

Effect of Cooking on Protein Digestibility, Fractions Content and Functional Characteristics of Defatted *Milletia ferruginea* Seed Flour

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Abstract: The effects of cooking on total protein digestibility, fractions and functional properties were investigated. Cooking significantly ($P > 0.05$) reduced the protein digestibility of the sample using pepsin and/or pancreatin. Pepsin digestibility of uncooked flour was $72.4 \pm 0.05\%$ and that of both pepsin and pancreatin was $97.2 \pm 0.05\%$. The major protein in uncooked defatted brebra seed flour was albumin (58.4%) followed by glutelin (8.5%). Cooking of the sample was significantly ($P > 0.05$) decreased the albumin (from 58.4% to 27.2%) content. The decrease was accompanied by significant increment in the glutelin (from 8.7% to 26.8%) fractions. In this study, cooking is not the best method to recover maximum amount of soluble protein. Emulsion capacity/stability, foam capacity/stability and gel formation of the studied sample was affected by cooking but water and oil retention significantly ($p > 0.05$) increased. Changes in protein conformation by cooking adversely affected some functional properties of the protein to a large extent. An understanding of protein digestibility, fraction content and functional properties as a result of cooking method would help in better utilization of these proteins in food systems.

Key words: Cooking • Functional properties • Protein digestibility • Protein fractions

INTRODUCTION

Food legumes, distinctively termed as best source of protein appear to be the most potential solution to overcome the crisis of protein-calorie malnutrition in the less developed countries. As the result of inadequate supplies of food proteins, there has been a constant search for unconventional protein sources, for use as both functional food ingredients and nutritional supplements [1].

Legumes have to be processed prior to consumption due to their content of antinutritional factors in order to use as food ingredient and to improve protein functionalities. Protein functionalities such as emulsification, gelation and foaming are highly affected by cooking [2]. Therefore, the relationship of protein quality with processing parameters that affect the functional performance of protein products is worthy of extensive investigation. Cooking is probably the oldest

treatment for making legumes edible. Usually it includes a previous soaking of the seeds and a subsequent cooking in boiling water until they become completely soft. Generally, food application on protein source or functional ingredient depends on the knowledge of its functional properties and the factors, which influence functionalities, i.e. intrinsic, environmental and process treatment factors [3].

Most legumes digestibility and functional characteristics were found to be associated with different factors. Mostly the digestibility of legume protein is dependent on protein structure. Heating is responsible for protein denaturation, eventually followed by aggregation of the unfolded molecules [4], which results in loss of solubility. Thermal denaturation involves an initial stepwise dissociation of subunits and subsequent re-association of only partially unfolded molecules with formation of either soluble or insoluble complexes [5]. The protein quality of a food or feed depends on its amino

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acid composition and digestibility; protein digestibility primarily determines the availability of its amino acid [6]. The existence of folding of polypeptide chains in the native protein structure as well as the subunits in some proteins facilitates studying the denaturation phenomena.

In this study, the defatted brebra seed flour was used as protein source. Brebra, *Milletia ferruginea* (Hochst.) Baker; Leguminaceae is a N₂ fixing leguminous tree species that is known to have significant positive effect for intercropping crops in the southern parts of Ethiopia [7]. It is an endemic agro-forestry tree to Ethiopia. Its cooked and uncooked defatted seed protein is not yet investigated for production of economically important proteins for different purposes (microbial media, human food and animal feed). The objective of this study was to investigate the effect of cooking on total protein digestibility, fraction content and functionality properties of defatted brebra seed flour. The results from this investigation can contribute a lot for better understanding of the effects of these processes on legume composition and allow selection of the best conditions for improving the quality of products when they have to be processed for human or animal nutrition.

