

Uptake of thiol anti-browning agents by cabbage on blanching and reactivity during dehydration

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The uptake of the anti-browning agents, mercaptoethanesulphonic acid, cysteine, *N*-acetylcysteine, glutathione and leucylcysteine (leu-cys), by cabbage from blanch liquor at pH 5.8 is measured and used to obtain the amounts of these thiols which undergo reaction when the vegetable is subsequently dehydrated. This behaviour is compared with that of sulphite species S(IV). The most reactive thiol was leu-cys, which was also found to be a better anti-browning agent than S(IV). © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Non-enzymic browning is recognised to occur during the dehydration and subsequent storage of fruit and vegetables, and sulphur(IV) oxospecies, S(IV), are widely used to inhibit such Maillard and ascorbic acid browning reactions. The ability of S(IV) to inhibit both these reactions is the result of a nucleophilic reaction of the additive with α,β -unsaturated carbonyl intermediates (Wedzicha, 1984). These reactions lead to an irreversible binding of the additive to the intermediates. The much greater reactivity of S(IV) during dehydration than upon the subsequent storage of the dry product indicates that browning proceeds most rapidly during the dehydration step. The aim of the use of S(IV) in fruit and vegetable dehydration is twofold: to achieve a product with a desired colour but also with sufficiently high residual S(IV) content to prevent browning during storage.

Any powerful nucleophile is potentially capable of reaction with α,β -unsaturated carbonyl intermediates in browning. Thiols are similarly good nucleophiles to sulphite ion and are known to inhibit the Maillard reaction (Song & Chichester, 1967).

A need has arisen to develop alternative anti-browning agents as a result of recent concern over the safety of S(IV) in foods (Fan & Book, 1987) and a desire for more 'natural' additives. Thiols appear to be the most promising alternatives to S(IV) in preventing non-enzymic browning.

Cysteine is an effective inhibitor of non-enzymic browning in a variety of foods, such as egg albumen (Kato *et al.*, 1974), pear juice concentrate (Montgomery, 1983) and milk (Arnold, 1969). However, kinetic studies have shown that the reaction of cysteine with the key intermediate in browning, 3-deoxyhex-

osulose (DH), is complex and probably involves the Strecker degradation of the amino acid (Wedzicha & Edwards, 1991). More suitable anti-browning agents would be amino acid derivatives with an *N*-substituted amino group.

Workers at UDSA (Albany, California) have investigated a range of novel anti-browning agents and advocate a variety of thiol-containing amino acid derivatives, including *N*-acetylcysteine (NAC) (Friedman & Molnar-Perl, 1990; Molnar-Perl & Friedman, 1990). However, the instability of NAC in acidic solution and its less well defined reactivity towards DH suggests that NAC may not be the best choice as an anti-browning agent in food (Edwards & Wedzicha, 1992).

Dipeptides with a C-terminal cysteine are effective inhibitors of the Maillard reaction of glucose + glycine (Edwards *et al.*, 1994). The most effective dipeptide in terms of the length of time for which this browning reaction is inhibited, at a given concentration of inhibitor, is leucylcysteine (leu-cys). This compound is more effective than S(IV) in extending the induction time for browning to commence, although it is less effective in terms of the inhibition of the formation of small amounts of colour in the early stages of reaction.

The object of this investigation is to determine the uptake of thiols by cabbage during blanching. The gross reactivity of the thiol additives upon the dehydration of cabbage can then be assessed. In this study we compare the effectiveness of a selection of thiol compounds and S(IV) in preventing browning of cabbage during the dehydration process. The thiols chosen were mercaptoethanesulphonic acid (MESA) as a simple ionic water-soluble thiol, cysteine as the amino acid contributing many of the thiol groups present in foods, and NAC, glutathione (GSH) and leu-cys as potentially

useful anti-browning agents. Furthermore, the ionic character of the reaction product formed when MESA reacts nucleophilically with DH is similar to that when sulphite ion reacts with this intermediate. The scope of the present research does not extend to the behaviour of residues of these substances when the dehydrated vegetable is stored.

