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AFTER-COOKING DISCOLORATION OF POTATOES:  
POSSIBLE INVOLVEMENT OF POLYPHENOLIC  
CONSTITUENTS

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After-cooking discoloration of potatoes has been a serious problem in several growing areas east of the Mississippi River for more than 20 years. This problem was acute with the 1953 crop but occurred in only rare lots of the 1954 crop. Several hundred samples of potatoes from the 1954 Maine crop were screened by staff members of the Maine Agricultural Experiment Station but little incidence of this type of discoloration was found. Blackening of cooked potatoes was again troublesome with the 1955 crop.

The extensive literature on after-cooking discoloration of potatoes was recently reviewed by E. Yanovsky (22), and will not be considered in detail here. This type of discoloration usually occurs as greying or blackening, in greatest intensity at the stem end just beneath the skin. Flesh of affected tubers typically appears normal in the raw state.

After-cooking discoloration is not only encountered in home and restaurant cooked products but in all types of processed potato products. This discoloration can cause difficulties to processors, particularly in canned potatoes, frozen products, and dehydrated potato products. Caldwell *et al.* (3) reviewed thoroughly the various types of discoloration encountered in the processing and use of dehydrated potatoes.

Water blanching and acid dipping treatments of the raw, peeled stock are of limited value in inhibiting after-cooking discoloration. Sulfiting is of some help but cannot be used with potatoes to be processed with meat, since food-stuffs containing meat must be sulfite-free for shipment in interstate commerce.

In the past 10 years, at least two investigations have been made of a colorless, fluorescent substance extracted from potatoes with acidified alcohol. Lewis and Doty (12) believed this substance to be the precursor of the pigment causing potato blackening, since it was readily converted to a black pigment by heating above 35° C. However, Bowman and Hanning (2) concluded that there was no clear relationship between the extent of fluorescence obtained with this substance and the tendency for potatoes to blacken.

Many possible causes have been suggested for after-cooking discoloration, but few facts have been established and much of the published data are contradictory when compared. Some of the factors proposed as being related to after-cooking discoloration are: potato variety; soil pH and composition; growing conditions; storage conditions; ratio of protein nitrogen to non-protein nitrogen; and the amounts of tyrosinase, iron, phosphorus, and manganese in the tubers.

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If the reaction or reactions responsible for pigment formation can eventually be defined, then discoloration can possibly be prevented by use of certain methods in growing potatoes or by some permissible treatment during processing. One popular theory is that oxidation of polyphenolic compounds, perhaps catalyzed by the presence of iron, forms the pigment of after-cooking discoloration. Smith, Nash, and Dittman (17) stated that *o*-diphenolic compounds upon oxidation may produce the pigment that darkens potatoes. Juul (8) believes that bivalent iron in potato juice combines with *o*-diphenol to give a light-colored substance. Upon exposure to air, oxidation leads to formation of a dark-colored ferric compound.

Juul (8) found that cooking potatoes in a dilute solution of sodium hydrogen pyrophosphate prevented after-cooking discoloration, presumably because of sequestration or chelation of the iron. Smith and co-workers have obtained some success in the use of sequestering and chelating agents to prevent discoloration by applying foliar sprays during growth of the plants (18) and by dipping peeled potatoes that are to be kept at least 24 hours before cooking (6). Cheng and Hanning (4) recently assayed various lots of potatoes for total tannin content, in which study they found chlorogenic acid to be the only polyphenol present in detectable amounts in the flesh. Caffeic acid was found in the skin extract in addition to chlorogenic acid.

Kiermeier and associates (9, 10, 11) have quite recently studied chemical and enzymic changes as possible causes for the discoloration of steamed potatoes. Their data indicated that only a small amount of chlorogenic acid is present in the German potato variety "Ackersegen" in raw tissue but that about 10 times as much is present in steamed potatoes. The findings of these German workers suggest that chlorogenic acid is hard to release physically from raw tissue or is bound in some sort of chemical complex from which it is released during cooking. However, the results that we have on comparison of the chlorogenic acid contents of raw and cooked tissue do not agree with this.

Kiermeier and co-workers (9, 10, 11) found quinic acid present in potatoes but felt that it did not contribute to discoloration. Caffeic acid was present in too low a concentration for them to determine it quantitatively. They believe that bivalent iron, chlorogenic acid and caffeic acid, and oxygen are essential for pigment formation.

