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# Green colour development in potato cooking water

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The aim of this study was to determine the cause of green colour development in water used for cooking new potatoes. Evidence, from studies on rates of pigment formation, on spectral changes and on gel filtration chromatography, strongly favours chlorogenic acid and amino acids being involved. In particular, the reaction between chlorogenic acid and glutamine that occurs under mildly alkaline conditions, like those of the potato cooking water, led to a mixture of blue-green and brown pigments with properties similar to the green water colouring matter. Chlorogenic acid and asparagine gave a pigment mixture spectrally similar to the glutamine-derived pigment and may also contribute to the green cooking water colour.

## INTRODUCTION

In recent years there have been several incidents of green colour development in water used for cooking new potatoes. These have not been confined to specific potato cultivars or growing sites, nor have they been associated with the type of cooking vessel, whether stainless steel, aluminium or glass. The raw potatoes did not have green skins, and after cooking, little green coloration of the flesh was observed. Storage of the potatoes prior to cooking led to a marked reduction in the extent of green colour formation. Concern that the green colouring matter may have toxic properties has led to the present study into the chemistry of its formation.

Ferric ion and chlorogenic acid can form a green complex in potatoes, though only at pH ≤ 5·5 (Hughes and Swain, 1962). A grey-blue complex is formed at pH 6·5–7·5 which is the main cause of after-cooking-blackening. At the pH of the green cooking water (pH 8–9), the ferric–chlorogenic acid complex is brown. In the absence of metals, chlorogenic acid has been reported to cause green discolorations in sunflower kernels, sweet potatoes and fried burdock due to its reaction with amino acids (Matsui, 1981). As this occurs under mildly alkaline conditions, it may be a plausible explanation for the green colour development in potato cooking water. Pierpoint (1982) has reported chlorogenoquinone derivatives of proteins that are blue-green above pH 10·5. He has suggested that all such chlorogenoquinone-modified amino-containing compounds with absorption bands near 680 nm should be named 'allagochromes'.



The main objective of this study was to provide evidence for the cause of the green discoloration in potato cooking water by comparing some spectral and chromatographic properties of the green water with model systems of chlorogenic acid and amino acids.

## MATERIALS AND METHODS

Potatoes found to exhibit green colour development in the cooking water were harvested from a local Chipping Campden garden. Crystalline chlorogenic acid, L-glutamine and DL- asparagine, and Sephadex G-25 (superfine) were purchased from Sigma Chemical Company Ltd. All other reagents were of analytical grade and were purchased from Merck Ltd.

### Potato cooking

Potatoes were scrubbed clean or scraped and 2–5 whole tubers covered with tap water in a 1 litre glass beaker. The water was brought to the boil using a Bunsen burner and held at boiling point for 15 min. The potatoes were drained off immediately and the cooking water hot-filtered through Whatman No. 541. After standing several hours at ambient temperature, water samples were held at 4°C to complete the green colour development and then frozen for subsequent freeze-drying.

In order to study the effect of pH on pigment formation, 20 ml aliquots of cooking water were sampled immediately after the cooking process, followed by rapid cooling and freezing. After 2 weeks frozen storage, the samples were thawed and their pH values

adjusted with HCl or NaOH. The rate of increase in absorbance at 680 nm was then measured at 30°C.

### Model systems

Solutions of chlorogenic acid, glutamine and asparagine (1 mM) were made up in tap water. Chlorogenic acid was mixed with each individual amino acid in equal proportions and the mixture boiled for 15 min. A control was prepared by boiling chlorogenic acid with an equal volume of tap water. After allowing the solutions to cool at ambient temperature, they were held at 4°C and then frozen and freeze-dried.

In order to study the effect of pH on the rate of green pigment formation in chlorogenic acid–glutamine model systems, 20 ml aliquots were sampled immediately after boiling, and then rapidly cooled and frozen. The samples were thawed after 1 day frozen storage and the pH adjusted with HCl or NaOH. The rate of increase in absorbance at 680 nm was then measured at 30°C.

### Spectra measurement

All spectra and rates of absorbance increase were measured using a Pye Unicam PU 8800 double beam spectrophotometer.

