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Received for review October 2, 1978. Accepted January 29, 1979. Paper No. 5777 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, NC. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named nor criticism of similar ones not mentioned.

Role of Citric Acid in the After-Cooking Darkening of γ-Irradiated Potato Tubers

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With the aim of exploring the reasons for radiation-induced after-cooking darkening of potato tubers, organic acids from a naturally darkening ("Irmgard") and a nondarkening cultivar ("Hansa") were purified by ion-exchange chromatography and quantified by gas–liquid chromatography of the trimethylsilyl derivatives. Citric, malic, and pyroglutamic acids were the main components, citric acid forming 70–80% of the total acids. Major differences in citric and malic acid content were observed between the darkening and nondarkening cultivars. A significant decrease in citric acid content accompanied by increases in malic and pyroglutamic acids were noted in irradiated tubers during storage. The induction of after-cooking darkening in irradiated potatoes is attributed to decreased citric acid levels and enhanced polyphenols in the tuber tissues, both changes favoring the formation of iron–phenolic complexes responsible for the discoloration.

The bluish-grey discoloration usually referred to as "after-cooking darkening" appearing in certain potato cultivars shortly after cooking is attributed to the interaction of iron with chlorogenic and/or caffeic acids (Juul, 1949; Kiermeir and Rickerl, 1955a). The darkening is generally more intense at the stem end than at the bud end of the tuber. Various agronomic and climatic factors (Baerug and Enge, 1974; Smith et al., 1942), as well as the composition of the tubers, especially the content of iron, orthodiphenols, organic acids, and pH, are known to influence the darkening tendency (Bate-Smith et al., 1958; Hughes and Swain, 1962a,b; Heisler et al., 1963, 1964; Hunter et al., 1957; Kiermeir and Rickerl 1955b; Smith, 1959; Wurster and Smith, 1963, 1965; Vertregt, 1968).

Among the various factors known to affect the after-cooking darkening, the role of citric acid, because of its known ability to chelate iron, has been investigated by many workers. Juul (1949) showed that the discoloration was influenced by citric acid, which he attributed to the pH effect. Mulder (1949) and Bate-Smith et al. (1958) recognized the citric acid action as a chelating effect and stated that the distribution of blackening in individual cooked tubers is governed mainly by the competition between chlorogenic and citric acid for iron. Hughes and Swain (1962a) studied the distribution of the after-cooking darkening within an individual tuber and found correlation of blackening with the ratio of citric to chlorogenic acid. A correlation between citric acid content and after-cooking darkening was reported by Heisler et al. (1963). Based on in vitro experiments on the effect of citric, orthophosphoric, and malic acids on the color of various phenol–iron complexes, Hughes and Swain (1962b) concluded that citric acid was the most important of these factors in reducing the intensity of color of the chlorogenic acid–iron complex.

In an earlier study, it was observed that γ irradiation at sprout-inhibiting dose levels (10 krad) induces after-cooking darkening in several Indian potato cultivars which normally do not show this phenomenon (Thomas and Joshi, 1977; Thomas, 1977). Since irradiated tubers stored at 15 °C showed higher tissue pH (near neutral) in comparison to unirradiated tubers stored at 2 °C, it was postulated that the radiation-induced darkening might be due to a reduction in the organic acid level, especially citric acid.

The studies reported here were undertaken to gain more information on the effect of radiation on the organic acid content of potatoes and its relation to after-cooking darkening.

MATERIALS AND METHODS

Chemicals. Chemicals and reagents were obtained as follows: All organic acids and pyroglutamic acid (PCA) were from Sigma Chemical Co., St. Louis, MO. Dowex-1×8, Dowex-50 W×8, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and trimethylchlorosilane (TMCS) were from Serva, Heidelberg, FRG, and pyridine was from Merck, Schuchart, Darmstadt, FRG.

Potatoes. Two potato cultivars, one showing natural after-cooking darkening ("Irmgard") and the other with no darkening ("Hansa") were obtained from Versuchsfeld für Sortenprüfung, Rheinstetten, FRG. Tubers were about

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0021-8561/79/1427-0519$01.00/0 © 1979 American Chemical Society

2 months old from harvest at the time of their receipt in the laboratory.

