Ambient Temperature Acid Extraction Method for the Determination of Oxalic Acid Contents of Vegetables

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An ambient temperature extraction–precipitation method is described for the determination of oxalic acid in vegetables. The current method is compared with the original hot acid extraction method using glucose and five different types of vegetables. The recovery of pure oxalic acid from spiked vegetables by the current method is determined and is found to be 98.5% ± 1.3%.

INTRODUCTION

Recently, Libert and Franceschi (1987) have comprehensively reviewed the problem of oxalate in crop plants and have referred to methods of analysis. In recent years, gas chromatography (Charransol et al., 1978; Hesse et al., 1980), high-performance liquid chromatography (Libert, 1981; Wilson et al., 1982; Grun and Loewus, 1983), and isotachophoresis (Schwendtner et al., 1982) have been employed for oxalate determination, and enzyme-based methods (Beutler et al., 1980; Kohlbecker and Butz, 1981) have been described. The AOAC has published an official method for oxalate determination. The above authors have concluded that chromatographic and enzymic methods are more rapid and more precise than the original classical precipitation method that had been in use (Kohman, 1939; Andrews and Viser, 1951).

However, the classical method remains in the AOAC *Official Methods of Analysis* (AOAC, 1984), and this happens to be the most readily available technique for oxalate analysis in foods in most laboratories of developing countries.

The original method employed hot acid extraction in one stage of the analysis. However, Zarembski and Hodgkinson (1962) demonstrated the conversion of carbohydrate to oxalic acid by the hot acid extraction procedure. This demonstration raised the fear that erroneously high results might be obtained for foods analyzed by the original method. Clearly, the AOAC (1984) is unconcerned by or perhaps unaware of this possibility.

Given this background, I have developed a quantitative ambient temperature acid extraction–precipitation procedure for the determination of oxalic acid in vegetables. The current method is more appropriate and can be used for the analysis of oxalic acid in other food samples. This analytical aspect is interesting as oxalate in foods is certainly an underestimated antinutrient/toxicant (Kohman, 1939; Hoover and Karunairatnam, 1945; Hodgkinson, 1977; Swartzman et al., 1978; Kelsay, 1981, 1985; Gilloly et al., 1983; Wittwer et al., 1947; Sing et al., 1972; Hodgkinson, 1978; Chadwick et al., 1973).

MATERIALS AND METHODS

Materials. Five different types of vegetables and glucose were bought in the open markets in western Nigeria. The vegetables are from the same locality and grown under the same agronomic conditions. The vegetables were therefore kept in polythene bags prior to analysis. The period between purchase and further processing for analysis was no more than 24 h.

Methods. The vegetables were rinsed with distilled water, drained, and air-dried before weighing. Weighed fresh samples were oven-dried at about 60 °C to constant weight and then ground to fine powder with a clean pestle and mortar. Extraction of oxalic acid and subsequent determination in sample was carried out by the following procedure: 2 g of ground sample was transferred into a 25-mL glass-stoppered graduated test tube, and 20 mL of 30% HCl at ambient temperature (about 25 °C) was added and mixed by gently inverting the tube at regular intervals for 5 min. Then about 4 g of $(NH_4)_2SO_4$ was added to make the solution half-saturated. The tube was set aside for 30 min.

The supernatant (acid extract) was filtered by using a Büchner flask and funnel under pressure. The filtrate was poured into a 25-mL volumetric flask and made to the 25-mL mark with 30% HCl at ambient temperature. Aliquots (10 mL) of this extract were then pipetted into the inner tube of the extraction apparatus (Figure 1). The extraction apparatus is a modified one different from the conventional Soxhlet extractor in the mode of construction and in its operational procedure, which is liquid–liquid extraction (that is, the acid extract is extracted with diethyl ether), unlike the Soxhlet which employs solid–liquid

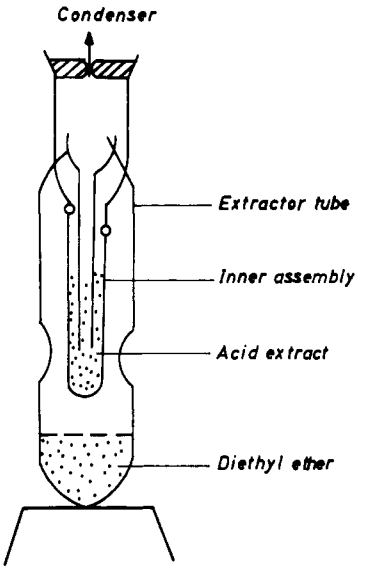

Figure 1. Extraction apparatus.

Table I. Recovery of Oxalic Acid Added to *B. oleracea* L. (Cabbage) as Control[a]

sample	amount of oxalic acid found in control after addition of 10 mg of pure oxalic acid/g of sample, mg g^{-1}	% recovery[b]
1	9.74	97.4
2	9.96	99.6
3	9.86	98.6

[a] Amount of oxalic acid found in control was negligible. [b] Mean = 98.5% ± 1.3.

