

Evaluation of the Yield, Protein Content and Functional Properties of Mungbean [*Vigna radiata* (L.) Wilczek] Protein Isolates as Affected by Processing

B.I. Akaerue and G.I. Onwuka

Department of Food Science and Technology, Michael Okpara University of Agriculture,
Umudike, Umuahia, Abia State, Nigeria

Abstract: The effect of processing on mungbean protein isolate yield, protein content and functional properties were evaluated. The functional properties evaluated were Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Emulsion Capacity (EC) and nitrogen solubility. The protein isolate yield and protein content from the raw mungbean flour were 10.52 g protein/100 g flour and 87.56% respectively. Processing had significant ($p < 0.05$) effect on the isolate yield, protein content and functional properties. The toasted flours yielded the highest protein isolates (10.68-8.48%), although there was progressive decrease in isolate yield as toasting time increased. Increase in sprouting time resulted to a significant ($p < 0.05$) increase in isolate yield, however, no significant ($p > 0.05$) decrease was observed for the protein content. Increase in boiling time markedly reduced both isolate yield (6.41-5.80%) and protein content (86.10-32.84%) respectively. The mungbean protein isolates from 60min boiled flour had the highest WAC (2.5 g/g), OAC (2.15 ml/g) and EC (22.16%) while the isolates from 90min toasted flour had the highest WAC (2.97 g/g), OAC (2.25 ml/g) and EC (18.92%). Isolates from 24 h sprouted flour gave the highest WAC (1.75 g/g), OAC (1.25 ml/g) and EC (10.96%). The functional properties of the mungbean protein isolates were significantly ($p < 0.05$) improved by processing and the high solubility indicates its suitability for industrial application as protein supplements.

Key words: Mungbeans, protein isolates, functional properties, processing

INTRODUCTION

Since legume seeds are important sources of protein, complex carbohydrate and dietary fibre in the diet, there has been a worldwide interest in searching for potential utilization of unconventional legumes (Siddhuraju *et al.*, 1996). Mungbean [*Vigna radiata* (L.) Wilczek] is one of the lesser known legumes which have not been fully utilized to alleviate the problem of protein malnutrition common in developing countries of the world. The consumption of mung proteins can fulfil the essential amino acids requirements with the exception of the sulphur-containing amino acids (Khalil, 2006). A large section of the population of most developing countries have inadequate protein intake. However, utilization of legume protein is below their potential partly due to the deficiency of some essential amino acids in their proteins and also due to the presence of some antinutritional factors associated with their proteins (Kavas and Nehir, 1992).

In order to produce a high protein food, the protein in a legume may have to be concentrated and isolated from the legume (Udensi and Okonkwo, 2006). Protein isolation and fractionalization is aimed at separating the protein from other components in such a form that they

remain (as much as possible) fully undenatured and thus retain their functionality (Zadow, 1993). Plant protein isolates are the most refined forms of proteins. Bean protein isolates are incorporated in food systems where heat treatment will be involved in order to eliminate or reduce significantly, the antinutrients contained in them. Isolated proteins often have improved appearance and taste compared with the original meal; therefore they can better be used as nutritional and functional ingredients in many food products (Mizrahi *et al.*, 1967). Furthermore, they contain much higher protein content than the flour, or meal so the same supplementation can be obtained with smaller admixtures (Thompson, 1977; Soestrino, 2007).

The protein isolates from legumes have been used in various food formulations: according to Thompson (1977), mungbean protein isolates were used as protein supplement in bread flour mixtures. Yellow pea, lentil and faba bean protein isolate both from germinated and ungerminated seeds, had been reported by Hsu *et al.* (1982) to be used for replacement of 3, 5 or 8% wheat flour in bread making. Soy isolates are mainly used to improve the texture of meat products, but are also used to increase protein content, enhance flavour and as an

emulsifier. It is also combined with other ingredients in the food industry (<http://wikipedia.org>, 3/5/2008), for instance, extruded rice flour fortified with soy isolates has been reported by Noguchi *et al.* (1981).