Irradiation and Storage Conditions. Tubers were irradiated with 10 ± 2 krad in a cobalt-60 gamma cell 220 (Atomic Energy Canada Ltd.) at a dose rate of 420 krad/h. They were stored at 15 °C, relative humidity 85–90%. Unirradiated tubers stored at 4 °C served as controls.

In some experiments potatoes were surface irradiated with a dose of 10 krad using a Van de Graff electron accelerator with a beam output of 1 MeV and stored at 15 °C. For comparative purposes, potatoes treated with the chemical sprout inhibitor Propham (isopropyl carbanilate; three dustings at 2-month intervals) were also stored at 15 °C.

Extraction and Purification of Organic Acids. Thomas and Joshi (1977) reported that radiation-induced after-cooking darkening was distributed throughout the tuber flesh, the intensity being greater in the peripheral region (cortex), with a maximum at periphery at stem end. Hence, in the present study organic acid content in cortex and pith tissues was estimated separately to find any correlation between the extent of darkening and distribution of organic acids.

Ten tubers selected at random after different storage periods were divided into two lots. One lot was used for organic acid extraction and the other lot was used for cooking and measurement of darkening.

Skin tissues were removed uniformly with a hand peeler. Cortex (outer 3–4 mm layer) and pith (central parenchymatous) tissues of 10 g each in duplicate, representative of five tubers, were plunged into boiling 80% aqueous ethanol (50 mL) and boiling continued for 3 min. The samples were homogenized for 3 min in a Waring Blendor, and the slurry was filtered on a Buchner funnel. The residue was reextracted with 50 mL of hot 80% ethanol, and the extracts were pooled and made up to 100 mL. To 25 mL of the centrifuged extract 2 mg of glutaric acid (Jadhav and Andrew, 1977) was added as an internal standard. The extract was freed of alcohol under reduced pressure at 40 °C in a rotary evaporator. The residue dissolved in 7 mL of distilled water was passed through a 10 × 100 mm cation-exchange column (Dowex 50 W×8 (H^+) 100–200 mesh) and the column washed with 20 mL of distilled water. The eluant was allowed to drip directly on to a 10 × 100 mm column of Dowex 1×8 (formate) 100–200 mesh anion-exchange resin. The column was washed with 40 mL of 8 M formic acid (Phillips and Jennings, 1976) and the column washed with 20 mL of water. The eluant was freed of formic acid under reduced pressure at 40 °C. The residue dissolved in 5 mL of water was taken in small reaction flasks, dried under reduced pressure, and taken to absolute dryness by storing over P_2O_5 in vacuo overnight at 20 °C.

Silylation. The dry residue was dissolved in 1 mL of anhydrous pyridine using a shaker for 20 min. To 100 μL of pyridine solution 30 μL of BSFTA containing 4% TMCS was added and shaken at 25 °C for 45 min. In separate experiments the above conditions were found to ensure complete silylation of the organic acids, and for routine analyses, samples were allowed to react for 45 min before gas chromatographic analysis.

Gas–Liquid Chromatography (GLC) of Organic Acids. Analyses were carried out using a Hewlett Packard Research chromatograph Model 5750 fitted with a flame ionization detector. Glass separation columns, 3 m long and 2 mm i.d., were packed with 10% silicone Gum Rubber UCC-W-982 (methyl-vinyl) as stationary phase on Chromosorb W-AW-DMCS, 80–100 mesh (Analabs).

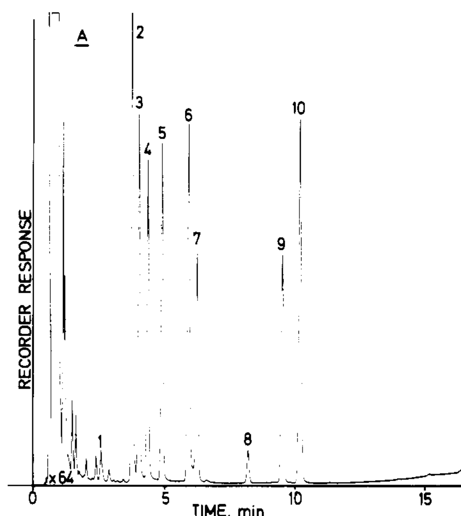

Figure 1. Gas chromatogram (FID) of trimethylsilyl (Me_3Si) derivatives of standard organic acids on a 3 m × 2 mm (i.d.) glass column packed with 10% silicone gum rubber UCC-W-982 on Chromosorb W-AW-DMCS (80–100 mesh). GLC conditions for separation of organic acids are given in the Materials and Methods section. The peaks are (1) oxalic, (2) phosphoric, (3) succinic, (4) fumaric, (5) glutaric (internal standard), (6) malic, (7) pyroglutamic, (8) *n*-heptadecane (a second internal standard), (9) citric, and (10) quinic acids.

