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PII: S0308-8146(97)00068-X

Food Chemistry, Vol. 61, No. 3, pp. 345–350, 1998

0308-8146/98 $19.00+0.00

Relationship between thiosulfinates and pink discoloration in onion extracts, as influenced by pH

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The influence of immediate and delayed pH control, and added *S*-methyl-L-cysteine sulfoxide (MCSO) on the progress of pink discoloration in relation to the fate of thiosulfinates in extracts of yellow onion (*Allium cepa*) bulbs was evaluated over an incubation period of about 3 weeks at 21–24°C. Greatest discoloration occurred in extracts prepared with immediate pH control at 6.1, and least discoloration occurred at pH 3.2, over the pH range of 3.2–6.1. Initial thiosulfinate levels, in descending order of abundance, occurred at pH 6.1 > 5.0 > 4.2 > 3.2 for extracts subject to immediate pH control. However, over 21 days incubation, the lowest residual levels (ranging 20–60% of maximum levels) were observed for extracts adjusted to pH 3.2 and 6.1. Extracts subject to delayed (10 min after tissue disruption) pH control (initial extract pH of 5.2–5.4) displayed the greatest extent of discoloration, in descending order, at pH 4.0 > 5.0, 6.0 > 3.0. In these samples, initial thiosulfinate levels were similar and the greatest residual thiosulfinate levels over the 21-day incubation period were observed at pH 3.0 and 4.0 (about 50% of maximum levels), with the lowest residual levels observed for extracts adjusted to pH 6.0 (about 20% of maximum levels). Tissue extracts supplemented with 9- to 18-fold excess MCSO were subject to modest increases in both extent of discoloration and thiosulfinate levels. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The discoloration of processed Allium tissues, and especially onion (*Allium cepa*), has been a long-standing problem, the basis of which is only partially understood (Schwimmer, 1981; Fenwick & Hanley, 1985*a*). This problem continues to serve as an obstacle to maintaining color quality in onion products, most recently in the development of minimally processed (diced) onions (Howard *et al.*, 1994). Pink discoloration of onion purees and dehydrated products proceeds in two phases (Lukes, 1959), the first of which is the action of endogenous alliin alkyl sulfenate-lyase (E.C. 4.4.1.4, cysteine sulfoxide lyase or CS-lyase) on alk(en)yl-L-cysteine sulfoxide(s) (ACSO) to yield an ether-soluble organosulfur component. This component (termed 'color developer' in the early literature) subsequently reacts, nonenzymically, with amino acid and carbonyl components to yield pigments ranging from yellow to red (Lukes, 1959; Joslyn & Peterson, 1958, 1960). A similar reaction sequence is believed to be responsible for a green–blue discoloration in garlic (*Allium sativum*) purees (Yamaguchi *et al.*, 1965; Shannon, *et al.*, 1967*a,b*; Lukes, 1986).

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Previous efforts sought to identify the ether-soluble organosulfur component that served as the 'precursor' to pigment formation. This ether-soluble component has an absorbance of 250 nm, and once isolated, is stable for several weeks in water at ambient temperature (Shannon *et al.*, 1967*a*). This precursor was speculated to contain a pyrrole nucleus and react with cysteine in a manner that prevents pink discoloration (Shannon *et al.*, 1967*b*). Although results from paper chromatography and specific stain reagents were inconclusive, it was suggested that if a thiosulfinate is the precursor, then it is most likely a propenyl thiosulfinate (Shannon *et al.*, 1967*a,b*). A subsequent study suggested the presence of multiple organosulfur compounds that served as precursors to pink discoloration in onion purees (Bandyopadhyay & Tewari, 1973). At least three compounds were reported to be separated and detected by thin-layer chromatography following reaction with a glycine-formaldehyde reagent to yield red pigments. These organosulfur compounds were suggested to be thiopropanal *S*-oxide (lachrymatory factor) and two other thioalkanal *S*-oxide-like compounds.

In the last several years, advances have been made in the understanding of the secondary reaction chemistry of organosulfur compounds in *Allium* species, with an

emphasis on the evolution, profile and properties of specific thiosulfinates (Block, 1992; Block *et al.*, 1992*a,b*). In this light, it seemed appropriate to revisit the nature of pink discoloration as related to the fate of thiosulfinates in disrupted onion tissues.

