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Effects of Salt (NaCl) Concentrations on the Functional Properties of Defatted Brebra (*Millettia ferruginea)* Seed Flour

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Abstract: The effect of NaCl on functional properties of defatted flour was investigated. The solubility of protein significantly ($p < 0.05$) increased with 2.0% NaCl solution. Addition of 0.5% NaCl significantly ($p < 0.05$) increased the emulsion capacity of protein. The emulsion stability of defatted flour was higher at zero NaCl concentration (84.6%). The highest foaming capacity of 13.7% and 17.6% were found in defatted flour at 5% NaCl. The foam stability of protein (60.2%) was higher at both 1 and 2% NaCl after 120 min. The water holding capacities of brebra and soybean flour were found to be 175% and 190%, respectively. The least gelation concentration of defatted flour was 16% (w/v), while maximum gel formation of soybean at 20% sample concentration is 65.5%. In conclusion, addition of NaCl to defatted flour up to certain limit improves its functionality and it was in line with functional properties of defatted soybean flour.

Key words: Defatted flour · Functional properties · NaCl concentrations

INTRODUCTION

Nutritional science is concerned with the research and discovery of new food sources in the context of improving the overall quality of humankind's life. In Africa and the rest of the developing world, where malnutrition due to inadequate protein intake is a prevalent problem, there is an urgent need to explore the utilization of plant proteins in the formulation of new food products from nonconventional food sources [1]. This predicted on the fact that animal protein such as the meat, milk and eggs are expensive and relatively difficult to acquire [2]. Legumes are leading candidates in this regard since they contain more protein than almost any other plant product. Many of the legumes have protein contents between 20 and 40% and a few ranges between 40 and 60% [3].

Among legumes, the protein of defatted seed flour of *Millettia ferruginea* (Hochst.) Baker (which is known as brebra in Amharic) is taken into account as best choice of candidate in this investigation. *Millettia ferruginea* is endemic N_2-fixing leguminous tree species that is known to have positive effects on associated crops in central-south part of Ethiopia [4]. It is also used as animal feed, for erosion control, household tool handles, fuel wood supply, shade provision for coffee trees and bee forage [5]. It is also used for mass fishing in rivers [6]. Apart from such economical use of the plant, the seed protein content and functional properties of the defatted flour are not yet studied. In our previous study, we have analyzed the proximate characteristics of the seed content of this plant. Determination of the functional properties of the defatted flour treated with NaCl is significant.

The ultimate success of utilizing plant proteins as ingredients depend largely upon the beneficial qualities they impact to foods which depend largely on their functional properties [7]. Functional properties are intrinsic physio-chemical characteristics which affect the behaviour of properties in food systems during processing, manufacturing, storage and preparation [8]. Nutritional and functional qualities of protein are largely determined by its amino acid content and nitrogen solubility [9]. The critical functional properties necessary in protein ingredients include gelation, emulsion and foam formation, which are important for function of proteins in food systems. Other paramount functionalities are

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proteins solubility, water and fat absorption capacity [8]. These characteristics are influenced by salt concentration during food formulation in modern food industry. The objective of this study was to investigate the functional characteristics of defatted brebra seed protein treated with different concentrations of NaCl.

MATERIALS AND METHODS

The effect of NaCl concentrations on some of the functional properties of the defatted brebra flour samples were determined as described by Ogungbenle *et al*. [10] and Oshodi and Ojokan [11]. The different concentrations of NaCl solutions used were prepared by weighing 0.5, 1, 2, 5, and 10 gm of the NaCl, which were dissolved in 99.5, 99, 98, 95 and 90 gm deionized water, respectively.

Protein Solubility along with Different Salt Concentration: Protein solubility was determined by using distilled water-flour and salt solution-flour suspensions through the slight modification of the method described by Padilla *et al*. (1996), where 6.6g [12] of the flour was suspended in 100 ml distilled water, the suspension stirred by laboratory magnetic stirrer at room temperature for 30 min and centrifuged at 4,000 rpm for 30 min. Supernatant liquid was then filtered through Whatman No. 1 filter paper to obtain a clear extract. The nitrogen contents of the supernatants were determined by the micro-kjeldal method [13]. Protein solubility was expressed as percent of the nitrogen content of the sample multiplied by the 6.25 factor. The distilled water was later replaced with appropriate salt solution. The salt (NaCl) concentrations used in this process were 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00 and 5.00%. In all the determinations, the suspensions were maintained at their "natural pH" (reconstituted pH of flour suspension which is between pH 6.1 and 6.8) [14].