Mustafa *et al.* (1986) succeeded in producing acceptable bread and cookies with 10 and 20% cowpea isolates fortified flour. Peanut protein isolates from three different varieties (Conkerton and Ory, 1976) were used as protein supplementation in pineapple juice, as an acid type beverage at 1% level and there was no difference in flavour, texture, or aroma although the turbidity was slightly increased. Nevertheless, the use of Mungbean as a protein supplement is limited by the beany flavour and dark colour it imparts on the final product. This problem could probably be overcome by the use of Mungbean protein isolates. Mungbean protein isolate has been shown to perform many desirable functions in processed foods, such as foaming, emulsification and water absorption (El-Adawy, 2000). However, improvements in those functions would make mungbean protein isolate more desirable as a food component (El-Adawy, 2000). Therefore the aim of this study is to evaluate the effect of processing on the yield, protein content and functional properties of mungbean protein isolates.

MATERIALS AND METHODS

The mungbeans [*Vigna radiata* (L.) Wilczek] seeds were obtained from the Crop Science Department of Michael Okpara University of Agriculture, Umudike, Nigeria. All chemical reagents used for the experiment were of analytical grade.

Seeds pretreatment: The dry cleaned mungbean seeds were dehulled. The dehulled sample was further subdivided into three sets; one was kept raw (untreated), the second set was boiled, the third set was toasted. The fourth set was germinated (sprouted) and then carefully dehulled. All these treatments were given to the mungbeans at different intervals after soaking for 12 h. The mungbean seeds were crushed to smaller fragments with the corona manual grinder after drying in the oven (65°C) and afterwards milled with a blender, using an 80 mesh sieve to sift the flour. The coarse particles were re-milled to obtain finer flour. All flour samples were stored in air-tight plastic bags until required for analysis.

Flour processing

Boiling: Three separate batches of the whole mungbeans [*Vigna radiata* (L.) Wilczek] weighing 800g each were soaked in distilled water (1:3w/v) for 12 h at room temperature (~25°C) according to Mubarak (2005) and Khalil (2006) with slight modification in time of

boiling. The seeds were drained and rinsed three times with 600 ml distilled water, dehulled and then boiled in tap water (100°C) in the ratio of 1:10 (w/v) on a hot plate for 30, 45, 60 and 90 min respectively. The water was drained off after each timing and the seeds dried in the oven at 65°C and cooled in a desiccator. The seeds were then dry milled, sifted with 80mesh sieve and packaged for analysis thereafter.

Dehulling: The hulls were removed manually after soaking the mungbean seeds for 12 h in distilled water (1:10w/v) according to El-Beltagy (1996).

Toasting: Three separate batches of the dehulled seeds weighing 800 g each were spread thinly in a pan and oven-dried at a fixed temperature of 120°C for time variables of 30, 45, 60 and 90 min. They were stirred intermittently to maintain uniform heating and then cooled in a desiccator after the toasting. The seeds were milled, sifted with 80mesh sieve and packaged for analysis thereafter (Emenalom and Udedibie, 2005) with slight modification in time of toasting.

Sprouting: The germination was carried out by spreading the unde-hulled seeds soaked in distilled water (1:3 w/v) for 12 h at room temperature (~25°C), weighing 800 g in between jute cloth and allowed to sprout in the dark for 24 and 36 h respectively. The seeds were kept wet throughout germination by spraying them with distilled water every 12 h. The sprouted mungbeans were harvested, rinsed twice in distilled water, carefully dehulled and oven dried at 65°C for 9 h and then cooled.

The dried seeds were subjected to dry milling and passed through 80 mesh sieve. The flour was cooled and packaged for analysis thereafter in air-tight plastic bags.

Preparation of mungbean protein isolates: Protein isolate was prepared using the methods described by El-Adawy (2000) with slight modification in the temperature and duration of shaking as shown in Fig. 2. Dispersions of 10% (w/v) mungbean flour in water were adjusted to pH of 9 with 0.1N NaOH at room temperature (~30°C), shaken for 1 hour and centrifuged for 15 min at 2000xg.

