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Effect of Autoclaving on Solubility and Functional Properties of Chickpea (*Cicer areitinum* L.) Flour as a Function of Salt Concentration

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Abstract: The effect of autoclaving on the functional properties of defatted chickpea flour as a function of NaCl concentration (M) was investigated. The protein solubility of both treated and untreated flour showed minimum solubility at 0.2 M and maximum solubility at 0.4 M. Higher emulsion capacity was observed at 0.6 M and then decreased. Maximum foaming capacity of the flour was obtained at 0.2 M and thereafter started to decrease. Foam stability of the flour was minimum at 0.2M and increased with increase in concentration of NaCl. The Emulsion Capacity (EC) of the flour was higher at 0.6M of NaCl. On either side of this concentration, EC gradually decreased. The emulsifying activity decreased slightly when NaCl was added. For both samples the emulsion stability was significantly decreased by addition of NaCl. Autoclaving had no significant effect on the functional properties of untreated chickpea flour.

Key words: Functional properties, *Cicer areitinum*, salt concentration

INTRODUCTION

Plant proteins play significant roles in human nutrition, particularly in developing countries where average protein intake is less than that required. Because of inadequate supplies of food proteins, there has been a constant search for unconventional legumes, as new protein sources, for use as both functional food ingredients and nutritional supplements[1]. Among legumes, chickpea is a good source of energy, proteins, some vitamins (thiamin, niacin and ascorbic acid) and mineral (Ca, P, Fe, Mg and K)[2]. Plant protein product, such as chickpea proteins, are gaining interest as ingredients in food systems throughout many parts of the word; the final success of utilizing plant proteins as additives depends greatly upon the favourable characteristics that they impart to foods. Therefore, the relationship of protein quality with processing parameters that affect the functional performance of protein products is worthy extensive investigation[3]. Solubility of protein is one of the critical functional attributes required for its used as food ingredient, because solubility greatly influences other properties, such as emulsification, gelation and foaming[4]. Thus, it determines the behavioursr of a protein food product. For plant proteins to be useful and successful in food application they should ideally possess several desirable characteristics, referred to as functional properties, as well as providing essential amino acids[4]. Proteins have unique surface properties due to their large molecular size and their amphiphilic properties. However, the industrial applications of food proteins are limited, because proteins are generally unstable to heating, organic solvent and proteolytic attacked[5]. Therefore, proteins could be converted into stable forms to broadened their applications. Attempts have been made to modify plant proteins to improve their physical functionality, i.e. gelation, viscosity, emulsification and foaming[5]. However, most chemical modifications are not applicable to the food industry. Therefore, in this study, we investigate the effect of autoclaving on the protein solubility and functional properties of chickpea flour as a function of NaCl concentrations (M) and to predict the possibility to apply both raw and autoclaved chickpea flours in the food industry.

MATERIALS AND METHODS

Materials: Chickpea seeds (*Cicer arinitum.)* of the cultivar Shendi grown at Dongla farm during the 2002/2003 season, obtained from the Arab Seeds Corporation, Sudan. Chickpea seeds were cleaned and freed from foreign matter and milled in a laboratory miller to pass through a o.4 mm screen and thereafter defatted. Refined ground nut oil was brought from Bittar Co. ltd., Khartoum. Sudan. Unless otherwise stated all chemicals used in this study were of reagent grade.

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Methods:

Preparation of autoclaved chickpea flour: Appropriate amount of defatted chickpea flour was placed in one liter conical flask and autoclaved for 15 min at 121°C. The autoclaved flour was stored at 4°C until used.

Proximate analysis: Moisture, oil, protein, fiber and ash contents were analyzed according to AOAC[6].

Determination of nitrogen solubility at different NaCl concentration: Nitrogen solubility of both raw and autoclaved flour was determined at different NaCl solutions by the procedure of Quinn and Beuchat[7] with a slight modification. About 0.2 grams material were dispersed in 10 mL distilled water or NaCl of a concentration ranged from 0.2 to 2M and mechanically shaken for 1 h at room temperature, centrifuged at 3000 rpm for 20 min at room temperature, and then the soluble nitrogen in the supernatant was estimated by micro-kjeldahl method. Nitrogen solubility was expressed as percent of the nitrogen content of the sample.