Defatted Brebra Flour Protein Functionality along with Different Salt Concentration: The functional activity of defatted brebra seed flour was determined along with defatted soybean flour. Defatted soybean flour was used as control since its protein functionality is currently well known.

Emulsion Capacity, Activity and Stability: The emulsifying capacity of the sample was estimated by the method of Beuchat *et al*. [15]. One gram sample was blended with 50 ml of distilled water or NaCl solutions ranged from 0.5-10 % (w/v) for 30 second in electric blender (WARING) at maximum speed. After complete dispersion, refined commercial 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 percentage of oil emulsified by one gram sample. Emulsifying capacity (%) = emulsified volume of oil/ total oil used (5ml) x 100

The procedure described by Volkert and Kelin [16] was used for both emulsification activity and emulsion stability. Two gm of sample was added to 50 ml of distilled water or 50 ml NaCl solution of a concentration ranged from 0.5-10 % (w/v) 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, content of centrifuge 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 [17]. EA (%) = (height of emulsion/ height of whole layer) x 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 leaved 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 (%) = (height of emulsion layer after heating/height of whole layer) x 100

Foaming Capacity and Stability: One gram of defatted brebra flour was suspended in 50 ml distilled water within the "natural pH" ranges indicated above; the suspensions were blended in a blender for 5 min [18]. The content was transferred into a 100 ml measuring cylinder and the volume of foam was read after 30 sec. for foam capacity. This was repeated for the various salt solutions at different concentrations, as discussed 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 [19]. All determinations were carried out at least in triplicate. FC (%) = (volume after whipping – volume before whipping/ volume before whipping) x 100. FS (%) = (Foam volume after time (t)/ Initial foam volume) x 100

Water Absorption Capacity: Two grams of the brebra defatted flour were thoroughly mixed, without pH adjustment, with 20 ml of distilled water or different salt concentration. Suspensions were shaked by rotary shaker (Gerhandt) over a 30 min period at 25°C and then centrifuged at 4000*g* for 30 min at 25°C [20]. The amount of water absorbed by the brebra protein was calculated as the number of gm of water held by 1 gm of protein sample and defined as the absorption capacity. Density of water assumed to be 1.000g/ml. The volume of water absorbed was converted to gram.

Gelation Capacity: Gelation capacity was determined according to the method of Coffman and Garcia, [21], as modified by Akintayo *et al*. [18]. Sample suspensions of 2–20% (w/v) were prepared from distilled water in test tubes. Five milliliter of each of the prepared dispersions was transferred into a test tube. The suspensions were heated in a boiling water bath for 1 h followed by rapid cooling in a cold water-bath. The samples were further cooled at 4±1°C for 2 h. The least gelation concentration was determined as the concentration when the sample from the inverted test tube did not slip or fall. The salt effect was studied by replacing distilled and deionized water with various salt concentrations. Results reported are means of triplicate determinations.

Statistical Analysis: Data were analyzed with SPSS 15.0 for windows. The results are given as means of triplicate samples. Appropriate statistical analysis of variance (ANOVA) was done to determine the significance differences among means followed by Duncan's multiple range test when the F-test demonstrated significance. The statistically significant difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Effect of NaCl Concentration on Solubility of Defatted Flour: Figure 1 shows the profile of defatted flour solubility at different NaCl concentrations. The solubility of defatted flour was observed to increase with increase in NaCl concentration; however the increase became statistically non-significant ($p < 0.05$) only at concentrations of 3%, 4% and 5% NaCl. Minimum (45.6%) and maximum (63.1%) defatted flour solubility was shown at 0.5% and 2% NaCl concentration, respectively. The solubility of the protein of flour under investigation increased with NaCl concentration up to 2.0%. The similar observation was reported by Arogundade *et al*. [22]. This may be due to the low ionic strength of the salt, which allows dissociation and consequent interaction with the proteins, thereby increasing solubility (salting in effect). However, at higher concentrations, NaCl produces a dehydrating effect on the protein, which tends to aggregate, resulting in decrease of solubility (salting out effect) [22].

