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Functional properties of protein isolate from fern fronds

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Abstract

The extraction of defatted fern protein isolate was conducted using 0.1 M NaOH and agitation time of 30 min. To ensure that the protein isolate could be utilized for food application, some functional properties such as water holding capacity, oil holding capacity, solubility, foaming capacity and stability were evaluated. *Nephrolepis biserrata* showed the highest foaming capacity of 65% and solubility of 55.9%. For each isolate, water and oil holding capacity were not significantly different ($p < 0.05$) although *Arthropteris orientalis* showed a better water and oil holding capacity than *Nephrolepis biserrata.* Thus, it can be used in food formulation systems.

Introduction

Ferns (Pteridophyta) are an important component of many ecosystems (Tryon, 1986) and potentially provide an abundant food resource for consumers. According to some perspectives, (Copeland, 1942; Thakur *et al.*, 1998; Shin, 2010; Wei, 2010) fronds (leaves) of fern may play a significant role as a potential source of food protein. Interest in this crop is developing due to its several advantageous properties. Babayemi *et al.* (2006) pointed out that the dry matter and crude protein content is higher in the leaves of aquatic fern than any part of the plant. In Ghana, fern leaves are added to palm nuts in soups preparation where the plant is a valued potherb and consumed mostly by mothers before and after childbirth. This is to ensure good health and also boost breast milk production, thus reflecting the local population's perception that the plant has nutritional value.

Functional properties of food protein are important in food processing and food product formulation. Protein isolates are intended to be used as additives in food products for improving functional properties such as foaming/emulsifying capacity, gel formation, viscosity, texture and water-binding capacity. The importance of these properties varies with the type of food products in which the isolated protein is to be used (Yada, 2004). Protein isolates with high oil and water binding capacities are desirable for use in meat, sausages and bread, while proteins with high emulsifying and foaming properties are good for salad dressing, confectionaries, frozen foods and soups (Ahmedna *et al.*, 1999). Proteins from defatted fern flour have considerable potential for use as a supplement in a variety of foods as new protein source.

Information on the functional properties of defatted fern frond protein isolates is almost non-existent although studies have been reported on various uses of fern fronds. Therefore, the present work was carried out to investigate functional properties such as water holding capacity, oil holding capacity, foaming capacity and stability in order to determine the potential application of the proteins extract in food processing.

Materials and Methods

Plant material

Fern frond, *Nephrolepis biserrata* was harvested from a stream along the mango road of Kwame Nkrumah University of Science and Technology, Ghana and *Arthropteris orientalis* was also harvested from palm trees along the Buroburo road within the same University community. Fern fronds were washed and solar-dried for two weeks, milled into flour using MPE roller mills (Model: GP-140 Grinder) and passed through 25 µm mesh sieve to obtain the flour.

Extraction of oil

Fern fronds oil from flour was extracted using the cold extraction method by soaking the flour (tied in a cheese cloth) in hexane using a ratio of 1:10 w/v, with respect to flour/solvent for 48 h in an air tight container at room temperature. The oil free fern fronds flour was then air-dried and stored in high

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density polyethylene bags under refrigeration until when needed.

Proximate analysis

Analysis of fern frond flour and its defatted fern flour was carried out as follows: Moisture, ash, crude lipid, crude fiber and nitrogen content were determined according to the method of AOAC (2005). Carbohydrate was calculated by difference using the formula [100 - (protein + fats + moisture + ash + fiber)].

Protein extraction and isolation

Protein was extracted by dispersing 0.5 g of defatted fern flour in 25 mL of 0.1 M NaOH. Samples were agitated in a centrifuge tube at room temperature for 30 min in a Gallenkamp orbital shaker at 150 rpm. The solubilized liquor was separated from insoluble material by centrifugation at 2500 rpm for 15 min at room temperature (25^{o}C). The supernatant was acidified to a pH range of 2.3 to 2.5 with concentrated HCL to precipitate proteins. Precipitated protein was recovered by centrifugal separation (2500 rpm for 15 min) and washed with an adequate amount of distilled water. The isolated protein was then freeze-dried and stored for analysis. The protein content of dried powdered sample was determined by Kjeldahl method (AOAC, 2005) using Kjeldahl factor of 6.25.