Having at our disposal a series of lots of potatoes obtained from different parts of the country and exhibiting varying degrees of blackening after cooking, there were 3 principal objectives in the work reported here:

1. To isolate as many polyphenolic constituents as possible from extracts of the tissue.
2. To search for differences in the number and relative amounts of various polyphenols in blackening tissue as compared with non-blackening or normal tissue.
3. To determine quantitatively the chlorogenic acid contents of potatoes of different blackening tendencies and in various sections of individual tubers.

All lots of blackening potatoes were selected after discoloration had been noted during the winter storage period. No data are presented on possible

changes in the polyphenol content during maturation of the tubers and storage of the mature potatoes.

We have used two-dimensional paper chromatography both to separate the polyphenolic constituents and to obtain data on the amounts of chlorogenic acid present in extracts.

#### EXPERIMENTAL

**Preparation of concentrated extracts.** Twelve samples of potatoes representing various degrees of blackening were examined to determine the number of polyphenols present. They were then assayed for chlorogenic acid. Samples of each lot of potatoes were taken from the stem end, bud end, skin, and whole potato. The stem and bud end samples were obtained by taking a 1/2-inch long by 7/8-inch diameter plug out of each end of the potato with a No. 15 cork borer. The skin samples were prepared by removing the skin with a potato peeling knife. About 1/8-inch of flesh adheres to the skin. The whole potato samples were prepared by taking a representative sample of cooked mash (including skin).

The preponderance of our data was taken on cooked potato samples. However, with 3 lots of blackening potatoes and one control lot, corresponding samples were taken of both cooked and raw tissue for chlorogenic acid assays.

Two methods of sample preparation were used. The first (Method A) is more time consuming, but results in a dry final product for assay. However, the second (Method B) required no drying step, and the final volume of purified concentrate more accurately represented the extract from a given weight of potatoes.

*Method A.* Method A was derived from that described by Johnson and Schaal (7), with modifications to make it quantitative. One hundred grams of the potato fraction to be assayed (stem end, bud end, skin, or whole potato) was steamed for 30 minutes and cooled. It was then ground in a Waring blender<sup>°</sup> for 5 minutes with 300 ml. of hot 95% ethanol. The material was diluted to 2 liters with 70% ethanol. After standing overnight, the mixture was filtered and 1800 ml. of the filtrate was concentrated to about 15 ml. in a rotating evaporator below 40° C. The concentrate was extracted in a separatory funnel with 300 ml. of hydrocarbon solvent (Skellysolve B<sup>°</sup>) to remove waxy and fatty material. The organic solvent in the aqueous layer was boiled off on a steam bath to prevent splashing during subsequent oven drying. A yellow or brown material, which was not removed, floated on top of the aqueous solution after removal of the organic solvent. The solution was then dried in a vacuum oven at slowly elevated temperatures not exceeding 40° C. for 5 hours. When almost dry, the material puffed up to give a friable texture that permitted drying to a moisture-free basis. Although quite hygroscopic, the dried substance could be weighed quickly without any difficulty. In early experiments the dried material was re-extracted with organic solvent, but this was later found to be unnecessary. The powder was stored in a desiccator because of its hygroscopicity. To prepare a batch of the final solution, 300 mg. of the powder was dissolved in 2 ml. of 30% isopropanol and this was allowed to stand in a refrigerator for the settling out of any sediment. Sample aliquots were then either filtered off or removed without shaking the solution. The sediment contained a considerable amount of asparagine in addition to fatty material.

*Method B.* Up to the point of drying the solution in a vacuum oven, *Method B* was the same as *Method A*, except that the material was concentrated down to at least about 8 ml. in the rotating evaporator. After expelling the Skellysolve on the steam bath, the material was not oven-dried, but was poured from its dish into a 25-ml. volumetric flask and the dish was washed with such amounts of water and isopropanol that the washings gave a final solution of about 30% isopropanol. The solution, at first cloudy because of emulsified droplets, was placed in a refrigerator until clear, then filtered or used without shaking.

Method A was developed first, and was used to prepare all the samples except one (57-3, Table 1), but Method B is preferred unless a stable, dried extract is desired for other assays.

<sup>°</sup> Mention of trade names does not imply that products are recommended or endorsed by the United States Department of Agriculture over others not mentioned.

