Effect of Autoclaving on the Basic Amino Acids and Proteins of the Chick Pea

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The work reported grew out of an interest in the effects of heat on the nutritive quality of plant proteins. Lysine is the limiting amino acid in most rations in which cereals are the source of energy. Because the chick pea, a typically starchy seed, is rich in lysine and is an important food crop, interest developed in the effects of heat on the nutritive quality of its proteins in comparison with typical oilseeds. About 25% of the chick pea is protein, rich in lysine (6.5 to 6.7%). The lysine content is reduced about 10% when the seeds are heated in the autoclave to 121° C. for 30 and 60 minutes. The reduction is greater the less the moisture content of the seed.

USUALLY the nutritional quality of oilseed meal proteins is improved when heating of the seed during processing for oil is moderate (*9*), but amino acids, especially lysine, cystine, and arginine, are destroyed when heating is severe. Renner *et al.* (*14*) reported the loss of 15% of the lysine and 9% of the arginine in sunflower seed during the processing for oil. Other workers (*5–7*) reported substantial losses in lysine, cystine, histidine, tryptophan, and arginine on heating soybean meal. A loss of cystine and arginine, and a serious loss of lysine, may occur during the processing of cottonseed (*3, 11*). Lysine is lost in the processing of peanuts for oil (*2*), and loss is severe when peas are autoclaved (*8*).

A study has been made to compare the effects of heat on chick peas (*Cicer arietinum*) with previously noted effects on typical oleaginous seeds. In contrast with the latter seeds, the chick peas are characterized by a low oil content (usually 4 to 7%), a relatively high proportion of starch (in the neighborhood of 45%), and protein at a 20 to 30% level, depending on the variety and prevailing ecological conditions (*4*). Changes induced by heat in the chemical properties and in the basic amino acids of the proteins of two varieties of chick peas are reported in this communication.

Experimental

Two varieties were used: a large white seeded variety (Garbanza) used for human food in southwestern areas of the United States, Mexico, India, Pakistan, and several European countries; and forage chick pea (garbanzo porquero), a small seeded variety used in the central valleys of Mexico (Bajio area) mostly for feeding swine.

Flour was prepared from each type by cracking the undecorticated seed in a Bauer mill and grinding the seed fragments in a ball mill to pass through a 40-mesh sieve.

A crude protein preparation was obtained from the flour by aqueous alkaline extraction (pH 8 to 8.5). Twenty grams of the flour from each variety were dispersed in 100 ml. of 0.025*N* aqueous sodium hydroxide, and the resulting suspension was agitated mechanically for 10 minutes. The suspension was centrifuged, the supernatant liquid decanted, and the residue suspended in 80 ml. of 0.0125*N* aqueous sodium hydroxide. The residue obtained on centrifuging was further extracted with 80 ml. of water. The combined extracts were dried by lyophilization. The crude protein thus obtained was extracted with diethyl ether at room temperature to reduce its oil content.

Purified proteins were also prepared from the aqueous alkaline extract of each variety, by precipitating them at their isoelectric point. These proteins were dried by lyophilization and the oil was removed from the dried protein by extraction with 95% ethyl alcohol. The purified protein from the large variety was subjected to four successive washings with water prior to drying, in order to obtain as pure a protein as possible. The purified protein from the small chick pea variety was not washed.

Samples of the flour and protein preparations were adjusted to 10 and 50% moisture and heated in a steam-jacketed autoclave (contact between sample and steam avoided) for 1 hour at 121° C. The higher moisture was selected for observation because a precooked chick pea food is produced by autoclaving soaked chick peas. The samples with a moisture content of 50% were dried by lyophilization; the samples autoclaved with 10% moisture were stored over anhydrous calcium sulfate.

The moisture, nitrogen, starch, total sugar, ash, crude fiber, and oil contents were determined by the methods of the Association of Official Agricultural Chemists (*1*). Nitrogen solubility was determined by the method of Lyman, Chang, and Couch (*10*).

In the determination of the basic amino acids, the sample of flour or protein fraction was hydrolyzed under refluxing conditions for 24 hours with twice-distilled constant boiling aqueous hydrochloric acid. Two milliliters of acid were used for each milligram of protein. The hydrolyzate was taken to dryness under reduced pressure, and the last traces of hydrochloric acid were removed by repeated evaporation to dryness under reduced pressure after addition of small quantities of water. The residue was then taken up in water,

Table I. Per Cent Composition of Chick Pea Products[a]

	Large Seeded Variety			Forage Variety		
Constituent	Flour	Crude protein	Purified protein	Flour	Crude protein	Purified protein
Protein (N × 6.25)	26.5	60.1	93.5	21.3	54.3	88.0
Total sugar	4.2	12.2	...	3.2	9.5	...
Glucose equiv. of reducing materials produced on mild acid hydrolysis	46.6[b]	9.3	3.4	45.7[c]	12.8	6.4
Oil	6.4	0.0	0.1	5.3	1.8	0.1
Ash	3.1	7.4	1.2	3.3	7.7	2.8
Crude fiber	2.6	0.0	0.0	7.7	0.0	0.0

[a] Moisture-free basis. [b] Starch, 41.9%. [c] Starch, 41.1%.

