

## Note on the Estimation of Choline in Plant Protein Sources

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The method commonly used for the estimation of choline in plant materials, AACC Method 86-45, involves Soxhlet extraction of a sample with methanol for 24 hr, hydrolysis of choline compounds by boiling in saturated solution of barium hydroxide, precipitation of choline as the reineckate salt at about pH 10, solubilization of this precipitate in acetone, and measurement of absorbance at 526 nm (Glick 1944).

As early as 1946, Street et al pointed out that under these conditions choline reineckate was unstable and tended to decompose during filtration. Ackerman and Salmon (1960) showed that hydrolysis of a mixed plant and animal tissue sample with barium hydroxide resulted in inconsistent and low values for choline as compared to direct hydrolysis with 25% nitric acid, followed by choline reineckate precipitation from 0.1-0.2*N* sodium hydroxide.

Some researchers have shown that the sensitivity of the method could be increased by measuring absorbance of choline reineckate in acetone solution at 327 nm, where the absorption maxima was  $5.8 \times 10^3$  (Dittmer and Wells 1969, Winzler and Meserve 1945). Ackerman and Chan (1960) found that choline reineckate in 50% ammonium hydroxide had a much higher molar absorptivity index ( $17.2 \times 10^3$ ) at 303 nm. Argoudelis and Tobias (1975) examined the ultraviolet spectrum of choline reineckate in a number of solvents and concluded that acetonitrile was the best solvent. In this, solubility of choline reineckate at 20°C was 1.63% and the molar extinction coefficient at 312 nm was  $24.9 \times 10^3$ .

In the present study a number of vegetable protein sources were examined for total choline content by adapting the method of Ackerman and Chan (1960). Amounts of water-soluble choline and the bitterness scores of some plant proteins were also examined in order to determine the contribution of water-soluble choline to bitter taste.

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## MATERIALS AND METHODS

### Total Choline

A 1-g sample of food material was digested by simmering in 70 ml of 15% nitric acid for 3 hr. The volume was adjusted to 70 ml and the digest filtered through a sintered glass funnel of medium porosity under suction. A known volume of filtrate was adjusted to pH 13 by adding sodium hydroxide pellets and then 10N NaOH. Volume was noted, and 5-ml samples in duplicates were taken in 15-ml centrifuge tubes. To each tube, 2 ml of freshly prepared 5% ammonium reineckate solution in methanol was added, and tubes were chilled overnight at 5°C. The choline reineckate precipitate was collected by centrifugation, washed three times with 1.5 ml of cold 1-propanol, dried in a desiccator containing sulfuric acid for 2 hr, and dissolved in 2 ml of acetonitrile. The solution was centrifuged to remove the undissolved material, and choline reineckate was quantified by measuring absorption either at 312 nm after further dilution with acetonitrile or at 303 nm after 20-fold dilution with 50% ammonium hydroxide. Sigma grade choline chloride was recrystallized four times from hot anhydrous ethanol, dried over sulfuric acid, and used as standard.

### Water-Soluble Choline

One gram of ground foodstuff was extracted with 25 ml of water with a Polytron (Type PT 10203500, Kinematical, GmbH., Lucerne, Switzerland) for 4–5 min and the residue removed by centrifugation (13,000 × g for 10 min). Choline was precipitated from 5 ml of supernatant as choline reineckate at pH 13 and quantitated.

### Purity of Choline Reineckate

*Ion Exchange Chromatography of Hydrolysis Product.* Choline reineckate prepared from water extract of dehulled rapeseed was hydrolyzed in 0.25N NaOH at 50°C under a tungsten lamp light for 6 hr. The sediment was removed by centrifugation and the supernatant placed on a 15 × 0.92-cm (diameter) column packed with Dowex 50W-X8 (100–200 mesh). The column was eluted with a gradient of water and 2N HCl (100 ml each). Eluate fractions (6.5 ml each) were examined for choline by complexing with hexanitrodiphenylamine (Schill and Danielsson 1959).

*High Pressure Liquid Chromatograph (HPLC).* Choline reineckate preparations from a number of foodstuffs were examined by elution from a reverse phase column (Bond Pack RP 18) with mixtures of acetonitrile and water at 3,000 psi and flow rate of 1 ml/min, using HPLC model M-6000A of Waters Associate Inc., Milford, MA.

### Bitterness Scores

Bitterness scores for water slurries (2% solids, w/v) were assigned by six trained judges in accordance with the procedures described by Malcolmson et al (1978), using four replications.

