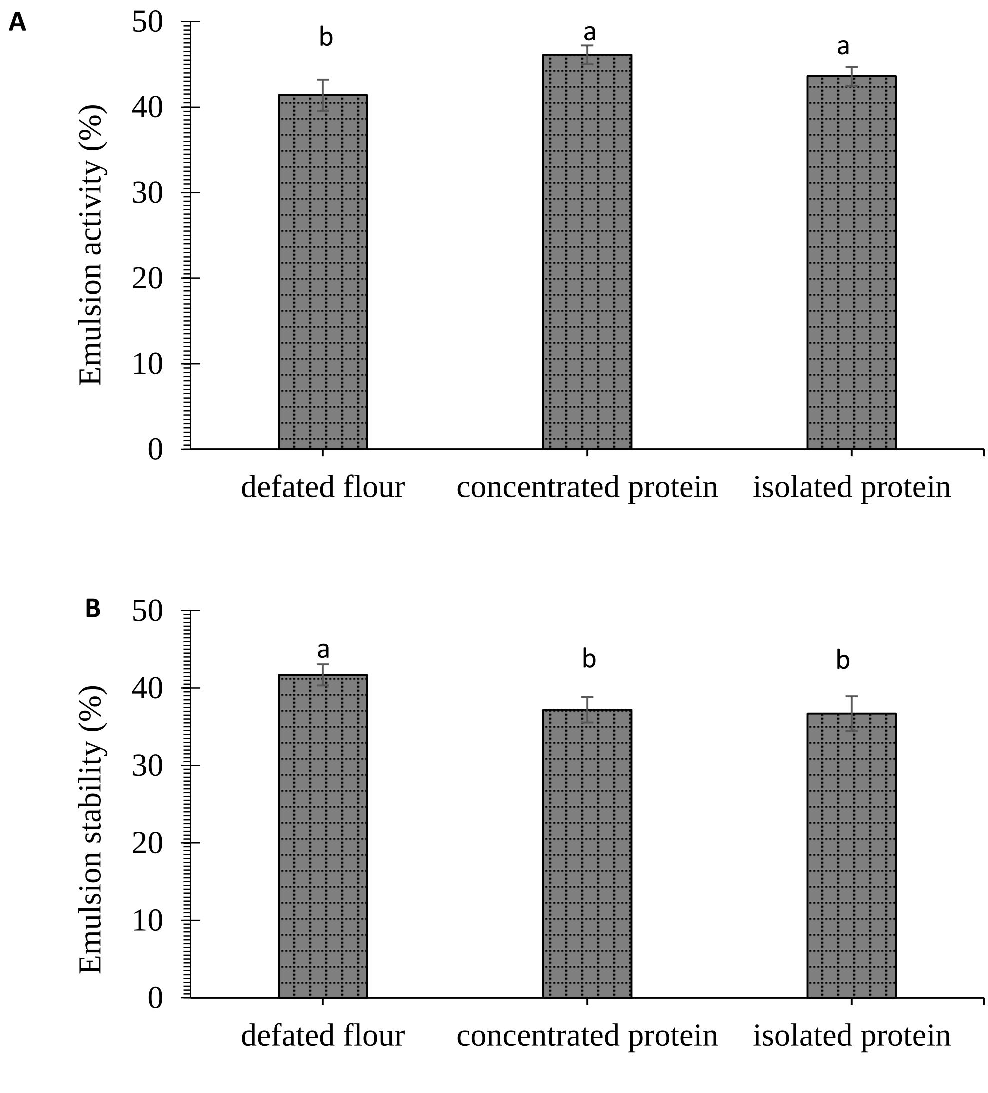
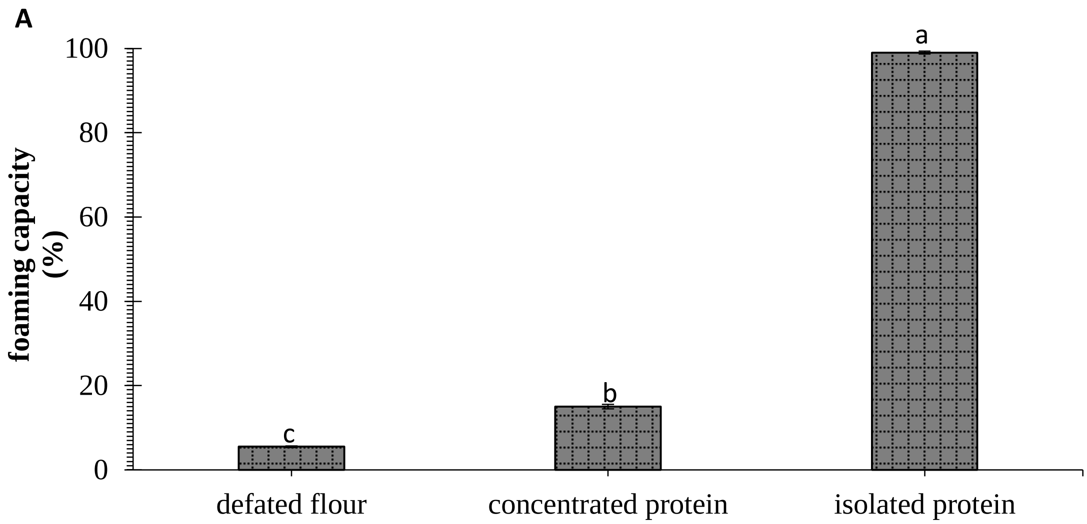