MATERIALS AND METHODS

Materials

Yellow onion bulbs were purchased from a local market and stored at 21–24°C prior to use. Chemicals were reagent or high performance liquid chromatography (HPLC) grade. Citric acid and 30% H_2O_2 were obtained from Baker Chemical Co. (Philipsburg, NJ); sodium phosphate, and sodium and potassium hydroxides were obtained from Mallinckrodt Specialty Chemicals Co. (Paris, KY); hydrochloric acid was obtained from Fisher Scientific (Fair Lawn, NJ); ethyl and isopropyl alcohols were obtained from Aldrich Chemical Co. (Milwaukee, WI); all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Pure *S*-methyl-L-cysteine sulfoxide (MCSO) was prepared by the method of Synge and Wood (1956) as described by Thomas (1992). Water was distilled and deionized.

Preparation of onion extracts

Trial A: Extracts with initial pH adjustment
The dry outer scales and root initials were removed or excised from about 600 g bulbs, and the remaining material was quartered. The quarters from each onion were separated into each of four groups and each group was homogenized with 0.5 volume (v/w) 0.5 M sodium citrate-phosphate buffer (pH range 3–6) in a Waring blender for 1 min. Homogenates were held at 21–24°C, and after 2 days, filtered through Whatman No. 42 paper, and then a 0.22 μm acetate filter (Micron Separations, Inc., Westborough, MA). The filtered homogenates were then re-filtered through pre-sterilized 0.2 μm Supor-200 filters (Gelman Science, Inc., Ann Arbor, MI) into aseptic culture tubes in a laminar flow hood and allowed to incubate for several weeks at 21–24°C. Preparation of homogenates was done in duplicate for two separate experiments, and samples that were subject to obvious microbial growth were discarded.

Trial B: Extracts with delayed pH adjustment
About 600 g peeled and trimmed onion bulbs were passed through a juicerator (pulp was largely retained by cheesecloth). The resulting extract was divided into four portions and allowed to stand at 21–24°C. After 10 min, each portion was adjusted to pH 3, 4, 5 or 6 with 5 N sodium hydroxide or hydrochloric acid. After 2 days incubation at 21–24°C, subsequent procedures of filtration, aseptic transfer and incubation were carried out, as described for Trial A, as was experimental replication.

Trial C: Extracts supplemented with MCSO and subject to initial pH adjustment to 4.9
Extract preparation and other experimental details were as described in Trial A except that three different buffer solutions (all at pH 4.9) were prepared and contained synthetic MCSO to yield either no (control, 1X), 9.4-fold or 17.9-fold excess MCSO in the resulting extract. The amount of MCSO added was calculated based on 1.6 μmol MCSO g^{-1} fresh weight (gfw) (Thomas, 1992) and assuming the onion bulb is 90% moisture (Fenwick & Hanley, 1985*b*).

Pigment determination

Extracts were filtered through 0.45 μm Nylaflo filters (Gelman Science, Inc.) to remove turbidity prior to spectrophotometric analysis. Absorption spectra were scanned between 350 and 750 nm (model DU-65, Beckman Instruments, Inc., Fullerton, CA). Absorption maxima ranged from 520 to 530 nm, and $A_{525\,nm}$ following baseline subtraction was used routinely for measuring pigment formation in onion extracts.

Thiosulfinate determination

A colorimetric procedure based on reaction with *N*-ethylmaleimide (NEM) was used (Carson & Wong, 1959; Nakata *et al.*, 1970), following dilution of onion extracts with ten volumes of isopropanol. The assay mixture (21–24°C) contained, in sequence, 1 ml diluted extract, 1 ml 50 mM NEM, 1 ml 0.25 M isopropanolic KOH and 1.5 ml 1% ascorbic acid, with vortexing after addition of each reagent. After 2 min incubation, $A_{515\,nm}$ was determined.

