STUDIES ON THE DEVELOPMENT OF INFANT FOODS FROM PLANT PROTEIN SOURCES. PART II. EFFECT OF PROCESSING CONDITIONS ON THE CHEMICAL AND NUTRITIVE PROPERTIES OF CHICKPEA *(Cicer arietinum)*

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SUMMARY

In order to improve the taste, flavor and nutritional quality of chickpea *(Cicer arietinum)*, various processing conditions were studied. The decorticated samples were processed under various conditions, either by presoaking or non-soaking in water or sodium carbonate solution. The proteins were also isolated from water or carbonate-presoaked chickpea and subjected to various processings. Carbonate-presoaked samples gave slightly lower protein and ash values. No major changes in other constituents were observed. Subjective analysis of the intensity of characteristic chickpea flavor in processed samples was carried out, indicating some improvement in the carbonate-presoaked samples. Carbonate-treated samples exhibited a lighter color. The carbonate presoaking procedure had no adverse effect on the availability of lysine and nitrogen solubility index (NSI), as compared to the water-presoaking procedure. The time required to inactivate trypsin inhibitors in carbonate-presoaked chickpea at boiling temperature, was half that required in the case of water-presoaked ones. Under the conditions used in treating chickpea with sodium carbonate, no beneficial effect was observed in reducing the tannin content. No significant differences were observed in net protein ratio (NPR) among the various processed chickpea samples, even though in some cases isolated protein gave significantly lower NPR values. Digestibility values were higher for isolated protein than for whole chickpea samples.

Manuscrito original recibido: 13–7–85.

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Publication INCAP/UNU-37.

INTRODUCTION

Chickpea *(Cicer arietinum)* has been grown in large quantities in the tropics, subtropics and the Mediterranean countries (1). The nutritional quality of chickpea proteins is known to be the highest of all the pulses (2). As a consequence, various attempts have been made to combat protein malnutrition using chickpea products, and the results have been most encouraging (3). Nevertheless, the quantity of the products required to provide adequate protein cannot be consumed by children because of the problem of indigestibility (1).

It has long been recognized that legume proteins are, in general, of poor digestibility attributed to the presence of trypsin inhibitors (4). Recent studies have shown, however, that antitryptic factors are not solely responsible for this low digestibility, and that only 40% of improvement of the nutritive value from raw to heat-processed soybean is due to inactivation of the trypsin inhibitors (5). Other factors which have been suggested to be responsible for the low digestibility of food legume protein include protein structure (1, 6-8), processing conditions (8-11), and the digestibility of the food legume starch (12-15).

In our earlier communication on the subject (16), the effect of chickpea germination on the nutritive value and digestibility of its proteins was reported. The present paper presents the results of studies on the quality of chickpea flour and on the changes in nutritive value of its proteins, by processing chickpea or its isolated protein under various conditions.

MATERIALS AND METHODS

Materials

Chickpea used in the present study was grown in Guatemala and purchased from the local market. This was a large-seeded variety with salmon-white seed coat. The chickpea was decorticated by using a Rural Industries Innovation Center (RIIC) dehuller. The decorticated cotyledons were used for further studies.

Processing of Chickpea

A portion of the decorticated chickpea (known as *dal* in the Indian subcontinent) was soaked in water overnight (17-18 hr) at a temperature of 4° - 5°C. After discarding the soaking water, the cotyledons were boiled in water for 40 min; the cooking water was also discarded, and the cooked cotyledons, freeze-dried. A second portion of the decorticated chickpea was boiled for 40 min in water without any presoaking. After boiling, the cotyledons were freeze-dried. The third portion of the chickpea cotyledons was soaked in 1% sodium carbonate solution overnight (17-18 hr) at room temperature (27° - 29°C). The soaking solution was discarded and the cotyledons were boiled in water for 20 min. The cooked cotyledons were washed thoroughly with water and then soaked in 0.05 N hydrochloric acid solution for 1 hr, to bring down the pH to near neutrality. The neutralized cotyledons were washed again with water. The

