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Photo-induced changes in the concentrations of individual chlorogenic acid isomers in potato (*Solanum tuberosum*) tubers and their complexation with ferric ions

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Summary

Changes in the concentrations of 3-, 4- and 5-caffeoylquinic acids in potato tubers exposed to light have been determined by high-performance liquid chromatography. In the first 24 to 48 hours the observed increases in total chlorogenic acid content was due primarily to an increase in 5-caffeoylquinic acid content but thereafter the rate of accumulation of the other isomers increased gradually. After 24 hours exposure 4-caffeoylquinic acid accounted for 10% of the total chlorogenic acid content of the tubers compared with 33% after 168 hours. The significance of this change in isomeric ratio on the spectral characteristics of potential ferri-chlorogenic acid complexes was investigated in vitro. It was concluded that potato quality, as reflected in the development of after-cooking blackening, was dependent on total chlorogenic acid content and was unaffected by the relative concentrations of the individual isomers.

Introduction

Quinic acid contains three readily accessible hydroxyl groups which may form depside bonds with the carboxyl group of caffeic acid resulting in the formation of three positional isomers, namely chlorogenic acid, kryptochlorogenic acid and neochlorogenic acid, more correctly referred to under current IUPAC convention as 5-, 4- and 3-caffeoylquinic acid, respectively (Clifford, 1985). All three isomers have been found in various plant families (Molgaard & Ravn, 1988), but many of the methodologies commonly used for chlorogenic acid quantification do not differentiate between the isomers and consequently the values given refer to total chlorogenic acids rather than 5-caffeoylquinic acid specifically.

The role of chlorogenic acids within the plant has not been fully elucidated but in some species these phenolic compounds may be involved in defensive strategies against attack by insects (Stevenson et al., 1993) or phytopathogens (Ghanekar et al., 1984). In vitro experiments have shown that chlorogenic acid may damage chromosomes and after oxidation by phenol oxidase the resulting quinone may react with amino acids thus reducing nutritional value as well as producing potentially toxic compounds (Dao & Friedman, 1992).

Chlorogenic acids have been shown to account for up to 90% of the total phenolic compounds present in potato tubers (Malmbergand & Theander, 1985), with 5-caffeoylquinic acid having been identified as the predominant isomer (Brandl & Herrman, 1984). The ability of these esters to form complexes with ferric ions during

the cooking process has been shown to be the primary cause of the development of after-cooking blackening (ACB) in susceptible potato cultivars (Hughes & Swain, 1962a,b). Although high concentrations of citric acid may reduce the amount of the iron-chlorogenic acid complex formed by acting as an alternative ligand for the ferric ions present, a good correlation between total chlorogenic acid content of potato tubers and visual assessment of ACB has been demonstrated (Griffiths et al., 1992). The development of ACB is believed to be of little nutritional significance and indeed may reduce any potential deleterious effects of free chlorogenic acid. However, environmental and/or genetic factors leading to high levels of chlorogenic acid and subsequently considerable discoloration of potato tubers post-cooking are clearly undesirable and are generally unacceptable to the consumer.

Early chromatographic studies (Hasegawa et al., 1966) have shown that storage of potato tubers particularly at temperatures below 5 °C results in a significant increase in chlorogenic acid content. The magnitude of the observed increase appears to be dependent on the physiological state of the tubers at harvest with immature potatoes showing the greatest increases (Leja, 1989). The effect of storage on the chlorogenic acid content of tubers from some 20 cultivars was studied by Griffiths et al. (1995) who using a non-specific quantitative assay confirmed earlier observations and also demonstrated that exposure to light resulted in a cultivar-dependent response. In three of the six cultivars studied, total chlorogenic acid increased almost linearly over 168 hours, whilst in the other cultivars a rapid increase in chlorogenic acid content was seen in the first 48 hours but thereafter further exposure to light produced only minor increases.