Sample size, 0.5 μL; H_2 flow rate, 30 mL/min; carrier gas (helium), 30 mL/min; air flow rate, 370 mL/min; injection port temperature, 270 °C; detector temperature, 290 °C; column temperature, 150–300 °C; program rate, 10 °C/min; input attenuation, 10^2; output attenuation, ×64, variable; final hold, 10 min; recorder speed, 0.2 in./min. Individual acids were identified by comparison of retention times with standard acids. For quantitative determinations peak areas of identified acids were compared with the internal standard.

Total Phenolics. Total phenolic constituents in the 80% alcohol extracts were determined by the modified Folin-Denis method (Swain and Hillis, 1959).

Tissue pH. Cell sap from 1 cm^3 tissue blocks was pressed out with a hand press and pH was determined with microelectrodes using a Knick digital pH meter.

Measurement of Darkening. Tubers were cooked with skin on for 30 min in boiling water, cut into two longitudinal halves, and exposed to air for 24 h for development of maximum after-cooking darkening. The intensity of darkening in five individual tuber halves was measured on a Photovolt Photoelectric Reflection Meter Model 610 (Photovolt Corp., New York) using a green filter. The reflectance values were taken directly as a measure of the degree of darkening: the lower the value, the more darkening in the potato.

RESULTS

A typical chromatogram depicting the separation of a mixture of standard organic acids as silyl derivatives is shown in Figure 1. A final hold time of 10 min at 300 °C was found necessary to obtain a symmetric peak in case of citric acid without which an occasional shoulder at the leading edge of the peak was noticed. A temperature program rate of 10 °C/min yielded rapid and reproducible separation of organic acids. Figure 2 shows a typical separation of organic acids extracted from potatoes. Using standard acids, the recovery of the major organic acid fractions, i.e., citric, malic, and pyroglutamic acids, after the ion-exchange cleanup and silylation procedure was found to be 96–98%.

Table I. Concentration of Organic Acids[a] in Darkening ('Irmgard') and Nondarkening ('Hansa') Potato Tubers as Determined by GLC

	'Irmgard'				'Hansa'			
	cortex		pith		cortex		pith	
organic acid	mg/100 g	as % of total	mg/100 g	as % of total	mg/100 g	as % of total	mg/100 g	as % of total
phosphoric	2.6 ± 0.5	0.9	3.5 ± 0.2	1.2	11.4 ± 1.5	3.2	2.8 ± 0.4	0.8
succinic	2.7 ± 0.2	1.0	2.7 ± 0.1	0.9	2.6 ± 0.5	0.8	2.0 ± 0.5	0.5
fumaric	Tr		Tr		Tr		Tr	
malic	41.2 ± 2.6	15.7	53.1 ± 3.1	17.6	89.4 ± 6.2	25.5	76.3 ± 3.5	20.5
pyroglutamic (PCA)	8.3 ± 0.5	3.2	23.9 ± 2.3	7.9	2.2 ± 0.3	0.6	8.7 ± 0.3	2.3
citric	208.3 ± 17.5	79.2	217.7 ± 19.2	72.4	244.4 ± 11.0	69.9	283.0 ± 13.3	75.9
total	265.1	100.0	300.8	100.0	350.0	100.0	372.8	100.0

[a] Expressed on fresh weight basis. Average of four determinations, mean value, and standard deviation. For details of GLC, see Figure 1.

Table II. Effect of Irradiation and Storage on pH of 'Hansa' and 'Irmgard' Potatoes[a]

	'Hansa'			'Irmgard'		
		after 7 months of storage			after 7 months of storage	
tissue	initial	control	irrad.	initial	control	irrad.
stem end	5.88 ± 0.05	6.14 ± 0.16	6.52 ± 0.14	6.36 ± 0.30	6.62 ± 0.20	6.42 ± 0.04
cortex	5.84 ± 0.02	6.00 ± 0.09	6.49 ± 0.11	6.20 ± 0.10	6.56 ± 0.20	6.54 ± 0.06
pith	5.58 ± 0.11	5.85 ± 0.06	6.23 ± 0.10	6.08 ± 0.01	6.16 ± 0.18	6.35 ± 0.05
bud end	5.75 ± 0.08	5.93 ± 0.09	6.42 ± 0.07	6.01 ± 0.05	6.35 ± 0.13	6.45 ± 0.04

[a] Mean and standard deviation of five determinations on cell sap.