cooked material was then divided into two portions: one portion was freeze-dried, and the other was dried in a hot-air oven at a temperature of 70°C. Another portion of the raw *dal* was boiled in 0.5o/o Na_2CO_3 solution for 40 min, without any presoaking. The cooked cotyledons were separated from the cooking solution, washed thoroughly with water, and then neutralized in the same way as the previous sample. After neutralization, the cooked *dal* was washed with water and freeze-dried. The last portion of the decorticated chickpea was also presoaked in 1o/o Na_2CO_3 solution in a similar manner as described above, but the presoaked cotyledons were autoclaved at 10 psi for 10 min. The use of sodium carbonate in concentrations higher than 1o/o in the soaking solution was not possible because the presoaked cotyledons became too soft during cooking, resulting in high losses of the cotyledon materials in the cooking water. The autoclaved chickpea cotyledons were washed with water, neutralized with 0.05 *N* HCl solution, washed again with water and then freeze-dried. All dried chickpea samples were ground into a flour using a Raymond screen mill No. 82, fitted with a 0.031 inch mesh screen.

Isolation and Processing of Chickpea Proteins

A weighed amount of the decorticated chickpea was soaked in water overnight (17 - 18 hr) at a temperature of 4° - 5°C. The presoaked cotyledons were washed with water and ground into a slurry after adding a small amount of water, in order to facilitate grinding in a commercial Waring blender (Dynamics Corp. of America, New Hartford) for 4-5 min. The slurry was then diluted with water in the ratio of one part of chickpea cotyledons to six parts of water. The diluted mass was stirred manually for 15 min and then filtered first through a coarse cloth and subsequently through a double layer of fine cloth. The filtrate was allowed to stand for 1 hr to settle the starch particles at the bottom of the container. The supernatant was decanted and divided into two parts: the first part was boiled for 40 min, and the second part was autoclaved at 15 psi for 15 min. The protein was precipitated at its isoelectric point (pH 4.5) by adding 0.5 *N* HCl solution with constant stirring at a temperature between 65° and 70°C. The protein curd was then separated by filtration through a double layer of fine cloth. The process for isolating the protein from sodium carbonate-presoaked chickpea was similar to that used in the case of water-presoaked chickpea, except that the extract was boiled for 20 min in this case, instead of 40 min as in the case of the water presoaking procedure. All protein curds were freeze-dried.

Chemical Analyses

Moisture, fat, and ash content in the various chickpea flours and protein isolates were determined by the standard AOAC methods (17). Nitrogen was determined by the macro-Kjeldahl technique, and the protein content was calculated by using the conversion factor 6.25. The crude fiber content in the chickpea flours was determined as the residue left after sequential hot digestion in 1.25o/o sulphuric acid and 1.25o/o sodium hydroxide. The carbohydrate level was estimated by difference. The trypsin inhibitor activity was then evaluated by the procedure of Kakade,

Simmons and Liener (18). Tannins and polyphenols were estimated by the Folin-Denis method (19) and expressed as tannic acid. Available lysine was assayed by the dinitrofluorobenzene method of Conkerton and Frampton (20) as modified by Carpenter (21). Nitrogen solubility index (NSI) was determined by the AOCS procedure (22).

Color Measurement

The intensity of the color of different chickpea flours was determined by using the Lovibond tintometer (The Tintometer Ltd., Salisbury, England).

Flavor Evaluation

The intensity of the characteristic chickpea flavor in flours prepared both from water-presoaked, and carbonate-presoaked chickpeas, was evaluated by a semitrained taste panel of eight judges who were well acquainted with the chickpea flavor prior to tasting the test samples. The judges were asked to score samples within a range from 8 points for very strong chickpea flavor to 0 for no chickpea flavor.

Biological Trials

Weanling rats of the Wistar strain from the INCAP's animal colony were used for evaluating proteins in various processed chickpea flours and protein isolates. At the beginning of the experiments, the rats were weighed and divided into groups consisting of four males and four females each. The average weight of the rats in each group did not differ by more than ± 0.5 g from that of any other group. The rats were individually housed in all-wire cages provided with screen bottoms. Each group received a 10% protein diet contributed by either chickpea flours, protein isolates or casein. One group was fed a nitrogen-free diet. Diets and water were provided *ad libitum*. The partial composition of diets prepared from various test materials is given in Table 1. All diets were supplemented with 4% salt mixture (23), 5% cottonseed oil, 1% cod liver oil, and cornstarch to make 100%. Five ml of a vitamin solution (24) were added to each 100 g diet. The rats were fed for a period of 10 days to determine the net protein ratio (NPR). The digestibility of proteins was evaluated at the end of the NPR experiments. For this purpose, the same diets were dyed with bone charcoal and offered to the same rats for another four days. Feces were collected daily and stored in a cold room at a temperature of 4° - 5°C, they were then dried, cleaned, weighed and ground into powder. Nitrogen content in the powdered feces was determined by the Kjeldahl method (17), and NPR and apparent and true protein digestibilities were calculated using the standard formulae (25).