Pyruvate determination

To evaluate the extent of CS-lyase action, pyruvate content in the extracts (diluted 1:1 with isopropanol) was determined (Schwimmer & Mazelis, 1963) by the lactate dehydrogenase/NAD(H) method of Schwimmer and Weston (1961), as described by Thomas and Parkin (1991).

RESULTS AND DISCUSSION

Pink discoloration in onion products is widely believed to involve CS-lyase action on ACSO (Lukes, 1959; Bandyopadhyay & Tewari, 1973) and secondary, non-enzymic reaction products, such as thiosulfinates (Shannon *et al.*, 1967*a,b*). Thus, the origin of the factors promoting discoloration lies within the chemical changes that take place in freshly disrupted tissue. Also, because both CS-lyase and thiosulfinates (Block *et al.*, 1992*a,b*) are thermally sensitive, we confined our studies to selected changes in freshly minced tissues and the extent of pink discoloration. The pH range of 3–6 was

selected based on a prior report that pigment formation occurs between pH 2.5–5.5 and the pigment is unstable under alkaline conditions (Joslyn & Peterson, 1958), and because the range of pH 3–6 is most relevant to foods or products in which onion tissues (pH of onion purees were 5.2–5.4) would probably be used.

Differences in the rate and extent of pink discoloration were observed in different batches of onions (i.e. experiments) used in this study. Therefore, the results presented are representative of the general trends that were observed for at least two experiments.

Trial A

Trial A was designed to evaluate the effect of pH on the total process (combined enzymic and nonenzymic steps) leading to pink discoloration in onion extracts. Discoloration was more rapid and pronounced as the pH of the extracts was increased from 3.2 to 6.1 (Fig. 1). One effect of pH is likely on CS-lyase activity, as the onion enzyme is known to have a pH optimum of 7–8, with about 50, 20 and 10% relative activity at pH 6, 5, and 4, respectively (Schwimmer & Mazelis, 1963). We found the level of pyruvate formed at pH 3.2 to be about 65% after 24 h, and about 91% after 3 days, relative to pyruvate levels observed at pH 4.2–6.1 at respective time periods. However, there was little influence of pH on the extent of CS-lyase action over the pH range of 4.2–6.1, where similar levels of pyruvate (8.3–10.4 μmol gfw^{-1}) were formed in the extracts within the first 3 days. After 13 days incubation, pyruvate levels ranged between 6.2 and 8.0 μmol gfw^{-1} for all samples.

While these results are consistent with the involvement of CS-lyase in the discoloration process (Lukes, 1959; Shannon *et al.*, 1967*b*), the trends observed can also be attributable to pH-dependency of nonenzymic reactions, especially in the pH range of 4.2–6.1, where similar extents of enzymic ACSO transformation were allowed to occur.

Over the range of pH evaluated, thiosulfinate levels in onion extracts transiently increased and decreased over the initial 5-day incubation period (Fig. 2). Greatest maximum and residual thiosulfinate levels were found in extracts prepared at pH 5–6. Residual thiosulfinate levels ranged between 20 and 60% of the maximum observed at each pH, with the greatest relative declines being observed at pH 5–6. These declines may reflect the instability and/or reactivity of thiosulfinates as a function of pH.

Trial B

Delayed pH adjustment was designed to allow for a normalized extent of initial CS-lyase action and focus on pH effects on the presumed secondary, nonenzymic reactions leading to discoloration. The pH of the initial extract was 5.2–5.4 and nearly complete action of CS-lyase on endogenous ACSO was expected during the 10 min period following tissue disruption (Schwimmer & Weston, 1961; Lukes, 1971; Lawson *et al.*, 1991; Thomas, 1992; Block *et al.*, 1992*a*). Analysis after 1–3 days of incubation indicated that nearly identical levels of pyruvate (6.2–6.7 μmol gfw^{-1}) in the extracts adjusted to pH 4–6, with the extract adjusted to pH 3.0 having slightly less pyruvate (5.5–5.6 μmol gfw^{-1}). Pyruvate levels declined slightly to 4.9–5.6 μmol gfw^{-1} after 12 days incubation.