Fig. 3. Emulsion activity (A) and Emulsion stability (B) of Al-Samh seed protein. Values are means (±SD) of triplicate samples. Values without superscript are not significantly different (P < 0.05) as assessed by LSD. Values followed by the same letter are not significantly different (P < 0.05) as assessed by LSD.



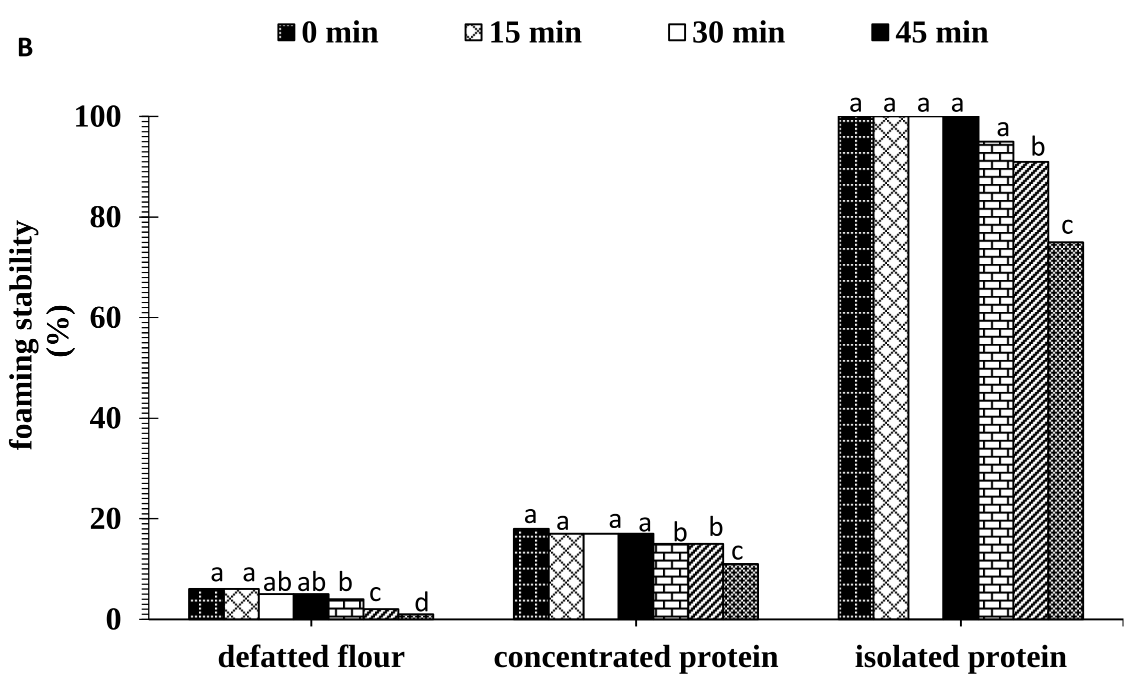


Fig. 4 Foaming capacity (A) and foaming stability (B) of Al-Samh seed protein. Values are means (±SD) of triplicate samples. Values without superscript are not significantly different (P < 0.05) as assessed by LSD. Values followed by the same letter are not significantly different (P < 0.05) as assessed by LSD.

