Effect of Autoclaving and pH on Solubility and Functional Properties of Chickpea (Cicer arietinum) Flour

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Abstract: The effect of autoclaving on the functional properties of defatted chickpea flour as a function of pH was investigated. The treated and untreated samples were least soluble at pH 4 (isoelectric region); and on either side of this pH, solubility started to increase with a maximum value (88.28%) obtained at pH 12. The foaming capacity was low at pH 4 and increased gradually on either side of this pH. The foam of the flour was stable at pH 2 and gradually declined as the pH increased. The emulsifying properties of the treated and untreated flour were poor at pH 4 compared to other pH values. Maximum value (72.0 ml/g) of the emulsion capacity was obtained at pH 12 and that of the emulsifying activity (67.0%) at pH 2, while the emulsion stability was high at pH 6. Autoclaving improved both water absorption capacity and bulk density and decreased fat absorption capacity. Both treated and untreated flours showed high dispersibility at neutral and alkaline pH levels compared to acidic conditions. The least gelation concentration for both treated and untreated flours at all pH values was 8%. It is concluded that autoclaving had no adverse effect on solubility and functional properties of chickpea flour.

INTRODUCTION

Chickpea flour is a good source of protein, carbohydrates, fats and some minerals (Kaur and Singh 2005); however, the presence of some antinutritional factors reduces its nutritive value. It consists of 26.2% protein, 59.5% carbohydrates, 6% lipids, 5.5% crude fibre and 2.8% ash (Ulloa et al. 1988).
The ultimate success of utilizing plant proteins as ingredients depends largely upon the beneficial qualities they impart to foods. In recent years, there has been increasing interest in the functional potential of plant proteins. Legumes have been the focal point of this interest since they contain 18%–25% protein (Pawar and Ingle 1988). In view of the increasing utilization of grain legumes in composite flours for various food formulations, their functional properties are assuming greater significance (Singh 2001).

Functionality has been defined as any property of a food ingredient, except its nutritional value, that has a great impact on its utilization (Mahajan and Dua 2002). Functional properties constitute the major criteria for the adoption and acceptability of proteins in food systems. The physical and chemical characteristics and interactions of proteins with other components in the food are the major contributors to the usefulness and success of proteins in food systems. These characteristics influence processing, preparation and quality attributes of foods. Moreover, Protein functional properties are determined to a large extent by a protein’s physicochemical and structural properties (Kinsella 1981).

Protein solubility is an important prerequisite for food functional properties, and it is a good index of potential applications of proteins (Kinsella 1981). Hydration properties, dispersibility, water absorption, binding, swelling and viscosity are known to directly influence the characteristics of a food system (McWatters 1983). Hydration of proteins is vital for several functional properties, such as emulsion capacity, foaming, viscosity and gelation (Sathe and Salunkhe 1981).

The functional properties of legume flours are provided not only by proteins but also by the complex carbohydrates and other components, such as pectins and mucilages. Autoclaving process reduces some antinutrititional factors, trypsin inhibitor and hemagglutinin activities and improves the in vitro protein digestibility of chickpea flour (Bansal et al. 1988). Therefore, the objective of this study was to investigate the effect of autoclaving on the physicochemical and functional properties of chickpea flour as a function of pH.
MATERIALS AND METHODS

Chickpea (*Cicer arietinum*) seeds of Shendi cultivar were obtained from the Arab Sudanese Seeds Company, Khartoum, Sudan. Refined groundnut oil was brought from Bittar Company Ltd., Khartoum, Sudan. Unless otherwise stated, all chemicals used in this study were of reagent grade. About 0.5 kg of defatted chickpea flour was placed in a conical flask and autoclaved for 15 minutes at 121°C and thereafter stored at 4°C for further analysis.

Nitrogen solubility of both raw and autoclaved flour was determined at different pH values (2, 4, 6, 8, 10, 12) using the procedure of Quinn and Beuchat (1975). The soluble nitrogen in the supernatant was determined following the AOAC (1985) method. Nitrogen solubility was expressed as percent of the total nitrogen content of the sample.