Determination of functional properties

Water holding capacity (WHC)

To determine the water holding capacity of defatted fern flour, the method outlined by Diniz and Martin (1997) was followed. Triplicate samples (0.5 g) of each isolate were dissolved with 10 mL of distilled water in a graduated centrifuge tubes and vortex for 30 sec to disperse the proteins. After a holding period of 30 min at room temperature, dispersions were centrifuged at 3000 rpm for 25 min. The supernatant was filtered with cheese cloth and the volume of released fluid was accurately measured. The difference between initial volumes of distilled water added to the protein samples and the volume retrieved was calculated. The results were reported in triplicate as volume (mL) of water absorbed per gram of protein sample.

Oil holding capacity

Oil holding capacity of defatted fern protein was determined as the volume of edible oil held by 0.5 g of fern protein according to the procedure described by Shahidi *et al*. (1995). The protein sample in 10 mL of oil was shook using orbital shaker at 150 rpm and then centrifuged at 3000 rpm for 25 min. The volume of the supernatant was measured. The oil-holding capacity was expressed as volume (mL) of oil held by 0.5 g of protein sample.

Foaming capacity and stability

The foaming capacity was determined using the method outlined by Aruna and Parakash (1993) with minor modification. One hundred milliters of deionised water (V_1) at different pH (2-14) were separately added to 1 g of defatted fern protein isolates. The suspension was blended for 3 min using a high-speed blender (Aruna and Parakash, 1993), poured into 250 mL graduated cylinder and the volumes of foam (Vt) were immediately recorded. Foam capacity (FC) was calculated as shown below.

$$FC = \frac{Vt - V_1 \times 100}{100}$$

The foam stability was determined by measuring the decrease in volume of foam as a function of time at 10, 20, 30, 40, 50 and 60 min using the pH of the protein suspension that recorded the highest FC %.

Protein solubility

The solubility of protein isolates as a function of pH was determined according to the method described by Dench *et al*. (1981) with slight modification. One-hundred milligram samples of each freeze-dried protein isolate were suspended in 20 mL of distilled water and the pH of the suspension adjusted from 2.0-14.0 with an interval of 2 units using 0.1N HCl or NaOH solution. The suspension was agitated with orbital shaker for 30 min at room temperature and then centrifuged at 4000 rpm for 30 min. The quantity of protein dissolved in supernatant was determined by Biuret method according to AOAC (1995) using egg albumin as standard. Protein solubility was calculated as:

Solubility (%) = (protein content of supernatant / protein content of samples) x 100

Statistical analysis

The experimental results for chemical composition and functional properties were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and the difference between samples were determined by Tukey's test using Minitab 16 program. P-values < 0.05 were regarded as significant.

Results

Chemical composition of fern frond

The chemical composition of whole fern frond flour and defatted flours are reported in Table 1. There was a general decreased in all the chemical composition after defatting for both *Nephrolepis biserrata* and *Arthropteris orientalis* except for carbohydrate and protein content. The defatted flour of *Nephrolepis biserrata* also showed an increase in ash content.

Functional properties of fern frond

There was no significant difference between the two fern types with respect to water and oil holding capacity as shown in Table 2 although *Arthropteris orientalis* protein isolate showed better water and oil holding capacity than *Nephrolepis biserrata.* Foaming capacity was low for both *Arthropteris orientalis* and *Nephrolepis biserrata* within the pH 2-8 as shown in Figure 1. Increased in foaming capacity was observed above pH 8 with *Nephrolepis biserrata* recording the highest foaming capacity at pH 14. There was a sharp drop in foaming stability within the first 10 min with *Arthropteris orientalis* maintaining stable foam at the end of the 60 min (Figure 2). There was a decrease in solubility for both fern types within the isoelectric point but increased above pH 4 as shown in Figure 3. *Nephrolepis biserrata* recorded the highest solubility at pH 8 with *Arthropteris orientalis* showing a better solubility at pH 14 than *Nephrolepis biserrata.*