Statistical Analysis

NPR, digestibility and food consumption data were subjected to an analysis of variance and a multiple comparison was performed following the Tukey procedure (26). Data for sensory evaluation were analyzed by Student's "t" test to determine the significance of treatment difference.

TABLE 1

PARTIAL COMPOSITION OF EACH EXPERIMENTAL DIET

Diet No.	Source of protein	Amount of protein source (%)	Cornstarch (%)
1	Chickpea, raw	50.35	39.65
2	Chickpea, presoaked in water, boiled, freeze-dried	43.92	46.08
3	Chickpea, boiled in water, freeze-dried	42.90	47.10
4	Chickpea, presoaked in carbonate solution, boiled, freeze-dried	47.04	42.96
5	Chickpea, presoaked in carbonate solution, boiled, oven-dried	47.73	42.27
6	Chickpea, boiled in carbonate solution, freeze-dried	45.83	44.17
7	Chickpea, presoaked in carbonate solution, autoclaved, freeze-dried	46.40	43.60
8	Protein isolate from water presoaked chickpea, boiled, freeze-dried	15.70	74.30
9	Protein isolate from water presoaked chickpea, autoclaved, freeze-dried	15.29	74.71
10	Protein isolate from carbonate presoaked chickpea, boiled, freeze-dried	23.74	66.26
11	Casein	11.31	78.69
12	—	—	90.00

RESULTS AND DISCUSION

The results of the analysis for proximate composition of various processed chickpea samples showed that protein content varied from 20.9 to 23.3% (average 21.9%); crude fat, from 6.8 to 8.8% (average 8.1%); ash, from 1.1 to 2.3% (average 1.6%); crude fiber, from 2.1 to 2.6% (average 2.3%); and CHO obtained by difference, from 64.4 to 67.2% (average 66.1%). The protein content in the isolated protein was 63.7, 65.4 and 42.1%, with a fat content of 23.2, 22.5 and 12.0% and CHO 11.1, 10.3 and 44.0%, respectively.

The composition of the protein isolates from water-presoaked chickpea was found to be similar to that reported by Deschamps (27). The protein and fat contents were comparatively lower in the isolate prepared from carbonate-presoaked chickpea, due to the presence of a higher amount of carbohydrates. During soaking and grinding, the protein and

starch particles probably swelled by the action of alkali. Consequently, the extract was more viscous and the sedimentation of starch particles was not as complete as in the case of water-presoaked chickpea during the specified time period. Therefore, the suspended starch particles in the supernatant were also separated with the proteins during precipitation.

The results, reported in Table 2, indicate that boiling for 20 min was more effective in inactivating trypsin inhibitors in carbonate-presoaked chickpea than boiling the water-presoaked chickpea for 40 min. Since trypsin inhibitors are proteins, it is probable that they were denaturated more quickly by the combined action of heat and alkali. Results of other studies on the activation of trypsin inhibitors in soymilk (10, 14) support these findings. Presoaking was found advantageous in inactivating trypsin inhibitors during the subsequent boiling process.