During incubation, the rate and extent of discoloration was greatest at pH 4 and least at pH 3 (Fig. 3). Thus, a pH of about 4 appeared to be most supportive

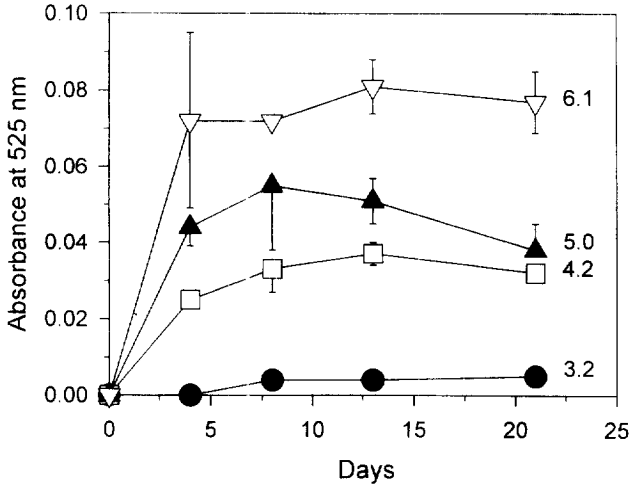

Fig. 1. Effect of immediate pH adjustment on pink pigment formation in onion extracts. Extracts were prepared by homogenizing onion bulb tissue with 0.5 volumes (v/w) 0.5 M sodium citrate-phosphate buffer. Pigment was determined as $A_{525\,nm}$. Results are expressed as means ± SD for two duplicate determinations from each of two homogenates at each time interval.

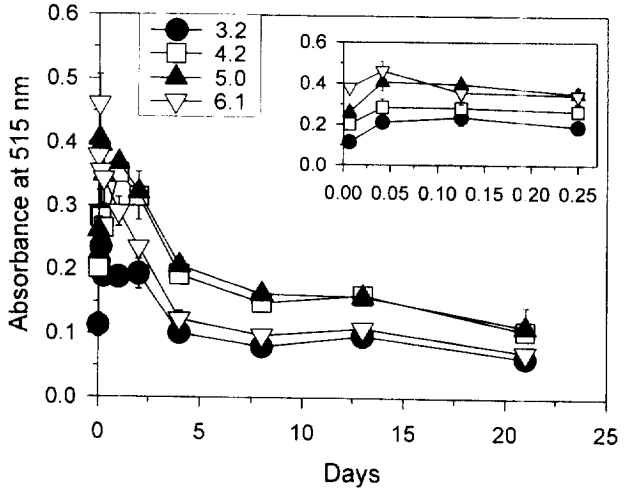

Fig. 2. Effect of immediate pH adjustment on thiosulfinate levels in onion extracts. Extracts were prepared as described in Fig. 1. Thiosulfinate levels are expressed as $A_{515\,nm}$ ml^{-1} diluted (1 volume in 10 volumes isopropanol) extract. Results are expressed as means ± SD for two to four determinations from each of two homogenates at each time interval. Insert represents expanded time scale.

of the nonenzymic reactions that collectively lead to pink pigment formation. This is somewhat consistent with the pH-dependence of reddening in White Globe onion bulb purees, where discoloration was most evident at pH 3–4 within 48 h at 20–25°C (Joslyn & Peterson, 1958). The slight difference in optimum pH range between the present and previous studies may be founded on the difference in the variety of onions used (Joslyn & Peterson, 1958; Yamaguchi *et al.*, 1965), differences in the time elapsed between tissue disruption and pH adjustment, and the use of acetic acid as acidifying agent in the previous study. Discoloration at pH 3–4 could have been accelerated by formaldehyde, a trace impurity in the acetic acid reagent used (Joslyn & Peterson, 1958, 1960).

Thiosulfinate levels were nearly equal among extracts at the beginning of the incubation period (Fig. 4), and this is likely because of the rapid conversion of sulfenic acids (the initial product of CS-lyase action on ACSO) to thiosulfinates after tissue disruption (Freeman & McBreen, 1973; Thomas, 1992; Block, 1992). During extended incubation, thiosulfinate levels declined by 50–80% of the maximum, with greatest residual levels observed at pH 3–4, and least residual levels observed at pH 6. These trends in thiosulfinate levels are probably reflective of the pH-dependence of stability and patterns of reactivity in the onion extracts.