Emulsion measurements: The Emulsification capacity (EC) of the sample was estimated by the method of Beuchat *et al.*[7]. One gm material was blended with 50 mL of distilled water or NaCl of a concentration ranged from 0.2 to 2 M for 30 sec. in a Braun electric blender; after complete dispersion, refined groundnut oil was added cautiously (0.4 mL sec^{-1}) from a burette and blending continued until there was a phase separation (visual observation/change in shaft sound). EC was expressed as milliliters of oil emulsified by one gram material. The emulsifying activity (EA) and emulsion stability (ES) was measured by the procedure of Yasumatsu *et al.*[8]. About 0.2 gm of material was added to 10 mL of distilled water or 10 mL of NaCl of a concentration ranged from 0.2 to 2 M and mixed well before adding to it 10 mL of refined groundnut oil. The mixture was blended in Broun electric blender for 5 min, poured into centrifuge tubes and centrifuged at 3000 rpm for 5 min and then poured into 50 mL measuring cylinders and stay a few minutes until the emulsified layer was stable. Emulsion Stability (ES) was measured by recentrifugation followed by heating at 80°C for 30 min and subsequently cooled to 15°C. After centrifugation, the emulsion poured into 50 mL measuring cylinders and stays a few minutes until the emulsified layer was stable. ES was expressed as the percent of the total volume remaining emulsified after heating.

Foam measurements: Foaming capacity of the sample was determined by following the procedure described by Lawhon *et al.*[9]. About 0.2 grams of the sample were blended with 10 mL distilled water or 10 mL NaCl of a concentration ranged from 0.2 to 2M in a moulinex blender at "hi" speed for 2 min. The mixture was poured into a 250 mL measuring cylinder and the foam volume was recoded after 30 sec. The foam stability (FS) was conducted according to Ahmed and Schmidt[10] method. The FS percent was recorded for 30 min after pouring the mixture in a cylinder.

Least gelation measurement: Least gelation concentration of the flour was measured by the method of Coffman and Garcia[11]with a slight modification. Appropriate sample suspensions of 2, 4, 6, 8 and 10% were prepared in 10 mL of distilled water or 10 mL NaCl of a concentration ranged from 0.2 to 2 M. The test tubes containing these suspensions were then heated for one hour in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were further cooled for 3 h at 4°C. The least gelling concentration was determined as that concentration at which the gel did not fall down or slip when the test tube was inverted.

Statistical analysis: Each determination was carried out in a triplicate and the figures were then averaged. Data was assessed by the analysis of variance (ANOVA)[12]. Duncan's multiple rang test was used to separate means. Significance was accepted at p = 0.05[27].

RESULTS AND DISCUSSION

Proximate composition of treated and untreated chickpea flour: The proximate composition of both treated and untreated chickpea samples are illustrated in Table 1. The moisture content of defatted untreated samples (7.83%) was approximately similar to that of untreated ones (8.1%) reported by Sánchez-Voique *et al.*[13]. The protein and carbohydrate contents were found to be 20.15 and 67.92%, respectively, which is lower than that of chickpea flour reported by Sánchez-Voique *et al.*[13] and Milán-Carillo *et al.*[14]. This difference may be due to variation between seeds and growing location of the cultivars. Ash content was found to be 2.9%, which is similar to that obtained by Milán-Carillo *et al.*[13] (2000). Fiber content

Table 1: Proximate composition of treated and untreated chickpea flour

Parameter (%)	Untreated samples	Autoclaved samples
Moisture	07.83 (± 0.08)ª	07.42 (± 0.06)ª
Protein	20.15 (± 0.13)ª	19.95 (± 0.30)ª
Carbohydrate	67.92 (± 0. 31)ª	67.10 (± 0.23)ª
Ash	02.90 (± 0.13)ª	02.85 (± 0.21)ª
Fiber	00.50 (± 0.07)ª	00.45 (± 0.05)ª
Oil	00.70 (± 0.22)ª	N D

Values are means (± SD), Values not sharing a common superscript in a raw are significantly (p =0.05) different was found to be 0.5% which is lower than that of chickpea (8.8%) reported by Sánchez-Voique *et al.*[13], this possibly due to variation between varieties, growing location and preparation conditions. Oil content was found to be 0.7%. Although chickpea flour was extracted with hexane, lipids were not removed completely and parts of them were remained in the flour and were associated with the protein isolates. Similar explanation reported by Sánchez-Vioque *et al.*[15]. Autoclaving slightly reduced the protein, fiber, moisture, and ash contents of untreated samples. This findings agreed with the observation of Venktesh and Prakash[16] who found that autoclaving of sunflower flour at 1 kg/cm^3 increased moisture content from 8% to 11% , but decreased the protein content from 49% to 46%, residual fats from 3.4% to 1.8% and carbohydrates from 13.6% to 10.4%.