MATERIALS AND METHODS

All chemicals were of AnalaR grade wherever possible and were obtained from BDH, Aldrich and Sigma Chemicals. Leucylcysteine hydrochloride was synthesised by the method described by Edwards *et al.* (1994). White cabbage was obtained locally and cut into slices ($\approx 2 \text{ mm} \times 10 \text{ mm} \times 10 \text{ mm}$).

Uptake of thiol during blanching

Blanching liquors contained thiol (5–150 mM) and were adjusted to pH 5.80 using NaOH (1 M). Cabbage samples (10 g) were blanched in boiling liquor for 2 min, after which time they showed a negative peroxidase test. Samples were quickly drained, dried between paper towels and homogenised (5 min) in water (50 ml). The slurry was filtered, washed and the filtrate made up to 200 ml with water. Aliquots were withdrawn and analysed for thiol content. Analysis was by the spectrophotometric method of Ellman (1959), in which the thiol reacts with 5,5'-dithiobis(2-nitrobenzoic acid), DTNB reagent, to form a new thiol which absorbs at 412 nm. Moisture content was determined by oven-drying to a constant weight at $103 \pm 1^\circ\text{C}$.

Dehydration of cabbage samples

Cabbage samples (10 g) were blanched as described previously. Dehydration was carried out in aluminium wire mesh trays in a fluidised-bed dryer at a temperature of 80°C for 3 h. The end of drying was taken as the point at which the samples came to a constant weight. Dried samples ($\approx 0.2 \text{ g}$) were ground to a powder and allowed to rehydrate in water (10 ml) for 5 min with stirring. Mixtures were filtered and washed; the combined filtrates and washings were made up to 25 ml with water and their absorbances (420 nm) measured. Aliquots were withdrawn and analysed for thiol content with DTNB reagent.

RESULTS AND DISCUSSION

Thiol content after blanching

Cabbage samples were blanched in solutions of different thiol compounds at the same pH (5.80) and temperature ($98\text{--}100^\circ\text{C}$). The pH chosen was that of a slurry made

up of rehydrated freeze-dried cabbage (2 g in 25 ml water). Commercially dehydrated cabbage is often pre-blanching at pH 9.0 to enhance the colour of the dried product (Greensmith, 1971). A lower pH was preferred in this work as the stability of the thiol compounds under alkaline conditions is not known. The constancy of pH was important since the amount of additive absorbed may be pH-dependent; the pH of blanching solutions has a significant effect on the absorption of S(IV) by apricots (Stafford & Bolin, 1972), which is explained in terms of the varying distribution of ionic species at different pH values.

The moisture content of cabbage, determined by oven-drying, was $92.0 \pm 0.5\%$ and was used to calculate the thiol content in terms of dry matter. The extraction procedure used to determine the uptake of thiol gave complete recovery of the thiol; no further thiol could be extracted from homogenised samples by repeating the extraction procedure. A background of $0.016 \text{ mmol thiol g}^{-1}$ (dry weight) was detected immediately after cabbage had been blanched in water containing no additive; this is a measure of naturally occurring extractable thiols. One of the contributors to thiol content is GSH, which is reported to be present at levels of $0.005 \text{ mmol g}^{-1}$ (recalculated from Nakagawa *et al.*, 1986), although the level present in blanched cabbage is likely to be lower since a proportion of this compound will have been leached from the vegetable during blanching. The background thiol content was subtracted from the total thiol content after blanching to give the amount of added thiol present at this stage.

The effect of the concentration of thiol in blanching liquor on the amount of thiol present immediately after blanching is illustrated in Fig. 1 for GSH and leu-cys. The behaviour and quality of the data for GSH are typical of those observed for the other thiols (cysteine,