In order to obtain increased yields, the extraction and centrifugation procedures were repeated on the residue. The extracts were combined and the pH adjusted to 4.5 with 1N HCl to precipitate the protein. The proteins were recovered by centrifugation at 2000xg for 15 min followed by removal of the supernatant by decantation. Protein curd was washed with distilled water and the curd was re-dispersed in distilled water (El-Adawy,

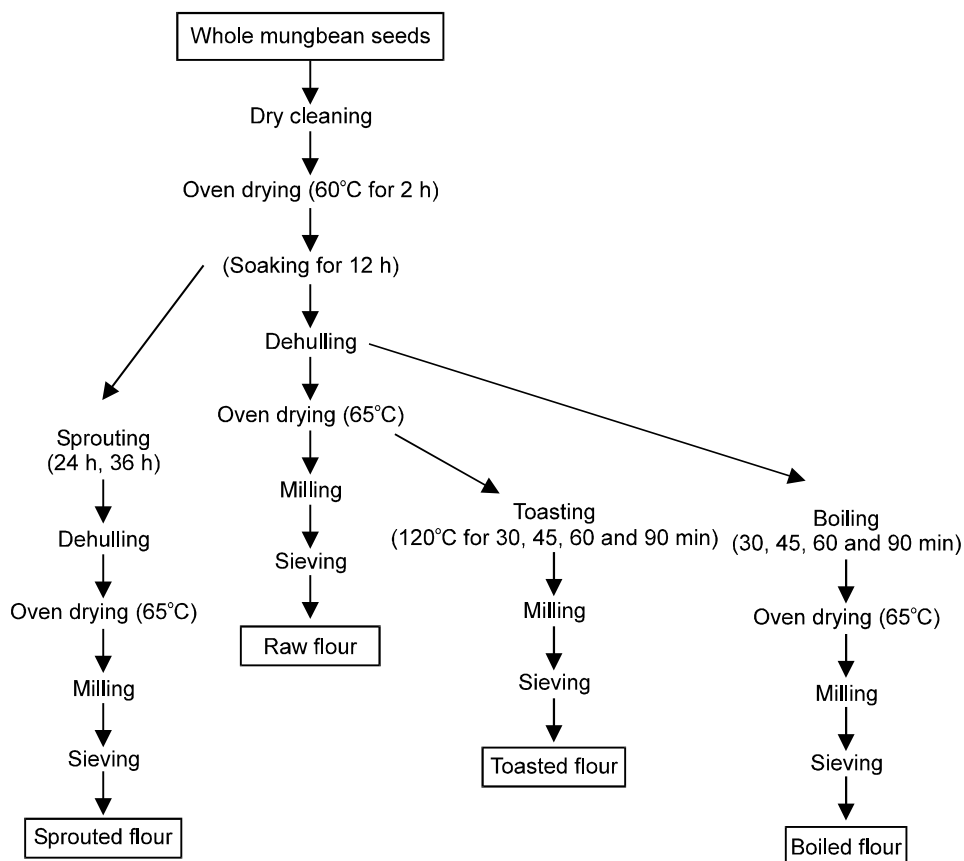


Fig. 1: Flow chart of the dehulled mungbean flour samples

2000). The resulting protein curd was separated by filtration with filter paper and washed three times using distilled water. Later the slurry was scraped out and placed in a moisture drying can and dried for 3 hours in a low temperature dryer (40°C). After drying, it was ground into fine powder using ceramic mortar and pestle (Udensi and Okonkwo, 2006). The protein isolate samples were analyzed for protein content using the micro Kjeldhal method reported by AOAC (1990). Oil and Water absorption capacities were determined using the method of Okezie and Bello (1988). Emulsion capacity was determined with the method described by Onimawo and Egbekun (1998). For nitrogen solubility studies, 1% water extract of each protein isolate sample was prepared by weighing 1g into 10ml of distilled water in a beaker, at 24°C and used for the assay. Each sample was adjusted to a given value - pH 2, 4, 6, 8 using either 1.0N NaOH or 0.1N HCl as appropriate (Fan and Sosulski, 1974). Samples were extracted at 24°C for 30 min using a magnetic stirrer and centrifuged for 15 min at 2000 rpm. Then, the nitrogen in the supernatant was estimated using the standard micro-Kjeldahl technique (AOAC, 1990). Results represent analysis of duplicate protein isolate samples.