Effect of NaCl concentration on the protein solubility of treated and untreated chickpea flour: As shown in Fig. 1, both treated and untreated flour showed lower extractability of protein at 0.2 M compared to that treated with distilled water. Highest extractability was observed at 0.4 M of NaCl due to salting in effect, thereafter extractability decreased due to salting out effect. There was no significant difference in protein solubility by dissolving the flour in NaCl of a concentration ranged from 0.6 to 2 M. Lower protein solubility at 0.2 M NaCl likely to be due to the formation of ionic bonds within the protein molecule and between adjacent proteins leading to the formation of aggregates. Similar trend was observed by Shehata and Tahnnoun[17] who reported that NaCl retarded protein solubility of Iragi mung bean at lower concentration. They also observed that increase in NaCl concentration from 0.0 to 0.05M caused nitrogen extractability to drop from 79 % to 55.45% with a maximum extractability of 78.78% at 0.5 M NaCl compared to 79% when mung bean dissolved in water alone. Also Hang *et al.*[28] reported similar results on mung beans, red kidney beans and pea beans. No apparent variation observed between untreated and autoclaved flour.

Effect of NaCl concentration on foaming properties of treated and untreated chickpea flour: Figure 2 shows the effect of NaCl concentration on Foaming Capacity (FC) of treated and untreated chickpea flour. Addition of NaCl of a concentration up to 0.4 M improved the FC of untreated chickpea flour. The maximum improvement was observed at 0.2 M NaCl and then decreased gradually due to salting out effect. Higher FC at low salt concentration may be due to improvement of protein solubility at the interface of the colloidal suspensions during foam formation, thus improving foaming capacity[18].

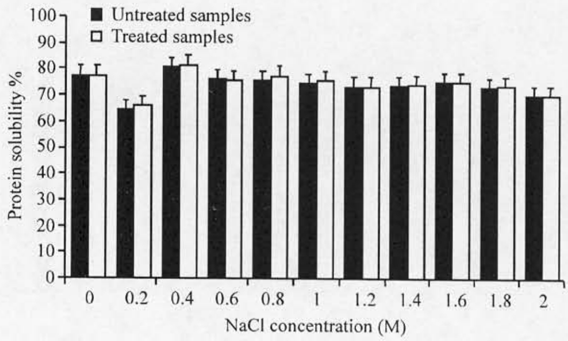

Fig. 1: Effect of NaCl concentration (M) on protein solubility (%) of treated and untreated chickpea flour

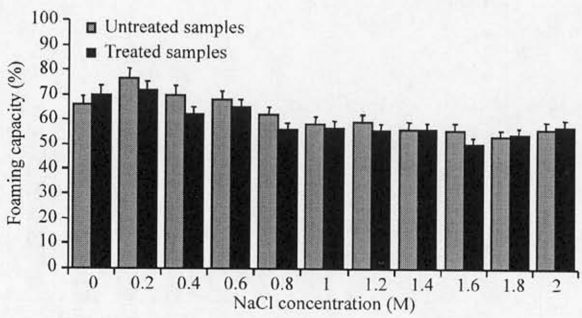

Fig. 2: Effect of NaCl concentration (M) on foaming capacity of treated and untreated chickpea flour.

The present findings supported the findings of Narayana and Narasinga Rao[19] who observed that the FC of both raw winged bean flour and soy bean flour at 0.2 M NaCl increased to a maximum and then dropped beyond this salt concentration. Also similar results were observed by Sathe *et al.*[20] on lupin seed protein. Autoclaving had no significant ($p = 0.05$) effect on the FC of untreated flour at all NaCl concentrations. However, the FC of the treated flour in distilled water was observed to be higher than that of untreated one. There was no significant difference between flour treated at 1.8 and 2 M NaCl. This observation was similar to the findings of Narayana and Narasinga Rao[19] who reported that addition of NaCl up to 0.2 M concentration increased the FC of autoclaved winged bean flour and considerably decreased at higher NaCl concentration. Moreover, they reported that the FC of autoclaved flour in distilled water was observed to be higher than that of untreated one. The effect of NaCl concentration on the Foam Stability (FS) of treated and untreated flour each are shown in Figure 3. For both treated and untreated flour stand for 30 min the minimum FS was observed at 0.2 M NaCl. As the salt concentration increased the FS significantly improved with a maximum