Effect of Concentration on Emulsifying Properties of Defatted Brebra Seed Flour in Comparison on Soybean Defatted Flour: Figure 2 shows the emulsifying capacity of defatted brebra and soybean seed flour at different NaCl concentrations. The emulsion capacity of defatted brebra and soybean seed flour was significantly ($P < 0.05$)

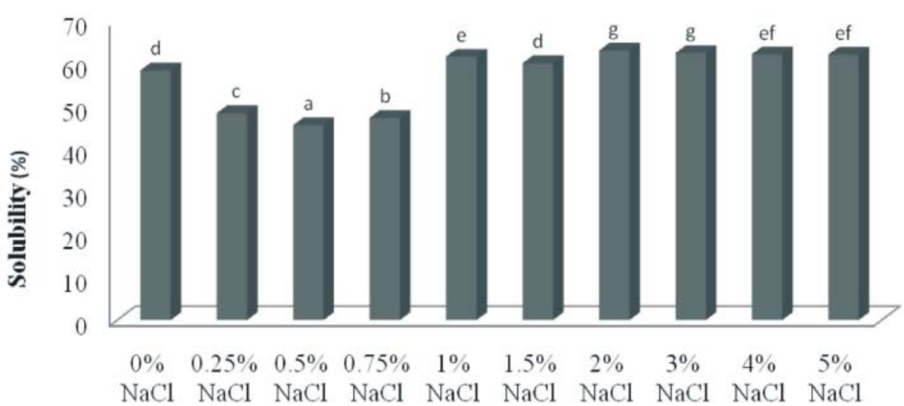

Fig. 1: Effect of NaCl concentration (%) on protein solubility of defatted brebra flour Values are means of triplicate determinations; Values within bars followed by different superscripts are significantly different at ($p < 0.05$)

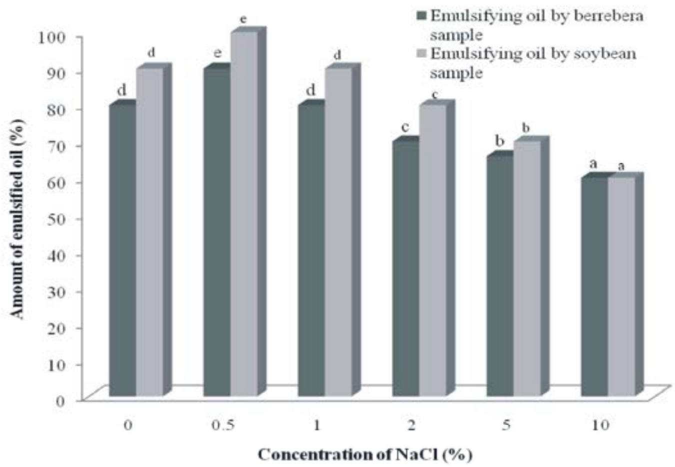

Fig. 2: The emulsifying capacity of defatted brebra flour in comparison with defatted soybean flour: Values are means of triplicate determinations; Values within the same colour of bars followed by different superscripts are significantly different at ($P < 0.05$)

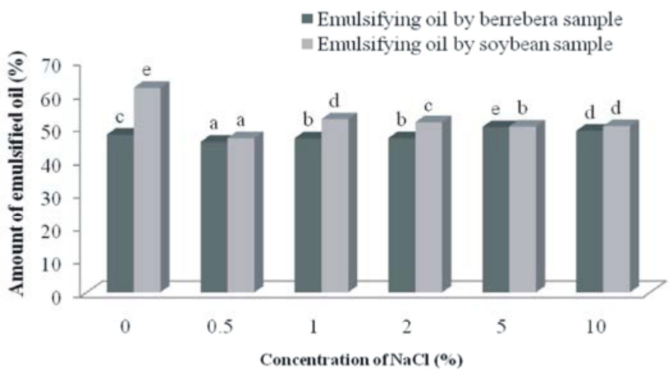

Fig. 3: The emulsifying activity of defatted brebra flour in comparison with defatted soybean flour: Values are means of triplicate determinations; Values within the same colour of bars followed by different superscripts are significantly different at ($P < 0.05$)