The software package used for the statistical analysis was the version 15 of SPSS while all the analyses were carried out in three replicates. The data were evaluated for significant differences ($p = 0.05$) in their means using Analysis of Variance (ANOVA). Differences between means were separated using Duncan's Multiple Range Tests (DMRT).

RESULTS AND DISCUSSION

The effect of some processing treatments on the yield of mungbean protein isolates are presented in Table 1. The average yield of protein isolated from the raw mungbean flour was 10.52 g protein/100 g of flour. Similar result was obtained for raw mungbean protein isolate (13 g protein/100 g flour) by El-Adawy (2000). Increase in boiling time of the mungbean seed resulted to progressive decrease in the protein isolate yield from the flour. It is however, noteworthy that the toasting treatments yielded higher protein isolates than boiling and sprouting treatments. Although, increase in toasting time resulted to a progressive reduction in the protein yield, the decrease in protein yield may be mainly due to thermal degradation of the proteins (Adebowale, 2008). Sathe *et al.* (1982) also reported the formation of insoluble aggregates with sulphur-rich proteins in soybean flour when heated to 70°C and above.

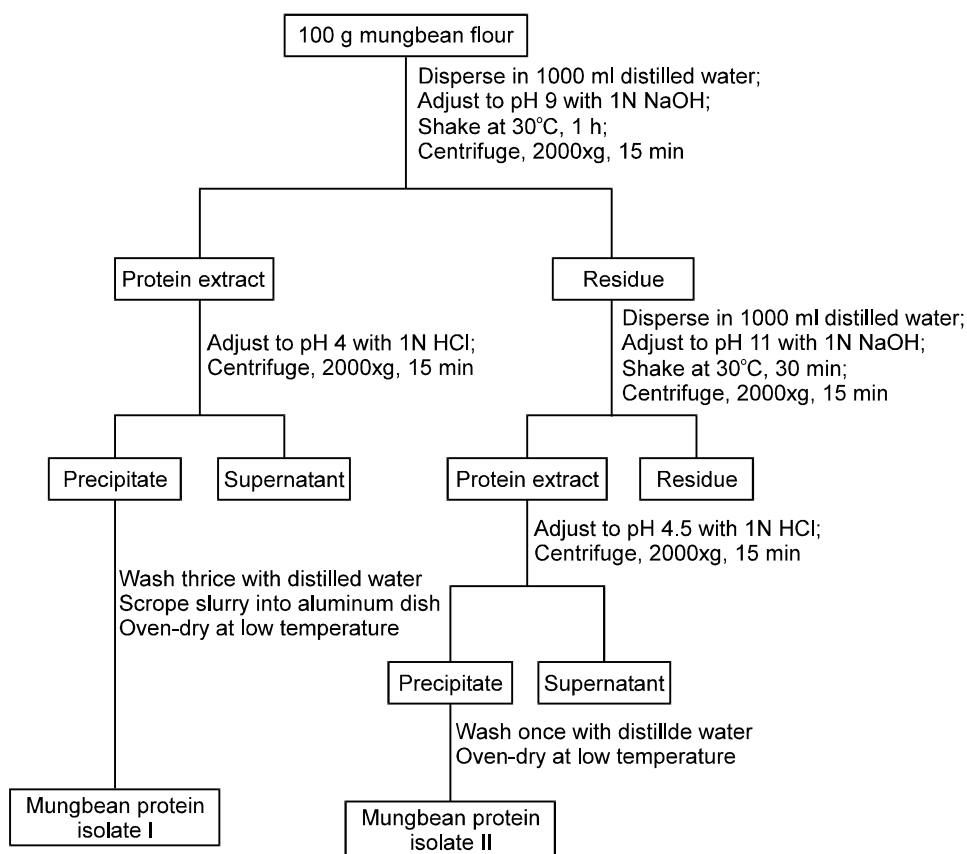


Fig. 2: Preparation of mungbean protein isolates

Table 1: Effect of some processing treatments on the protein isolation from mungbean flour at pH 9.0^a and pH 4.0^b