Water absorption capacity of chickpea flour was measured by the centrifugation method of Sosulski (1962), and the least gelation concentration was determined by the method of Sathe and Salunkhe (1981). The dispensability of the flour at selected pH levels (3, 7, 10) was measured according to the method of Kulkami *et al.* (1991). For the determination of fat absorption capacity, the method of Lin *et al.* (1974) was used.

The flour wetability was determined for both treated and untreated samples according to the method of Regenstein and Regenstein (1984). The emulsifying properties of the samples were determined by the method described by Beuchat *et al.* (1975) using groundnut oil. The emulsion stability was expressed as a percentage of the emulsifying activity remaining after heating as described by Yatsumatsu *et al.* (1972). The capacity and stability of the flour foams were determined by the method of Ahmed and Schmidt (1979).

Each determination was carried out on three separate samples and analyzed in triplicate. The data were subjected to the analysis of variance, and the Duncan's multiple range test was used to separate means.
RESULTS AND DISCUSSION

Protein solubility of treated and untreated chickpea flour
The protein solubility of both treated and untreated flour at various pH values is shown in Fig. 1. Treated and untreated samples had a minimum protein solubility of 11% at pH 4 (isoelectric point). On either side of this pH, solubility progressively increased specially at the alkaline region at which more protein was extracted compared to the acidic one with a maximum solubility value (88.28%) obtained at pH 12. The occurrence of minimum solubility near the isoelectric pH is primarily due to the lack of electrostatic repulsion, which promotes aggregation and precipitation via hydrophobic interactions.

The lower protein solubility at the acidic region may be due to the occurrence of phytates in chickpea flour. It has been reported that phytases are water-soluble, predominate in the acidic solution and can readily form insoluble complexes with cationic protein (Bera and Mukherjee 1989). Autoclaving had no significant effect on the protein solubility of the flour, except at pH 2 at which the solubility was slightly increased from 43.85% to 46.49%. These results are at variance with those of Naryana and Narasinga-Rao (1982) who observed reduction in protein solubility of raw winged bean flour due to autoclaving.

Foaming properties of treated and untreated chickpea flour
As shown in Fig. 2, the foaming capacity (FC) of treated and untreated flour was minimum at pH 4 and maximum at pH 12. At pH 2, the FC was slightly higher than that at pH 6, 8 and 10. This result agrees with Sathe and Salunkhe (1981) finding that the FC of lupin protein concentrate is highest at pH 2 and least at pH 4. However, it disagrees with the findings of Narayana and Narasinga-Rao (1982) who reported that the FC of raw winged bean flour is minimum (76%) at pH 4.6 and maximum (150%) at pH 9.8. Autoclaving didn’t change the FC of the flour significantly, except at pH 8 at which the FC was decreased from 58.33% to 54.17%. The effect of autoclaving on FC of chickpea flour in this study disagrees with the observation reported by Narayana and Narasinga-Rao (1982). However, at pH above 10, autoclaved flour had higher FC than the untreated one. These differences may be due to differences in the protein content of the seeds of the cultivars as well as the treatment conditions.
Fig. 1. Effect of pH on solubility (%) of treated and untreated chickpea flour

Fig. 2. Effect of pH on foaming capacity (%) of treated and untreated chickpea flour
For both treated and untreated flours, the foam volume stands for 30 minutes decreased significantly as the pH of the mixture increased up to pH 10, and thereafter it started to increase again (Fig. 3). At pH 6 and 12, the foam stability of autoclaved flour was higher than that of untreated one. The foam was very stable at acidic pH due to the formation of stable molecular layers in the air-water interface, which impart texture, stability and elasticity to the foams. Sathe and Salunkhe (1981) observed that the molecular stabilizing effect was in the acidic range and found that the FS of lupin protein concentrates was maximum at pH 4 and progressively decreased at alkaline pH.