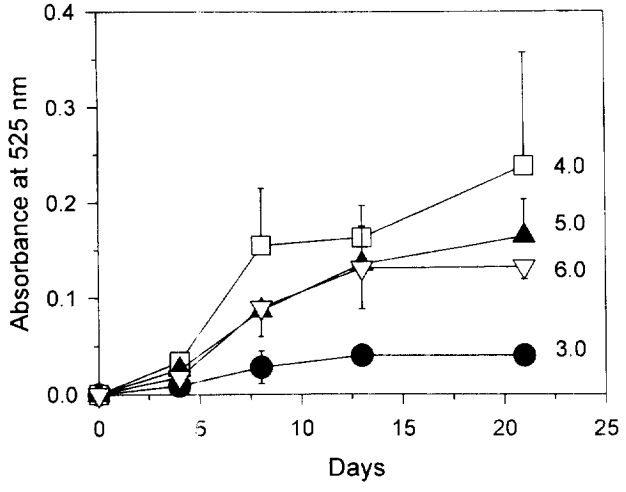

Fig. 3. Effect of delayed pH adjustment on pigment formation in onion extracts. After tissue disruption, 10 min elapsed prior to pH adjustment with 5 N NaOH or HCl. Pigment was determined as in Fig. 1.

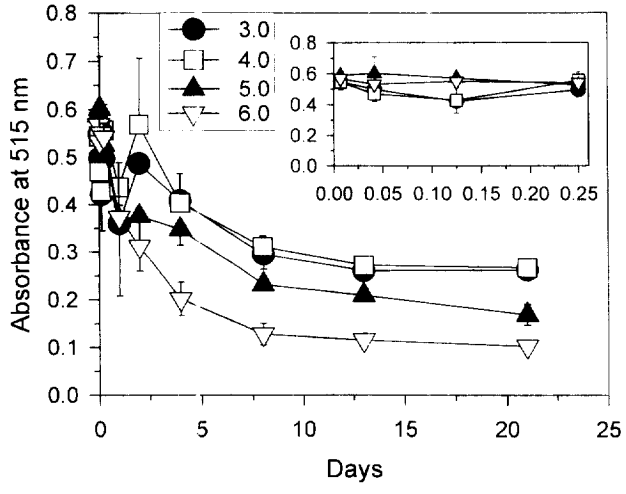

Fig. 4. Effect of delayed pH adjustment on thiosulfinate levels in onion extracts. Extracts were prepared as in Fig. 3, and thiosulfinates determined as in Fig. 2.

Trial C

The intensity of pink discoloration increased 2- to 3-fold when onion extracts (pH 4.9) were supplemented with MCSO (Fig. 5). This appeared to reaffirm the role of CS-lyase-transformed ACSO as the organosulfur components serving as the putative precursors to pink pigment formation in tissue extracts (Lukes, 1959; Shannon *et al.*, 1967*b*). However, there appeared to be a maximum level of involvement of MCSO in discoloration, as no difference in effect was noted between the two levels of MCSO supplementation. The lack of a more profound effect of added (synthetic, ±) MCSO may be attributable to a reduced rate or extent of conversion by onion CS-lyase relative to other, endogenous ACSO (Schwimmer *et al.*, 1964; Schwimmer, 1969), or a limited role of MCSO-derived organosulfur components in reactions leading to discoloration.

Enhanced thiosulfinate levels were initially formed and persisted during incubation in extracts supplemented with MCSO (Fig. 6). The magnitude of effect of MCSO supplementation on thiosulfinate levels was similar to that observed for discoloration. However, since multi-fold supplementation of extracts with MCSO had a modest influence on the extent of thiosulfinate formation and pink discoloration, the participation of