MATERIALS AND METHODS

Sample Preparation: Seeds were cleaned, dehulled, ground to a fine powder (0.18mm sieve) and defatted by hexane to 1:6 ratio (w/v). The hexane was recovered by vacuum evaporation under reduced pressure using Rota vapour (Buchi, Switzerland). For cooking, the flour was suspended in distilled water (1:10 w/v) and boiled in water bath for 30 min the cooked gruel was then dried at 65°C for 24 hrs and ground to pass a 0.4mm screen. All reagents used in this study were reagent grade.

Determination of *In vitro* Protein Digestibility

Pepsin Digestibility: The *In vitro* protein digestibility was carried out according to the method of Saunders *et al.* [8]. From defatted sample, 250 mg was suspended in 15ml of 0.1 N HCl containing 1.5mg pepsin and then the mixture was incubated at 37°C for 3 h. The reaction was stopped by addition of 15ml of 10% trichloroacetic acid (TCA), the mixture was finally filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method [9]. By using the same procedure, all reagents and sample without enzyme were used as control in order to determine degree of enzyme digestibility. The same procedure was

used in pepsin-pancreatin digestibility test.

Pepsin-pancreatin Digestibility: The method was carried out according to Saunders, Connor [8]. About 250 mg sample was suspended in 15ml of 0.1 N HCl containing 1.5mg pepsin, the mixture was incubated at 37°C for 3 h, then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 ml 0.2 M phosphate buffer (pH 8.0) containing 0.005 M sodium azide, then the mixture was incubated at 37°C for 24 h. Ten ml of 10% trichloroacetic acid (TCA) were added to stop the reaction then centrifuged at 5000 rpm for 5 minute. From the supernatant, 5ml was taken for nitrogen analysis using the micro-Kjeldahl method [9]. Digestibility of pepsin and pepsin-pancreatin were determined by using the following equation:

$$\text{Protein digestibility \%} = \frac{(T-B) \times N \times 14.00 \times TV}{(X) \times a}$$

Where:

$$X = \frac{250 \times \text{CP \%}}{100 \times 6.25}$$

N = Normality of HCl, T = ml of titer, B = ml of blank, a = Number of ml of aliquot, TV = Total volume of the mixture, 14 = Equivalent weight of nitrogen, 250 = Sample weight in mg, CP%= Percent crude protein.

Determination of Protein Fractionations: Protein fractions were extracted according to their solubility in different solvents, as described by Landry and Moureaux [10]. Defatted cowpea flour (6.6 g) was extracted twice with 50 ml distilled water for 30 min at room temperature. The extract was centrifuged at 3000 rpm for 30 min and the supernatant was used for the determination of a water-soluble protein (albumin). The residue was then extracted successively in a similar manner with 1.0 M NaCl, 70% ethanol or 0.2% NaOH. The supernatant of each extract was collected separately and used to estimate the salt-(globulin), alcohol- (prolamin) or alkali-(glutelin) soluble fraction. The residue remaining after successive extractions represents the insoluble proteins.

SDS-polyacrylamide Gel Electrophoresis: SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli [11] method. Half gm sample was prepared from both cooked and raw brebra flour in 1:16 (w/v) ratio of flour and distilled water, respectively and then sonicated by using (Soniprep 150,

MSE). The portion of the sample was diluted into 1:1 ratio with distilled water in order to see clear bands in the electrophoresis gel. The whole sample was centrifuged at 10,000 rpm for 10 min. Sample was taken from the supernatant and mixed with Laemmli sample buffer in 1:1 ratio. The prepared sample in this manner was denatured in boiling water for 10 min and 25µl injected to SDS-PAGE using a 5% (v/v) stacking and 12.5% (v/v) resolving gel. Electrophoresis was done at a voltage of 200 for 1 hrs. After electrophoresis, the gel sheets were stained for proteins with 0.2% Coomassie brilliant blue-R250 for an hour. Protein stain was destained with 7% acetic acid containing 30% methanol. Unstained protein molecular weight marker (Fermentas, SM0431) was used to determine the molecular weight of the samples.