Flour sample code	Sample	Average weight of P.I.	
		25 g of mungbean flour	Protein isolates (P.I) yield (%)
RD	Raw dehulled	2.63 ^b ±0.01	10.52 ^b ±0.01
BD ₃₀	Boiled for 30 min	1.61 ^a ±0.02	6.41 ^a ±0.01
BD ₄₅	Boiled for 45 min	1.59 ^a ±0.01	6.32 ^a ±0.01
BD ₆₀	Boiled for 60 min	1.45 ^b ±0.03	5.81 ^b ±0.01
BD ₉₀	Boiled for 90 min	1.45 ^b ±0.03	5.80 ^b ±0.01
TD ₃₀	Toasted for 30 min	2.67 ^a ±0.02	10.68 ^a ±0.01
TD ₄₅	Toasted for 45 min	2.29 ^c ±0.01	9.16 ^c ±0.02
TD ₆₀	Toasted for 60 min	2.28 ^c ±0.02	9.12 ^c ±0.02
TD ₉₀	Toasted for 90 min	2.12 ^d ±0.01	8.48 ^d ±0.02
SpD ₂₄	Sprouted for 24 h	1.65 ^a ±0.01	6.60 ^a ±0.01
SpD ₃₆	Sprouted for 36 h	1.88 ^a ±0.02	7.52 ^a ±0.02

Means are values of triplicate determinations;

^aUnless specified, the extractions were at 30°C for 30 min using a mungbean flour to solvent ratio of 1:15.

^bUnless specified, isoelectric precipitation of the extracted proteins were at the pH of 4.0.

Means on the same column with different superscripts are significantly different (p<0.05)

Since the extraction was carried out in aqueous medium, the decrease in the extraction of protein might be due to reduced solubility of the protein with increase in temperature due to coagulation. Thompson (1977) observed a decrease in solubility at 80°C due to partial coagulation of the proteins.

However, increase in sprouting time led to a significant (p<0.05) increase in the protein isolate yield of the flour. This may be explained on the basis of increased solubility of the protein with increase in sprouting time. Also, increase in sprouting time was observed to increase extraction of more proteins. The protein

extraction obeys the Well mechanism for solid-liquid mass transfer which assumes that solid extraction from a solid-liquid ratio increases until a position of equilibrium is attained, after which the extraction of solute remains relatively constant (Prabhudesal, 1988). Many factors affect the extractability of protein, including particle size and quality of flour, solvent-to-flour ratio, pH and temperature during extraction, ionic strength or addition of salts into extractant (Kinsella, 1979). However, it is important to recover as much protein as possible during extraction in order to get maximum protein content in the concentrate or isolate products.

Table 2 shows the effect of some processing treatments on the protein content of the mungbean protein isolates. The protein content of the raw mungbean protein isolate was 87.56% which was similar to the result (88%) obtained by Fan and Sosulski (1974) and lower than the 92% protein content observed by Thompson (1977) after a repeated extraction. Increase in boiling treatments resulted to a progressive reduction in the protein content of the protein isolates (86.10-32.48%). This might be due to protein denaturation and leaching during the boiling of the mungbean seeds. The toasted mungbean protein isolates had lower protein contents with further reduction (74.43-22.62%) as the toasting time was increased. Lower protein solubilities as a result of thermal denaturation and polymerization of amino acids (Kato *et al.*, 1985) may be accountable for the lower protein content of the toasted isolates. The sprouting treatments (24 h and 36 h) increased the protein content of the isolates (85.37% and 83.18% respectively). The sprouted flours had high protein contents and solubilities hence the high protein content of the isolates. This study has proved mungbean protein isolates to have good effect in food applications.

In Table 3, the effect of some processing treatments on the functional properties of mungbean [*Vigna radiata* (L) Wilczek] protein isolates was presented.

Table 2: Effect of some processing treatments on the protein composition of the mungbean protein isolates

Protein isolates (P.I) from mungbean flour	Protein composition of the protein isolates
Raw dehulled	87.56 ^a
Boiled	
30 min	86.10 ^a
45 min	81.72 ^a
60 min	44.51 ^b
90 min	32.84 ^{bc}
Toasted (120°C)	
30 min	74.43 ^a
45 min	39.41 ^{bc}
60 min	32.84 ^{bc}
90 min	22.62 ^c
Sprouted	
24 h	85.37 ^a
36 h	83.18 ^a

Values are means of triplicate determinations.