### Gel filtration chromatography

A 1 cm diameter glass column was filled to a height of 28 cm with either Sephadex G-10 or Sephadex G-25 (superfine). The Sephadex columns were equilibrated with 0·1 M $Na_2CO_3$–$NaHCO_3$ buffer (pH 9·0). The following samples were applied to each column and eluted at 0·5 ml $min^{-1}$ with $Na_2CO_3$–$NaHCO_3$ buffer:

1. Freeze-dried potato cooking water (20 mg) dissolved in 0·2 ml $Na_2CO_3$–$NaHCO_3$ buffer.
2. Freeze-dried chlorogenic acid-glutamine model system (5 mg) in 0·2 ml $Na_2CO_3$–$NaHCO_3$ buffer, with 0·02 ml glycerol added to increase sample density.
3. Blue Dextran (5 mg) in 0·2 ml $Na_2CO_3$–$NaHCO_3$ buffer with 0·02 ml glycerol added.

## RESULTS AND DISCUSSION

### Green colour formation in potato cooking water

The potato cooking water changed from colourless to lemon yellow on coming to the boil and then to yellow/green as cooking time increased. On removing the heat source, the cooking water gradually turned greener and after several hours standing the water was green with no hint of yellow. The green colour became more intense on holding the cooking water for 24 h at 4°C. On complete colour formation, the visible spectrum showed a strong absorbance maximum at 680 nm (Fig. 1).

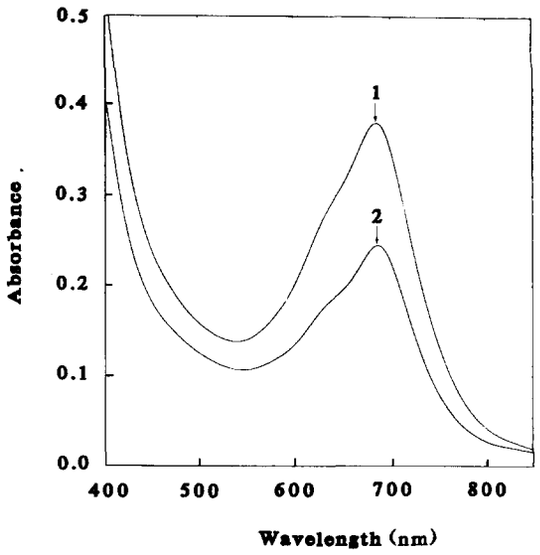


**Fig. 1.** Visible light spectra of green cooking water and chlorogenic acid–glutamine model system, 1 cm light-path. Freeze-dried preparations in $Na_2CO_3$–$NaHCO_3$, pH 9·0 (1, green cooking water (2 mg/ml); 2, model system (0·4 mg/ml)).

Raw potato to tap water ratios in the range 0·2–0·5 g/ml gave rise to the green discoloration. The initial pH of the water before cooking the potato was 7·7 rising to 8·1–9·1 after cooking and cooling. Expulsion of carbon dioxide on boiling the water was presumably the cause of the pH increase, the final pH value depending on the potato to water ratio. Only cooking water with a pH ≥ 8·5 at ambient temperature turned green.

Cooking potatoes in distilled water, initially at pH 5·8, gave no colour change either during cooking or on cooling and holding the cooking water. The final pH value in this case had risen to only 6·3.

### Green colour formation in model systems

Chlorogenic acid gave a yellow colour on dissolving in buffer at pH 8–9 under aerobic conditions or on heating in tap water, which resembled the colour initially formed on cooking the potatoes. This is expected as most of the chlorogenic acid is concentrated in the outer layers of potatoes (van Es and Hartmans, 1987), implying that it would be easily released into the cooking water. Under mildly alkaline conditions, chlorogenic acid is known to react with various amines and amino acids to give green pigments (Horikawa, 1979; Matsui, 1981). Model experiments were therefore carried out which involved boiling chlorogenic acid solutions with the major potato amino acids, glutamine and asparagine. The final level of chlorogenic acid in the model system (0·5 mM) was selected to be somewhat higher than the highest published levels for potato (0·4 mM; Griffiths *et al.*, 1992) in order to test whether the green colour development was due to excessive chlorogenic acid biosynthesis. An equimolar level of amino acid was chosen although the levels in mature potatoes are known to be much higher than this (van Es and Hartmans, 1987).