The objective of this study was a) to confirm that the increases in chlorogenic acid concentration produced in response to light exposure were due to an increase in chlorogenic acids rather than to other *ortho* dihydroxy phenols, which could also react with the non-specific reagents used in the initial study (Griffiths et al., 1995), b) to determine the relative effect of light on the concentration of the three positional isomers of chlorogenic acid and c) to assess in vitro whether changes in the proportion of the three isomers affected their potential for the development of after cooking blackening.

Materials and methods

Plant material. The potato tubers from the six named cultivars used in this study were grown in a field trial at Mylnefield (Dundee, UK), which has a Carpow soil described as a free draining sandy loam. The trial was planted in April 1991, subjected to normal agronomic practices and harvested at the end of September. Prior to the commencement of the light exposure experiment the tubers were stored at ambient humidity (c. 85–95% rh) in the dark at temperatures of 6–8 °C.

The tubers were exposed to light as outlined in Griffiths et al. (1995) and immediately after sampling, diced, immersed in liquid nitrogen and freeze dried. The samples were then ground in a mill (Glen Creston Ltd., Stanmore, UK) fitted with a 0.5 mm sieve and stored at -20 °C until required for analysis.

Chemical analysis. Total chlorogenic acid content of the freeze-dried tubers was determined by the sodium nitrite method (Griffiths et al., 1992) and the results expressed as mg per 100 g freeze-dried matter (mg 100 g^{-1} FDM). The freeze dried samples contained on average 95.6 (±1.07)% dry matter as determined by placing 1 g of sample in an oven at 105 °C for 18 h. Consequently the approximate values in terms of mg per 100 g dried matter (mg 100 g^{-1} DM) can be calculated by using a factor of 1.05 (i.e. mg 100 g^{-1} DM=1.05xmg 100 g^{-1} FDM).

The concentration of the individual isomers was determined by a high-performance liquid chromatographic (HPLC) method based on that of Brandl & Herrmann (1984). The identities of the peaks corresponding to the individual chlorogenic isomers were determined as outlined in Fernandes et al. (1996).

The response factor for 5-caffeoylquinic acid (5-CQA) was determined using standard solutions (Sigma Ltd., Poole, UK) and the same factor was then used for all three isomers. The validity of this was checked using a partially isomerised mixture (see below) whose total chlorogenic acid concentration was determined by two colorimetric methods which both depend on the presence of *ortho*-dihydroxy phenol groups, namely the sodium nitrite method (Griffiths et al., 1992) and the molybdate method (Mapson et al., 1963). The concentration of total chlorogenic acid obtained by these methods were 279 and 286 μg ml^{-1}, respectively, and they were in close agreement with the value of 280 μg ml^{-1} obtained by hlpc when the individual concentrations of the three constituent isomers (5-CQA: 196; 4-CQA: 52 and 3-CQA: 32 μg ml^{-1}), calculated using the same response factor for all three isomers, were summed.

For HPLC analysis the chlorogenic acids were extracted from the milled freeze-dried tubers using 0.1% hydrochloric acid-methanol as outlined by Malmberg & Theander (1984).

Iron complexation studies. Isomeric mixtures of the chlorogenic acids were prepared by dissolving 5-caffeoylquinic acid in 0.2 M tris maleate buffer adjusted to pH 5.5 or pH 6.0 with 0.2 M sodium hydroxide and heating at 90 °C for 30 mins. The resulting mixture was then suitably diluted with the appropriate buffer to give a final concentration of approximately 0.8 μmoles ml^{-1}.

The ferric ion solution was prepared daily by dissolving 0.482 g of ferric ammonium sulphate dodecahydrate (Sigma Ltd, Poole, UK) in 100 ml distilled water, a 10 ml aliquot was then made up to 100 ml with the appropriate tris maleate buffer to give a final concentration of 1 μmoles ml^{-1}. This stock solution was further diluted as required.