**Paper chromatographic methods.** The most satisfactory solvents tried were those used by Cartwright, Roberts, Flood and Williams (3a) in their study of tea. We used their solvents in the reverse order, i.e., 2% acetic acid in the first direction and butanol, acetic acid, water (4:1:2.2) in the second which gave better results with potato extracts. The descending technique was employed, using a Chromatocab<sup>o</sup> and Whatman No. 1<sup>o</sup> paper, 46 by 57 cm. Large sheets of filter paper were used because substances having high  $R_f$  values in the first solvent were thereby separated sufficiently from the polyphenols so as not to interfere with the development in the butanol-acid system. Two methods of extract preparation were used, as described below. A spot of concentrated extract about 2 cm. in diameter was applied at the starting line and developed in the long direction first. About 7 hours was taken for development in acetic acid as solvent and about 17 hours (overnight) for the second solvent system.

The completed chromatogram was observed under ultraviolet light. Several spots fluoresced blue-white. After exposure of the paper to  $\text{NH}_3$  vapor, other spots appeared under ultraviolet light. The paper was then sprayed with Benedict's solution, which was used by Gage, Douglass, and Wender (5) for flavonoid-type compounds. We found this solution useful for color differentiation of certain types of polyphenols, as outlined in the Results and Discussion section.

Some of the spots obtained on the chromatogram were very faint. In order to concentrate further the polyphenols before regular two-dimensional chromatography, the following scheme was used. An extract of potato peel was applied as a streak across the starting line and the streak developed with 2% acetic acid. Four UV-fluorescent bands were obtained, the fastest running one being discarded since it contained most of the inorganic salts and amino acids. The other 3 bands were eluted separately, the eluates concentrated, applied to large sheets of paper, and chromatograms developed using the two solvent systems mentioned above. The papers were then sprayed with Benedict's solution. The procedure was used for chromatograms representing the fluorescent polyphenols found in potatoes. Tyrosine was not included in consideration of the polyphenols since it is non-fluorescent.

**Chlorogenic acid assay.** The concentrated extracts prepared by either of the two methods were applied to large sheets of Whatman No. 1 filter paper in suitable amounts, about 30 or 40 mcl. for samples extracted from skin and 60 to 80 for the others. As was the case with formation of the complete chromatogram, about 7 hours was required for development with the first solvent and about 17 hours for the second solvent system. Sufficient equilibration time was allowed with each solvent. The papers were not sprayed in the quantitative determination. The main chlorogenic acid spot of the chromatogram was located under ultraviolet light and circled. It is assumed that the quantity of the principal isomer of chlorogenic acid bears a definite relation to the amounts of other isomers present or formed in the experimental procedures and that it is valid to compare relative amounts of chlorogenic acid in different samples of potatoes on this basis. The area of paper comprising the spot was cut into pieces and eluted with 6 ml. of 50% *n*-propanol in a stoppered tube. The color was developed with 2 ml. of a solution containing 1%  $\text{NaNO}_2$  in 1% acetic acid. This reagent was used in 10% acetic acid by Paech and Ruckebrod (14). The stoppered tubes containing the paper were heated at 60° C. for ½ hour, cooled, and the liquid transferred to calibrated tubes. These were read in a spectrophotometer at 445  $\mu$ . The readings were compared with a standard curve prepared according to Ayres (1). The most accurate reading range is between 40 and 300 mcg. of chlorogenic acid. This is, of course, limited by the amount of potato extract which can be applied to the paper without resulting in streaking during subsequent chromatography. Most situations permitted application of 50 to 100 mcg. of chlorogenic acid. Occasionally, however, only about 30 mcg. of chlorogenic acid was obtained from as much as 80 mcl. of potato extract.

The chlorogenic acid sample used for the standard was dried in a vacuum oven at 70° C. before use. It was found to be a hemihydrate, as reported by Moores, McDermott, and Wood (13), after drying to constant weight over  $\text{P}_2\text{O}_5$  at 135° C. The values in Table 1 are reported as anhydrous chlorogenic acid.