Green colours in the model systems followed a

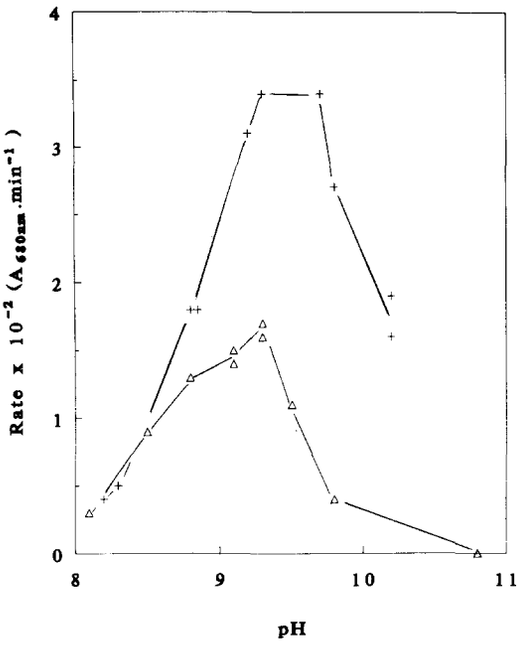


**Fig. 2.** The effect of pH on the rate of absorption increase at 680 nm, (+, green cooking water; Δ, chlorogenic acid and glutamine).

similar pattern of colour development to that in potato cooking water. The chlorogenic acid–glutamine model system had a strong absorption at 680 nm, the spectrum matching very closely the potato cooking water spectrum in the visible region (Fig. 1). Similarly, the chlorogenic acid–asparagine spectrum showed a maximum at 680 nm. Boiling chlorogenic acid alone gave a yellow-brown colour with no absorption peaks in the visible region. The final pH of the tap water-based model systems was 8·9(±0·1).

Further evidence for the cause of the green colour formation on cooking new potatoes was obtained by comparing rates of absorbance increase, spectral and colour properties, and gel chromatographic behaviour of the green cooking water with the chlorogenic acid–amino acid model systems.

**Table 1. Variation of cooking water and model system colours with pH value**

| Sample [a] | pH | $A_{680\ nm}/A_{540\ nm}$ | Colour |
|---|---|---|---|
| Cooking water | 9·0 | 2·7 | Green |
| Model system | | 2·9 | |
| Cooking water | 7·0 | 2·4 | Green |
| Model system | | 2·8 | |
| Cooking water | 5·0 | 1·7 | Light-green |
| Model system | | 1·8 | |
| Cooking water | 3·0 | 0·4 | Yellow-brown |
| Model system | | 0·3 | |
| Cooking water | 1·0 | 0·2 | Red-brown |
| Model system | | 0·1 | |

[a] Cooking water: freeze-dried preparation.
Model system: boiled mixture of chlorogenic acid and glutamine.

**pH effect on rate of green pigment formation**

The rate of increase in absorbance at 680 nm reached a maximum for both the potato cooking water and the chlorogenic acid–glutamine model system at approximately pH 9·3 (Fig. 2). The observation of a maximum suggests that the chromophore is produced in a reaction between oppositely charged species whose relative charges are influenced by pH. The phenolate form of chlorogenic acid (possibly as the chlorogenoquinone carbanion) and the amino acid zwitterion are feasibly the reactive species. From the p$K$ of the more acidic phenolic hydroxyl group of chlorogenic acid (8·5; Timberlake, 1959) and the p$K$ of the alpha amino group of glutamine (9·1; Dawson *et al.*, 1986), a maximum rate of chromophore production would be expected to occur between pH 7·5 and 10·1. Below pH 7·5, the level of chlorogenoquinone carbanion would be rate-limiting, whilst above pH 10·1 the zwitterion level would be significantly lower, thereby reducing the reaction rate.

**pH effect on spectrum and colour**

The potato cooking water spectrum changed on pH modification in a similar way to the spectrum of boiled solutions of chlorogenic acid and glutamine, with close agreement between maximum to minimum absorbance ratios (see Table 1). The colour of the cooking water and model system also varied with pH in a closely similar fashion; the change to red at low pH appears to be typical of the 'allagochromes' defined by Pierpoint (1982). Lowering the pH to 7 with ascorbic acid caused both cooking water and model system to turn yellow, indicating reduction of the chromophores. Bubbling air through the solutions gave a rapid return of green coloration.

