Ipomeamarone in Blemished and Diseased Sweet Potatoes (*Ipomea batatas*)

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A simple and efficient procedure is described for the quantitative determination of ipomeamarone in sweet potato products. The method is rapid and sensitive to 1 ppm. Sweet potato shreds or mash are comminuted and extracted in a blender with a mixture of chloroform, methanol, and water. After filtering the homogenate, the chloroform layer is dried and evaporated under vacuum. The residual oily material is dissolved in a known volume of chloroform and analyzed quantitatively by gas chromatography. The method was applied to blemished and diseased sweet potatoes before and after processing. Although the method indicates that blemished and diseased sweet potatoes have a relatively high concentration of ipomeamarone, it also reveals that peeling and trimming raw, baked, or boiled sweet potatoes leaves healthy tissue with little or no detectable ipomeamarone. Neither baking nor boiling appears to promote diffusion of ipomeamarone into the healthy tissue; however, baking appears to reduce the concentration of this hepatotoxin.

American and Japanese investigators have isolated several furanoterpenoid toxins from blemished and diseased sweet potatoes and have shown that ipomeamarone, the most common of these metabolites, produces liver necrosis in mice and other animals (Wilson and Hayes, 1973). Since most sweet potatoes are consumed as human food or animal feed, it is essential to determine the presence of this hepatotoxin in sweet potato products. Although several procedures are available for the determination of ipomeamarone (Boyd and Wilson, 1971; Wood and Huang, 1975; Coxon et al., 1975), we needed more practical methodology for our survey. A modified method of analysis was developed that permits rapid and efficient quantitative determination of ipomeamarone in sweet potato products. This procedure also indicates that ipomeamarone is concentrated primarily in the blemished and diseased tissue of the sweet potatoes.

MATERIALS AND METHODS

Sweet Potato Samples. Blemished and diseased sweet potatoes were selected either from discarded potatoes at the grading line of packing sheds or from washed potatoes that became blemished and diseased in storage.

Preparation of Sample. Blemished and diseased sweet potatoes were either quartered with peel intact or peeled and trimmed 3–10 mm beyond the affected areas, depending upon the degree of infection. Some of the potatoes were peeled and trimmed raw, some after baking at 200 °C for 45–80 min, and some after boiling in a covered container for 30–50 min.

The quartered sections or the peelings and trimmings from either individual potatoes or from samples weighing 3–6 lb were shredded or mashed in a Hobart Food Grinder and thoroughly mixed by hand. Some of the raw shreds were baked before analysis. Samples weighing 5, 10, or 25 g were comminuted and extracted with a total of 125 ml of a mixture of ACS Reagent chloroform, ACS Certified methanol, and water, in a ratio of 2:2:1 (Bligh and Dyer, 1959). Unless otherwise specified, 25-g samples were evaluated.

The same volume of solvent was used with the smaller samples to better extract ipomeamarone from severely blemished and diseased sweet potatoes. Each sample was first comminuted for 2 min in a Waring Blendor with 25 ml of chloroform and 50 ml of methanol. An additional 25 ml of chloroform was added, and the mixture comminuted further for 30 s. After adding 25 ml of water the mixture was comminuted for 30 s and filtered under a 12-in. vacuum into a 4-l. filter flask, using Whatman No. 1 paper contained in a large (151 mm i.d.) Coors Büchner funnel. The residual cake was washed three times with 25-ml portions of chloroform, and the filtrates were combined into a 250-ml separatory funnel. The chloroform layer, which separated quickly from the aqueous layer, was dried with anhydrous sodium sulfate powder.

After filtering through Whatman No. 1 paper, the chloroform extract was poured into a round-bottomed flask and evaporated under vacuum at 40 °C with a rotary evaporator. The residual oily material from 5-, 10-, and 25-g samples was dissolved in 0.2, 0.4, and 1.0 ml of chloroform, respectively. For analysis by gas chromatography, 1 μl of each of the respective solutions was injected into a gas chromatograph, fitted with an injection port glass liner packed with glass wool. If the concentration of ipomeamarone was found to be higher than 400 ppm, the solution was diluted accordingly and reanalyzed.

To evaluate the effect of processing on removal of ipomeamarone, sweet potatoes that were severely infected with soil rot fungi were washed and divided in three portions. One portion, unpeeled, was used for control samples; the second portion was lye-peeled in 10% sodium hydroxide at 100 °C for 6 min; the third portion was lye-peeled and trimmed to remove scar or infected tissue. Each portion was packaged in 303 × 406 enamel-lined cans, covered with 30% cane sugar syrup, exhausted for 3.5 min in live steam at 82 °C, sealed, retorted at 118 °C for 45 min, and cooled in running water for 15 min. Six cans from each portion of sweet potatoes were analyzed quantitatively for ipomeamarone. The solids and the syrup were analyzed separately.