**Sample calculation for chlorogenic acid assay of extract prepared by Method A.** Upon extracting 100 g. of potato tissue in 2000 ml. of 70% alcohol, 1800 ml. of the filtered extract was taken for concentrating and preparation of the dry powder. Let us

**TABLE 1**  
Chlorogenic acid contents of potatoes of different varieties,  
sources, and blackening tendencies

Sam- ple	Blackening extent	% Solids	Variety, source	Chlorogenic acid, mg./100 g. fresh tissue			
				Stem	Bud	Skin	Whole
N-2	None	20.5	White Rose, Calif., 1956	12	32	30	8
57-3	V. slight	18.6	Katahdin, Pa., 1956	7	10	42	11
5	Slight	20.0	Rus. Burbank, Mich., 1955	13	23	57	11
4	Slight	21.9	White Rural, Mich., 1955	14	38	95	18
9	Mod.	18.5	Katahdin, Maine, 1955	13	19	42	13
1	Mod.	23.0	Unnamed, <sup>1</sup> N.D., 1955	24	37	167	32
2	Mod.	20.2	Kennebec, Maine, 1955	23	39	50	16
7	Mod. severe	19.6	Var. unknown, <sup>2</sup> Maine, 1955	13	17	35	6
8	Severe	23.2	Katahdin, Pa., 1955	18	26	....	....
3	Severe	21.2	Sebago, Maine, 1955	13	12	32	9
57-1	Severe	22.4	Kennebec, Maine, 1956	13	20	....	....

<sup>1</sup> Obtained from W. G. Hoyman, North Dakota Agr. Expt. Sta., Fargo, N. D.  
<sup>2</sup> Obtained through Maine Agr. Expt. Sta., Orono, Maine.

say that 2.74 g. of dry powder was recovered from the 70% alcohol extract after extraction with Skellysolve B. From this dry powder, 0.300 g. was weighed out and dissolved in 2 ml. of 30% isopropanol. In 50 mcl. pipetted from this concentrated extract 48 mcg. of chlorogenic acid was found. Then, the quantity of chlorogenic acid present in 100 g. of the potato tissue is computed in the following manner:

$$\frac{2000 \times 2.74 \times 2 \times 48 \times 10^{-6}}{1800 \times 0.300 \times 0.050} = 0.02 \text{ g. chlorogenic acid}$$

Data in the tables are given in terms of milligrams of chlorogenic acid per 100 g. of potato tissue.

This calculation disregards the volume occupied by the insoluble material in the 2-liter flask in which the alcoholic extraction was made.

**Determination of total polyphenols.** Total polyphenolic constituents were determined by the method of Rosenblatt and Peluso (16), using standard chlorogenic acid as the basis for expressing total polyphenols. The same powdered extracts were used for the total polyphenols determinations as were employed for the determination of chlorogenic acid by paper chromatography. Spectrophotometric absorption measurements on these colored solutions were made at 650 m $\mu$ .

## RESULTS AND DISCUSSION

**Polyphenolic substances considered individually.** Figure 1 represents diagrammatically a chromatogram of the polyphenolic compounds typically extracted from potato peel by 70% alcohol. Peel extracts were used for observation because the polyphenols are most abundantly present in the skin and the flesh immediately beneath. Six of the spots that occur most commonly in chromatographing the extracts are denoted by underlining the numbers.

In semiquantitative work, the polyphenolic content of potato skin was compared with that of the flesh immediately underneath the skin. The skin of cooked potato was carefully separated manually from the flesh of the layer removed in paring with a potato peeling knife. Chromatographs of the purified, concentrated extracts of these two fractions showed that on the dry basis the concentration of polyphenols in the skin was about twice that in the fleshy tissue.