Table 1. Chemical composition of whole fern leaves flour and defatted flour obtained by the cold method of extraction

Composition	*Nephrolepis biserrata*		*Arthropteris orientalis*	
(Mean %)	Whole flour	Defatted flour	Whole flour	Defatted flour
Moisture	7.79±0.18^{a}	4.22±0.21^{b}	7.81±0.02^{a}	3.92±0.64^{b}
Fat	3.03±0.58^{a}	0.27±0.25^{b}	3.78±0.33^{a}	0.53±0.05^{b}
Fiber	17.14±1.40^{a}	11.21±0.91$^{b\,c}$	12.21±1.30^{b}	9.00±0.59^{c}
Ash	5.59±0.48^{c}	6.09±0.52^{c}	12.72±0.67^{a}	10.39±0.27^{b}
Protein	23.42±1.62^{b}	25.57±2.62^{a}	19.28±1.49^{c}	22.71±0.43^{b}
Carbohydrate	43.01±1.53^{c}	48.71±1.18^{b}	44.21±0.65^{c}	51.74±0.92^{a}
*Protein isolate content	-	39.33±0.32^{a}	-	35.26±0.54^{b}

Values are means of triplicates ± standard deviation. The superscript showed that at $P < 0.05$, a significant difference exists. Means in row that do not share a letter are significantly different. *Percentage protein after isolation.

Table 2. Water and oil holding capacity of defatted fern protein isolates

Protein isolates	Water holding capacity (mL/g protein)	Oil holding capacity (mL/g protein)
Nephrolepis biserrata	2.13±0.00^{a}	2.73±0.23^{b}
Arthropteris orientalis	2.39±0.23^{a}	2.93±0.42^{b}

Means within each column followed by the same letters are not significantly different ($p > 0.05$)

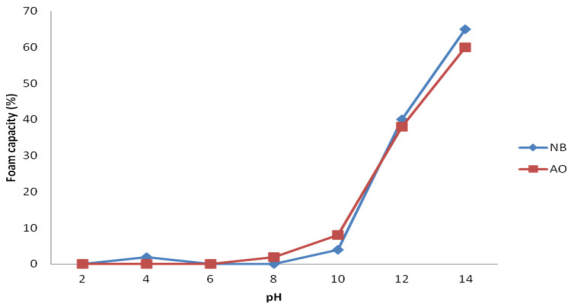

Figure 1. Foaming capacity of defatted fern protein isolate at different pH treatment

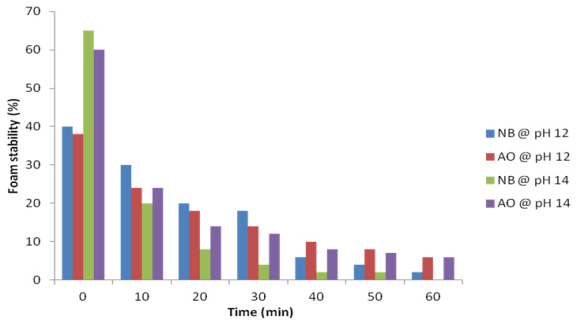

Figure 2. Foaming stability of defatted fern protein isolate at pH 12 and 14

Discussion

Chemical composition of fern frond

Results show no significant differences in moisture content between whole flour of *N. biserrata* and *A. orientalis* (Table 1). Moisture content of the flour decreased significantly ($p < 0.05$) from 7.79% to 4.22% and 7.81% to 3.92% for *N. biserrata* and *A. orientalis*, respectively after oil extraction. The trend in moisture content of the two fern types in this study was consistent with observation made by Tömösközi *et al.* (2008) who reported 13.36% of moisture content of full fat flour of *Amaranthus hypocondriatus* but reduced to 12.00% after defatting. Earlier report by Abu-Tarboush (1995) showed a different trend with increased in moisture content after defatting. According to Vaclavik and Christian (2008), controlling water level in foods is an important aspect of food quality as water content affects the shelf life of food.