Determination of Protein Functionality

Gelation Capacity: Gelation capacity of brebra proteins was determined according to the method of Coffman and Gracia [12] with a slight modification. Sample suspensions of 1-20 g/100 ml were prepared in 5 ml distilled water. The test tubes containing these suspensions were then heated for 1 h in a boiling water bath, followed by rapid cooling under running cold tap water. The test tubes were then further cooled for 2 h at 4°C. The least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall or slip

Water and Fat Absorption Capacity: Water absorption capacity was estimated by the method described by Wang and Kinsella [13]. Two gm of the protein samples from brebra flour was suspended in 20 ml distilled water, stirred for 5 min, shaken by rotary shaker (Gerhardt) over a 30 min period at 25 °C and then centrifuged at 4000 rpm for 30 min, the freed water was carefully decanted in a graduated measuring cylinder and the volume recorded. Water absorption capacity was expressed as ml water retained by one gm protein. Fat absorption capacity (FAC) was measured by the method described by Lin, *et al.* [14]. FAC expressed as ml oil retained by one g protein.

Foaming Capacity and Stability: One gram of defatted brebra flour was suspended in 50 ml distilled; the suspensions were blended in a blender (WARING) for 5 min [15]. The content was transferred into a 100 ml measuring cylinder and the volume of foam was read after 30 s for foam capacity. This was repeated for the various salt solutions at different concentrations, as indicated above. The foam stability was determined by measuring

the volume of foam at 10, 30, 60 and 120 min, after pouring the whipped suspension. The percentage ratio of the volume increase (to that of the original volume of protein solution) was calculated and expressed as foaming capacity [16]. All determinations were carried out in triplicate.

$$FC (\%) = (\text{volume after whipping} - \text{volume before whipping}) / \text{volume before whipping} \times 100.$$

$$FS (\%) = (\text{Foam volume after time (t)} / \text{Initial foam volume}) \times 100.$$

Emulsion Capacity, Activity and Stability along with Raw and Cooked Defatted Flour: The emulsifying capacity of the sample was estimated by the method of Beuchat *et al.* [17]. One gram sample was blended with 50 ml of distilled water for 30 second in electric blender at maximum speed. After complete dispersion, refined soya bean oil was added continuously (0.4ml per sec.) from 5 ml oil and blending continued until there was a phase separation (visual observation/change in shaft sound). Emulsifying capacity was expressed as ml of oil emulsified by one gram sample.

The procedure described by Volkert and Kelin [18] was used for both emulsification activity and emulsion stability. Two gm of sample was added to 50 ml of distilled water and mixed well before addition of 50 ml of oil at room temperature (25°C). The mixture was blended for 5 min. The emulsified sample was divided equally into 50 ml centrifuge tubes for the determination of emulsification activity and emulsion stability, respectively. To determine emulsification activity, the content of one tube was directly centrifuged at 4,000 rpm for 30 min and then poured into 50 ml measuring cylinders and stay a few minutes until the emulsified layer was stable. Results were expressed as percentage of the emulsified oil after separating the upper layer from emulsion [19].

$$EA (\%) = (\text{height of emulsion} / \text{height of whole layer}) \times 100.$$

To determine emulsion stability, the other portion was centrifuged and heated in a water bath at 80°C for 30 min and subsequently cooled to 15°C. After centrifugation, the emulsion was poured into 50 ml measuring cylinder and stays a few minutes until the emulsified layer was stable and emulsion stability expressed as the percent of the total volume remaining emulsified after heating.

$$ES (\%) = (\text{height of emulsion layer after heating} / \text{height of emulsion layer before heating}) \times 100.$$

of whole layer) x 100.

Bulk Density: The bulk density was determined according to Wang and Kinselle [13] method. Ten grams of the sample were placed in 25 ml-graduated cylinder and packed by gently, the volume of the sample was recorded and the bulk density is expressed as g protein/ml.