The emulsifying activity values of the studied samples were found higher compared to Akee apple dehydrated seed flour 25.7% (Akintayo et al., 2002), canola dehydrated seed flour 32.48% and canola protein isolate

39.8% (Aluko and McIntosh, 2001). The decrease in emulsion stability in comparison with the emulsifying activity of both the protein concentrate and isolate is consistent with several studies. Aluko and McIntosh, (2001) reported that the emulsified activity of canola dehydrated seed flour was 32.48% while the emulsion stability decreased to 15.71%. The study of El-Adawy and Taha (2001) showed that the stability of the fat emulsion of red paprika seeds was 18.6% while its emulsification activity was 41.7%. Stabilized Rapeseed dehydrated emulsion stability was 86% while the emulsifying activity of this product was 97.25% (Mahajan et al., 1999).

The greater emulsion activity observed in the isolated protein of Al-Samh could be due to its high protein solubility. On the other hand, lowering the emulsion stability of Al-Samh protein after the isolation might be related to the changes in protein as described above in protein solubility. Changes, such as protein aggregation as well as surface hydrophobicity and charge characteristics, affect emulsifying properties in different ways.

3.4. Foaming properties of Al-Samh flour, protein concentrate, and protein isolate

Foaming capacity and foam stability of the defatted flour, protein concentrate, and protein isolate of Al-Samh seeds were illustrated in figure 4 (A &B). It was observed that the foaming capacity increased significantly (P < 0.05) with the processing the Al-Samh seed into protein concentrate and isolate (fig 4A). The protein isolate showed the highest foaming capacity (99%) compared to defatted flour (15%) and protein concentrate (5.5%). The foam stability followed the same pattern of the foaming capacity (fig 4B). The foaming stability was determined by measuring the decrease in forming as functional of time. The isolated protein showed a higher foam stability than both concentrated protein and defatted flour of Al-Samh seed. Foam stability of the isolated protein maintained at 100% up to 45 min and it was dropped to 95, 91 and 75% when it was left to stand for 60, 90 and 120 min, respectively. The obtained result revealed that the foaming properties of Al-Samh greatly improved when isolated protein is prepared. This finding is in agreement with what was reported by Ogunwolu et al. (2009) who stated that both the foaming capacity and stability of cashew nut improved when protein is isolated. On the other hand, the foam stability of the isolated protein of the Al-Samh is much higher than those found by other researchers. The Foam stability of Mustard white and green mustard protein isolates was found as 37.5% and 37.8%, respectively (Aluko et al., 2005), amaranth 40% (Fidantsi and Doxastakis 2001) and tomato seed protein isolate 60.4% (Liadakis et al., 1998). Also, Gurner and Ismond (1997) found that the stability of the foam for canola protein isolate was 74.7%. The enhancement of foaming properties of isolated protein might be due to the changes in the protein nature and solubility.

3.5. Protein solubility of Al-Samh flour, protein concentrate, and protein isolate

Figure 5 shows the protein solubility profile of defatted flour, concentrated and isolated protein of Al-Samh seeds at different levels of pH (2.0 – 12.0). The solubility of isolated protein of Al-Samh seed was found to be 75.9% at pH 2.0 and sharply dropped to minimum values of 19.4, 15.5 and 15.5% at pH 4.0, 5.0 and 6.0, respectively. Thereafter, the solubility increased as the pH increased to reach the highest value of 98.1% a the pH 12.0. Likewise, the solubility of the defatted flour and concentrated protein of Al-Samh seed was found slightly high 31.9% at pH 2.0 and significantly (P < 0.05) decreased at pH levels of 4.0, 5.0 and 6.0 to 17.8, 15.5 and 15.6% for concentrated protein and 17.7, 16.8 and 21.2% for the defatted flour, respectively. For both samples, the maximum values of protein solubility was 70% for defatted flour and 61.9% for protein concentrate were recorded at pH 12.0. The maximum values of the protein solubility of the defatted flour, protein concentrate, and protein isolate was noted at pH values from 7.0 to 12.0, while the minimum solubility values of the samples were obtained at pH levels between 4.0 and 6.0. A similar observation was reported by several researchers. Ogunwolu et al. (2009) stated that the solubility of the defatted flour, concentrated protein and isolated protein of cashew nut is high in the alkaline pH level and low at the pH 4.0. Low solubility rate at pH between 4.0 to 6.0 indicated that Al-Samh protein is naturally acidic and its isoelectric point was in the range of the pH 4.0 – 5.0. It has been reported that the majority of plant proteins are acidic protein and have minimum solubility at the isoelectric point (Damodaran 1997). This phenomenon might be due to an increase of the net charge of the peptides when the pH values increased and hence become alkaline (Sorgentini and Wagner, 2002). In general, Al-Samh protein, particularly isolated protein, showed respectable solubility rate in both acid and alkaline pH, however, its solubility was found to be relatively high in alkaline pH compare to acidic pH. Hence, the isolated protein of Al-Samh seed has the potential application in food industries since the solubility is probably the most critical function which is related directly to many other functional properties of proteins.