There was a significant increase in protein and carbohydrate content after defatting by 2.14% and 5.70% for *N. biserrata* and 3.43% and 7.53% for *A. orientalis*, respectively. This observation could be explained by the fact that when fat is removed from a food sample, the ingredients available to replace it are protein, carbohydrates, minerals or air (Vaclavik and Christian, 2008). These items increase automatically and proportionally even if nothing new is added to the food sample (Glicksman, 1991). This phenomenon was consistent with observations Abu-

Tarboush (1995) and Govardhan-Singh *et al.* (2011) made who also recorded increased in protein and carbohydrate contents as a result of defatting. The high protein content of the fern leaves suggest that it can supplement cereal and tuber flours which are not only deficient in amino acids but also low in protein.

Functional properties of fern frond

Water/oil holding capacity

Interactions of water and oil with proteins are very important in food systems because of their effects on the flavour and texture of foods (Kanu *et al*., 2007). Water absorption is dependent on various parameters such as size, configuration, conformational characteristics, hydrophobic and hydrophilic balance of the protein (Chavan *et al*., 2001). Water holding capacity is an important processing parameter and has implications for viscosity, bulking and consistency of products, as well as in baking applications. The ability of a protein matrix, such as protein particles, protein gels or muscle to imbibe and retain water against gravity is known as Water Holding Capacity (WHC). *Arthropteris orientalis* protein isolate recorded higher water holding capacity of 2.39 mL/g than *Nephrolepis biserrata* which recorded 2.13 mL/g as shown in Table 2. According to Kinsella (1979), an increase in the water holding capacity is due to the ability of a protein isolate to swell and unfold, exposing additional binding sites, whereas the carbohydrate and other components of the protein concentrate may impair it. This support the views of Bandyopadhyay and Ghosh (2002), who reported that protein concentrate exhibits poor water-binding capacity compared to protein isolate.

Another important parameter that influences water holding capacity is pH. The pH of a system markedly influences its ability to bind water due to changes in the surface charges on a protein as the pH is altered. Increasing or decreasing the pH away from the isoelectric point will result in increased water holding capacity by creating a charge imbalance. Bora and Ribeiro (2004) reported best water absorption of 1.64 mL/g of defatted macadamia (*Macadamia integrofolia*) kernel flour protein isolate at pH 7.2, followed by 1.55 mL/g protein at pH 12.0 and least of 1.02 mL/g protein at pH 2.0 as a result of conformational difference in protein structure caused by the pH of extraction medium. These values were less than that observed in this study at a precipitated pH range of 2.3 to 2.5.

An important functionality that influences taste of a product that is required in various food industries is the ability of protein to absorb oil. The oil holding capacity of defatted fern protein was 2.73 mL/g and 2.93 mL/g for *Nephrolepis biserrata* and *Arthropteris orientalis*, respectively. Though the *Arthropteris orientalis* protein isolate showed a higher oil holding capacity than the *Nephrolepis biserrata* but were not significantly different ($p < 0.05$). The oil holding capacity of the isolate followed the same trend as that of water holding capacity for both *Arthropteris orientalis* and *Nephrolepis biserrata* (Table 2). The oil holding capacity for *Nephrolepis biserrata* and *Arthropteris orientalis* was higher than that reported by Bora and Ribeiro (2004) but compared favourably with that reported by El-Adawy (2000) who recorded oil absorption values of 1.60 mL/g, 2.72 mL/g and 2.71 mL/g - 2.81 mL/g for fava bean, mustard seed and lupin seed protein isolates, respectively.

Foaming capacity and stability

The capacity of proteins to form stable foams with gas by forming impervious protein films is an important property in the production of a variety of foods. Foam capacity is influence by the hydrophobic interactions of protein (Dia-Moukala and Zhang, 2011). Foam capacity of defatted fern protein as shown in Figure 1 was pH dependent and was found to be low at pH within the area of it isoelectric point (pH 2-8).