Chromatographic Conditions. Instruments used included a Varian 1800 gas chromatograph with dual independent hydrogen flame detectors, Varian 20 recorder, and a Hewlett Packard 3370B integrator. The column was a 1/8 in. (0.093 in. i.d.) stainless steel coiled tube, 6 ft long, packed with 10% UC-W98 (methyl vinyl silicone) on 80–100 mesh Gas-Chrom Q (Boyd and Wilson, 1971). Flow rates were 30 ml/min nitrogen carrier gas through each column, 30 ml/min hydrogen to each flame, and 300 ml/min air for both flames. The temperatures were 170 °C at inlet, 280 °C at detector, and 180 °C in column oven.

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Table I. Determination of Ipomeamarone in Sweet Potato Products

Sample size, g	1st extraction, ppm	2d extraction[a] ppm	2d extraction[a] %
5[b]	25 370	120	0.5
5	21 540	360	1.6
5	18 100	110	0.6
5	17 130	220	1.3
5	15 390	760	4.7
5	15 100	45	0.3
5	4 410	35	0.8
10	10 000	540	5.1
10	5 460	380	6.5
25	8 800	500	5.4
25	6 320	630	9.1
25	4 500	900	16.7
25[c]	1 500	300	16.7
25	1 100	180	14.1
25	210	5	2.3
25	170	5	2.9
25	40	ND	
25	10	ND	
25[d]	ND[e]	ND	
25	ND	ND	
25	ND	ND	
25[f]	ND	ND	
25	ND	ND	
25	ND	ND	

[a] Filter cake was reextracted to determine efficiency of the solvent system by measuring the percent of total ipomeamarone left in the filter cake. [b] Twelve samples of peelings and trimmings from severely blemished and diseased sweet potatoes. [c] Six samples of blemished and diseased sweet potatoes. [d] Three samples of sound and healthy sweet potatoes. [e] Nondetectable, or less than 1 ppm. [f] Three samples of processed sweet potatoes.

Under these conditions, the retention time for ipomeamarone was 7.5 min. The electrometer setting was 8 $\times 10^{-11}$, and chart speed was 20 in./h. After 25 min, when the analysis was completed, the temperature of the column oven was increased from 180 to 210 °C for 10 min to clear the column packing in preparation for the next run. A fresh liner filled loosely with glass wool was inserted in the injection port at the end of each day. To minimize the possibility of interferences, some analyses were done on a column packing of SE-30 (methyl silicone). For the preparation of a standard curve, a stock solution of ipomeamarone was diluted to give eight concentrations ranging from 40 to 0.1 mg/ml. Two analyses of each sample were made and averaged. Integration counts plotted against concentration yielded a straight line curve.

RESULTS AND DISCUSSION

A simple and efficient extraction procedure (Bligh and Dyer, 1959) was adapted for the quantitative determination of ipomeamarone in sweet potato products. After quartered sweet potatoes, peelings and trimmings, or processed sweet potato products were shredded or mashed in a Hobart Food Grinder, comminution and extraction were easily accomplished in a blender in the presence of a mixture of chloroform, methanol, and water. The homogenate filtered readily through Whatman No. 1 filter paper, and the chloroform layer separated within 1 min. Emulsion problems as experienced by the authors with the procedure of Boyd and Wilson (1971) for some samples of sweet potatoes were not encountered. Table I shows that, for efficient extraction of ipomeamarone in severely blemished and diseased sweet potato tissue, the normal 25-g sample should be reduced to 5 or 10 g; the volume of solvent should not be reduced. The 25-g sample, which is more representative, is preferred, and a second extraction is recommended for severely blemished and diseased sweet potato tissue. Since the method was found to be very efficient, in particular at low levels of ipomeamarone, pure ipomeamarone was not added to sweet potato tissue.

Table II. Ipomeamarone in Slightly Blemished and Diseased Sweet Potatoes

Peelings and trimmings,[a] ppm			Remaining pulps, ppm		
Raw	After baking	After boiling	Raw	After baking	After boiling
60	55	100	ND[b]	ND	ND
125	20	250	ND	ND	ND
135	360	125	ND	ND	ND
165	55	30	3	ND	1
185	30	55	10	ND	ND
205	15	70	ND	ND	ND
245	670	300	ND	1	13
320	335	500	ND	ND	3
345	95	800	6	ND	4
350	110	20	25	ND	ND
515	265	520	ND	ND	ND
840	130	360	ND	ND	4
900	660	540	ND	25	9

[a] Each 10-lb sample was divided into three portions based on appearance, not ipomeamarone content. One portion was peeled and trimmed raw, one after baking, one after boiling. [b] Nondetectable, or less than 1 ppm.

Table III. Ipomeamarone in Severely Blemished and Diseased Sweet Potatoes

Peelings and trimmings,[a] ppm			Remaining pulps, ppm		
Raw	After baking	After boiling	Raw	After baking	After boiling
545	2200	680	7	45	15
675	340	625	30	18	3
800	330	300	6	ND[b]	ND
830	95	125	4	ND	1
1 300	3500	2500	ND	2	40
1 350	130	120	ND	ND	2
1 800	2100	600	ND	ND	2
2 200	700		ND	ND	
2 300	2500	1100	ND	ND	8
2 375	1550	440	3	4	1
2 615	1140	910	15	6	4
2 735	2085	1145	6	25	25
6 920	700	1200	40	1	11
10 000	2450	460	15	1	2

[a] Each 10-lb sample was divided into three portions based on appearance, not ipomeamarone content. One portion was peeled and trimmed raw, one after baking, one after boiling. [b] Nondetectable, or less than 1 ppm.