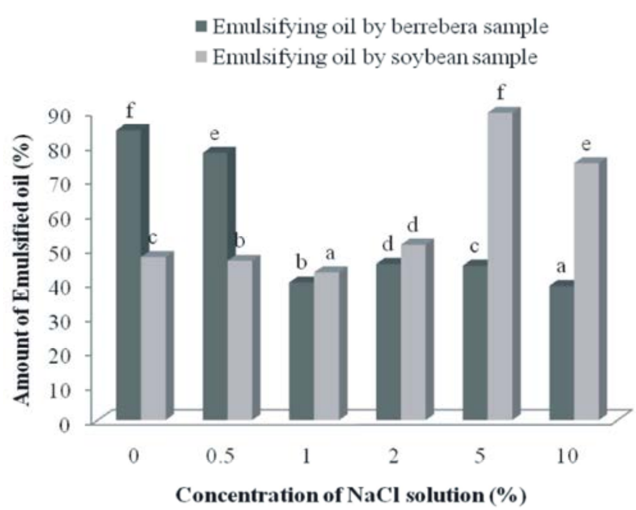

Fig. 4: The emulsifying stability of defatted brebra flour in comparison with defatted soybean flour: Values are means of triplicate determinations; Values within the same colour of bars followed by different superscripts are significantly different at ($P < 0.05$)

increased with the addition of 0.5% NaCl up to 90% and 100%, respectively. The result in this study is in the line with Ragab *et al*. [23] report, this due to the fact that addition of NaCl improved solubility of the proteins. Beyond this salt concentration, the emulsion capacity gradually decreased due to the salting effect of NaCl [23]. The same trend of emulsion capacity was also reported by Ogungbenle *et al*. [24]. On the other hand, significant ($P < 0.05$) differences were not observed for the emulsifying capacity obtained for defatted brebra and soybean seed flour at all NaCl concentrations.

Different concentrations of NaCl affect emulsion activity of defatted brebra and soybean flour (Figure 3). The emulsifying activity of defatted brebra flour, in this study, was not increased ($P > 0.05$) from 0 % to 2% concentration of NaCl solutions but higher after the addition of 5% NaCl solution. On the other hand, the emulsification activity of defatted soybean flour was higher at zero concentration of NaCl but lowered slightly by addition of NaCl. The results in this study agree with that of reported by Osman *et al*. [25], who stated that the emulsion activity of chickpea was higher than in distilled water and then decreased at 0.2M NaCl. In this study of soybean, no obvious reduction of emulsion activity was observed after 0.5% salt concentration.

The oil emulsion stabilities of defatted brebra and soybean flour are shown in Figure 4. The emulsion stability of defatted brebra flour was significantly ($p < 0.05$) higher at zero NaCl concentration (84.6%) and 0.5% NaCl concentration (78%) and then gradually decreased on the higher concentration side. On the other hand, the emulsion stability of soybean (89.7%) was significantly ($p < 0.05$) higher at 5% NaCl solution and then gradually decreased on either side of this concentration. This result was in agreement with that of reported by Osman *et al*. [25]. In case of defatted brebra flour, the quantity of water separated from emulsion produced increased with the concentration of the NaCl, indicating a decrease in emulsion stability in the presence of NaCl. The same trend of emulsion stability was observed in the report of Ogungbenle *et al*. [24]. The decrease in emulsion stability as observed in this study may be due to increase contact leading to coalescence which thereby reduces stability [10] and that is whey as the higher the concentration of the salt added the higher the volume of water separated in this study of defatted brebra flour.

Foam Capacity and Stability: The effect of NaCl on the foaming capacity is presented in Table 1. There was an increase in foaming capacity of the samples with increase in concentration of NaCl from zero to 5%. The highest foaming capacity of 13.7% and 17.6% were found in defatted brebra and soybean flour, respectively, at 5% concentration of NaCl and foam depression was observed with further increase in NaCl concentration in each sample. This sort of tread was also reported in Arogundade *et al*. [22]. The increment of foaming

Table 1: Foam capacity of defatted brebra flour in comparison with defatted soybean flour

	Concentration of NaCl (%)					
Sample	0	0.5	1	2	5	10
Defatted brebra flour (%)	7.7^a	7.8^a	9.8^b	9.8^b	13.7^e	11.8^c
Defatted soyabean flour (%)	13.7^a	13.7^a	13.7^a	13.7^a	17.6^c	15.7^b