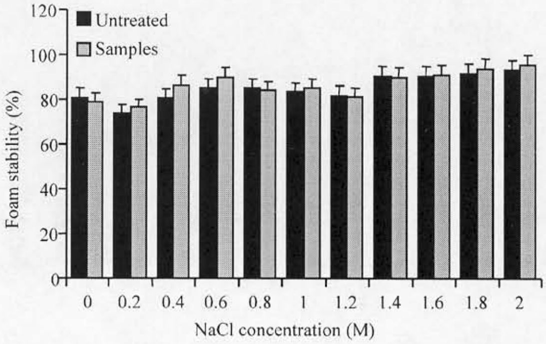

Fig. 3: Effect of NaCl concentration on foam stability (%) of treated and untreated Chickpea flour

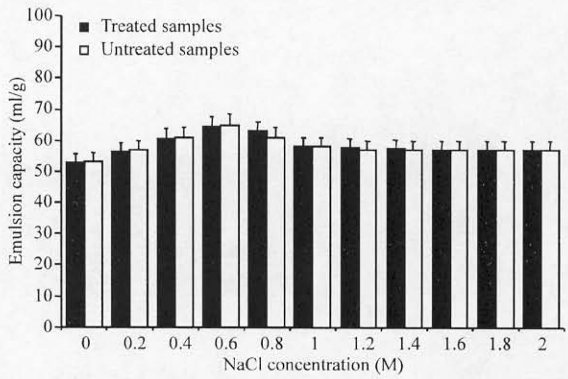

Fig. 4: Effect of NaCl concentration on the emulsion capacity (mL g^{-1}) of treated and untreated Chickpea flour

value at 2 M concentration. Minimum stability at 0.2 M NaCl possibly due to the fact that salt improved the protein solubility at the interface of the colloidal suspensions during foam formation, it retarded the partial denaturation of the surface polypeptides of the proteins that are necessary for protein-protein interaction and stability[18]. The improvement in FS as a result of addition of NaCl was also reported by Bera and Mukherjee[21] who observed that foaming stability of rice bran protein concentrates was slightly improved when salt concentration was increased from 0.1M to 1M NaCl. The improvement of FS by increase in NaCl concentrations might be due to cross linking of protein molecules and creation of films with better viscoelastic properties. Similar explanation was reported by Fennema[22] who reported that the FS of a protein improved by addition of divalent cations, such as Ca and Mg. Autoclaving (Fig. 3) had no effect on FS of the flour. As shown in Fig. 4 the Emulsion Capacity (EC) of both treated and untreated flour was higher at 0.6 M NaCl and then gradually decreased on either side of this concentration. Narayana and Narasiga Rao[19] found that incorporation of NaCl at concentration up to 0.4 M had an incremental effect on the EC of raw winged bean flour. Beyond this concentration EC decreased steadily, due to salting out effects of NaCl. The effect of NaCl on EC of untreated flour did not resemble the effect of NaCl on the protein solubility. This is possibly due to the fact that the emulsifying properties of flour cannot be solely attributed to the proteins but other food components such as carbohydrates and lipids may also contribute through protein-carbohydrate and protein-lipid interactions and also may be due to hydrophilic-lipophilic balance of a particular protein[23]. McWatters and Holmes[29] showed that high concentration of soluble nitrogen was not necessarily related to a maximum emulsifying capacity. Naki[23] reported that solubility, surface hydrophobicity and molecular flexibility influence emulsifying behavior of globular proteins such as pea proteins that have extensive quaternary structure. The EC of autoclaved flour was similar to that of untreated one. The results obtained were correlate with earlier findings of Narayana and Narasiga Rao[19] who reported that addition of NaCl to autoclaved winged bean flour did not cause an observable change in the EC and explained that it would be possibly due to possible denaturation of the protein. The solubility characteristics in water and salt solutions may be changed and reflected on emulsion capacity behavior.