0021-8561/91/1439-0293$02.50/0

Table II. Oxalic Acid Contents of Samples: Comparison of Ambient Temperature HCl Extraction Procedure Results with Hot HCl Extraction Procedure Results (Milligrams per Gram of Dry Weight)[a,b]

sample	common name	method I: ambient temperature HCl extraction procedure results			method II: hot HCl extraction procedure results		
		1	2	av[c]	1	2	av[c]
glucose		0.3	0.3	0.3	8.3	7.3	8.1
Talinum triangulare	water leaf	25.3	38.4	26.8	38.7	37.8	38.2
Celosia argentea (green varian)		16.3	22.0	19.2	23.4	25.2	24.2
Solanum macrocaroon	garden egg	12.7	16.7	14.7	15.7	18.7	17.2
Chlorchorus otitorus	Jew's mallow	13.0	10.0	11.6	12.4	11.9	12.1
Abelmoscus esculentus	okro	1.0	1.5	1.2	2.9	3.3	3.1

[a] Calculated Student t values for the analysis of vegetables by the two methods is 2.21. [b] Critical value for four degrees of freedom at 90% confidence level is 2.13. [c] Average of two replicate analyses.

extraction. The modified apparatus is cheaper and simpler in form and is easily blown in any glass-blowing workshop. Also, its simplicity not withstanding, it has excellent efficiency as demonstrated in the recovery work.

The acid solution was then extracted with 20–30 mL of diethyl ether for 5 h. The diethyl ether was transferred quantitatively into a 250-mL conical flask, and the outer extractor tube was rinsed with distilled water. Diethyl ether in the extract was evaporated off, and the remaining aqueous solution was filtered through a filter paper and funnel into a 50-mL centrifuge tube. The pH of the solution was adjusted to 7.0 with 0.75 M NH_4OH and CH_3COOH, and then saturated $CaCl_2$ was added in an amount that was double the equivalent expected oxalate contents of the vegetables. The solution was maintained at 60 °C overnight in an oven. After centrifugation, the supernatant liquid was decanted and the precipitate dissolved in 1 M H_2SO_4 at ambient temperature. The resulting solution was transferred quantitatively into a 250-mL conical flask and titrated hot (about 80 °C) against standardized 0.008 $KMnO_4$. The amount of oxalic acid was calculated in milligrams per gram of sample, dry weight.

Recovery experiments were carried out to determine the efficiency of the proposed procedure. Tem milligrams of pure oxalic acid was added per gram (dry weight) of *Brassica oleracea*, whose amount of oxalic acid had previously been determined by the above procedure. The oxalic acid recovered was again determined by the same procedure.

Glucose. Two grams (dry weight) of glucose was taken and analyzed by the ambient temperature acid extraction procedure described above.

Determination of Oxalic Acid in Vegetables and in Glucose by Hot Acid Extraction Procedure. This aspect of the work is necessary to find out how the oxalate figures compare by both methods on the same sample.

Two grams (dry weight) of sample was transferred into a 25-mL graduated test tube; 20 mL of hot (about 80 °C) 30% HCl was added and mixed by gently inverting the tube at regular intervals for 5 min. Then about 4 g of $(NH_4)_2SO_4$ was added to make the solution half-saturated, and the tube was set aside for 30 min. This was the hot extraction stage of the analysis. The supernatant (acid extract) was filtered into a 25-mL volumetric flask and was made to the mark with 30% HCl at ambient temperature. Aliquots (10 mL) of this extract were pipetted into the inner tube of the extraction apparatus. Subsequent steps after in the oxalic acid estimation were the same as described previously for the ambient temperature extraction procedure.

RESULTS AND DISCUSSION

The recovery of oxalic acid from three replicate addition experiments employing the ambient temperature acid extraction procedure and using standard oxalic acid and *B. oleracea* (cabbage) as control was 98.5 ± 1.3% (Table I). Hence, the method has shown good efficiency. The reproducibility of both methods was checked by carrying out analyses in duplicate samples.

The experiments on glucose showed that a substantial amount of oxalic acid was formed from glucose by the original hot acid extraction procedure. The amount of oxalic acid formed from glucose by the current method was negligible (Table II). This observation showed that the fear earlier raised of the possibility of the conversion of carbohydrate in food to oxalic acid by the original method is legitimate and that the procedure might lead to erroneously high results.

Comparison of the oxalate figures determined by both methods on the same sample shows that the original method values are greater than those by the current method. The Student t-test showed that there is significant difference between the two methods (Table II). There is circumstantial evidence, therefore, in support of the hypothesis that hot acid is inappropriate in the classical precipitation technique and that it may lead to erroneously high results.

The ambient temperature acid extraction procedure has been found appropriate, and therefore it is the one recommended.

CONCLUSION

An ambient temperature HCl extraction method is developed for the determination of oxalic acid in vegetables by a classical precipitation technique. The current method, when compared with the original hot acid extraction method, is found to be more appropriate as the latter method leads to the conversion of carbohydrate (glucose) to oxalic acid. Hence, the current method is the one recommended in precipitation technique for oxalate analysis in foods.

ACKNOWLEDGMENT

I acknowledge the assistance received from Miss Lola Solarin and Miss Desola Bello during the collection and analyses of samples.

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Received for review May 15, 1990. Accepted September 10, 1990.

Registry No. Oxalic acid, 144-62-7; glucose, 50-99-7