Wang and Kinsella (1976) found that the FS varies with pH, being minimum in the isoelectric range (pH 3-4) and maximum in narrow pH regions above the isoelectric range where the protein is slightly negatively charged and showed a rapid decrease at the alkaline pH values. The latter effect may be explained by the charge repulsion between proteins with a resultant lack of adhesion and thereby reducing the quantity of aggregated protein necessary to stabilize the foams (Wang and Kinsella 1976).

Autoclaving (Fig.3) considerably decreased the FS of untreated flour at pH 2 and 8. This finding disagrees with that of Narayana and Narasinga-Rao (1982) who reported that the FS of raw winged bean flour at pH values of 2.5, 6.6 and 8.7 decreases markedly within 10 minutes and then decreases gradually. However, at pH 4.5 it decreased steadily. They also reported that autoclaving markedly decreases the foam volume within 10 minutes at all pH values; at pH 2.5, it decreased from 50% to 45% while at pH 4.6 the FS of autoclaved flour collapsed immediately after its formation.

**Emulsifying properties of treated and untreated chickpea flour**

The emulsion capacity (EC) of treated and untreated chickpea flour as a function of pH is shown in Fig. 4. The EC of treated and untreated flour was markedly affected by pH. The EC was poor at pH 4 (isolectric pH), but was significantly (P ≥ 0.05) improved when the pH was shifted below or above pH 4. The behaviour of the EC of the flour versus pH values closely resembled the behaviour of the protein solubility versus pH values. The data showed that the EC was greatly affected by the protein solubility. For both soybean (McWatters and Holmes 1979) and winged
bean flours (Narayana and Narasinga-Rao 1982) similar relationship between EC and protein solubility versus pH has been reported. Autoclaved flour showed a slight decrease in EC at all pH values. The present findings are in agreement with the results reported by Narayana and Narasinga-Rao (1982) who indicated that autoclaving slightly affects the EC. The EC was greatly affected by the protein solubility, and there were no apparent differences between the treated and untreated flours.

The effect of pH on the emulsifying activity (EA) of treated and untreated chickpea flour is shown in Fig. 5. Untreated flour showed minimum value of EA (7.47%) at pH 4 due to increase in protein-protein interaction and decrease in the net charge as well as the solubility of the protein. On either side of pH 4, the EA was greatly improved. The EA was higher at acidic than alkaline pH values. At pH 12, the EA was lower than at pH 6, 8 and 10. This finding disagrees with that of Khalid et al. (2003) who observed higher EA at alkaline pHs than at acidic ones for sesame and peanut, respectively.

Untreated flour had minimum emulsion stability (ES) at pH 4 (Fig. 6). The ES was high (93.09%) at pH 6, and it decreased at alkaline pH values. Variations in ES at different pH values are possibly due to colloidal particles carrying an electrical charge that promotes the stability of the colloid itself as well as in emulsions formed by causing particles of similar charge to repel each other, thereby preventing precipitation.

Neutralization of the charge, which occurs at a protein's isoelectric point, causes colloidal particles to become both unstable and less soluble (McWatters and Holmes 1979). Similar trend was obtained by Khalid et al. (2003) who found that the ES of sesame protein isolate at pH 2 (75%) was higher than at pH 7 (70%) and pH 10 (62%) with a minimum stability at pH 4.9 (37.8%). No apparent differences in the ES were obtained between the treated and untreated flour.
Fig. 3. Effect of pH on foam stability (%) of treated and untreated chickpea flour

Fig. 4. Effect of pH on emulsion capacity (ml/g) of treated and untreated chickpea flour
Fig. 5. Effect of pH on emulsifying activity (%) of treated and untreated chickpea flour

Fig. 6. Effect of pH on emulsion stability (%) of treated and untreated chickpea flour
Physicochemical properties of treated and untreated chickpea flour