Means with different superscripts on the same column are significantly different (p<0.05)

The Water Absorption Capacity (WAC) of the raw mungbean protein isolates (1.12 g/g) was observed to be significantly different (p<0.05) from the protein isolates of the processed flours. Mesallam and Hamza (1987) reported a higher value of 2.26 g/g WAC for green gram (*Phaseolus aureus*) protein isolates. Much higher WAC values have been reported for some legumes protein isolates; winged bean isolate (5.00 g/g) and soy isolate (4.10 g/g) (Okezie and Bello, 1988). Mucuna bean protein isolates (6.00 g/g) (Udansi and Okonkwo, 2006). However, water absorption capacity, lower than the value 1.0 g/g, reported for succinylated and acetylated mungbean protein isolates has been observed for untreated mungbean protein isolate by El-Adawy (2000). The boiling and toasting treatments were observed to significantly (p<0.05) increase the water absorption capacity of the protein isolates with higher values obtained in the toasted protein isolates. This could be

Table 3: Effect of some processing treatments on the functional properties of mungbean [*Vigna radiata* (L) Wilczek] protein isolates

Samples	Water absorption capacity (WAC), g/g	Oil absorption capacity (OAC), ml/g	Emulsification capacity (EC), %
Raw dehulled	1.12 ^a ±0.25	1.05 ^a ±0.05	19.15 ^a ±0.03
Boiled			
30 min	1.55 ^d ±0.05	1.75 ^c ±0.25	21.45 ^b ±0.62
45 min	1.33 ^{de} ±0.29	2.15 ^{ab} ±0.05	18.76 ^d ±0.07
60 min	2.50 ^b ±0.17	1.88 ^{bc} ±0.08	22.16 ^a ±0.04
90 min	1.95 ^e ±0.05	1.12 ^d ±0.03	16.73 ^{cd} ±0.07
Toasted (120°C)			
30 min	2.45 ^b ±0.05	1.20 ^d ±0.00	9.48 ^e ±0.62
45 min	2.45 ^b ±0.05	2.25 ^a ±0.25	18.24 ^a ±0.08
60 min	1.93 ^c ±0.29	1.82 ^c ±0.10	17.87 ^a ±0.03
90 min	2.97 ^a ±0.29	1.63 ^c ±0.11	18.92 ^{cd} ±0.02
Sprouted			
24 h	1.75 ^c ±0.05	1.25 ^d ±0.25	10.96 ^b ±0.04
36 h	1.65 ^c ±0.05	0.75 ^e ±0.25	7.30 ^d ±0.04

Values are the means ± standard deviation of triplicate determinations.

Means with different superscripts on the same column are significantly different (p>0.05)

explained on the basis of the fact that when a protein is heated, the bonds that maintain its secondary and tertiary structures are weakened and at some temperatures, broken. This breaking of non-covalent bonds with its resulting alteration of protein structure is termed denaturation. The early stages of thermal denaturation cause most protein molecules to begin to unfold which often lead to slight increase in the amount of water to interact with the charged groups. At some temperature, the attractive forces will have been weakened enough to allow extensive water-ion interactions. This causes an unfolding of the molecule and an increase in water binding (Feeney *et al.*, 1982; El-Adawy, 2000)

The sprouting treatments were also observed to significantly ($p < 0.05$) increase the WAC of the protein isolates although there was no significant difference ($p > 0.05$) between the WAC of the 24 h and 36 h sprouted protein isolates (1.75 g/g and 1.65 g/g respectively). This increase in WAC might have resulted from the hydration of the mungbean seeds during soaking and sprouting which in turn unfolds the protein, thereby increasing its hydrophilic binding sites and exposing them to the aqueous phase. Udensi and Okonkwo (2006) reported a higher value (7.00 g/g) for 24 h germinated *Mucuna* bean protein isolates.