## RESULTS AND DISCUSSION

Precipitation of choline reineckate at pH 10 and 13 gave very similar recoveries of standard choline chloride. Calculations based on a molar extinction coefficient of  $24.9 \times 10^3$  for choline

TABLE I

Effect of Method of Estimation on Choline Content of Plant Proteins

Sample	Estimation Method, mg/g ± SEM <sup>a</sup>	
	AACC	Nitric Acid Digestion
Rye flour <sup>b</sup>	0.19 ± 0.07	0.70 ± 0.10
Wheat flour <sup>b</sup>	0.81 ± 0.15	1.34 ± 0.00
Fababean protein concentrate <sup>c</sup>	2.71 ± 0.30	4.65 ± 0.07
Rapeseed flour <sup>d</sup>	7.03 ± 0.20	10.13 ± 0.01

<sup>a</sup> Values are averages of 2–4 determinations in duplicates.

<sup>b</sup> Roller-milled with bench equipment.

<sup>c</sup> Dehulled, pinmilled, and air-classified.

<sup>d</sup> Dehulled and solvent-extracted.

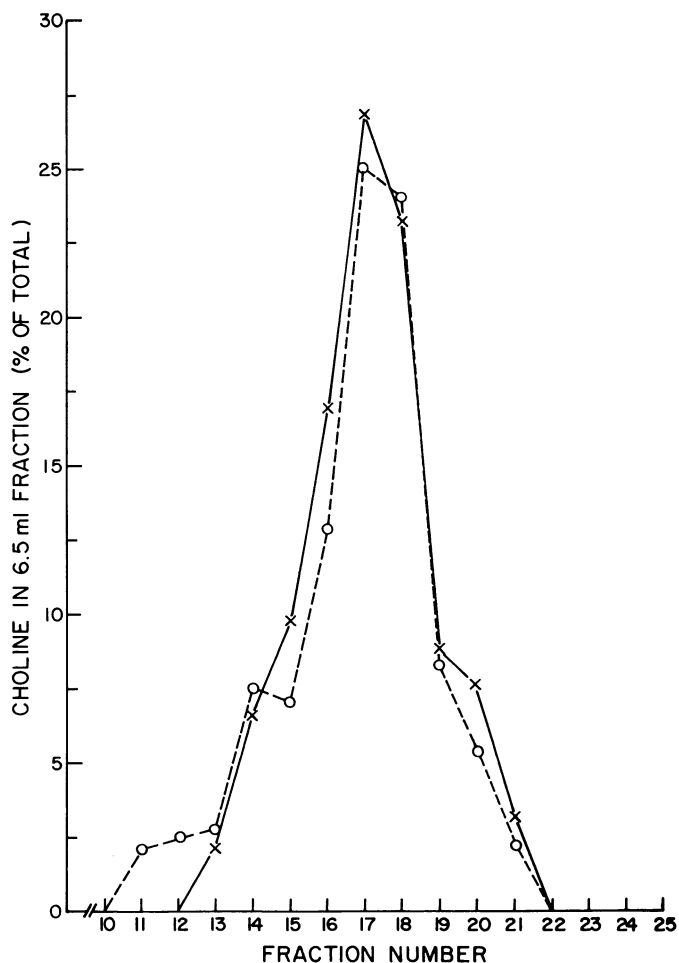


Fig. 1. Elution of standard choline chloride (—○—) and hydrolyzed choline reineckate (—×—) from a Dowex 50W-X8 column with a gradient of water and 2N HCl (100 ml: 100 ml).

reineckate (Argoudelis and Tobias 1975) showed that percent recoveries (± SEM) from 6 μmol of standard choline chloride at pH 10 and 13 were  $93.6 \pm 0.36$  and  $94.5 \pm 0.17$ , respectively.

The procedure we used resulted in higher recovery and much more consistent values for total choline in plant protein sources than did AACC Method 86-45 (Table I). For example, in rapeseed flour, choline estimated by the AACC method was 31% lower than that by the nitric acid hydrolysis method. About half of this decrease resulted from extraction and hydrolysis steps; the rest was due to the procedure for the precipitation of choline reineckate and its purification. Similarly, Ackerman and Salmon (1960) showed that hydrolysis of a sample (mixture of animal and plant tissues) with barium hydroxide resulted in inconsistent and low values for choline as compared to hydrolysis with 25% nitric acid.

Ion exchange chromatography of hydrolysis products from choline reineckate prepared from a water extract of dehulled rapeseed showed an elution pattern similar to that of standard choline chloride (Fig. 1). Total recovery of choline in fractions 13–21 (85–137 ml) was 106% of the choline in the choline reineckate complex. Also HPLC resolution of choline reineckate prepared from various foodstuffs showed that these preparations were similar to that prepared from standard choline chloride. Elution with acetonitrile/water (35:65) at pH 5.56 showed a retention time of 2.16 min for all preparations (Fig. 2).