Table II. Basic Amino Acids in Chick Pea Products

(Grams of amino acid per 16 grams of nitrogen)

Basic Amino Acid	Flour			Crude Protein			Purified Protein		
		Autoclaved 1 Hour at 121° C.			Autoclaved 1 Hour at 121° C.			Autoclaved 1 Hour at 121° C.	
	Control	50% moisture	10% moisture	Control	50% moisture	10% moisture	Control	50% moisture	10% moisture
					Large Seeded Variety				
Lysine	6.5	6.2	5.8	6.5	6.2	5.7	6.5	6.3	6.1
Histidine	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.9	2.9
Arginine	11.4	11.2	11.3	11.0	10.9	11.0	10.0	10.0	9.9
					Forage Variety				
Lysine	6.7	6.3	5.8	6.6	6.3	5.7	6.6	6.5	6.1
Histidine	2.9	2.9	2.9	3.0	2.9	3.0	2.9	3.0	3.0
Arginine	9.5	9.5	9.6	9.6	9.6	9.4	9.6	9.5	9.5

Table III. Effect of Autoclaving on Protein Solubility

	Nitrogen Solubility in 0.02N NaOH, %		
	Control	50% H_2O	10% H_2O
	Large Seeded Variety		
Flour	98	61	55
Crude protein	99	76	64
Purified protein	99	75	59
	Forage Variety		
Flour	98	39	39
Crude protein	95	54	39
Purified protein	98	55	38

filtered through a medium-porosity sintered-glass filter, and brought to a known volume (25 to 50 ml.) at pH 6. An aliquot of this solution was shaken with an equal volume of a 50% suspension of Dowex 2 resin for 45 minutes. The suspension was then filtered and washed, first with water and then with 0.04*M* acetate buffer at pH 3.7. The combined washings and filtrate were evaporated to dryness under reduced pressure, and the residue was taken up in a known volume of citrate buffer (25 ml.) at pH 4. An aliquot of this solution was placed on a 3 × 15 cm. Amberlite IR 120 column, and the basic amino acids were eluted, according to the procedure of Moore, Spackman, and Stein (*12*). The basic amino acids, which are resolved on the column, were determined by the photometric ninhydrin method of Moore and Stein (*13*).

Results and Discussion

The chick pea is used for food and feed by a large segment of the world population. Because it is a potential source of a high quality protein concentrate, compositional data recorded in Table I are of interest. The protein, oil, and total sugar contents of the flour from the large seeded variety are approximately 20% higher than those of the forage chick pea. The starch content of both varieties is approximately 42% of the weight of the seed. The crude fiber content of the forage variety, however, is about three times that of the large seeded variety.

The nitrogen content of the crude protein fraction from the large seeded variety was about 10% and the total sugar content about 20% greater than those of the forage variety. The total reducing materials produced on mild acid hydrolysis as determined by the Somogyi (*15*) method and calculated as glucose were 25% greater in the crude protein of the forage variety than of the large seeded variety. Comparable reducing materials in the purified protein fraction of the forage variety were about 50% higher than those of the large seeded variety.

The crude protein product of the large seeded variety represented 42% of the seed, and contained 94% of the total nitrogen, while the crude protein of the forage variety represented only 36% of the seed, and contained 93% of the total nitrogen.

The purified protein product of the large seeded variety represented 22% of the seed and contained 75% of the total nitrogen. The purified protein of the forage variety represented around 20% of the flour and contained 80% of the total nitrogen. The difference in carbohydrate content was due to the fact that the precipitated protein from the forage variety was not washed with water prior to lyophilization.

Data for the basic amino acids in the flour and in the crude and purified proteins of both varieties are listed in Table II. The lysine and histidine levels were about the same in both varieties; the arginine level was lower in the flour from the forage variety. The arginine contents of the forage chick pea flour and proteins remained constant; however, the crude and purified proteins of the large chick pea were, respectively, 3.5 and 12.3% lower in arginine content than the flour.

The destruction of lysine on autoclaving the products of the chick pea for 1 hour at 121° C. was not greater than 14% in the chick pea flours and crude proteins. This measured destruction was about 7.5% in the purified proteins, when the samples contained 10% moisture. The highest drop in the lysine value for the moist samples heated for 1 hour was 6%. Conkerton *et al.* (*3*) reported a reduction of 37% in the lysine content on autoclaving cottonseed meal for 2 hours. The reduction in the lysine level which occurred with peanuts cooked for 2 hours at 121° C. was 15% (*2*). Destruction of lysine by heat appeared more extensive in cottonseed than in peanuts or chick peas.

No measurable reduction was noticed for the arginine on autoclaving the chick peas. Destruction of this amino acid was reported after autoclaving cottonseed meal for 2 hours (*3*).

The data recorded in Table III show that the fraction of total nitrogen soluble in 0.02*N* aqueous sodium hydroxide decreases on autoclaving the flour, crude protein, and purified protein from both varieties of chick pea, but that the reduction is greater for the drier materials and is greater for the forage chick pea fractions than for those from the large seeded variety.

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