Absorbance values and spectra were recorded using a PU8800 UV/Vis spectrophotometer (Pye Unicam Ltd, Cambridge, UK). All samples were placed in 1 cm cuvettes and read against appropriate blank solutions.

Statistical analysis. Analysis of variance (ANOVA), correlation coefficients and linear regressions were calculated as outlined by Snedecor (1955).

Results and discussion

Total chlorogenic acid content. Previous studies (Griffiths et al., 1995) have shown that the total chlorogenic acid content, as determined by the sodium nitrite method, of potato tubers increased on exposure to light. As the reagent used was specific only with respect to the fact that it reacted to the presence of *ortho*-dihydroxy phenols the same material was re-analysed by HPLC and the total chlorogenic acid content determined by summing the values obtained for each chlorogenic acid isomer (Tables 1 and 2) Comparison of the results (Table 1) indicated good agreement between the results originally obtained by the sodium nitrite method and by HPLC indicating that

Table 1. The total chlorogenic acid content (mg 100 g^{-1} FDM) of light exposed tubers from two potato cultivars as determined by high performance liquid chromatography and the sodium nitrite method.

Period of light exposure (hours)	Cultivars			
	Eden		Torridon	
	HPLC	$NaNO_2$	HPLC	$NaNO_2$
8	44	47	107	92
24	82	69	142	111
48	84	85	189	156
96	96	87	229	188
168	81	100	286	268
Sed	7.2	3.7	5.3	11.2

Sed = Standard error of difference

Table 2. The total chlorogenic acid content (mg 100 g^{-1} FDM) of tubers from six potato cultivars exposed to light for periods of up to 168 hours.

Cultivar	Period of exposure (hours)						Mean
	0	8	24	48	96	168	
Ailsa	53	58	87	114	125	116	92
Brodick	76	97	141	191	222	308	172
Eden	42	44	82	84	96	81	71
P. Dell	73	98	151	165	234	217	156
Shula	77	77	157	196	202	206	152
Torridon	113	107	142	189	229	286	177
Mean	72	80	127	156	184	202	
Sed	5.3						5.3

Sed = Standard error of difference

the previously reported increases were indeed due to an increase in total chlorogenic acid content rather than to an increase in any other *ortho*-dihydroxy phenolic compounds. As shown in Table 2 total chlorogenic acid content as determined by HPLC increased rapidly in all six cultivars over the first 48 hours. Thereafter, further exposure to light resulted in only minor increases in total chlorogenic acid content of tubers from three of the cultivars namely Ailsa, Eden, and Shula, whilst in the other three cultivars, Brodick, Pentland Dell (P. Dell) and Torridon, total chlorogenic acid content continued to increase rapidly until the termination of the experiment after 168 hours of light exposure.

Chlorogenic acid isomers. Previous studies of the chlorogenic acid content of ten potato cultivars (Brandl & Herrmann, 1984) revealed the presence of three chlorogenic acid isomers (5-caffeoylquinic acid, 4-caffeoylquinic acid and 3-caffeoylquinic acid) in the tubers. In all cultivars the predominant isomer was under current IUPAC convention 5-caffeoylquinic acid, which on average accounted for 69% of the total chlorogenic acid present, whilst the 4- and 3-caffeoylquinic acids accounted for 19 and 12% of the total respectively. In this study in un-illuminated potatoes (Table 3) the predominant isomer was also found to be 5-caffeoylquinic acid which on average accounted for 85% of the total chlorogenic acid present in the tubers but in contrast to the work of Brandl & Herrman (1984) no 3-caffeoylquinic acid was detected in quantifiable amounts in any of the six cultivars studied.