Table 1 shows the physicochemical properties of treated and untreated chickpea flour. The fat absorption capacity (FAC) of untreated chickpea flour (141.25 ml oil/100 g) was higher than that observed by Sánchez-Vioque et al. (1999) for chickpea flour and lower than that reported by Pawar and Ingle (1988) for moth bean flour. Autoclaving significantly ($P \geq 0.05$) decreased the fat absorption capacity of the flour to 135 ml/100 g. The decrease in FAC after autoclaving could be due to the aggregation of the flour proteins as reported by Venktesh and Prakash (1993) who found that the FAC of the protein isolate of autoclaved meal (105 ml/100 g) was lower than that of defatted meal (108 ml/100 g) of sunflower protein. The FAC of flour is an important character as it improves the mouth feel and retains the flavour (Kinsella 1981). According to Kinsella (1981), more hydrophobic proteins show superior binding of lipids, implying that non-polar amino acid side chains bind the paraffin chains of fats. Based on this suggestion, it could be inferred that untreated chickpea flour, which showed higher FAC, had more available non-polar side chains in its protein molecules than the autoclaved one.

Untreated flour had water absorption capacity (WAC) of 130 ml/100 g, while that of the autoclaved one was 140 ml/100g (Table 1). These results agree with those of Quinn and Beuchat (1975) who found that the WAC of autoclaved flour was higher than that of defatted peanut flour. The increment in WAC may be due to the fact that the protein solubility of autoclaved flour decreases as a result of protein dissociation during heating and denaturation. This phenomenon can be minimized by a short-period treatment that would unmask the non-polar residues from the interior of the protein molecules (Abbey and Ibeh 1987). The degree of WAC is a useful indicator of performance in several food formulations, especially those involving dough handling.

The bulk density (BD) of untreated flour (Table 1) was 0.55 g/cm$^3$ which is lower than that reported by Kaur and Singh (2005) for raw chickpea flour and Padmashree et al. (1987) for raw cowpea flour. This is probably due to differences in particle size, preparation methods and seed composition. Autoclaving slightly increased the BD of chickpea flour to 0.57g/cm$^3$. The difference in BD between treated and untreated flour indicated that the autoclaved flour was denser than the untreated one.
This finding agrees with those of Venktesh and Prakash (1993) who reported that autoclaving of defatted sunflower flour increased the BD of the control flour from 0.263 g/cm$^3$ to 0.271 g/cm$^3$ and suggested that it could be due to the removal of the residual moisture of the flour, resulting in dense packing of the flour particles for the same unit volume. Higher BD is desirable since it helps to reduce the paste thickness, which is an important character in convalescent and child feeding (Padmashree et al. (1987).

For both treated and untreated flours, dispersibility was high at alkaline and neutral pH and low at acidic pH (Table 1). These results agree with those of Khalid et al. (2003) who reported that the dispersibility of sesame protein isolate was significantly higher at neutral and alkaline pH values than at acidic ones. There were no significant (P ≥ 0.05) differences in dispersibility between untreated and autoclaved flour. Kinsella (1979) observed that higher dispersibility enhances the emulsifying and foaming properties of proteins during making of bread, macaroni and cookies. According to the latter observation, the emulsifying and foaming capacities of chickpea flour were not changed even after autoclaving.

**Least gelation concentration of treated and untreated chickpea flour**

Untreated flour formed a weak gel at 6% (w/v) for the three pH values (Table 2), and a strong gel was formed at 8% and very strong one at 10%. No gel was formed at 2% and 4% flour for both treated and untreated flours. The pH had no effect on the least gelation concentration of the flour. Legume flours contain high protein and starch contents, and the gelation capacity of flours is influenced by a physical competition for water between protein gelation and starch gelatinization (Singh 2001). Flour from treated and untreated chickpea formed a relatively firm gel at a significantly lower concentration (8%). This may be attributed to the fact that constituents such as proteins, carbohydrates and lipids in the flour are not greatly affected by autoclaving. Moreover, autoclaving may enhance protein-polysaccharide complexation according to Schmidt's (1981) finding that gelation in legume flours involves the formation of a protein-polysaccharide complex.
Table 1. Physicochemical properties of treated and untreated chickpea flour