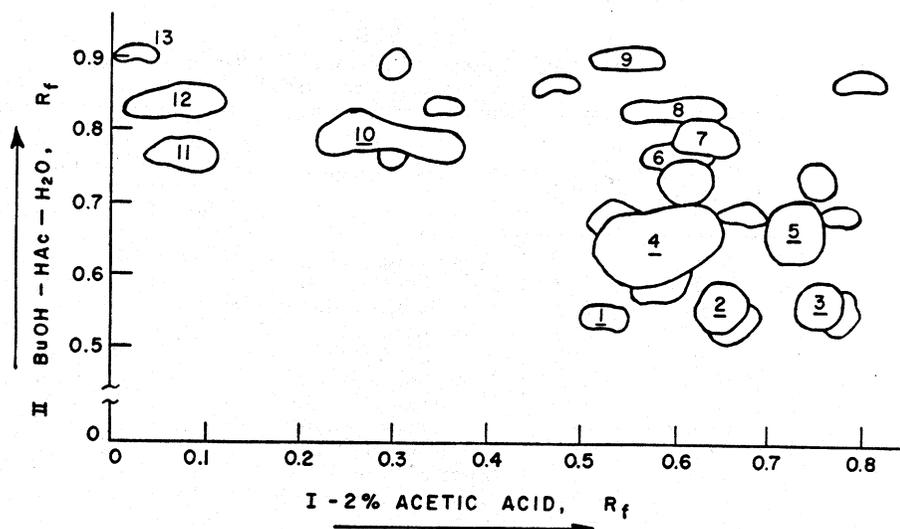


Figure 1. Diagrammatic chromatogram of polyphenolic compounds typically extracted from potato peel by 70% alcohol.

The polyphenolic substances were first separated into three fluorescent bands on paper as indicated under "Paper Chromatographic Methods." These bands, after elution and concentration, were resolved separately by two-dimensional paper chromatography. Figure 1 is a composite schematic representation of these individual chromatograms. The first band (that nearest the acetic acid starting line) yielded, after eluting, concentrating, and chromatographing, the group of spots numbered 11, 12 and 13. These appeared in the position expected of substances concentrated in the first band. Simultaneously, a second group of spots numbered 6, 8 and 9 appeared at a position well beyond that of the first group, as shown in Figure 1. It is believed that 6, 8 and 9 represent isomers of 11, 12 and 13. Williams (20) has expressed the belief that *cis*- and *trans*-isomers of cinnamic acid derivatives exist in equilibrium. Following separation of the cinnamyl isomers by chromatography with 2% acetic acid as solvent, Williams found that a second chromatographic treatment brought about further resolution in which each form yielded a portion of the other isomer. In addition to chlorogenic and caffeic acids, the other polyphenols of the potato may well be of the cinnamyl type, since many of these compounds are known to be present in plants (19, 20).

The second band contained what appeared to be caffeic acid (spot number 10) in the expected position, and three fluorescing spots. There also appeared in the chromatography of the extract from the second band a spot (number 7) believed to be an isomer of caffeic acid. Tentative identification of the caffeic acid isomers is based on the  $R_f$  values of Williams (20) and the color reactions. Benedict's solution can be used to differentiate caffeic acid, chlorogenic acid, and their isomers from the other polyphenolic compounds. Spots formed by the caffeic and chlorogenic acids are yellow in visible light and dark under ultraviolet light, while the other spots do not appear in ordinary sunlight but are blue under ultraviolet.

The third band contained the largest number of polyphenolic compounds of the three bands. Upon chromatographing this band, following elution and concentration of the constituents as done with the other bands, four forms of chlorogenic acid were obtained. Spot number 4 is the principal form of chlorogenic acid and 5 is its isomer. Spot number 2 is neo-chlorogenic acid and 3 is its isomer. These 4 constituents were identified by  $R_f$  values given in the literature (15). A number of blue-fluorescing spots also appeared as well as spot number 1, which may be another form of chlorogenic acid, since it exhibited the same color reactions as the chlorogenic acid group. Spots representing chlorogenic acid isomers were identified by the following color reaction: the paper was exposed to  $\text{NH}_3$  vapor and examined under ultraviolet light for spots colored "duck egg" green (greenish yellow), which is characteristic of the chlorogenic acids (3). Spots having  $R_f$  values below 0.50, in the butanol-acid system, are not shown in Figure 1 because they are either vary faint or not clearly defined. Except for the chlorogenic and caffeic acids, we have not identified up to the present time the other compounds whose spots appear in the chromatogram.

Quinic acid was not given attention in this paper since it is not a phenolic compound. However, its presence in the aqueous alcoholic extracts of potatoes was detected on paper chromatograms, as evidenced by a specific color test (3).

Table 1 gives values of chlorogenic acid found in whole, cooked potato and in various fractions. Confirming the results of others, chlorogenic acid content was found to be always higher in the skin than in the other parts of the potato. This was no more pronounced in potatoes that blacken severely than in those that do not. In any given section of potato, wide variation in chlorogenic acid content was found from lot to lot. Counter to blackening tendency, chlorogenic acid content was greater in the bud than in the stem end. However, this was not true with sample number 3, one that exhibited severe blackening.