TABLE 2

EFFECT OF VARIOUS PROCESSING CONDITIONS ON THE TRYPSIN INHIBITOR ACTIVITY AND TANNIN CONTENT IN CHICKPEA AND PROTEIN ISOLATE

Processing conditions	Trypsin inhibitors[a] (TUI/ml extract)	Tannin (as tannic acid) (%)	Lysine g/16 gN	NSI
Chickpea, raw	8.12	0.34	6.92	82.24
Chickpea, presoaked in water boiled, freeze-dried	2.52	0.16	5.36	14.83
Chickpea, non-soaked, boiled, freeze-dried	5.20	0.35	5.88	26.88
Chickpea, presoaked in carbonate solution, boiled, freeze-dried	0.91	0.17	5.32	14.28
Chickpea, presoaked in carbonate solution, boiled, oven-dried	0.31	0.16	5.17	14.12
Chickpea, non-soaked, boiled in carbonate solution, freeze-dried	1.93	0.23	5.82	23.56
Chickpea, presoaked in carbonate solution, autoclaved, freeze-dried	0.32	0.19	5.15	15.70
Protein isolate from water presoaked chickpea, boiled, freeze-dried	1.93	0.23	4.99	4.71
Protein isolate from water presoaked chickpea, autoclaved, freeze-dried	0.83	0.17	4.99	3.82
Protein isolate from carbonate presoaked chickpea, boiled, freeze-dried	0.00	0.17	4.96	5.64

[a] Trypsin units inhibited (TUI) as defined for the BAPA method.

Table 2 also shows that chickpea flours prepared from either water-presoaked or carbonate-presoaked chickpeas, processed either by boiling or autoclaving, contain almost the same amount of tannins. Muindi, Thomke and Ekman (28) showed that treatment of sorghum grains with a concentration as low as 4 g liter^{-1} of a sodium sesquicarbonate salt ($Na_2CO_3 \cdot NaHCO_3 \cdot H_2O$) for three days, reduced the level of assayable tannins by 40 to 57%. Other alkalis such as ammonium hydroxide, sodium hydroxide and potassium carbonate were found to be effective in removing tannins from sorghum grains when treated for three days (29). It is probable that the soaking period (17-18 hr) used in the present study was too short to get any measurable effect of alkali on chickpea tannins. In the processed samples, tannin content was lower than that in raw chickpea flour. This was probably due to diffusion of the polyphenolics in the soaking and cooking waters, as evidenced by the fact that the samples which were not presoaked contained more tannins (Table 2). This Table also shows that the tannin content in protein isolates was similar to that present in chickpea flours.

Available lysine and water-soluble nitrogen contents in various processed chickpea samples and protein isolates were determined, with the results detailed in the same Table 2. These indicate that no appreciable reduction in available lysine and in the solubility of the nitrogenous constituents occurred with the carbonate treatment. Processing of chickpea, presoaked either in carbonate solution or water, resulted in slightly lower available lysine values as compared to the corresponding values of the non-soaked ones. Walker and Kochhar (30) also found lower available lysine in water-presoaked cowpea than in non-soaked ones under identical processing conditions.

Available lysine in the isolated proteins was slightly lower than that in the protein in flours. Pobably, total lysine content in the isolated protein was lower than in the protein in chickpea flours. Lysine content in isolated soy protein was lower than that in soy protein concentrate (31). As expected, the water solubility of nitrogen in isolates was very much lower as compared to that in flours.

The flours prepared from sodium carbonate presoaked chickpea (boiled for 20 min and oven-dried at 70°C) was subjected to evaluation of the intensity of the characteristic flavor, in comparison to that of the flour prepared from water-presoaked chickpea (boiled for 40 min and oven-dried at 70°C); the results are presented in Table 3. The data therein indicate a slight improvement in flavor in the flour prepared from carbonate-treated chickpea, but the statistical analysis revealed that the mean scores for flavor of the two flours tested were not significantly different. Treatment of soybeans with sodium carbonate, however, was most effective in reducing the characteristic beany flavor in the resulting soy milk, in comparison to that prepared from water-presoaked soybean (14). A detailed study is necessary to evaluate the effect of sodium carbonate on the chickpea flavor.

Results of the measurement of color intensity in flours prepared from carbonate-treated and water-presoaked chickpeas, indicate that the former is lighter in color than the latter (Table 3). This is presumably due to either more coloring matters leached out in the alkaline soaking solution and cooking water, or to the fact that the color components reacted with sodium carbonate or were broken down by the action

TABLE 3

EFFECT OF SODIUM CARBONATE TREATMENT OF CHICKPEA ON THE FLAVOR AND COLOR OF THE RESULTING FLOURS

Chickpea	Mean flavor scores[a] (n = 8)	Lovibond tintometer color description	
		Yellow	Orange
Water presoaked, boiled and oven-dried	4.25	2.2	0.8
Sodium carbonate presoaked, boiled, neutralized, oven-dried	2.25	1.3	0.7

a Two means are not significantly different.

of the alkali during heat processing.