<table>
<thead>
<tr>
<th>Physicochemical property</th>
<th>Untreated samples</th>
<th>Autoclaved samples</th>
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<tbody>
<tr>
<td>Fat absorption capacity (ml/100 g)</td>
<td>141.25 (± 4.79)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.00 (± 5.00)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water absorption capacity (ml/100 g)</td>
<td>130.00 (± 0.31)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140.00 (± 0.74)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Bulk density (g/ml)</td>
<td>0.55 (± 0.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57 (± 0.21)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Dispersibility (%):</td>
<td></td>
<td></td>
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<tr>
<td>distilled water</td>
<td>59.23 (± 0.24)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.21 (± 0.13)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 3</td>
<td>48.67 (± 0.94)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.33 (± 1.41)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>pH 7</td>
<td>73.83 (± 0.24)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.50 (± 1.18)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>pH 10</td>
<td>71.67 (± 2.36)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.50 (± 1.65)&lt;sup&gt;a&lt;/sup&gt;</td>
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Values are means (± SD). Means not sharing a common superscript letter in a row are significantly different at P ≥ 0.05.

Table 2. Effect of pH on the least gelation concentration of treated and untreated chickpea flour

<table>
<thead>
<tr>
<th>pH</th>
<th>Untreated flour concentration (%)</th>
<th>Treated flour concentration (%)</th>
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<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
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<td>3</td>
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<td>7</td>
<td>-</td>
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<td>10</td>
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- No gel; + weak gel; ++ strong gel; +++ very strong gel
The data obtained in this study disagree with those of Wiseman and Price (1987) who found that less protein was required for gelation at neutral and alkaline pHs than at acidic pHs for the protein concentrates of pressed jojoba meal. These differences may be due to high content of starch of untreated flour that induced gelation due to starch or starch-protein interactions.

In conclusion, autoclaving of chickpea flour had no adverse effect on flour functionality even under different pH levels. Therefore, autoclaving could be a useful mean to inactivate enzymes that interfere with the processing and storage of the flour.

REFERENCES


تأثير التسخين الرطب والأس الهيدروجيني على الذوبانية والخصائص الوظيفية لدقٍق الحمص

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موجز البحث: تمّت دراسة تأثير التسخين الرطب على دقيق الحمص المنزوع الزيت تحت تأثير محاليل مختلفة الأس الهيدروجيني. كانت العينات المعاملة والغير معاملة أقل ذوبانًا عند الأس الهيدروجيني 4 (نقطة التعادل الكهربي) ، وعند أس أقل أو أكثر من 4 زادت ذوبانية العينات ، وأقصى قيمة للذوبانية (88.28%) كانت عند الأس 12. كانت السعة الرغوية للعينات عند أس هيدروجيني 4 منخفضة وزادت تدريجياً مع نقص أو زيادة الأس عن 4 ، ورغوية الدقيق كانت ثابتة عند أس هيدروجيني 2 وقلت تدريجياً مع زيادة الأس. الخواص الاستحلابية للدقيق المعامل وغير المعامل كانت ضعيفة عند أس 4 مقارنة بقيم الأس الأخرى. تم الحصول على القيمة القصوى (مل/ج) للسعة الإستحلابية عند أس 12 وللنشاط الاستحلابي (67.0%) عند أس 2 بينما كان التثبات الاستحلابي عاليًا عند أس 6. أدي التسخين الرطب لتحسين القدرة على امتصاص الماء والكثافة والانخفاض القدرة على امتصاص الدهون. أظهراً دقيق كل من العينات المعاملة وغير المعاملة درجة انتشار عالية عند الأس الهيدروجيني المتعادل والقلوي مقارنة بالأس الهيدروجيني الحمضي. وجد أن أقل تركيزاتتكوين الهلام لدقيق العينات المعاملة وغير المعاملة هو 8% عند جميع قيم الأس الهيدروجيني. يستطيع منهج أعلاه أن التسخين الرطب ليس له تأثير سلبي على الذوبانية والخصائص الوظيفية لدقٍق الحمص.