The effect of light exposure on the concentration of the individual isomers was similar for all cultivars (Table 3). The concentration of 5-caffeoylquinic acid increased rapidly in the first 24 to 48 hours but thereafter the rate of accumulation of this isomer declined in all six cultivars. In contrast the rate of accumulation of 4-caffeoylquinic acid was low during the first 24 hours of light exposure but subsequently its rate of accumulation increased. This was reflected in the fact that meaned over all cultivars, 4-caffeoylquinic acid accounted for 10% of the total chlorogenic acid content of the tubers after 24 hours exposure as compared with 33% after 168 hours. Quantifiable amounts of 3-caffeoylquinic acid were detected in all cultivars after 8 hours exposure to light. In comparison with the other two isomers its rate of accumulation was low, only increasing by 2 mg 100 g^{-1} FDM as the time of exposure increased from 8 to 96 hours, however after 168 hours its concentration had increased to an average value of 7 mg 100 g^{-1} FDM accounting for over 3% of the total chlorogenic acid content of the tubers as compared with 2% at the earlier sampling times.

From the experiment reported here it is not possible to determine whether the increase in the relative amounts of the three isomers results from enzymatic conversion, direct synthesis or chemical isomerisation. Consequently in order to determine whether the major mechanism was chemical isomerisation, a buffered solutions of 5-caffeoylquinic acid were placed under identical conditions to that experienced by the tubers and analysed chromatographically after fixed periods of time (Table 4).

No isomerisation could be detected under acidic conditions (pH 3.1), but at pH 5.8, which coincided with the pH of reconstituted potatoes, a small but significant amount

Table 3. The effect of light exposure on 3-, 4- and 5-caffeoylquinic acid content (mg 100 g^{-1} FDM) of tubers from six potato cultivars.

Cultivar	Period of exposure (hours)						Mean
	0	8	24	48	96	168	
a) 5-Caffeoylquinic acid							
Ailsa	46	53	76	96	94	78	74
Brodick	66	83	121	153	149	186	126
Eden	33	35	69	66	71	56	55
P. Dell	60	83	131	135	173	143	121
Shula	65	72	143	161	154	133	121
Torridon	100	96	126	155	170	182	138
Mean	61	70	111	127	135	129	
Sed	4.3						4.3
b) 4-Caffeoylquinic acid							
Ailsa	8	5	9	17	28	38	17
Brodick	10	12	18	35	69	113	43
Eden	9	8	10	15	22	23	14
P. Dell	13	12	17	27	55	71	32
Shula	12	6	11	35	48	67	30
Torridon	15	10	14	34	55	95	37
Mean	11	9	13	27	46	68	
Sed	2.0						2.0
c) 3-Caffeoylquinic acid							
Ailsa	nd	1	2	3	3	5	2
Brodick	nd	2	2	2	5	10	3
Eden	nd	2	3	3	3	3	2
P. Dell	nd	4	4	4	6	8	4
Shula	nd	1	1	2	2	5	2
Torridon	nd	2	3	3	4	10	4
Mean		2	2	3	4	7	
Sed	0.4						0.4

nd = not detected
Sed = standard error of difference

(6%) of 4-caffeoylquinic acid was detected after 168 hours. This was however significantly less than was detected in any of the six cultivars studied. At pH 7.0 all three isomers were detected after only 48 hours, suggesting that at higher physiological pH values chemical isomerisation may be important. However a higher

Table 4. The effect of pH and length of light exposure on the isomerisation of 5-caffeoylquinic acid (CQA) solutions

Light exposure (hours)	Isomers (g 100 g^{-1} total chlorogenic acid)								
	pH 3.1			pH 5.8			pH 7.0		
	5-CQA	4-CQA	3CQA	5-CQA	4-CQA	3CQA	5-CQA	4-CQA	3CQA
0	100	0	0	100	0	0	100	0	0
24	100	0	0	100	0	0	100	0	0
48	100	0	0	100	0	0	71	18	11
72	100	0	0	100	0	0	64	20	16
168	100	0	0	94	6	0	44	26	30

concentration of 3-caffeoylquinic acid relative to that of 4-caffeoylquinic acid would be expected in the light-exposed tubers if this was the major process leading to their formation in vivo.

