COMMUNICATIONS

A Rapid Method for the Extraction and Quantitation of Total Anthocyanin of Cranberry Fruit

A time-consuming method for the extraction and quantitation of total anthocyanin of cranberry fruit was modified to produce the same results in 5 to 6 min. The rapid method can be applied to fresh and frozen fruit and requires only 100 g of fruit and 154 mL of extracting solvent. The principle of the method may be applicable to other small fruit.

A method for extraction and determination of total anthocyanin (TAcy) of cranberry fruit was developed by Fuleki and Francis (1968) and later modified to be less time consuming (Starr, 1978). In an attempt to reduce the time required for one determination without loss of accuracy, we succeeded in modifying the original method to determine TAcy in fresh fruit within 5 to 6 min. The principle of the modification may be applicable to other small fruit.

EXPERIMENTAL SECTION

Reagents. Extracting solvent: EtOH 95%–1.5 M HCl (85:15).

Fruit. Since we were interested in the analysis of fresh fruit (1–2 days after harvest), and the study extended over several weeks, fruit from different batches had to be used in the evaluation of the different techniques. Fruit from one batch was used for one set of determinations. Pigmentation of early harvested fruit is incomplete, and weaknesses of the method were easier to recognize.

Procedure. One hundred grams of fresh fruit were pulped in a Waring blender with the addition of 100 mL of extracting solvent. Blending time was 3 min at 16 100 rpm (free running). Of the resultant slurry, 10 mL was transferred to a 11-cm wet No. 44 Whatman filter disc in a Buchner funnel. The filter was wetted with a small amount out of a total of 50 mL of extracting solvent. The remaining solvent was poured over the slurry while suction was applied. The extract was collected in a 250-mL suction flask. An aliquot of the extract was diluted 1:8 with extracting solvent. Measuring of absorbance and quantitation followed the method of Fuleki and Francis (1968), taking the new dilution ratios into consideration.

RESULTS AND DISCUSSION

It was the main purpose of the blending step to obtain small but representative subsamples for TAcy determination. In preliminary tests, these subsamples were transferred to 160-mL flasks of a VirTis 45 homogenizer and homogenized in extracting solvent at high speed for 2 min. Since at 45 000 rpm, 34.9 (32.2–37.3, n = 10) mg of TAcy/100 g of fruit was determined, and at 16 000 rpm 34.8 (33.1–38.3, n = 10) mg of TAcy/100 g of fruit, the lower speed was considered acceptable for blending.

Although the berries are small, we had doubts about the adequacy of 100 g of fruit for a determination because the distribution of pigments in a sample varies. To determine the effect of sample size on the precision of the method, six replicate samples of 100, 200, and 300 g of fruit were analyzed. The 100-g samples yielded 15.9 mg of TAcy/100 g of fruit (s = 1.44), the 200-g samples yielded 16.9 mg of TAcy/100 g of fruit (s = 1.31), and the 300-g samples yielded 16.6 mg of TAcy/100 g of fruit (s = 0.60). As expected, standard deviations decreased with increasing sample weight.

In juice production, berries are frozen to increase yield and improve the taste of juice. In this method, freezing did not increase pigment yields. Fresh fruit gave 17.2 mg of TAcy/100 g of fruit (s = 1.24, n = 10), and fruit frozen for 5 days at −20 °C gave 15.9 mg of TAcy/100 g of fruit (s = 0.94, n = 10). There was no statistically significant difference between the means.

Blending time was expected to affect TAcy determinations. We found 15.6 mg of TAcy/100 g of fruit (s = 0.95, n = 6) after 2 min and 18.2 mg of TAcy/100 g of fruit (s = 1.32, n = 6) after 3 min blending time. Although the difference between 15.6 mg and 18.2 mg was statistically not significant, it was decided to take 3 min blending time as acceptable. The decision was also based on the finding that increased blending time did not increase pigment yields (4 min, 19.1 mg/100 g, s = 1.12, n = 6; 5 min, 18.2 mg/100 g, s = 1.24, n = 6).

To facilitate the transfer of slurry from the blender jar to the filter, a 10-mL measuring pipet with the tip removed at the 10-mL mark was used. The average weight of 10 mL slurry was found to be 8.92 g (s = 0.31, n = 30). In converting volume to weight, we did not consider the specific weight of fresh fruit. The average TAcy content of 20 subsamples taken from a slurry with this pipet was 17.3 mg/100 g of fruit (s = 0.67).

Wetting of the filter paper with several milliliters out of 50 mL of extracting solvent prior to filtration assured faster and more uniform distribution of slurry on the filter during the first seconds of filtration. Filtration was discontinued after about 90% of the filtrate was obtained. The use of a slow paper (Whatman No. 44) became necessary to avoid turbidity observed when Whatman No. 1 was used.

Filtration was introduced instead of centrifugation recommended in the original method. A comparison showed that both techniques produced the same results. With filtration, 19.7 mg of TAcy/100 g of fruit (s = 0.75, n = 10) were found, whereas with centrifugation (3000 rpm, 3 min) 19.4 mg of TAcy/100 g of fruit (s = 1.24, n = 8) were obtained.