Statistical Analysis: The data were analyzed using SPSS version 15.0. Means and standard deviations of the triplicate analysis were calculated using analysis of variance (ANOVA) to determine the significance differences between the means using Duncan's Multiple range test ($p < 0.05$) when the F-test demonstrated significance.

RESULT AND DISCUSSION

In Vitro Protein Digestibility: The effect of cooking on *In vitro* protein digestibility of defatted brebra flour is shown in Table 1. When defatted flour was digested with only pepsin, 74.4± 0.05% of the uncooked flour was digested, while cooking of the flour significantly ($p < 0.05$) decreased the *In vitro* protein digestibility and it was found to be 55.1 ± 0.08%. When pepsin together with pancreatin were used, the protein digestibility of uncooked was significantly ($p < 0.05$) increased and found to be 97.2 ± 0.05% while cooking significantly ($p < 0.05$) decreased the protein digestibility and was found to be 69.1 ± 0.05%. The same trend of protein digestibility was reported by Yagoub and Abdalla [20], in which uncooked bambara groundnut *In vitro* pepsin digestibility was 78.75±0.35 while cooked treatment significantly ($p < 0.05$) decreased in protein digestibility and was found to be 51.57± 0.1%. It was clear that the *In vitro* protein digestibility obtained was significantly ($p < 0.05$) affected by cooking even when the flour was digested in both with pepsin and pancreatin. Similar results were observed by Sulieman *et al.* [21] who attributed the lack of improvement in digestibility of lentil and fabea bean to be related in part to protein aggregation that is a consequent to the thermal treatment. According to Carbonaro *et al.* [22] report, formation of aggregated protein on heat treatment is due to the oxidation of sulfhydryl groups and also interactions between acidic and basic residues and would be more resistant to proteases. According to Otterburn *et al.* [23] suggestion, the formation of a three dimensional network on severe heating of proteins, as a result of Ca^{+2} mediated electrostatic bonds, hydrophobic

Table 1: Effect of cooking on *In vitro* protein digestibility of defatted brebra flour using pepsin and pepsin with pancreatin (%)

Sample	Digestibility (%)		
	None	Pepsin	Pepsin + pancreatin
Cooked	1.37 ± 0.10	55.1 ± 0.08	69.1 ± 0.06
Uncooked	1.17 ± 0.19	72.4 ± 0.05	97.2 ± 0.05

Values (n=3) in each row with different superscript differ at $P < 0.05$.

Means were tested by ANOVA and ranked by Duncan's multiple range test

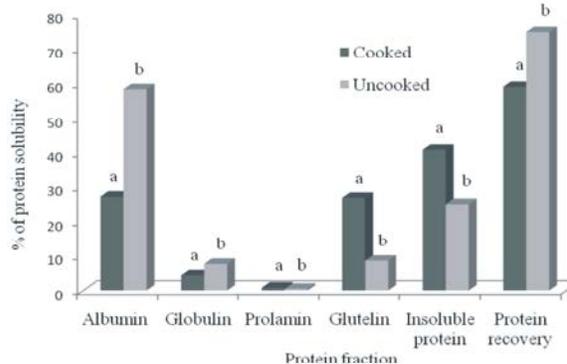


Fig. 1: Protein fraction (%) of cooked and uncooked defatted brebra flour. Values are means ± (SD). Means not sharing a common letter in the same protein fraction, for the same parameter, are significantly different at $P < 0.05$.

interactions and the involvement of cross links, preventing enzyme penetration or masking the sites of the enzyme attack. The negative effect of cooking on the *In vitro* protein digestibility also observed by Abdel Rahim [24] for the faba bean who attributed the reduction of *In vitro* protein digestibility to the formation of disulphide bonds resulting in folding of protein molecule and hence prevent access to and restrict digestion of more digestible protein by digestive enzymes. Moreover, it proposed that cooking may lead to oxidation of plant polyphenols that leads to formation of quinones and highly reactive peroxides, which are oxidizing agents. These peroxides may then bring about oxidation of amino acid residues and subsequently, polymerization of proteins. This may then lead to reduced protein digestibility [25]. On the other hand, during cooking proteins undergo a change in secondary structure from an alpha-helical to antiparallel, intermolecular beta-sheet conformation and thereby reduced protein digestibility [25]. The results agree with relative increase in glutelins (Fig. 1), which is increased from 8.55% in uncooked to 26.8% in cooked defatted flour. Thus, protein digestibility has been reported to decrease with increased highly polymer glutelins [20].