The similar spectral and colour changes which occur on pH modification strongly suggest that the formation of pigments from chlorogenic acid and glutamine make a major contribution to the green colouring matter in potato cooking water.

**Gel filtration chromatography**

Application of either the potato cooking water or the chlorogenic acid–glutamine model system to a column of Sephadex G-10 gave a single green band that eluted in the void volume (13 ml), indicating that the pigments have molecular weights exceeding the exclusion limit of 700.

In contrast, Sephadex G-25 chromatography resulted in the separation of two coloured bands; the first, eluting in the void volume, was brown (nominal molecular weight $\geq 5 \times 10^3$) whilst the second, incompletely resolved from the first, was blue-green (nominal molecular weight $1–5 \times 10^3$) (Fig. 3). The spectra of the brown fractions contained no absorption peaks in the visible region, only inflexions at 600–700 nm, whereas the blue-green fractions from both the cooking water

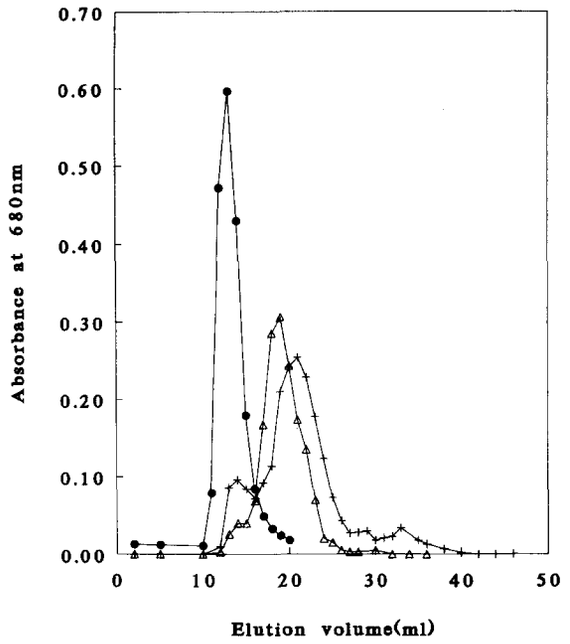


**Fig. 3.** Gel filtration chromatography of green cooking water and chlorogenic acid—glutamine model system (+, green cooking water; Δ, model system; ● Blue Dextran).

and model system samples gave well-defined absorption peaks at 680 nm. Acidification with hydrochloric acid to pH ≤ 1 caused no significant change of colour in the brown fractions whilst the blue-green fractions turned purple with accompanying shifts in absorption maxima to 512 nm. Ascorbic acid addition caused no colour loss in the brown fractions but almost complete decoloration of the blue-green fractions

Despite differences in their proposed pigment structures, both Horikawa (1979) and Pierpoint (1982) suggest that 2 mole of polyphenol react with 1 mole of amino acid. This would lead to a chlorogenic acid–glutamine adduct of molecular weight 855 which is consistent with the behaviour of the blue-green pigment on Sephadex G-10 and G-25. The slightly greater elution volume of the blue-green pigment from the cooking water compared with that from the model system may be due to the presence of significant amounts of the chlorogenic acid-asparagine pigment with an adduct molecular weight of 841. The brown pigment may arise as a result of polymerisation, either of the chlorogenic acid–amino acid adducts or of chlorogenic acid alone.

## CONCLUSIONS

The green colour development in potato cooking water is probably due to reactions between the naturally-occurring chlorogenic acids and amino acids. This conclusion is strongly suggested by (1) the comparable rates of green pigment formation in cooking water and the chlorogenic acid–amino acid model systems (2) the closely similar spectral behaviour of the cooking water and the chlorogenic acid–glutamine model system and (3) the presence of blue-green and brown components in the green colouring matter from cooking water and the chlorogenic acid–glutamine model system. The increase in pH on boiling the water during cooking probably gave suitable conditions for the reactions to occur.

It may be speculated that potatoes which give rise to green cooking water are abnormally high in chlorogenic acid and probably also glutamine and asparagine, due to excessive nitrogen fertiliser application. The manifestation of the phenomenon would then depend mainly on the final pH of the cooking water (in itself dependent on such factors as water hardness and the ratio of potatoes to water) whilst the temperature of the water and holding duration after cooking would influence the intensity of green colour development.

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