Values are means of triplicate determinations Values within the same row followed by different superscripts are significantly different at ($p < 0.05$)

Table 2: Foam stability of defatted brebra flour in comparison with defatted soybean flour in different NaCl concentration

		Foam stability (%) at different minutes			
Sample	Concentration of NaCl (%)	10 min	30 min	60 min	120 min
Defatted brebra flour	0	100	42	42	42
	0.5	100	48.7	25.6	0
	1	100	60.2	60.2	60.2
	2	100	60.2	60.2	60.2
	5	71.5	43.1	27.7	27.7
	10	66.1	50	32.2	16.9
Defatted soybean flour	0	71.5	64.2	64.2	21.2
	0.5	86.1	86.1	71.5	43.1
	1	100	100	100	86.1
	2	86.1	86.1	71.5	71.5
	5	89.2	72.7	61.4	61.4
	10	75.2	68.8	62.4	49.7

Table 3: Water holding capacity of defatted brebra flour in comparison with defatted soybean flour

	Concentration of NaCl (gm H_2O/ gm sample)					
Sample	0	0.5	1	2	5	10
Defatted brebra flour (gm of water/gm sample)	1.75^{a}	2.1^{b}	2.0^{b}	1.65^{a}	1.7^{a}	1.6^{a}
Defatted soyabean flour (gm of water/gm sample)	1.9^{a}	2.0^{ab}	2.25^{b}	2.0^{ab}	2.0_{ab}	1.9_{a}

Values are means of triplicate determinations. Values within the same row followed by different superscripts are significantly different at ($p < 0.05$)

Table 4: The effect of NaCl on gelation of defatted brebra flour in comparison with defatted soybean flour (w/v)

	Concentration of NaCl (w/v)					
Berrebera sample (w/v)	0 %	0.5 %	1 %	2 %	5 %	10 %
2 %	11.4	1.6	1.6	5.5	9.4	9.4
4 %	22.5	22.5	22.5	18.6	10.9	10.9
6 %	27.5	23.7	23.7	23.7	14.1	12.2
8 %	39.8	36.1	36.1	28.6	24.8	21.1
10 %	53.7	42.6	40.7	33.3	25.9	20.4
12 %	72.6	48.9	47.1	45.3	37.9	30.7
14 %	82.0	60.4	53.2	49.6	42.4	32.4
16 %	100	100	64.5	64.5	48.9	35.5
18 %	100	100	100	68.5	51.0	35.3
20 %	100	100	100	75.9	58.6	41.4
Soybean sample (w/v)						
2 %	5.5	3.5	3.5	5.5	19.7	3.5
4 %	10.9	18.6	14.7	7.0	12.8	14.7
6 %	14.1	23.7	19.8	10.3	14.1	12.2
8 %	38.0	39.8	24.8	19.2	21.1	24.8
10 %	40.7	40.7	29.6	20.4	22.2	33.3
12 %	50.7	45.3	34.3	27.0	41.6	45.3
14 %	51.4	47.8	42.4	35.3	42.4	47.8
16 %	57.4	64.5	53.9	43.3	53.9	53.9
18 %	58.0	58.0	58.0	47.6	54.5	49.3
20 %	65.5	67.2	55.2	55.2	65.5	48.3

capacity up to certain NaCl concentration limit is may be due to the fact that the salt reduces surface viscosity and rigidity of protein films but increase spreading rate, thereby weakening interpeptide attractions and increasing foam volume for certain protein [25]. In this study, the foam capacity of defatted soybean is better than defatted brebra flour. In general, the improved foaming capacity of these samples studied in the presence of NaCl may consequently improve their functionalities to be useful for the production of cakes [26].