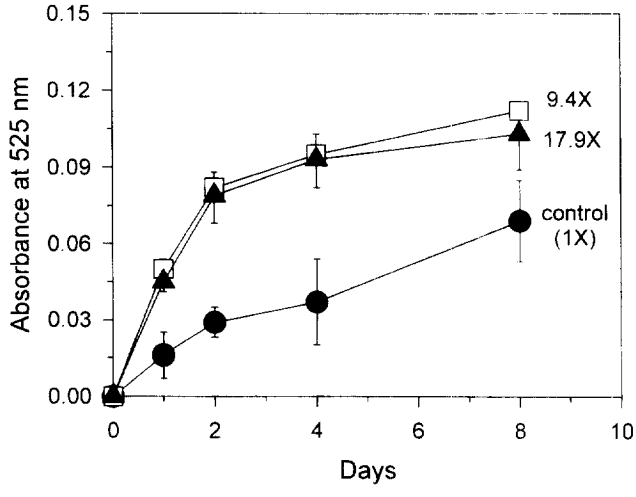

Fig. 5. Effect of MCSO supplementation on pink pigment formation in onion extracts. Extracts were prepared and pigment determined as in Fig. 1, except that all extracts had a pH of 4.9 and contained either no added MCSO (control) or 9.4-fold or 17.9-fold excess MCSO, supplied by the homogenizing buffer.

MCSO in reactions leading to discoloration is probably limited.

General discussion

The suggested role of CS-lyase and endogenous ACSO on pink discoloration in disrupted onion tissues (Lukes, 1959; Shannon *et al.*, 1967*b*) was supported by two observations in these studies. First, a pH of 6.1 imposed during tissue disruption resulted in greater pink discoloration relative to extracts adjusted to lower pH (3–5) values. Onion CS-lyase is optimum at slightly alkaline pH (Schwimmer & Mazelis, 1963; Tobkin & Mazelis, 1979), whereas we found the presumptive nonenzymic steps involved in discoloration after CS-lyase action on ACSO were favored by a pH range of 4–5. Second, extracts supplemented with MCSO were subject to enhanced discoloration, although the effect was modest relative to the levels of supplementation.

The precise effect of pH on the process leading to discoloration is not clear from this or previous studies. Although tissue disruption with immediate adjustment to pH 6 was most conducive to discoloration, the extent of pyruvate formation over the pH range of 4–6 was similar. Therefore, pH 6 must also be more conducive than pH 4–5 to some secondary, nonenzymic reaction step(s) leading to discoloration. In contrast, under conditions of delayed pH adjustment, allowing initial and nearly complete CS-lyase action on endogenous ACSO at pH 5.2–5.4, subsequent pH adjustment to pH 4 most favored discoloration in the pH range of 3–6. We conclude that an early non-enzymic step in the reactions leading to pink pigment formation in onion extracts is favored by pH 6 (or greater), and subsequent steps are favored by more acidic conditions. We also suspect that determining the temporal changes in *specific species* of thiosulfinates in the time frame immediately following tissue disruption may be critical to understanding the

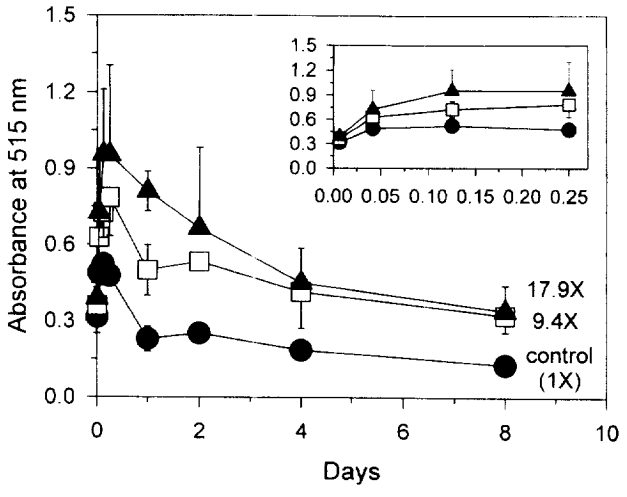

Fig. 6. Effect of MCSO supplementation on thiosulfinate levels in onion extracts. Extracts were prepared as in Fig. 5, and thiosulfinates determined as in Fig. 2.

reactions leading to discoloration, if indeed, thiosulfinates are involved. Although Shannon *et al.* (1967*b*) have proposed a reaction sequence of the organosulfur component first reacting with a 1° amine functional group, and this adduct then reacting with a carbonyl component to yield the pigment, this sequence has not been scrutinized by detailed studies.