The lower foaming capacity could mainly be attributed to reduction in molecular flexibility as a result of high disulfide bonds (Kim and Kinsella, 1987). Away from pH 8, foaming capacity significantly increased but higher for *Nephrolepis biserrata* than *Arthropteris orientalis*. Foaming capacity of 65% and 60% was observed for *Nephrolepis biserrata* and *Arthropteris orientalis*, respectively at pH 14. The higher foaming capacity at this pH could be attributed to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allows the protein to diffuse more rapidly to the air-water interface to encapsulate air particles (Aluko and Yada, 1995; Wierenga and Gruppen, 2010) and the more easily it is denatured there, the more it is able to foam (Belitz *et al.*, 2009). Kim and Kinsella (1987) have also reported that the reduction of disulfide bonds would increase the molecular flexibility of protein and then result in the improvement of its foaming properties.

To have foam stability (Figure 2), protein molecules should form continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are important to produce stable foams (Kamara *et al.*, 2009). A sharp drop of foam stability was observed within 10 min for both *Nephrolepis biserrata* and

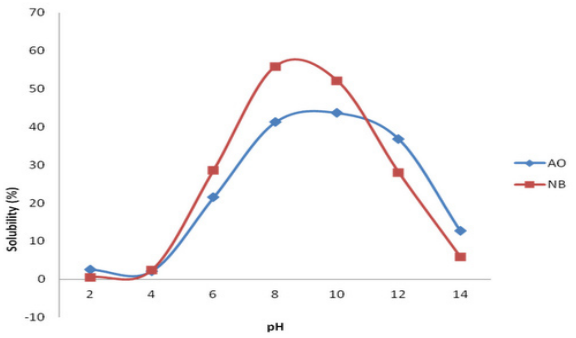

Figure 3. Solubility of fern protein isolates at various pH levels

Arthropteris orientalis but was more stable for *Arthropteris orientalis* within the time range of 20 min to 60 min.

Protein solubility

Solubility is considered the most important functional attribute for its contribution to other functional properties like gelling and emulsification (Sikorski, 1997). The general shape of solubility curves (U-shaped) when solubility is plotted against pH (Sikorski, 1997; Hu *et al.*, 2010) disagree with the solubility curve observed in this study as depicted in Figure 3. *Nephrolepis biserrata* and *Arthropteris orientalis* isolates showed a minimum solubility of 0.5% and 2.2% at pH 2- 4, respectively. This is lower than the reported isoelectric points (pI) of pH 4-5.5 of sesame protein isolate (Kanu *et al.*, 2007) and the pH of minimum solubility of most vegetable protein isolates (Vani and Zayas, 1995). The low solubility at pH 2-4 observed in this study was due to the isoelectric point (pI) of the fern protein isolates since at that pH there is no electrostatic repulsion between the molecules.

Above the pI region which was observed to be above pH 4, the solubility for the *Arthropteris orientalis* protein isolate increased but < 45% between pH 4-10. Within that same pH (pH 4-10), *Nephrolepis biserrata* protein showed a solubility profile of up to 55.9% at pH 8 and drop to 52.1% at pH 10. The observed increased in protein solubility is dependent on pH. At pH values above the pI of the protein isolates, the proteins carry a net charge (due to repelling of the positive or negative ions) and ionic hydration promote solubilization of protein.

Other research works have observed increases in solubility at pH values below or above the pI of a protein. This was contrary to the observation in this study where only increase in solubility was observed above the pI of the fern protein. Solubility was low at the extreme ends of the pH tested and the maximum solubility was shown at pH 8 and 10 for *Nephrolepis biserrata* and *Arthropteris orientalis*, respectively. This outcome disagrees with observations made by Tömösközi *et al.* (2008) and Hu *et al.* (2010). The decreased in solubility above pH 8 and 10 might be due to denaturation of the proteins in the fern at that pH.

Conclusion

The demand for low-cost vegetable protein supplements to increase the nutritional value of cereal and other food has encouraged research on improved products from defatted fern flour. Fern protein has the potential for use in food industry because of its high protein content and functionality. Fern protein isolates showed good functional properties that confirmed that the procedure used in it production was good enough and it could be used in protein supplementation in various food systems particularly for developing countries where protein deficiencies remain a major health problem for children.

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