Table III. After-Cooking Darkening in Unirradiated and Irradiated 'Irmgard' and 'Hansa' Tubers[a]

	'Irmgard'			'Hansa'		
		after 7 months of storage			after 7 months of storage	
tuber position	initial	control	irradiated	initial	control	irradiated
stem end	35.2 ± 3.7	30.8 ± 3.1	14.8 ± 5.8	44.8 ± 2.5	41.2 ± 5.2	27.6 ± 5.4
middle	37.7 ± 3.8	34.2 ± 5.1	17.5 ± 3.4	45.7 ± 3.4	43.0 ± 5.1	29.7 ± 6.9
bud end	39.8 ± 2.9	36.0 ± 5.8	21.3 ± 1.7	46.4 ± 2.9	42.8 ± 4.3	34.5 ± 2.7
surface (after removing the skin)	35.7 ± 3.1	28.0 ± 3.8	15.6 ± 1.5	43.9 ± 3.8	41.6 ± 3.8	27.1 ± 3.4

[a] Mean value and standard deviation of five separate readings. The figures denote reflectance value of cooked tubers after 24-h exposure to air. A lower reflectance value indicates more darkening.

Major differences in the content of citric and malic acids were noted between the naturally darkening cultivar Irmgard and the nondarkening cultivar Hansa (Table I). The nondarkening cultivar contained more citric and malic acid and less pyroglutamic acid (2-pyrrolidone-5-carboxylic acid, PCA) in comparison to the darkening cultivar. In both cultivars, citric acid was predominant (70–80% of the total acids). Phosphoric, succinic, fumaric, and quinic acids were found in traces in both varieties. Cortex tissues contained lower concentrations of acids than the pith.

The differences in organic acids content between cultivars and tissues were also reflected in the pH of the cell sap (Table II). In "Irmgard" the pH ranged from 6.08 in pith to 6.20 in cortex, while in "Hansa" the respective values were 5.58 and 5.84.

Irradiation at 10 krad was found to induce after-cooking darkening in "Hansa" during storage at 15 °C, while in "Irmgard" the extent of darkening was further enhanced (Table III). In "Hansa", the tendency to darken appeared initially after a storage of 2 to 3 months and thereafter continued to increase with advancing storage. The darkening was more intense along the periphery of tubers (cortex region) with maximal intensity at periphery at stem end. The tendency to darken was also found to increase in unirradiated "Irmgard" tubers stored at 4 °C, while in unirradiated "Hansa" no noticeable darkening was observed even after 7 months storage at 4 °C. After this storage period, irradiated "Hansa" tubers showed approximately the same degree of darkening as unirradiated "Irmgard" tubers.

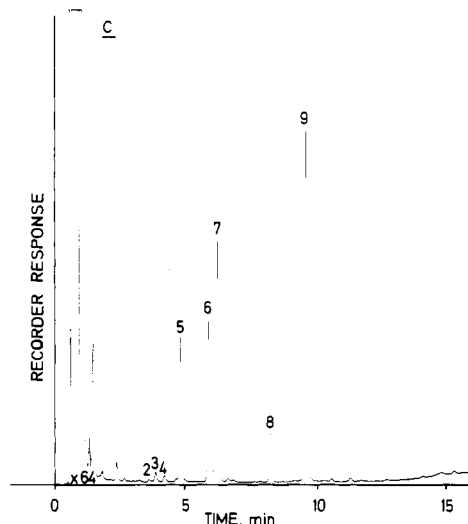

Figure 2. Gas chromatogram of Me_3Si derivatives of the organic acid fraction of "Irmgard" potatoes (pith tissues of 4-month stored, irradiated tubers). For GLC conditions and numbering of peaks, see Figure 1.