In view of the possibility that an early step in the reaction sequence following CS-lyase action on ACSO is important in controlling the extent of discoloration, the fate of thiosulfinates in the extracts was followed. Thiosulfinates are formed by condensation of sulfenic acids (Block, 1992), and have been speculated to be the organosulfur compound(s) involved in pink discoloration (Shannon *et al.*, 1967*b*). The difficulty in attempting to draw correlations between thiosulfinate levels and discoloration is founded on the inherent instability and reactivity of thiosulfinates, especially in a tissue milieu (Block *et al.*, 1992*a*,*b*). Furthermore, if thiosulfinates are involved in discoloration, they must be intermediate reactants and thus, their levels might be expected to be transient and/or fugitive.

In spite of the aforementioned limitations, some correlations could be made regarding thiosulfinate levels in tissue extracts and the extent of discoloration. For extracts subject to immediate pH control, levels of thiosulfinates were correlated ($r^2 = 0.983$) with the degree of discoloration (descending levels were observed in the order of: pH 6.1 > pH 5.0 > pH 4.0 > pH 3.2). For extracts subject to delayed pH control, and where initial thiosulfinates levels were similar, greater residual thiosulfinate levels during extended incubation were associated with greater degrees of discoloration (pH 4 > pH 5 > pH 6), with an exception to this trend noted at pH 3 (this exception rendered a regression/correlation analysis meaningless). Also, supplementation of extracts with MCSO led to increases in initial and residual thiosulfinate levels and extent of discoloration.

In considering thiosulfinates (R-S(O)-S-R′) as organosulfur components involved in reactions leading to discoloration, several factors must be accommodated. Thiosulfinates are generally stabilized in acidic environments (Block, 1992). Thus, their ability to contribute to discoloration must represent a balance of being stable enough to persist in tissue extracts, but not to the point of being unreactive with other components for the balance of reactions involved in pigment formation. The anomalous behavior of extracts with delayed adjustment to pH 3 may result from a stabilization of thiosulfinates, perhaps coupled with a lack of reactivity with other components under these acidic conditions. Another factor is that there are at least eight thiosulfinates known to exist in onion homogenates (Block *et al.*, 1992*a*), and it is likely that not all thiosulfinates have similar reactivity in processes leading to discoloration, if they are involved at all. Furthermore, different thiosulfinates have different stabilities in a tissue matrix (Block *et al.*, 1992*a*,*b*),

and analysis for total thiosulfinates may not be representative of the fate of the thiosulfinate(s) involved in discoloration. The potential relationship between thiosulfinates and pink discoloration in onion bulb extracts, with specific reference to the issues just described, is being addressed in continuing studies on this topic in this laboratory.

ACKNOWLEDGEMENTS

This work was supported by the College of Agricultural and Life Sciences of the University of Wisconsin-Madison. The technical assistance of Ms Cunxi Shen is also greatly appreciated.

REFERENCES

Bandyopadhyay, C. & Tewari, G. M. (1973). Thin-layer chromatographic investigation of color developer involved in pinking of white onion purees. *J. Agric. Food Chem.*, **21**, 952–954.

Block, E. (1992). The organosulfur chemistry of the genus Allium. Implications for the organic chemistry of sulfur. *Angew. Chem. Int. Ed. Engl.*, **31**, 1135–1178.

Block, E., Naganathan, S., Putman, D. & Zhao, S-H. (1992*a*). Allium chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (Ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J. Agric. Food Chem.*, **40**, 2418–2430.

Block, E., Putman, D. & Zhao, S.-H. (1992*b*). Allium chemistry: GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive and Chinese chive. *J. Agric. Food Chem.*, **40**, 2431–2438.

Carson, J. F. & Wong, F. F. (1959). A colour reaction for thiosulphinates. *Nature*, **183**, 1673.

Fenwick, G. R. & Hanley, A. B. (1985*a*). The genus Allium. Part 1. *CRC Crit. Rev. Food Sci. Nutr.*, **22**, 199–271.