Effect of isomerisation on iron complex formation. In order to assess the potential effects of changing the relative concentrations of the individual chlorogenic acid isomers on the development of after cooking blackening, a series of experiments were undertaken in vitro to compare the intensities of the ferric ion complexes formed with 5-caffeoylquinic acid with those produced in the presence of all three isomers. The total chlorogenic acid concentration and the relative proportions of each of the individual isomers present in the solutions used are given in Table 5.

The absorbance spectra of the resulting complexes formed in the presence of excess ferric ions (0.4 μM chlorogenic acid: 0.5 μM Fe^{3+}) were compared from 450 to 700 nm. Increasing the pH from 5.5 to 6.0 resulted in an increased intensity at all wavelengths and a shift in the maximum absorbance(λ_{max}) from 625 to 600 nm. These results were in good agreement with those previously published by Hughes & Swain (1962b) who in a similar study using 5-caffeoylquinic acid reported λ_{max} values of 620 and 600 nm for ferri-chlorogenic acid complexes at pH 5.5 and 6.0 respectively.

Table 5. The effect of heat and pH on the isomerisation of 5-caffeoylquinic acid (CQA) solutions.

pH	Temperature (°C)	Total[1] (μg ml^{-1})	Isomeric ratio (g 100 g^{-1} total chlorogenic acid)		
			5-CQA	4-CQA	3-CQA
5.5	24	288	100	0	0
	90	279	70	19	11
6.0	24	282	100	0	0
	90	261	53	26	21

[1] = Total chlorogenic acid concentration

Comparison of the spectra at both pH 5.5 and 6.0 of ferric complexes produced by the mixture of isomers with that produced by 5-caffeoylquinic acid alone revealed no significant difference in the λ_{max} and the slightly lower intensity (less than 10%, e.g. see results Table 6) observed in the complexes produced in the presence of all three isomers could be almost completely accounted for by the slightly lower total chlorogenic acid content (Table 5) of the isomerised solutions.

In order to determine whether the presence of the three isomers resulted in any change in the ratio of iron to organic ligand in the ferri-chlorogenic acid complexes formed, the effect of Fe^{3+} concentration on absorbance was determined. The results (Table 6) did not reveal the presence of major quantities of either the di or tri chlorogenic acid complexes which would have resulted in a reduced response to increasing Fe^{3+} concentration when the molar ratio of ferric ions to chlorogenic acids fell below 1:2 and 1:3 respectively. At both pH 5.5 and 6.0 the complexes formed by both 5-caffeoylquinic acid and the isomerised mixture were similar and a linear response to increasing ferric ion concentration was found as the molar ratio decreased from 1:8 to 1:1. The consistently lower absorbance values seen in the ferri-chlorogenic acid complexes formed by the isomerised solutions were again compatible with the difference in total chlorogenic acid contents of the solutions.

It can therefore be concluded that the development of after cooking blackening in potato tubers will be dependant on total chlorogenic acid content rather than the relative concentrations of the individual isomers. However it is possible that the activities of the three isomers may differ with respect to their effects on potential pathogens and clearly further studies are required to compare their effectiveness as plant defence compounds.

Table 6. The effect of pH, degree of isomerisation and ferric ion concentration on the absorbance (580 nm) of the ferri-chlorogenic acid complex.

Fe^{3+} Conc ($\mu M\ ml^{-1}$)	Ratio Fe:CA[a]	pH 5.5		pH 6.0	
		Control[1]	Isomerised[2]	Control[1]	Isomerised[2]
0.05	1:8.0	0.218	0.203	0.291	0.273
0.10	1:4.0	0.319	0.298	0.445	0.413
0.15	1:2.7	0.410	0.388	0.565	0.528
0.20	1:2.0	0.493	0.457	0.655	0.597
0.25	1:1.6	0.561	0.516	0.718	0.654
0.30	1:1.3	0.616	0.570	0.769	0.699
0.40	1:1.0	0.698	0.650	0.825	0.755
0.50	1:0.8	0.757	0.708	0.861	0.794
Sed		0.0027			
Mean		0.509	0.473	0.641	0.589
Sed		0.0010			

Sed = Standard error of difference; [a] = Molar ratio of ferric ions to total chlorogenic acid
[1] = 5-Caffeoylquinic acid; [2] = Ratio of isomers as in Table 5 (90°C)

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References

Brandl, W. & K. Herrmann, 1984. Über das Vorkommen der Chlorogensäuren in der Kartoffel. *Zeitschrift für Lebensmittel Untersuchung und Forsch*ung 178: 192–194.