Analyses of organic acid levels at 4 (not reported here) and 7 months of storage revealed more citric acid losses

Figure 3. Gas chromatogram of Me_3Si derivatives of the organic acid fraction of "Hansa" potatoes (pith tissues of 4-month stored, irradiated tubers). For GLC conditions and numbering of peaks, see Figure 1.

in irradiated tubers in comparison to controls. Table IV shows the organic acid content in irradiated and unirradiated potatoes stored for 7 months at 15 and 4 °C, respectively. In unirradiated "Hansa" potatoes, the citric acid level in cortex and pith remained practically unchanged, while in irradiated tubers a decrease of 35 and 28% was recorded in cortex and pith, respectively. In irradiated "Irmgard" tubers, citric acid content in cortex and pith decreased by 61 and 65%, respectively, as compared to 29 and 35% in unirradiated tubers. Malic and pyroglutamic acids showed an increase with storage in both irradiated and unirradiated tubers, the increase being greater in irradiated tubers. The increase in pyroglutamic acid was more pronounced in pith than in cortex and among the cultivars Irmgard exhibited a marked increase as compared to Hansa (Figures 2 and 3). The changes in citric acid content were also reflected in the pH of cell sap (Table II). Both storage and irradiation lead to higher pH values.

Total phenolic constituents were increased by storage and by irradiation (Table V). Levels were higher in "Irmgard" tubers, as compared to "Hansa", both initially and after irradiation and storage.

DISCUSSION

Earlier studies have shown that the citric acid content in the tuber plays a significant role in the manifestation of after-cooking darkening (Hughes and Swain, 1962a; Heisler et al., 1963) and consequently any factor which alters the citric acid content could be expected to influence the tendency to darken as well as the extent of darkening. Using the more sensitive gas–liquid chromatographic technique we have confirmed the earlier reports on the differences in citric acid content between darkening and nondarkening potato cultivars.

Examination of data shows a strong tendency for the radiation-induced after-cooking darkening to be associated with the low citric acid levels in such tubers. The decrease in citric acid accompanied by increases in malic and pyroglutamic acids in irradiated potatoes implicate an enhancement in citric acid catabolism and pathways leading to formation of malic and pyroglutamic acids. Maxie et al. (1964) reported a marked decrease in total acidity in irradiated lemons and Romani (1964) presented data suggesting that the acid loss is not due to direct irradiation decarboxylation of acids. A similar radiation-induced

Table IV. Changes in Organic Acid Content[a] of Irradiated and Unirradiated 'Hansa' and 'Irmgard' Potatoes after 7 Months of Storage

	control						irradiated (10 Krad)					
	cortex			pith			cortex			pith		
	mg/100 g	as % of total	% change over initial	mg/100 g	as % of total	% change over initial	mg/100 g	as % of total	% change over initial	mg/100 g	as % of total	% change over initial
'Hansa'												
phosphoric	7.0 ± 0.6	1.7	38.5	6.2 ± 0.3	1.6	122	12.2 ± 0.9	3.1	7.0	7.5 ± 0.6	1.8	169
succinic	2.3 ± 0.1	0.6	−11.5	3.5	0.9	76	2.5 ± 0.3	0.6	3.8	2.4 ± 0.1	0.6	20
malic	117.3 ± 13.0	28.8	31.2	103.8 ± 9.6	26.7	36	187 ± 18	47.8	109.2	181.4 ± 10.6	45.5	137.7
pyroglutamic	9.2 ± 2.3	22.6	318	11.9	3.1	36.7	25.1 ± 1.7	6.4	1041	27.1 ± 3.5	6.8	211.5
citric	266.7 ± 25.3	65.3	9.1	259.0 ± 12.0	66.7	8.5	158.5 ± 7.9	40.5	−35.0	176.2 ± 9.9	44.1	−27.9
total	407.3			388.3			390.8			399.9		
'Irmgard'												
phosphoric	8.3 ± 1.1	2.6	223	4.7 ± 1.9	1.5	34.2	6.6 ± 0.2	2.76	156.8	2.76 ± 0.05	0.84	−21.1
succinic	3.3 ± 0.7	1.0	22.2	3.9 ± 1.1	1.3	44.4	2.5 ± 0.3	1.04	−7.4	1.40 ± 0.17	0.42	−48.1
malic	85.4 ± 7.6	26.9	107.3	79.5 ± 8.2	26.4	50.0	67.9 ± 2.9	28.4	64.8	90.2 ± 6.7	27.6	70.2
pyroglutamic	66.6 ± 2.3	21.0	153.0	66.7 ± 2.4	22.1	179.0	75.7 ± 9.0	31.6	812.0	151 ± 15.2	46.2	531
citric	148.3 ± 4.2	46.8	−28.8	142.2 ± 11.9	47.2	−34.7	81.5 ± 6.9	34.1	−60.8	76.2 ± 14.3	23.34	−64.9
total	316.9			301.3			238.95			326.36		

[a] Mean value and standard deviation. Expressed on fresh weight basis, determined by GLC.