Table 2 presents comparable data on the chlorogenic acid contents of bud and stem end sections of raw and cooked potatoes. Determinations to be compared were made on potatoes of a given lot and on the same day. The values obtained before and after cooking were nearly the same.

**TABLE 2**  
Comparison of chlorogenic acid contents of raw and cooked potato tissue

Sample	Blackening extent	Section	Chlorogenic acid expressed as mg./100 g. of fresh potato	
			Raw	Cooked
N-1.....	None	Bud	18	16
		Stem	9	8
7.....	Moderate-severe	Bud	20	17
		Stem	10	13
9.....	Moderate	Bud	19	19
		Stem	12	13

**TABLE 3**  
Precision of the method of determining chlorogenic acid in high and low ranges

Number of replicates	Mg. chlorogenic acid/100 g. fresh potato		
	Range	Mean	Standard deviations <sup>1</sup>
10.....	164-182	172.7	5.3
10.....	34- 38	36.6	1.6

$$^1 \text{ Standard deviation} = \sqrt{\frac{\sum \text{squares of deviations}}{\text{number of replicates} - 1}}$$

The precision in the estimation of chlorogenic acid by the paper chromatographic method is indicated in Table 3.

Standard amounts of chlorogenic acid were added to extracts of potato before the concentrating step in order to check percentage recovery. The recovery of added chlorogenic acid ranged from 88-98%. This indicates a fairly high degree of accuracy in determining the amount of chlorogenic acid present.

**Polyphenolic constituents considered as a group.** In addition to the chlorogenic acid determinations, analyses were made for the total polyphenolic content of extracts of the bud, stem, skin, and whole potato tissue from the same series of potatoes with varying blackening tendencies. Table 4 shows the data obtained on the stem-end sections. These data, as well as those for every sample from the other sections of the tuber and from the whole potato, show that the total polyphenolic content of potatoes, in addition to their constituent chlorogenic acid content, is appreciable. Comparison of Table 4 with Table 1 shows that the ratios of total phenols to chlorogenic acid in the stem ends of the tubers ranged from 5 to 12. In the skins the total polyphenolic content ranged from 2 to 6 times that of the chlorogenic acid content.

In Table 4 no correlation is apparent between total polyphenolic content

**TABLE 4**  
Total polyphenolic contents of stem-end sections of cooked potatoes

Sample	Blackening extent	Total polyphenolics <sup>1</sup> expressed as mg./100 g. of:	
		Fresh tissue	Dry tissue
N-2.....	None	122	595
57-3.....	V. slight	87	468
5.....	Slight	104	520
4.....	Slight	170	777
9.....	Moderate	138	745
1.....	Moderate	107	464
2.....	Moderate	109	540
7.....	Moderate-severe	134	683
8.....	Severe	146	628
3.....	Severe	92	432
57-1.....	Severe	92	411

<sup>1</sup> Determined by Rosenblatt and Peluso method, with chlorogenic acid as standard of reference.

and blackening tendency. As a rule, the stem ends of the potatoes, where blackening is most acute, were not found to contain any more polyphenols than the bud end.

#### SUMMARY AND CONCLUSIONS

Ten lots of potatoes exhibiting after-cooking discoloration were examined in comparison with normal, non-blackening potatoes regarding the possible relationship between the amount of polyphenolic compounds present and the extent of blackening. In two-dimensional chromatography of concentrates prepared from 70% alcohol extracts of potato tissue, about 27 spots were obtained representing substances having certain properties of polyphenols. In agreement with the results of others, the polyphenols were found to be concentrated in the skin and immediately-underlying tissue. However, counter to blackening tendency, the polyphenols were present in no greater amount in the stem end than in the bud end of the tuber. In general, potatoes from blackening lots did not have a greater variety of polyphenols present or a higher concentration of chlorogenic acid than those from nonblackening lots. Chlorogenic acid assays were made on tissue from various sections of the potato, using a paper chromatographic method. Chlorogenic acid is believed to be the principal polyphenol in potatoes, but determinations of total polyphenols by the method of Rosenblatt and Peluso showed that an appreciable fraction of the polyphenols consists of compounds other than chlorogenic acid. Although polyphenols may be involved in the after-cooking discoloration of potatoes, the tendency toward discoloration apparently is not proportional to the concentrations of chlorogenic acid and other polyphenols present.

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