Protein Fractional Properties: In our previous study, the protein content of defatted brebra seed flour was found to be 48.5%. Fig. 1 shows the effect of cooking on total protein fractions of defatted brebra seed flour. The total protein of defatted flour was fractionated on the basis of solubility into albumins, globulins, prolamins and glutelins. For uncooked defatted brebra seed flour, the content of albumin and globulins were 58.4% and 7.6%, respectively. When defatted flour was cooked, the content of albumin and globulins were significantly ($p < 0.05$) decreased and were found to be 27.2% and 4.3%, respectively. Similar results were obtained by Sulieman *et al.* [21] for cooked lentil cultivars who attributed this loss on cooking to high susceptibility of albumin and globulin to heat treatment. On the other hand, the uncooked defatted flour content of glutelin and prolamin were found to be 8.5% and 0.5%, respectively and they were significantly ($p = 0.05$) increased after cooking and were found to be 28.8% and 0.8%, respectively. The increment in glutelin after cooking was reported for different lentil cultivars by Sulieman *et al.* [21].

The results obtained indicated that about 59.1% of the total protein could be extracted from cooked sample by solvents and the remaining percentage (40.9%) accounted for the non-protein nitrogen and insoluble proteins. On the other hand, the soluble protein for uncooked sample was 75% and the remaining 25% accounted for non-protein nitrogen and insoluble proteins. Thus, large amount of soluble protein can be recovered from uncooked sample or other words, cooking can decrease the protein solubility of the sample. In conclusion, compared to the control uncooked defatted flour, a significant reduction in the level of albumin in the heat treated samples was observed. As a result a relative increase in glutelins and insoluble proteins was found. The variation in the protein fractions observed is the consequences of changes of the molecular mass of the different proteins. The changes in protein conformation and complexation of proteins due to heat [26] may modify their solubility.

Electrophoresis: SDS-PAGE analysis on both raw and cooked defatted protein of this study represents on Fig. 2. There is no apparent molecular masses difference of proteins in the electrophoresis gel of both raw and cooked samples. In both case, SDS-PAGE of the sample proteins have major bands at around 35 and 25 kDa. The purpose of conducting SDS-PAGE was to identify the major bands of proteins in the sample and to observe the

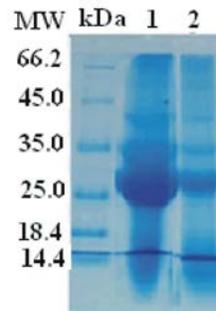


Fig. 2: SDS-PAGE pattern of raw and cooked defatted brebra protein bands of different MW (1= raw, 2 = cooked)

Table 2: Selected functional properties of defatted brebra flour

Functional properties	Cooked	Uncooked
Emulsion capacity (%)	36.00 ± 0.10 ^a	47.7 ± 0.02 ^b
Emulsion activity (%)	48.50 ± 0.04 ^a	80.0 ± 0.03 ^b
Emulsion stability (%)	52.40 ± 0.05 ^a	84.6 ± 0.07 ^b
Water holding capacity (ml/gm)	1.75 ± 0.02 ^a	1.5 ± 0.00 ^b
Oil holding capacity (ml/gm)	1.50 ± 0.00 ^a	1.0 ± 0.05 ^b
Bulk density (gm/ml)	0.70 ± 0.03 ^a	0.6 ± 0.02 ^a

Values are means ± (SD). Means not sharing a common superscript letter in the same protein fraction, for the same parameter, are significantly different at $P < 0.05$. Defatted brebra flour protein content, as determined in our previous study, was 48.5%.

effect of cooking. The SDS-PAGE pattern of proteins from samples exhibited high-density bands corresponding to the 25 kDa. In this study, cooking might not be affected the molecular mass of the protein.