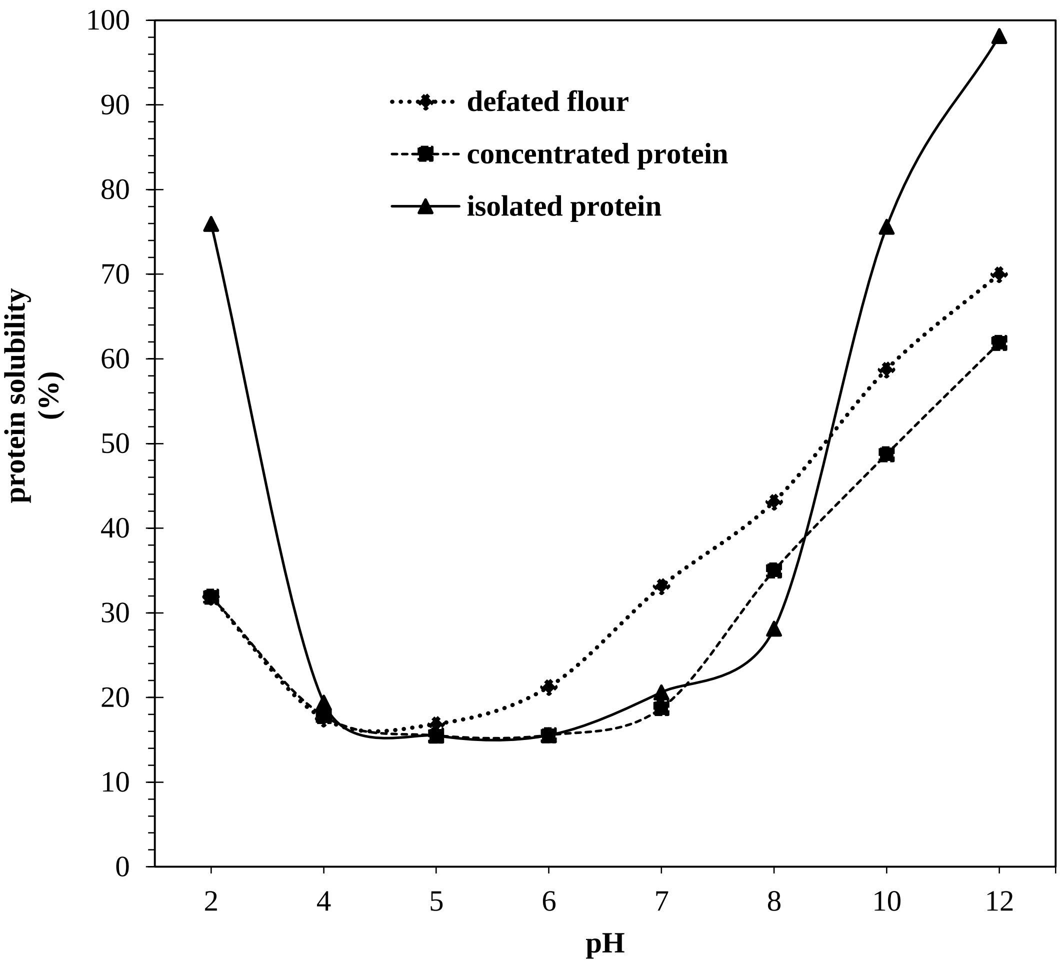


Fig. 5 Solubility of Al-Samh seed protein. Values are means (±SD) of triplicate samples. Values without superscript are not significantly different (P < 0.05) as assessed by LSD. Values followed by the same letter are not significantly different (P < 0.05) as assessed by LSD.