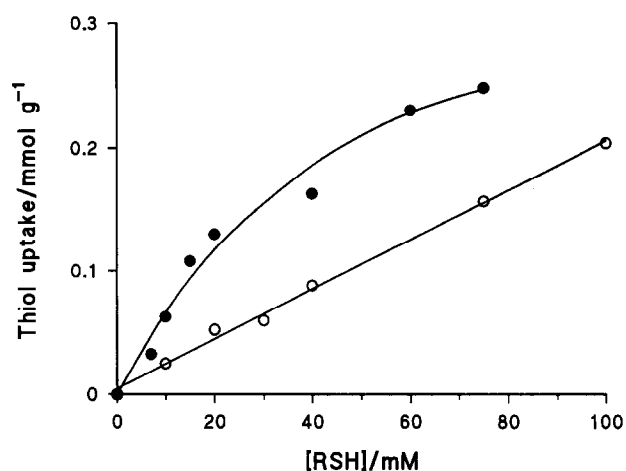


Fig. 1. Relationship between the concentration of GSH and leu-cys in the blanching liquor and the thiol uptake in cabbage. Conditions: pH 5.8, blanching time 2 min. (○) GSH; (●) leu-cys. The uptake of the other thiol compounds, NAC, MESA and cys is within the limits of the two sets of results shown.

MESA, NAC) investigated, and S(IV). Thus, with the exception of leu-cys, the amounts of the thiols and S(IV) present in the cabbage after blanching are linearly dependent upon their concentration in the blanching liquor. For the different anti-browning agents, the order of increasing uptake is $\text{GSH} < \text{cys} \approx \text{MESA} < \text{S(IV)}$; the slopes/ $(\text{dm}^3 \text{kg}^{-1})$ of the straight line graphs are 2.06, 2.45, 2.79, 2.91 and 3.63, respectively. In all these cases, the uptake is probably dependent on the concentration gradient as one goes from the bulk solution to the interior of the vegetable structure. On the other hand, the uptake of leu-cys appears to show 'saturation' behaviour. A consequence of this behaviour is that, whilst the uptake of leu-cys by the cabbage is greater than the uptake of any of the other anti-browning agents at low concentration, the uptake of S(IV) exceeds that of leu-cys when their concentrations exceed $\approx 60 \text{ mM}$. A linear relationship between uptake and the concentration of blanching solution is reported for the treatment of apricots with S(IV) (Stafford & Bolin, 1972). However, Wedzicha & Adamu (1987) found that the uptake of S(IV) by vegetables, including cabbage, did not always correlate with the concentration of S(IV) in the blanching solution.

The extent of thiol uptake is comparable to that of S(IV) at pH 5.80. It is essential that thiols are effectively absorbed during blanching if they are to be considered as potentially useful anti-browning agents. The fact that, at low concentrations, leu-cys shows greater uptake is a possible advantage regarding its use as an anti-browning agent.

Loss of thiol on dehydration of cabbage

The amount of unreacted thiol, after dehydration, was determined by extraction of the reconstituted vegetable into water and determination of the thiol content therein. The amount of thiol measured includes that which is leached during reconstitution. The extraction procedure gave complete recovery of the unreacted thiol; no further thiol could be extracted by repeating the extraction procedure on the residue from the first extraction. The moisture content of dehydrated samples was $3.8 \pm 0.6\%$. The background thiol present after dehydration and reconstitution of cabbage that had been blanched in water containing no additive was $0.0017 \text{ mmol g}^{-1}$, implying significant reactivity of the naturally occurring thiol in the cabbage. It could be speculated that the reactions of low molecular weight thiols could well contribute to flavour and colour development in heat-treated cabbage.

The thiol content after dehydration was used to obtain the relationship between the amount of thiol which was not recoverable after dehydration and the amount present in freshly blanched cabbage. This is illustrated in Fig. 2. The amounts of thiol reported have been calculated after taking into account the measured moisture content of dehydrated cabbage. At low initial

concentrations, all thiol compounds and S(IV) behave similarly and there is a high loss of additive ($\approx 90\%$). As the concentration of thiol or S(IV) after blanching is increased, different behaviour is seen for different compounds. The reactivities of GSH, NAC, MESA and S(IV) become almost independent of concentration and the results suggest that the vegetable shows a given capacity to react with each compound, and no more. In the case of S(IV), the fractional loss (0.73) of additive is seen to be constant (graph shown in Fig. 2 is linear; $r = 0.9993$, $n = 5$) to an initial S(IV) content of nearly 0.1 mmol g^{-1} (i.e. 6400 ppm SO_2) and a final S(IV) content in the region of 2000 ppm SO_2 . This level of additive approaches the legal limit (2500 ppm) for this dehydrated vegetable in the UK. It has been reported (Wedzicha & Adamu, 1987) that the loss of S(IV) during the dehydration of cabbage under similar conditions to those used here is $51 \pm 6\%$ for initial S(IV) contents up to 0.06 mmol g^{-1} (nearly 4000 ppm). The available data provide no reason for this difference between the two sets of very consistent data.