Clifford, M.N., 1985. Chlorogenic acids in coffee. In: R.J. Clarke & R. Macrae (Eds), Coffee (Vol. I: Chemistry). Elsevier Applied Science, London, pp. 153–202.

Dao, L. & M. Friedman, 1992. Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. *Journal of Agricultural and Food Chem*istry 40: 2152–2156.

Fernandes, J.B., D.W. Griffiths, H. Bain & F.A.N. Fernandes, 1996. The development and evaluation of capillary electrophoretic methods for the determination of the major phenolic constituents of potato tubers. *Phytochemical Analysis* 7: 253–258.

Ghanekar, A.S., S.R. Padwal-Desai & G.B. Nadkarni, 1984. The involvements of phenolics and phytoalexins in resistance of potatoes to soft rot. *Potato Research* 27: 1189–1199.

Griffiths, D.W., H. Bain & M.F.B. Dale, 1992. The development of a rapid colorimetric method for the determination of chlorogenic acid in freeze dried potato tubers. *Journal of the Science of Food and Agriculture* 58: 41–48.

Griffiths, D.W., H. Bain & M.F.B.Dale, 1995. Photo-induced changes in the total chlorogenic acid content of potato (*Solanum tuberosum*) tubers. *Journal of the Science of Food and Agriculture* 68: 105–110.

Hasegawa, S., R.M. Johnson & W.A. Gould, 1966. Effect of cold storage on chlorogenic acid content of potatoes. *Journal of Agricultural and Food Chem*istry 14: 165–169.

Hughes, J.C. & T. Swain, 1962a. After cooking blackening in potatoes II. Core experiments. *Journal of the Science of Food and Agriculture* 13: 229–236.

Hughes, J.C. & T. Swain, 1962b. After cooking blackening in potatoes III. Examination of the interactions of factors by in vitro experiments. *Journal of the Science of Food and Agriculture* 13: 358–363.

Leja, M., 1989. Chlorogenic acid as the main phenolic compound of mature and immature potato tubers stored at low and high temperature. *Acta Physiologiae Plantarum* 11: 201–206.

Malmberg, A. & O. Theander, 1984. Analysis of chlorogenic acid, coumarins and feruloylputrescine in different parts of potato tubers infected with *Phoma*. *Swedish Journal of Agricultural Research* 14: 63–70.

Malmberg, A.G. & O. Theander, 1985. Determination of chlorogenic acid in potato tubers. *Journal of Agricultural and Food Chem*istry 33: 549–551.

Mapson, L.W., T. Swain & A.W. Tomalin, 1963. Influence of variety, cultural conditions and temperature of storage on enzymic browning in potato tubers. *Journal of the Science of Food and Agriculture* 14: 673–684.

Molgaard, P. & H. Ravn, 1988. Evolutionary aspects of caffeoyl esters distribution in dicotyledons. *Phytochemistry* 27: 2411–2421.

Snedecor, G.W., 1955. Statistical Methods Applied to Experiments in Agriculture and Botany. Iowa State College Press, Ames IA, USA.

Stevenson, P.C., J.C. Anderson, W.M. Blaney & M.S.J. Simmonds, 1993. Developmental inhibition of *Spodoptera litura* (Fab.) larvae by a caffeoylquinic acid from wild ground nut, *Arachis paraguariensis* (Chod and Hassl.). *Journal of Chemical Ecology* 19: 2917–2933.