3.6. Principal components analysis (PCA)

Multivariate analysis using PCA demonstrated the interrelationship among the functional properties of Al-smah flour, protein isolate, and protein concentrate (Fig. 6). In the biplot, the axes of the principal component (PC1 and PC2) accounted for 100% variability of the plotted components suggesting their great contribution to the variability of the data. Acute angles between vectors of traits indicated great positive correlations between water holding capacity (WHC), foaming capacity (FC), foam stability FS (0-120 min), and protein solubility at extreme pH conditions (2, 4, 10, and 12). These functional properties were higher in protein isolate compared to defatted flour and protein concentrate. The great correlation between foaming properties with protein solubility at extreme pH conditions, at which high protein solubility was evident, could be attributed to the fact that high protein solubility would allow proteins to be more flexible by unfolding/refolding at the air and water interface and thereby enhancing their ability to encapsulate air particles and foam (Naiker et al., 2019). Besides, acute angles between the vectors of oil holding capacity (OHC), emulsification activity (EA), and WHC demonstrated positive correlation among these properties. Among these traits, OHC and EA were greater in the protein concentrate than in protein isolate and defatted flour. Similar to our findings, a positive correlation between OHC and EA was reported previously in ginkgo

Biloba seeds protein isolates (Deng et al., 2011). Moreover, the emulsion stability (ES) and protein solubility (PS) at neutral pH (5, 6, 7, and 8) were positively correlated suggesting that increase in one property will result in an enhanced level of the other. These functional properties were higher in defatted flour compared to protein isolate and protein concentrate. Similarly, positive correlations between protein solubility and EC of chickpea, faba bean, lentil and pea protein isolates were also reported (Karaca et al., 2011). Overall, PCA analysis demonstrated that Al-Samh protein isolates outscore the defatted flour and protein concentrate in the foaming properties, protein solubility, and WHC.

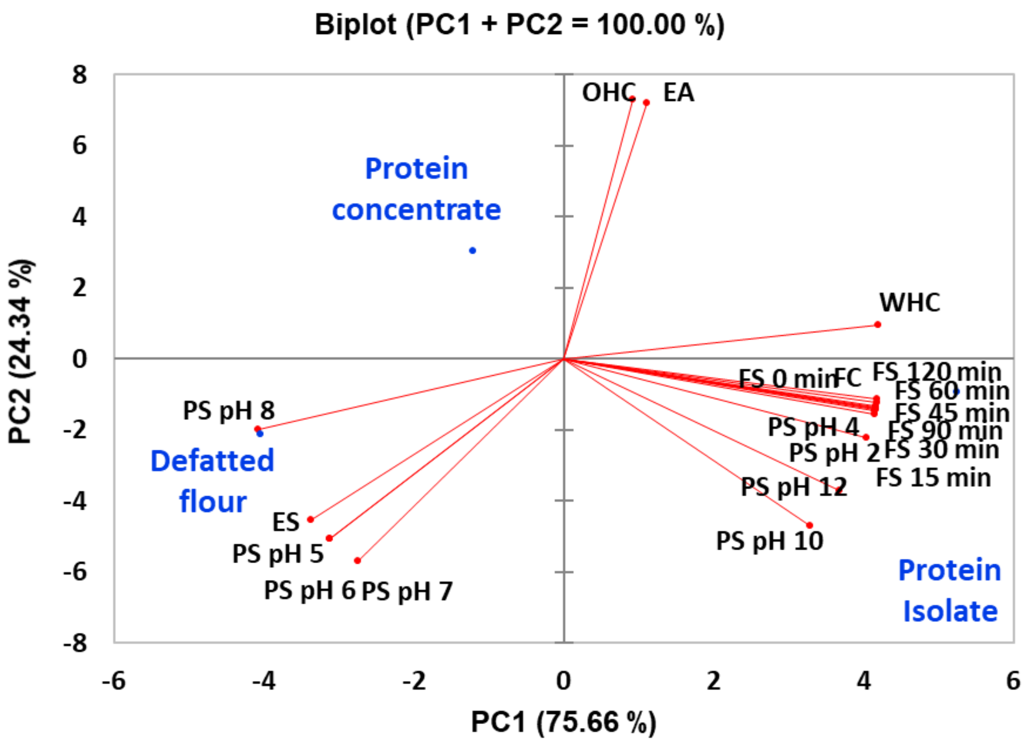


Fig. 6. Principle component analyses of Al-Samh flour, protein isolate and protein concentrate.

4. Conclusion

The functional properties of Al-Samh seed protein were enhanced by the processing of Al-Samh flour into protein isolate. Isolated protein from the seeds showed higher water and oil holding capacity, emulsion capacity and foaming properties than concentrated protein and defatted flour of the seeds. Moreover, the protein solubility of the protein isolate is high particularly at the acidic and alkaline pH levels. Regarding the obtained results, isolated protein form Al-Samh seeds have appropriate functional properties; hence, it has the potentiality to be a respectable source of protein element in food processing.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interests.

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