The life of the gas chromatographic column packing was easily extended for a few months by inserting a glass liner filled loosely with glass wool in the injection port, which was heated to only 170 °C. This temperature was adequate for rapid and complete volatilization of ipomeamarone and retained more of the less volatile impurities on the glass wool. For best performance, a liner with fresh glass wool was inserted in the injection port at the end of each day. After ipomeamarone was eluted and resolved, the column packing was cleaned of impurities within 10 min by heating to only 210 °C. These simple modifications eliminated the need to clean up the sweet potato extracts by column chromatography prior to gas chromatographic analysis (Wood and Huang, 1975). As can be seen in Figure 1, suitable chromatograms were obtained with varying concentrations of ipomeamarone in sweet potatoes down to the parts per million level.

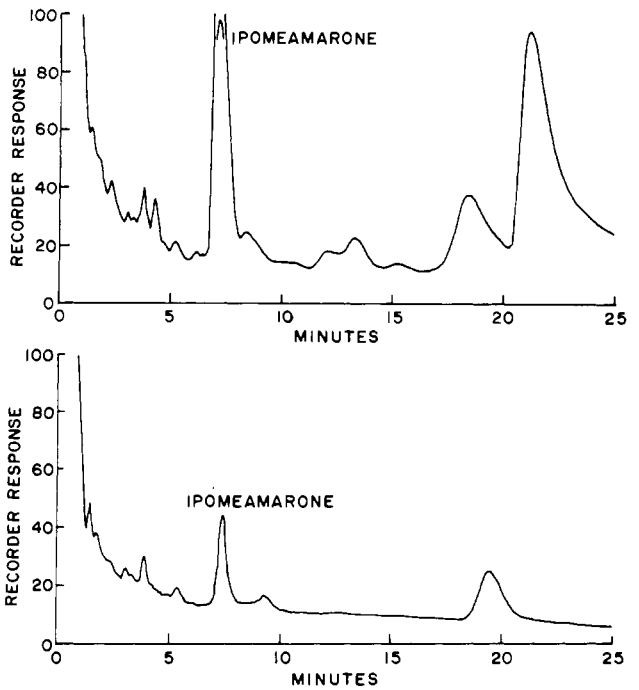

Figure 1. Typical gas chromatograms of sweet potato extracts. Upper chromatogram was of an extract from a sweet potato that contained 150 ppm of ipomeamarone; lower chromatogram, from a sweet potato that contained 15 ppm of ipomeamarone.

Table IV. Effects of Baking on Ipomeamarone Content of Peelings and Trimmings of Blemished and Diseased Sweet Potatoes

Raw, ppm	After baking	
	ppm	Change, %
60	35	-42
125	110	-12
135	115	-15
165	285	+73
185	90	-51
205	125	-39
245	160	-35
320	165	-48
345	245	-29
350	265	-24
515	440	-15
840	415	-51
900	615	-32
2615	1230	-53
2735	2200	-20

Slightly blemished and diseased sweet potatoes contain significant amounts of ipomeamarone, but peeling and trimming them 3–10 mm beyond the infected areas leaves healthy tissue that contains little or no detectable ipomeamarone, as is shown in Table II. Also, this procedure is effective with highly blemished and diseased sweet potatoes that have thousands of parts per million of ipomeamarone. Little or no ipomeamarone remains in the healthy tissue after peeling and trimming (Table III), which can be effectively accomplished on raw, baked, or boiled sweet potatoes, since neither baking nor boiling appears to promote diffusion of ipomeamarone into the healthy tissue. Although it is very difficult to obtain homogenous samples of peelings and trimmings, representative samples were prepared and analyzed for ipomeamarone before and after baking. Although baking does not eliminate ipomeamarone, it does appear to reduce its concentration, as can be seen in Table IV.

Table V. Effects of Processing on Ipomeamarone Content of Sweet Potatoes Infected with Soil Rot Fungi

Control, ppm		Lye peeled, ppm		Lye peeled and trimmed, ppm	
Solids	Syrup	Solids	Syrup	Solids	Syrup
1100	150	200	80	1	ND[a]
ND	ND	120	10	ND	ND
30	9	ND	ND	ND	ND
ND	ND	1	ND	ND	ND
50	25	125	50	ND	ND
7	ND	80	25	ND	ND

[a] Nondetectable, or less than 1 ppm.

Cody and Haard (1976) observed a much greater reduction upon baking, but the concentration of ipomeamarone increased with prolonged baking. It is significant to note that normal commercial processing conditions, which involve lye-peeling and trimming, effectively remove the tissue containing ipomeamarone in sweet potatoes that have been contaminated with soil rot fungi as shown in Table V.

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