Protein Functional Properties

Emulsion Properties: The emulsion properties were presented in Table 2. The emulsion capacity of defatted brebra seed flour was significantly ($p < 0.05$) decreased from 47.7 ± 0.02% uncooked sample to 36 ± 0.1% after cooking of the sample. The same results were observed in both emulsion activity and stability of defatted flour. Moreover, emulsion stability was significantly ($p < 0.05$) lower than that of the uncooked sample. This finding agrees with that reported by Osman *et al.* [27] who concluded that application of high heat decreased the emulsifying activity and capacity of plant proteins. The same trend of emulsification capacity (from 46.06 ± 2.30 uncooked to 34.71 ± 1.50% cooked) from conophor nut protein were also reported by Enujiugha [2]. The results obtained in this study was in line with that reported by Pawor and Ingle [28] who stated that applications of high heat decreased the emulsion stability of north bean flour. Sever heat treatment yields undissolved, denatured proteins that are unable to orient and migrate to form an

interfacial film at the oil-water interface, thus preventing or reduced emulsion formation [29], as is the case in this study. In conclusion, the emulsion activity and emulsion stability were found to be correlated with hydrophobicity and solubility of the proteins. Changes in protein conformation adversely affected the functional properties of the protein to a large extent. An understanding on the variation in functional properties as a result of domestic processing methods would help in better utilization of these proteins in food systems.

The Water and Oil Holding Capacity: The water and oil retention capacity of defatted brebra seed flour is shown on Table 2. The water holding capacity was significantly ($p < 0.05$) increased after cooking of defatted flour. In agreement with this study, heat-processed flours of cowpea had greater water retention than did flours from uncooked seed flours [30]. The increase may be due to changes in concentration and structure conformation of proteins. According to Cheftel and Cuq [29], water absorption of proteins can be improved by partial denaturation, dissociation, unfolding and insolubilization of the protein. Improvement of water retention of cowpea flour by heat treatment has been reported [31]. According to Hutton and Campbell [32] report, cooking of cowpea flour may have caused conformational changes of proteins from globular to random coil, exposing buried amino acid side chains, thereby making them available to interact with water. Protein water interaction occurs at polar amino acid sites on the protein molecules.

Oil retention capacity of defatted brebra seed flour significantly ($p < 0.05$) increased from 1 ± 0.05 ml/gm (uncooked sample) to 1.5 ± 0 ml/gm (cooked sample). In this study, the effect of heat treatment on oil retention of defatted flour was less compared to water retention. The same trend of results was reported from work done on cowpea flour [30]. According to Kinsella [33] report, fat absorption entirely depends on physical entrapment of oil and several authors have related the oil absorption capacity to the non-polar side chains of the protein as well as to the different conformational features of the proteins. According to this finding, cooked defatted flour has both good water and oil retention capacity. These characteristics may help to improve binding of the structure, enhance flavour retention, improve mouth-feel and reduce moisture and fat losses of extended meat products [34].

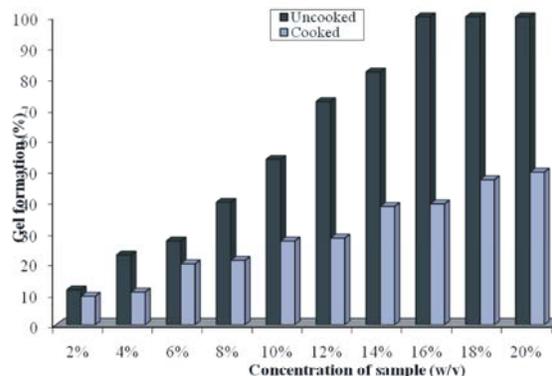


Fig. 3: The effect of cooking of defatted brebra flour in gel formation in comparisons with that of uncooked defatted brebra flour (w/v).