Total choline, water extractable choline, and bitterness scores estimated for a number of foodstuffs are shown in Table II. Values for total choline in wheat preparations were at least three to four times higher than those reported by Wilson and Lorenz (1979). Oilseeds and legumes were much higher in choline content than were cereals. In rapeseed, lupine, fababeans, and soybeans, 70–80% of the total choline was extractable with water. This was reflected in a much lower choline content for protein concentrates and isolates

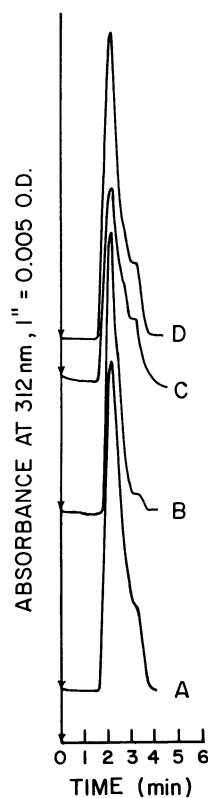


Fig. 2. Elution of choline reineckate from a reverse phase high pressure liquid chromatographic column. Reineckate complexes prepared from: A, standard choline chloride; B, wheat flour; C, fababean protein concentrate; and D, rapeseed flour.

prepared by water extraction of some of these materials.

Choline is known to be bitter in taste (Sessa et al 1974), and high levels of water-soluble choline in oilseed and legume preparations may be partially responsible for their bitter taste. Degree of bitterness was much less in water-extracted protein isolates of fababean and soybean than in the original materials. However, no direct relationship between choline content and degree of bitterness was apparent, indicating that some other compounds were also contributing to the bitter taste.

The method reported here for the estimation of choline in foodstuffs is very sensitive, specific, and much less time-consuming than the AACC method.

#### ACKNOWLEDGMENTS

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TABLE II  
Total Choline, Water-Soluble Choline, and Bitterness Scores of Vegetable Protein Sources

Sample	Choline, mg/g $\pm$ SEM <sup>a</sup>		Bitterness Score <sup>b</sup>
	Total	Water-Soluble	
Durum flour <sup>c</sup>	1.24 $\pm$ 0.11	0.03 $\pm$ 0.03	1.6 e
Wheat flour <sup>c</sup>	1.34 $\pm$ 0.00	0.15 $\pm$ 0.02	1.9 e
Triticale flour <sup>c</sup>	1.31 $\pm$ 0.01	0.20 $\pm$ 0.05	1.6 e
Rye flour <sup>c</sup>	0.70 $\pm$ 0.10	0.09 $\pm$ 0.03	1.8 e
Oat flour <sup>d</sup>	1.44 $\pm$ 0.18	0.08 $\pm$ 0.00	1.8 e
Fababean flour <sup>e</sup>	1.93 $\pm$ 0.15	1.24 $\pm$ 0.18	4.8 abc
Protein concentrate <sup>f</sup>	4.65 $\pm$ 0.07	3.03 $\pm$ 0.10	4.1 bcd
Protein isolate <sup>d</sup>	0.51 $\pm$ 0.07	0.38 $\pm$ 0.07	1.4 e
Pea flour <sup>e</sup>	0.88 $\pm$ 0.05	0.25 $\pm$ 0.01	5.7 ab
Protein concentrate <sup>f</sup>	2.63 $\pm$ 0.06	0.86 $\pm$ 0.05	6.6 a
Lupine flour <sup>e</sup>	2.41 $\pm$ 0.07	1.68 $\pm$ 0.18	6.4 a
Sunflower protein concentrate <sup>g</sup>	0.64 $\pm$ 0.17	0.14 $\pm$ 0.01	3.2 cde
Soybean flour <sup>h</sup>	2.86 $\pm$ 0.07	2.05 $\pm$ 0.06	3.2 cde
Isolate <sup>d</sup>	0.58 $\pm$ 0.07	0.05 $\pm$ 0.003	2.0 de
Mustard concentrate <sup>i</sup>	4.06 $\pm$ 0.08	1.38 $\pm$ 0.06	...
Rapeseed, dehulled	4.67 $\pm$ 0.11	3.49 $\pm$ 0.10	...
Flour <sup>j</sup>	10.13 $\pm$ 0.01	8.06 $\pm$ 0.10	...
Concentrate <sup>i</sup>	3.76 $\pm$ 0.02	0.52 $\pm$ 0.07	4.38 abc

<sup>a</sup> Values are averages of 2-4 determinations in duplicates.

<sup>b</sup> Mean values for 6  $\times$  4 tests. Higher scores indicate more bitterness. Values with the same letters are not significantly ( $P < 0.05$ ) different by Duncan's multiple range test.

<sup>c</sup> Roller-milled with bench equipment.

<sup>d</sup> Experimental commercial sample.

<sup>e</sup> Dehulled and pinmilled.

<sup>f</sup> Dehulled, pinmilled, and air-classified.

<sup>g</sup> Diffusion-extracted for 4 hr at pH 4.5 and solvent-extracted.

<sup>h</sup> Flaked, solvent-extracted, and ground (untoasted).

<sup>i</sup> Dehulled, heated, washed with water, and solvent-extracted.

<sup>j</sup> Dehulled and solvent-extracted.

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