Fenwick, G. R. & Hanley, A. B. (1985*b*). The genus Allium. Part 2. *CRC Crit. Rev. Food Sci. Nutr.*, **22**, 273–377.

Freeman, G. G. & McBreen, F. (1973). A rapid spectrophotometric method of determination of thiolsulphinate in onion (*Allium cepa*) and its significance in flavour studies. *Biochem. Soc. Trans.*, **1**, 1150–1152.

Howard, L. R., Yoo, K. S., Pike, L. M. & Miller, G. H. (1994). Quality changes in diced onions stored in film packages. *J. Food Sci.*, **59**, 110–112,117.

Joslyn, M. A. & Peterson, R. G. (1958). Reddening of white onion bulb purees. *J. Agric. Food Chem.*, **6**, 754–765.

Joslyn, M. A. & Peterson, R. G. (1960). Reddening of white onion tissue. *J. Agric. Food Chem.*, **8**, 72–76.

Lawson, L. D., Wood, S. G. & Hughes, B. G. (1991). HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. *Planta Med.*, **57**, 263–270.

Lukes, T. M. (1959). Pinking of onions during dehydration. *Food Technol.*, **13**, 391–393.

Lukes, T. M. (1971). Thin-layer chromatography of cysteine derivatives of onion flavor compounds and the lacrimatory factor. *J. Food Sci.*, **36**, 662–664.

Lukes, T. M. (1986). Factors governing the greening of garlic puree. *J. Food Sci.*, **51**, 1577–1582.

Nakata, C., Nakata, T. & Hishikawa, A. (1970). An improved colorimetric determination of thiolsulfinate. *Anal. Biochem.*, **37**, 92–97.

Schwimmer, S. (1969). Characterization of *S*-propenyl-L-cysteine sulfoxide as the principal endogenous substrate of L-cysteine sulfoxide of onion. *Arch. Biochem. Biophys.*, **130**, 312–320.

Schwimmer, S. (1981). Enzyme action and food color quality, part V. In *Source Book of Food Enzymology*. AVI Publishing, Westport, CT, pp. 265–342.

Schwimmer, S. & Mazelis, M. (1963). Characterization of alliinase of *Allium cepa* (onion). *Arch. Biochem. Biophys.*, **100**, 66–73.

Schwimmer, S., Ryan, C. A. & Wong, F. F. (1964). Specificity of L-cysteine sulfoxide lyase and partially competitive inhibition by *S*-alkyl-L-cysteines. *J. Biol. Chem.*, **239**, 777–782.

Schwimmer, S. & Weston, W. J. (1961). Enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agric. Food Chem.*, **4**, 301–304.

Shannon, S., Yamaguchi, M. & Howard, F. D. (1967*a*). Reactions involved in formation of a pink pigment in onion purees. *J. Agric. Food Chem.*, **15**, 417–422.

Shannon, S., Yamaguchi, M. & Howard, F. D. (1967*b*). Precursors involved in the formation of pink pigments in onion purees. *J. Agric. Food Chem.*, **15**, 423–426.

Synge, R. L. M. & Wood, J. C. (1956). (+)-(*S*-methyl-L-cysteine *S*-oxide) in cabbage. *Biochem. J.*, **64**, 252–259.

Thomas, D. J. (1992). Studies on the flavor biochemistry of Alliums. Ph.D. Thesis, University of Wisconsin, Madison, Wisconsin.

Thomas, D. J. & Parkin, K. L. (1991). Immobilization and characterization of C-S lyase from onion (*Allium cepa*) bulbs. *J. Food Biotechnol.*, **5**, 139–159.

Tobkin, H. E. & Mazelis, M. (1979). Alliin lyase: preparation and characterization of the homogeneous enzyme from onion bulbs. *Arch. Biochem. Biophys.*, **193**, 150–157.

Yamaguchi, M., Shannon, S., Howard, F. D. & Joslyn, M. A. (1965). Factors affecting the formation of a pink pigment in purees of onion. *Proc. Am. Soc. Hort. Sci.*, **86**, 475–483.