The Oil Absorption Capacity (OAC) of the raw mungbean protein isolates (1.05 ml/g) was observed to significantly ($p < 0.05$) differ from the protein isolates of the processed flours. A higher value of oil absorption capacity (1.24 g/g) was reported for raw green gram (*Phaseolus aureus*) protein isolate by Mesallam and Hamza (1987). The values obtained for winged bean isolate (9.65 g/g); soy isolate (4.88 g/g) (Okezie and Bello, 1988) and *Mucuna* bean isolate (2.20 g/g) (Udensi and Okonkwo, 2006) were much higher than the OAC of mungbean protein isolates.

Oil absorption by the mungbean protein isolates were found to be significantly ($p < 0.05$) increased by the boiling and toasting treatments. The difference in oil absorption could be attributable to temperature-protein interaction. The highest values of oil absorption capacity were observed in 45 min toasted protein isolates (2.25 ml/g) and 45 min boiled protein isolates (2.15 ml/g) (Table 3).

The hydrophobic portions of protein can interact with lipids during protein unfolding causing increased absorption of lipids. The oil absorption capacity is affected by several factors, such as the protein content, the surface area, the hydrophobicity, the charge and topography, the liquidity of the oil and the method used (El-Adawy, 2000). Also, oil absorption capacity of protein may depend on its capacity to entrap the oil (Kinsella, 1976).

The 24 h sprouting treatment significantly ($p < 0.05$) increased the oil absorption capacity (1.25 ml/g) of the mungbean protein isolate whereas a slight reduction was observed in the 36h sprouted protein isolate (0.75 ml/g). Udensi and Okonkwo (2006) reported a significant ($p < 0.05$) reduction in the OAC of the 24 h germinated protein isolate.

Significant differences ($p < 0.05$) existed among the emulsifying capacity of the raw (19.15%) and processed mungbean protein isolates. Mesallam and Hamza (1987) reported an emulsion capacity of 31.4g/g for raw green gram protein isolates. The emulsion capacity of the mungbean protein isolates was markedly increased by 30 min and 60 min boiling but slightly reduced by 45 min and 90 min boiling treatment (Table 3).

However, the toasting treatments significantly ($p < 0.05$) reduced the emulsion capacity of the protein isolates. Sprouting treatments also significantly reduced the emulsion capacity of the mungbean protein isolates.

A contrary observation was made by Hsu *et al.* (1982) on the emulsion capacity of the protein isolates from germinated yellow peas, lentils, and faba beans. They reported that protein isolates from those germinated legumes had higher emulsion capacity although the protein isolate from germinated pea or lentils gave severe syneresis. This could be adduced to the increases in small subunit proteins after germination (Soestrino, 2007).

Lower value (12.5 mg/g) obtained for *Mucuna* bean protein isolate (Udensi and Okonkwo, 2006) was similar to that (12.9%) reported for winged bean protein isolate by Okezie and Bello (1988) but higher than that obtained for soy isolate (8.00%).

The observed increase in the emulsifying properties of the boiled protein isolates could be due to the unfolding of protein chains, thereby exposing hydrophilic residues of peptides (Feeney *et al.*, 1982) which causes an improvement in emulsifying capacity. The value of some treated protein isolates could be as a result of insufficient exposure of the hydrophilic protein to the aqueous phase or due to insufficient unfolding for the hydrophobic groups of the protein to contact the lipid phase. Once a protein molecule reaches the surface (fat/water interface), it must be able to unfold enough to expose hydrophobic groups if it is to function as an emulsifier. In theory, a measure of relative hydrophobicity of a protein should be related to its ability to function as an emulsifying agent (<http://www.yahoo.com-protein-functionality-11/06/2009>).

The industrial application of proteins such as in the production of fibers, adhesives, ingredients of coating, emulsifiers, food additives and different food products depend upon bringing proteinous materials into solution. Hence, the knowledge of protein solubility will be an important factor in selecting particular vegetable proteins for possible industrial application (Adebowale, 2008).