The results of foam stability at 10, 30, 60 and 120 min for NaCl at different concentrations are shown in Table 2. The foam stability of defatted brebra seed flour was found to be high at 1 and 2% NaCl concentrations after 120 min. When then foam stands after 30 min significant changes were observed in foam stability along with the increment of NaCl concentrations. The results of this study is not in line with the result reported by Osman *et al*. [25] and by Mahajan *et al*. [37] who found that, as the concentration of NaCl increased the foam stability of chickpea flour also increased significantly. However, the results are supported by the findings of Bera and Mukherjee [28] who stated that the foam stability of rice bran concentrates, slightly improved when the salt concentration was increased up to certain limits. On the other hand, the foam stability of soybean was 100% up to 60 min and 86.1% at 120 min in 1% NaCl concentration. Thus, 1% NaCl is the best concentration used to stabilize foam of soybean. Beyond 1% NaCl solution the foam stability gradually decreased up to 49.7% at 10% NaCl concentration. In this investigation, both tested samples show similar trends. Foam stability of the samples tested in this study was found to be depending on NaCl concentration. The foam stability of a protein is governed by the cross linking of protein molecules and creation of films [25].

The improved foam capacity and foam stability in the presence of NaCl at appropriate concentration will enhance its functionality and thus its application in food such as whipped toppings, cake-mixes and frosting [9], where foaming is of paramount importance.

Water Absorption Capacity: The water holding capacity of defatted brebra and soybean seed flour is presented in Table 3. The water holding capacity of defatted brebra and soybean flour are found to be 175% and 190%, respectively, which are higher than the values reported for some sun flower (107% and 137%) protein concentrates [29], ground seed (100%), yellow melon (117%) [30], quinoa (147%) [24] and bovine plasma protein concentrate (94%) reported by Oshodi and Ojokan [11] but lower than the value reported for the protein concentrate of *Adenopus breviflours* benth (201%), white melon (200%) [30] and seed flour [31]. The high water holding capacity of defatted brebra and soybean flour may make them more susceptible to heat denaturation. [32]. The water holding capacity of defatted brebra (210%) and soybean (225%) flour increased at 0.5% and 1% NaCl solution, respectively but in both the water holding capacity decreased after 0.5% and 1% NaCl solution concentrations. This trend may be due to masking of charges as concentration increased, which can reduce electrostatic interaction and hydration but increase hydrophobic interaction [11, 25, 32]. Thus, the water absorption capacity observed for both tested samples decreased with increased in salt concentrations after 0.5% NaCl solution, the lowest water absorption capacity values observed at 10% NaCl concentration.

Gelation Capacity: The gelation capacity of defatted brebra and soybean flour (Table 4) was assessed by determining the least gelation concentration which is operationally defined as the lowest concentration at which gel remains in the inverted tube. The least gelation concentration of defatted brebra flour is 16% (w/v) that similar to quinoa, 16% [24], while the maximum gel formation of soybean at 20% sample concentration in the same procedure is 65.5%. In this case, the gel property of brebra is better than soybean. Addition of salt solution in defatted brebra flour is not improved the least gel formation since distilled water and 0.5% NaCl have the same least gel formation at 16% sample concentration. The least gel capacity of 1% NaCl was 18% of the sample. When defatted soybean dissolved in distilled water and tested for gelation capacity, it was found to be 65.5% at 20% sample concentration but addition of 0.5% and 1% NaCl were increased capacity by 67.2% and 66.8% on the same concentration, respectively. However, the gel capacity of soybean in this study is by far better than cowpea protein isolate in distilled water [23]. The ability of protein to form gels and provide a structural matrix for holding water, flavours, sugars and food ingredients is useful in food applications and in new product development. The results obtained suggested that, 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; a similar conclusion was reached by Sathe and Salunkhe [33] in their study on the great northern bean.

CONCLUSION

The functional properties of defatted brebra seed flour is more or lease in line with functional properties of defatted soybean flour in the function of different NaCl concentrations in this study. The improved foaming capacity of these samples studied in the presence of NaCl may consequently improve their functionalities to be useful for the production of different food types in the food formulation systems. The improved foam capacity and foam stability in the presence of NaCl at appropriate concentration will enhance its functionality and thus its application in food such as whipped toppings, cake-mixes and frosting. The defatted flour could be a useful replacement in viscous food formulation such as soups and baked goods due to high values of water absorption and oil emulsion capacities. The results further showed that protein solubility, emulsion properties, foaming properties, water absorption capacity and gelation capacity were affected by NaCl concentrations. Therefore, concentration of NaCl may selectively used to improve or inhibit the functional properties of defatted brebra seed flour. Defatted brebra seed flour functional properties with appropriate concentration of NaCl suggest that it has potential for use as functional ingredients in food formulations.

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