The loss of S(IV) during the dehydration of cabbage is accounted for mainly by organic sulphur compounds, although small amounts of inorganic sulphate are formed (Gilbert & McWeeny, 1976). The oxidation of thiols to disulphides is also likely to be minimal because cabbage contains sufficient amounts of simple reducing sugars to act as effective antioxidants.

The loss of thiol (MESA, GSH, NAC) is likely to include the irreversible binding of the additives to browning intermediates, such as DH. Thiols are known to react with DH by way of a mechanism similar to the DH-S(IV) reaction (Wedzicha & Edwards, 1991), although the exact nature of the product is not known. The various thiol anti-browning agents used in this work have other functional groups, some of which are reactive in food; a direct comparison between these substances is not feasible. It is possible that the

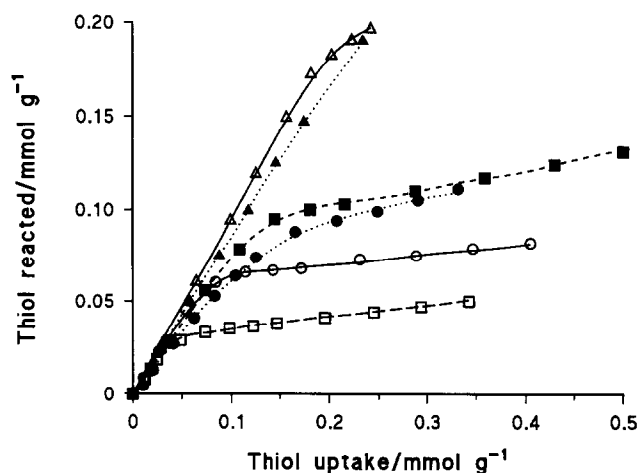


Fig. 2. Theoretical curves for the relationship between the amount of thiol and S(IV) taken up by cabbage and the amount lost during dehydration at 80°C for 3 h. (●) GSH; (□) NAC; (▲) cysteine; (○) MESA; (△) leu-cys; (◇) S(IV).

anti-browning agents could themselves affect the rate of the browning reactions; for example, S(IV) is known to catalyse the early stages of the browning of glucose (Wedzicha & Vakalis, 1988), fructose (Swales & Wedzicha, 1992) and the degradation of ascorbic acid (Davies & Wedzicha, 1992). Those anti-browning agents that have amino groups within their structures could also take part in non-enzymic browning.

Added amounts of S(IV) above 0.06 mmol g^{-1} in cabbage are reported to lead to a reduction in the amount of C-sulphonates formed during dehydration (Wedzicha *et al.*, 1984). Reversible binding of S(IV) to carbonyl intermediates in browning, which ultimately lead to products containing irreversibly bound S(IV), reduces their reactivity towards S(IV) and is thought to lead to a reduction in yield of C-sulphonate. This might explain the reduction in reactivity of S(IV) at higher concentration, as illustrated in Fig. 2. A similar argument could be applied to thiols which too can bind reversibly to carbonyl intermediates forming hemimercaptals (Lienhard & Jencks, 1966).

Cysteine and leu-cys exhibit a high level of reactivity during dehydration. The fractional losses, up to an initial amount of 0.2 mmol g^{-1} , are 94% and 86% for leu-cys and cysteine, respectively. This high reactivity is not surprising as a similar fast loss of these additives is observed in model Maillard systems of glucose + glycine + thiol (Edwards *et al.*, 1994); this has been explained by the ability of these inhibitors to bind with carbonyl groups to form thiazolidines (Schubert, 1936) which are stable under the conditions of thiol analysis. Thus, the loss of leu-cys and cysteine on dehydration of cabbage could be the result of their reaction with carbonyl compounds.