Net protein ratio (NPR) values of flours prepared from raw and variously processed chickpeas, as well as those of isolated proteins, were determined, with the results given in Table 4. These show that the NPR's of all flours tested were equivalent to each other and to that of casein. Processing of either presoaked or non-soaked chickpea, however, gave slightly higher NPR valuen than raw samples. Drying the processed chickpea in a hot air oven at 70°C, or freeze-drying, did not affect nutritive value. Autoclaving had no effect in improving the nutritive value of proteins over the boiling of carbonate-presoaked chickpea. In our previous communication on the subject (16), no significant difference in NPR values was detected between autoclaved and boiled water-presoaked chickpeas. The results reported by Geervani and Theophilus (32) also indicate no improvement of the PER values of chickpea by autoclaving than by boiling. The NPR's of isolated proteins from water-presoaked chickpea, processed either by boiling or autoclaving, were slightly lower than those of proteins in the flour prepared from boiled water-presoaked chickpea. Isolated soy protein also gave a lower PER value compared to the protein in soybean (33) and soy protein concentrate (31). This lower PER value of isolated protein was attributed to the lower amount of S-containing amino acids, which are already limiting in soy protein. As the data in Table 4 reveal, the NPR's of isolated proteins from carbonate-presoaked chickpea were significantly lower than those of protein isolates prepared from water-presoaked chickpea. This was probably due to destruction of the S-amino acids to some extent during precipitation, as evidenced by the fact that Kon *et al.* (13) reported a significantly lower PER value of bean protein cooked at pH 3.5, as compared to that obtained when cooked at pH 6.7 under identical conditions. The same authors proved that the loss of methionine during cooking at the acidic pH is responsible for these lower PER values. In the present study, the pH during precipitation of protein from the extract prepared from carbonate-presoaked chickpea was 3.8, and the temperature, 65°–70°C. These conditions were found suitable for optimum protein precipitation. Sathe, Deshpande and Salunkhe (34), also observed a lower solubility of

TABLE 4

EFFECT OF VARIOUS PROCESSING CONDITIONS ON THE NUTRITIVE VALUE OF PROTEINS IN CHICKPEA AND PROTEIN ISOLATES

Source of protein	Average weight gain (g/10 d)[a]	Net protein ratio ratio (NPR)	Apparent digestibility (AD)	True digestibility (TD)
Chickpea, raw	36.6	$3.65^{a} \pm 0.26$	$70.11^{d} \pm 2.00$	$78.38^{c} \pm 2.23$
Chickpea, presoaked in water, boiled, freeze-dried	44.0	$4.02^{a} \pm 0.25$	$80.75^{bc} \pm 1.40$	$82.84^{b} \pm 1.32$
Chickpea, presoaked in carbonate solution, boiled, freeze-dried	40.4	$3.97^{a} \pm 0.30$	$79.56^{c} \pm 1.36$	$81.74^{bc} \pm 1.37$
Chickpea, presoaked in carbonate solution, boiled, freeze-dried	38.5	$3.83^{a} \pm 0.41$	$80.18^{c} \pm 1.33$	$82.58^{b} \pm 1.50$
Chickpea, presoaked in carbonate solution, boiled, oven-dried	40.9	$3.82^{a} \pm 0.37$	$78.58^{cd} \pm 1.62$	$80.99^{bc} \pm 1.77$
Chickpea, boiled in carbonate solution, autoclaved, freeze-dried	40.7	$3.68^{a} \pm 0.40$	$79.66^{c} \pm 2.31$	$81.97^{b} \pm 2.41$
Chickpea, presoaked in carbonate solution, autoclaved, freeze-dried	38.9	$3.97^{a} \pm 0.41$	$80.27^{c} \pm 0.93$	$82.94^{b} \pm 1.03$
Protein isolate from water presoaked chickpea, boiled, freeze-dried	36.7	$3.64^{a} \pm 0.17$	$85.21^{a} \pm 2.68$	$87.49^{a} \pm 2.64$
Protein isolate from water presoaked chickpea, autoclaved, freeze-dried	33.9	$3.64^{a} \pm 0.45$	$84.96^{a} \pm 2.42$	$87.61^{a} \pm 2.74$
Protein isolate from carbonate presoaked chickpea, boiled, freeze-dried	23.0	$2.85^{b} \pm 0.28$	$83.73^{ab} \pm 1.69$	$86.42^{a} \pm 2.06$
Casein	44.0	$4.06^{a} \pm 0.30$	$85.73^{a} \pm 2.56$	$88.31^{a} \pm 2.65$