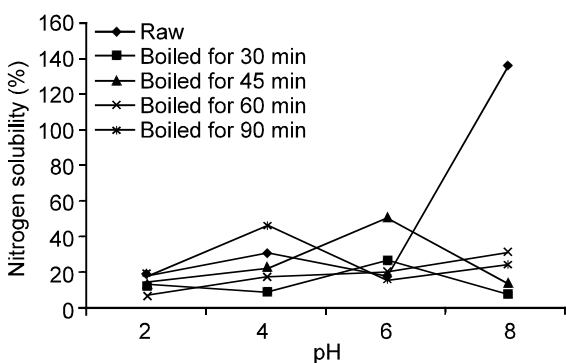


Fig. 3: Effect of boiling at different intervals on the nitrogen solubility of mungbean protein isolates

The pH-dependent protein solubility profile of the mungbeans protein isolate samples is presented in Fig. 3-5. Apparently, the isoelectric point of the proteins was found to be at pH 4-4.5. For the raw protein isolates, the pattern showed highest nitrogen solubility at the alkaline than acid pH with minimum solubility at the pH 2. Earlier reports of Hang *et al.* (1970) and Thompson (1977) had indicated similar results in the nitrogen solubility of mungbean protein isolates. The boiling treatments had varying effects on the nitrogen solubility of the protein isolates (Fig. 3). They had higher solubility at alkaline pH except the protein isolate from 90 min boiled flour which had higher solubility at pH 4 and least solubility at pH 6. However, it was observed that protein isolates from 30 and 45 min boiled flours had their least nitrogen solubility at pH 8 where as the protein isolate from 60 min boiled flour has its least solubility at acidic pH of 2. The effect of toasting treatment at varying times on the nitrogen solubility of protein isolates was shown in Fig. 4. For protein isolates from 30 minutes and 45 min toasted flours, the solubility reduced as pH increased and approached the isoelectric point; this was followed by progressive increase in solubility with further increase in pH.

Similar observation was reported for wing bean, chickpea and Mucuna bean protein isolates (Sathe *et al.*, 1982; Sanchez-Vioque *et al.*, 1999; Adebowale *et al.*, 2005). The protein isolates from 60 min toasted flour had its highest solubility at an acid pH 2 and the least at alkaline pH 8 while the protein isolate from 90 min toasted flour had its highest solubility at alkaline pH 8 and the least at acid pH 2. As shown in Fig. 5, the 24h sprouted flour gave protein isolates with highest nitrogen solubility at pH 4 whereas the 36 h sprouted flour yielded protein isolates with its highest solubility at alkaline pH 6. The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which might have occurred during the isolation process. It also gives

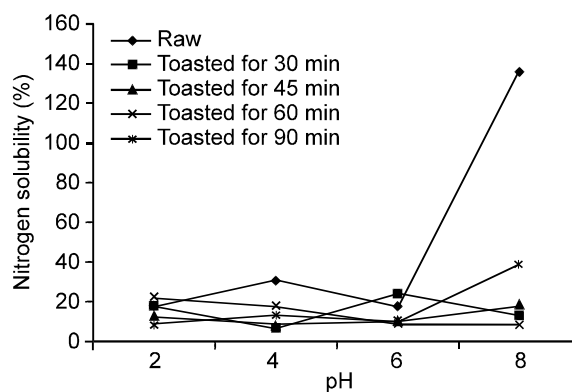


Fig. 4: Effect of Toasting at different intervals on the nitrogen solubility of mungbean protein isolates

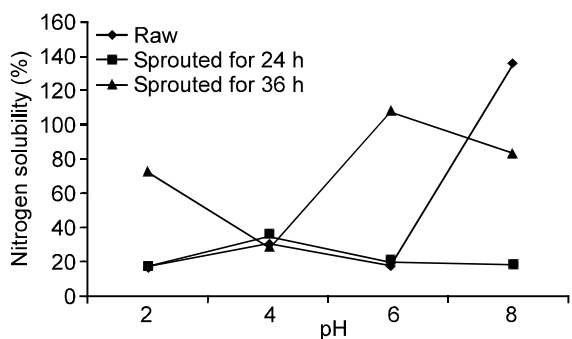


Fig. 5: Effect of Sprouting at different intervals on the nitrogen solubility of mungbean protein isolates

an indication of how the protein could be incorporated. According to Kinsella (1976), since protein solubility affects other functionalities like emulsification, foaming and gelation, the high solubility of the mungbean protein isolates indicates that they have good functionality and could have promising food application in Nigeria for instance as protein supplements.

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