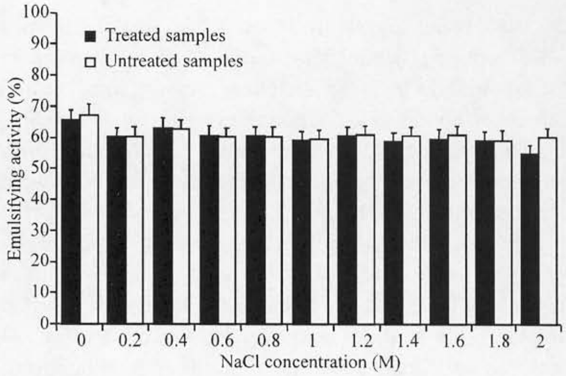

Fig. 5: Effect of NaCl concentration on emulsifying activity (%) of treated and untreated chickpea flour

Figure 5 shows the Emulsifying Activity (EA) of treated and untreated chickpea flour. The EA of untreated flour was higher in distilled water and then it decreased slightly by addition of NaCl. At all levels of NaCl concentration, EA remained constant for both treated and untreated flour. Contradictory results were reported by Monteiro and Prakash[24] who reported that the EA of the peanut protein isolate decreased considerably at 0.3 M NaCl. However, after 0.3 M NaCl concentration, EA

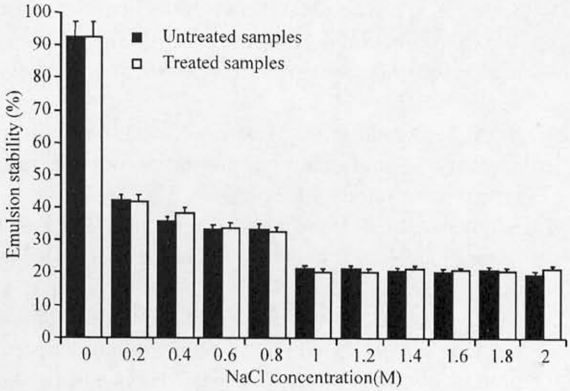

Fig. 6: Effect of NaCl concentration on emulsion stability (%) of treated and untreated chickpea flour

Table 2: Effect of NaCl concentration on least gelation concentration of untreated flour

	Flour concentration (%)				
NaCl concentration (M)	2	4	6	8	10
0.0	-	-	+	++	+++
0.2	-	-	+	++	+++
0.4	-	-	+	++	+++
0.6	-	-	+	++	+++
0.8	-	-	++	+++	+++
1.0	-	-	+	++	+++
1.2	-	-	+	++	+++
1.4	-	-	+	++	+++
1.6	-	-	+	++	+++
1.8	-	-	±	+	++
2.0	-	-	±	+	++

- no gel, ± very weak gel, + weak gel, ++ strong gel, +++ very strong gel

increased with increase in NaCl concentration. No obvious variation observed between untreated and autoclaved flour. This is agreed with the findings of Pawar and Ingle[30] who found that the EA of cooked moth bean flour (17%) was lower than the uncooked flour (20%). Venktesh and Prakash (1993) reported that autoclaving of defatted sunflower flour at 1 kg/cm^2 increased the EA from 15.5 to 20.2 as an absorbance at 500 nm.

Figure 6 shows the effect of NaCl concentration on Emulsion Stability (ES) of chickpea flour stands for 30 min. Addition of NaCl significantly decreased the ES of both treated and untreated flour. Higher ES value (92.67%) was observed when distilled water was used without addition of NaCl and then decreased significantly as NaCl concentration increased. Similar observation was reported by Jane *et al.*[3] who found that emulsions prepared in NaCl significantly reduced the emulsion stability compared to that dissolve in water. The higher emulsion stability in distilled water might be due to the globular nature of the major proteins of chickpea flour. Also Sathe *et al.*[25] reported similar results on winged bean. Autoclaving had no adverse effects on the ES of untreated flour.

Effect of NaCl concentration on the least gelation concentration of treated and untreated chickpea flour: The effect of NaCl concentration on the least gelation concentration of treated and untreated chickpea flour is shown in Table 2. No gel was obtained at 2% and 4% flour in distilled water and all NaCl solutions. This is might be due to a certain degree of protein solubility which is necessary for protein gelation as reported by Venktesh and Prakash[16]. At 6% untreated flour also formed a weak gel in NaCl solution and formed a strong gel at 0.8 M. A hard gel was obtained at 0.8 M NaCl and 8% flour might be due to globulin which account for 60-80% of the total protein in chickpea seeds as reported by Narayana and Rajagopal[26] or it might be due to charge neutralization by NaCl, which promote hydrophobic aggregation upon heating[22]. The decrease in the hardness after 0.8 M NaCl might be due to the differences in boiling temperature of the solutions. It was observed that NaCl concentration up 0.8 M have a higher boiling temperature than lower concentration or might be resulted from the presence of too many ions, which interfere with the formation of protein-protein bonds, as reported by Wiseman and Price[31]. No significant difference was observed on the effect of NaCl on the least gelation concentration of treated and untreated flour (data for untreated not shown).

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