Table V. Total Polyphenols[a] in γ-Irradiated and Unirradiated 'Irmgard' and 'Hansa' Tubers

tissue	'Irmgard'			'Hansa'		
		after 7 months of storage			after 7 months of storage	
	initial	control	irradiated	initial	control	irradiated
skin	310.0 ± 33.0	347.0 ± 11.3	376.0 ± 2.1	262.5 ± 11.9	399.0 ± 12.7	329.0 ± 6.3
cortex	73.0 ± 16.0	95.0 ± 0.7	141.2 ± 0.3	47.0 ± 0.9	90.2 ± 6.7	115.5 ± 7.8
pith	37.2 ± 2.8	78.7 ± 3.2	108.5 ± 2.8	33.5 ± 1.9	76.0 ± 5.6	93.6 ± 6.5

[a] Mean value and standard deviation of four separate estimations.

decline in the percentage of citric acid in lemons was reported by Dennison and Ahmed (1966). However, in the above-mentioned studies the radiation doses employed were higher (200–400 krad) and the effects were noticed during relatively short storage periods and hence a comparison with our findings may not be valid. Rumpf (1972) reported a gradual decrease in citric acid and an increase in malic acid in untreated as well as in potatoes subjected to γ and electron irradiation or exposed to chemical sprout inhibitor during storage at 10 °C. In preliminary studies we have observed that after-cooking darkening was less in tubers treated with chemical sprout inhibitor Propham or subjected to surface irradiation with electrons and stored at 15 °C, or γ irradiated and stored at 4 °C as compared to γ-irradiated tubers stored at 15 °C.

No correlation seems to exist between the distribution of pyroglutamic acid (PCA) and after-cooking darkening since darkening is more pronounced in the cortical regions while maximum PCA levels were detected in the central pith region. The presence of PCA in uncooked potatoes has been reported as probably or wholly due to the method of sample preparation (Schwartz et al., 1962) and its formation by nonenzymatic cyclization of glutamine is known to be rapid above 65 °C (Shallenberger et al., 1959). However, in the present studies the observed accumulation of PCA in irradiated tubers during storage does not appear to be an artifact of sample preparation since tissues extracted by hot or cold alcohol did not show appreciable differences in the PCA content. Thus the reported values of PCA apparently reflect naturally occurring acid.

The observed pH increase alone cannot explain increased darkening in irradiated tubers. After irradiation and storage, "Hansa" and "Irmgard" tubers had approximately the same pH (Table II) although they differed considerably in their darkening tendency (Table III).

The data on the increased levels of total phenolics in cortex and pith tissues of irradiated potatoes confirm the earlier findings of Thomas and Joshi (1977). Berset and Sandret (1976) reported a persistent increase in the polyphenols and chlorogenic acid all over the tubers irradiated with γ rays and only in cortex when irradiated with the less penetrating electrons. Increased polyphenolic levels alone cannot explain increased darkening: in cortex and pith of stored controls the two cultivars had about the same polyphenolics concentration, while in skin the concentration was even higher in nondarkening "Hansa" (Table V).

The results of the present study suggest that the decrease in the content of citric acid, together with an increase in polyphenols in irradiated tubers provide conditions favorable for the formation of ferrous–phenolics complexes on cooking which on exposure to air turn to the bluish dark ferric–phenolics complexes. In unirradiated tubers of the nondarkening cultivar Hansa, the complex formation is inhibited, probably due to the higher citric acid content which chelates the iron, thus making it less available for the reaction.

ACKNOWLEDGMENT

Paul Thomas thanks the Alexander von Humboldt Foundation, Federal Republic of Germany, for a fellowship for the duration of this study.

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Received for review September 21, 1978. Accepted December 8, 1978.