a Initial weight = 45.0 g.
Means carrying the same superscript are not significantly ($p < 0.05$) different. (Mean ± SD).

alkali-extracted proteins from lupine seed at pH 3-4, although the isoelectric point was at pH 4.5. Statistical analysis of food intake data indicated that the lower NPR obtained in the case of alkali-extracted protein may not be due to a lower food consumption. The slightly lower food intake in this case was probably due to the lower pH of the diet.

The apparent and true digestibilities of protein in the various chickpea flours and protein isolates analyzed, are also presented in Table 4. Our findings indicated that the digestibilities of proteins in boiled chickpea presoaked either in water or carbonate solutions, were similiar but significantly higher than that of the raw chickpea protein. This was probably due to either inactivation of the trypsin inhibitors, removal of tannins, or structural changes during processing. Presoaking either in water or carbonate solution had a slight beneficial effect in improving digestibility. Oven drying at 70°C reduced digestibility to some extent, compared to freeze-drying. There was no difference in digestibility between carbonate-presoaked chickpea samples subjected to boiling for 20 min, or autoclaving at 10 psi for 10 min. The digestibility of isolated proteins was significantly higher than that of proteins in chickpea flours and equal to that of casein. The lower digestibility of proteins in chickpea flours was probably due to the presence of cellulosic materials (35).

From the results herein reported, it can be concluded that from the point of view of better product quality and shortening of cooking time, carbonate presoaking has many advantages over water presoaking for the preparation of chickpea flour as a component of infant foods to be developed. The saving of fuel by shortening of the cooking time, is an important factor for developing countries because of its scarcity.

RESUMEN

ESTUDIOS SOBRE EL DESARROLLO DE ALIMENTOS INFANTILES A BASE DE FUENTES DE PROTEINA VEGETAL. PARTE II. EFECTO DE LAS CONDICIONES DE PROCESAMIENTO EN LAS PROPIEDADES QUIMICAS Y NUTRICIONALES DEL GARBANZO

Se sometieron a estudio diferentes condiciones de procesamiento con el propósito de mejorar el gusto, sabor y calidad nutricional del garbanzo. Las muestras de garbanzo decorticado se procesaron bajo diferentes condiciones, tales como ausencia de remojo o remojo en agua y solución de carbonato de sodio. Se aislaron también las proteínas del garbanzo remojado en agua y solución de carbonato de sodio, y se sometieron a diferentes procesamientos. Se encontró que las muestras remojadas en carbonato de sodio acusaban valores más bajos de proteína y cenizas. No se observó cambio alguno en los demás constituyentes. Se analizó subjetivamente la intensidad de las características de sabor del garbanzo procesado, análisis que indicó que el remojo se traducía en un mejor sabor. Las muestras tratadas con carbonato exhibieron un color más pálido.

El procedimiento de remojo en carbonato no tuvo ningún efecto adverso en la disponibilidad de lisina ni en el índice de solubilidad de nitrógeno, en comparación con el procedimiento de remojo en agua pura. El tiempo requerido para inactivar los inhibidores de tripsina en garbanzos remojados en carbonato a la temperatura de ebullición, fue la midad del requerido por las muestras remojadas en agua pura. Bajo

las condiciones usadas en el tratamiento de garbanzos con carbonato de sodio, no se observó ningún efecto benéfico en cuanto a reducir los niveles de taninos. Tampoco se constataron diferencias significativas en la razón proteínica neta (NPR) entre las muestras procesadas con los diferentes tratamientos, aunque en algunos casos los aislados de proteína arrojaron valores de NPR significativamente menores. Los valores de digestibilidad fueron de mayor magnitud para los aislados proteínicos que para las muestras de garbanzo.

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