Table 3: Foaming capacity and stability of cooked and uncooked of defatted brebra flour

Functional properties	Cooked	Uncooked
Foaming capacity (%)	3.9 ± 0.05	7.7 ± 0.40
Stability (%)		
10 min	75.0 ± 0.01	100 ± 0.03
30 min	25.0 ± 0.07	85.7 ± 0.05
60 min	0	64.3 ± 0.04
120 min	0	57.1 ± 0.2

Values are means \pm (SD).

Bulk Density: Bulk density of cooked and uncooked defatted brebra seed flour was shown on Table 2. The bulk density of cooked defatted flour was significantly ($p < 0.05$) increased (0.7 g/ml) over that of uncooked defatted flour (0.6g/ml). Bulk density depends on combined effects of interrelated factors such as intensity of attractive inter-particle forces, particle size and number of contact points. Higher bulk density is desirable since it helps to reduce the paste thickness which is an important factor in convalescent and child feeding [35]. Thus, cooked defatted flour might be important for formulation in child food system.

Foaming Capacity and Stability: Table 3 shows that foaming capacity of uncooked defatted seed flour ($7.7 \pm 0.4\%$) was significantly ($p < 0.05$) decreased after cooking ($3.9 \pm 0.05\%$). Heat treatment resulted in the highest reduction in foam ability of cooked flour. Foam stability of uncooked and cooked samples is shown in Table 3. The cooked defatted flour has poor foam stability in comparison with that of uncooked one. The cooked defatted flour lost stability after 30 min. The same trend of foam stability of bambara groundnut was reported by Yagoub and Abdalla [20]. The increase in the fraction of

the insoluble proteins after cooking of the sample (Fig. 1) may partially explain this reduction. Foam capacity and stability of proteins increased due to increased solubility and surface activity of the soluble protein [20]. Thus, the differences in the foaming properties may result from the differences in protein solubility.

Least Gelation Capacity: The gel formation between uncooked and cooked defatted brebra seed flour was shown on Fig. 3. The least gelation capacity of uncooked defatted flour was 16% concentration of sample (100% gel formation take place), while cooked defatted flour maximum gel formation were 47.2% and 49.6% at 18% and 20% of sample concentration, respectively. Heat processing brought about a reduction in the gel forming properties of defatted brebra flour. The same trend of gel formation on codnophor nut was reported by Prinyawiwatkul *et al.* [30]. The gel forming ability is known to be influenced by the nature of the protein, starch and gums in the sample as well as their interaction during heat treatment [2]. According to our previous study, the amount of starch in brebra seed was very low ($0.473 \pm 0.09\%$). Raw flour lowered the least gelation concentration of defatted brebra seed flour, implying that the uncooked one is a better binder. Gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to the non-protein components and protein solubility.

CONCLUSION

Cooking resulted in a significant reduction *In vitro* digestibility using pepsin or pepsin with pancreatin and also reduced the level of albumin and globulin fraction while the content of glutelin and prolamin were increased. The major protein in both cooked and uncooked samples were albumin and glutelin. Cooking is not the best method to recover maximum amount of soluble protein. The results further showed that emulsion capacity, activity/stability, foam capacity/stability and gel formation of the studied sample was affected by cooking but water and oil retention of the sample significantly ($p < 0.05$) increased by cooking. Changes in protein conformation by cooking adversely affected some functional properties of the protein to a large extent. An understanding of the variation in functional properties as a result of domestic processing methods would help in better utilization of these proteins in food systems. This would also help in deeper understanding on the role of individual processing methods on the seed proteins present with other constituents.

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