With the use of automatic dispensers to measure constant volume of extracting solvent, these modifications allow the analysis of one sample in 5 to 6 min. Results of this method are comparable with those of the original method. Whereas 16.8 mg of TAcy/100 g of fruit (s = 1.37, n = 5) were determined with the original method, 17.8 mg of TAcy/100 g of fruit (s = 2.00, n = 10) were determined with the modified method. There was no statistically significant difference between the two means.

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Thin-Layer Chromatographic System for Identification and Quantitation of Potato Tuber Glycoalkaloids

A system involving thin-layer chromatography and densitometry is described for quantitating the individual glycoalkaloids of potato tubers. The procedure is simple and inexpensive and could easily be used to assay individual glycoalkaloids in a large number of tuber samples.

While several methods are known for the determination of total glycoalkaloids in potato tissue (Fitzpatrick and Osman, 1974; Smittle, 1971), quantitation of individual glycoalkaloids has been accomplished only by rather lengthy and complicated gas (Herb et al., 1975) and high-pressure liquid (Hunter et al., 1976) chromatographic procedures. These methods are not well-suited for the screening of large numbers of potato varieties for the levels of individual glycoalkaloids.

We developed the methods described in this paper for the routine analysis of potato glycoalkaloids in our breeding program.

EXPERIMENTAL SECTION

Extraction of Tissues. Potato tubers were washed and sliced, and the outer 5 mm, including the peel, was extracted according to the method of Shih and Kúc (1974). The extracted glycoalkaloids were dissolved in the solvent used for thin-layer chromatography (TLC) at a level of 2 mL/10 g fresh tuber weight.

Total Glycoalkaloid Assay. Glycoalkaloids were assayed by a modification of the method of Wang et al. (1972). A 0.5-mL aliquot of each glycoalkaloid extract was placed in a test tube, evaporated to dryness, and then dissolved in 0.5 mL of 5% acetic acid. Analogous tubes containing up to 0.5 mg of standard α-solanine were also prepared. Each tube then received 1.5 mL of 85% H_3PO_4 with mixing and 1 mL of paraformaldehyde reagent (0.2 g of *p*-formaldehyde dissolved in 15 mL of H_2O and then diluted to 100 mL with 85% H_3PO_4). The tubes were incubated at 60 °C for 5 min, and the absorbance was then read at 600 nm in a Hitachi Model 124 spectrophotometer.

Assay of Individual Glycoalkaloids. The identities of the glycoalkaloids isolated in this study were confirmed using standard 20 cm × 20 cm silica gel TLC plates in several solvent systems (Shih and Kúc, 1974). For quantitative assays, the glycoalkaloid extracts and solutions containing various amounts of standard α-solanine were spotted on Kontes 1 in. × 3 in. Q6F silica gel plates and developed in the organic layer of $CHCl_3$–95% ethanol–1% NH_4OH (2:2:1, v/v). The developed plates were air-dried, dipped in $CHCl_3$ saturated with $SbCl_3$, and then heated at 150 °C for 4 min. The intensities of the colored spots which appeared were determined with a Kontes densitometer using a single-beam mode, reference head, medium light intensity, and a long-wavelength ultraviolet light source.

Table I. Glycoalkaloid Contents of Tubers from Three Cultivars of Potato[a]

cultivar	glycoalkaloid content, mg/100 g fresh weight		
	α-solanine	α-chaconine	total[b]
B 5141-6	22.9 ± 0.8	42.9 ± 2.7	60.8 ± 0.3
B 6039-WV6	16.4 ± 0.3	25.0 ± 0.5	46.8 ± 0.3
B 6039-WV9	21.3 ± 0.7	24.9 ± 0.7	40.6 ± 0.5

[a] Each value is the average of three assays ± the standard error of the mean. [b] Total glycoalkaloid values were determined by the *p*-formaldehyde–H_2SO_4 method.

RESULTS AND DISCUSSION

The levels of total glycoalkaloids in the tubers of three potato cultivars are shown in Table I. The amounts of α-solanine and α-chaconine, the only glycoalkaloids isolated from these tissues, are also summarized in Table I. It can be seen that the sums of the concentrations of the individual glycoalkaloids are in close agreement to the values for total glycoalkaloid determined by the paraformaldehyde method. Thus the TLC method reported here can be used as a quantitative assay for total as well as individual glycoalkaloids.

The R_f values for α-solanine and α-chaconine were considerably different on the small TLC plates used in this study than they were on standard silica gel TLC plates. Apparently the hardness of the adsorbent and the length of the Kontes microplates contributed to significant variations from the recorded R_f values.

The small TLC plates used in this study were convenient because of their cost and the speed of the development step. Standard plates could have been used and quantitated with equal ease, and they may be desirable in some studies. For example, other tubers or potato foliage may contain glycoalkaloids that are more difficult to separate than the two obtained in this study. In addition, if a densitometer with lower sensitivity than the Kontes instrument is used, greater sample sizes might be needed and it is unlikely that the microplates used in this study would completely resolve the individual alkaloids. It is also possible that the distribution of glycoalkaloids in foliage or tubers of different cultivars of potato might necessitate

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