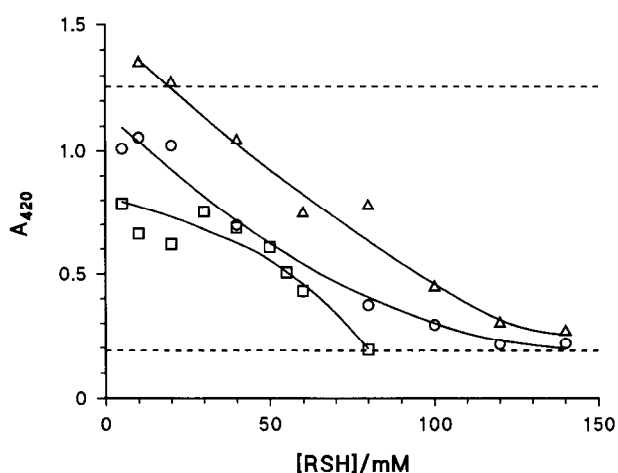


Fig. 3. Absorbance at 420 nm of aqueous extracts of dehydrated cabbage, as a function of the concentration of anti-browning agent in blanching liquor. (Δ) GSH; (\square) leu-cys; (\circ) S(IV). The upper broken line indicates the absorbance of an extract of dehydrated cabbage prepared with no inhibitor; the lower broken line indicates the absorbance of an extract of cabbage dehydrated after blanching in 0.25 M S(IV) solution.

Anti-browning behaviour of thiols on dehydration

The effectiveness of thiol compounds as inhibitors of browning during dehydration was determined by measuring the absorbance of the colour extracted into water. Two standards were used: cabbage blanched in water and dehydrated to represent uninhibited browning, and cabbage blanched in an excess of S(IV) (0.25 M) to represent maximum inhibition.

The effect of the concentration of thiol in the blanching solution on the browning of dehydrated cabbage, extracted into water, is illustrated in Fig. 3 for GSH and leu-cys, and for S(IV). The absorbance values (420 nm) for maximum and minimum browning were 1.257 and 0.189, respectively. These spectrophotometric data also correlate well with conclusions drawn from visual inspection of the dehydrated cabbage samples.

The most effective inhibitor, in terms of inhibitor concentration in the blanching solution, is leu-cys. If its effect is expressed in terms of the amount of inhibitor taken up by the vegetable, one finds that complete inhibition of browning is achieved when this amount is 0.26 mmol g^{-1} (dry weight). In this situation, the amount of leu-cys that is irreversibly bound, and so becomes unavailable after dehydration, is $\approx 0.20 \text{ mmol g}^{-1}$, and the residue in the dehydrated cabbage is, therefore, some 0.06 mol g^{-1} . Whilst this high reactivity of leu-cys during dehydration offers excellent protection against browning, the low residual level may not be sufficient to prevent spoilage of the vegetable in subsequent storage. It is not known if this anti-browning agent is as efficient at a low temperature as it is during dehydration. We see, however, that leu-cys is a better inhibitor of browning than S(IV) during the dehydration of cabbage, even taking into account the higher uptake of S(IV) when the concentration of the anti-browning agents in the blanching liquor is $> 60 \text{ mM}$.

CONCLUSION

The uptake of thiol compounds by cabbage during blanching is comparable to that of S(IV) in terms of moles of additive per gram (dry weight), which is an important factor when considering thiols as alternative anti-browning agents.

Thiols show significant reactivity during the dehydration process; presumably these compounds react with intermediates in the browning reactions. Thiols are effective inhibitors of the browning of cabbage during dehydration. At a given concentration in blanch liquor, the most effective anti-browning agent is leu-cys, but it is also the most reactive of the anti-browning agents investigated. Whilst such high reactivity means that the residual levels in the dehydrated vegetable are low, it leads to toxicological implications which must be investigated before such an additive can be considered for food use.

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