Allium Discoloration: The Nature of Onion Pinking and Garlic Greening

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Abstract: Precursors involved in the formation of pink and green-blue pigments during onion and garlic processing, respectively, have been studied. It has been confirmed that the formation of both pigments is of a very similar nature, with (*E*)-*S*-(1-propenyl)cysteine sulfoxide (isoalliin) serving as the primary precursor. Upon disruption of the tissue, isoalliin and other *S*-alk(en)ylcysteine sulfoxides are enzymatically cleaved, yielding prop-1-enyl-containing thiosulfinates [$CH_3CH = CHS(O)SR$; R = methyl, allyl, propyl, 1-propenyl], among others. The latter compounds subsequently react with amino acids to produce the pigments. Whereas the onion and leek-related propyl, prop-1-enyl and methyl derivatives can form pink, pink-red and magenta compounds, those containing the allyl group yield dark blue products after reacting with glycine at pH 5.0.

Keywords: *Allium*; garlic; onion; discoloration; pigment

INTRODUCTION

During processing of garlic (*Allium sativum* L.), onion (*A. cepa* L.) and leek (*A. porrum* L.) intensely colored pigments are often formed. In the case of garlic, green, blue-green or blue compounds are generated, whereas onion and leek homogenates may turn pink or red within several hours [1–9]. The pigments are, however, quite unstable and eventually turn into yellow or brown compounds which significantly lower the organoleptic quality of the product. Thus, effective controlling of this discoloration process is apparently of great economic importance for the food industry.

Despite having been studied for almost 50 years, this phenomenon is still very poorly understood. It has been shown that the discoloration is a multistep process. The first step is considered to be an enzyme catalyzed degradation of an amino acid precursor, leading to formation of an ether-soluble colorless compound. The latter compound, called the 'color developer', subsequently reacts with various amino acids and carbonyl compounds, yielding the pigments. SHANNON *et al.* [8, 9] proposed that the primary amino acid precursor involved in the pinking of onion is (*E*)-*S*-(prop-1-enyl)cysteine sulfoxide (isoalliin, **1**). Isoalliin is the major free amino acid occurring in onion and it is also present as a minor *S*-substituted cysteine sulfoxide derivative in garlic. The key role of isoalliin in the discoloration of garlic was later confirmed by LUKES [5]. Although BANDYOPADHYAY and TEWARI [10] tentatively identified the major color developing compound to be (*Z*)-thiopropanal *S*-oxide (**5**), the lachrymatory factor (LF) of onion, this finding has never been confirmed.

Described herein are our investigations into the identification of the precursors involved in the discoloration of onion, leek and garlic during processing. Our attention was mainly focused on the roles of isoalliin and its analogues, together with their primary decomposition products, i.e. (*Z*)-thiopropanal *S*-oxide and thiosulfinates.

EXPERIMENTAL

Material and methods: Detailed description of chemicals and instrumentation used as well as syntheses of reference compounds and alliinase isolation can be found in KUBEC *et al.* [12].

Model experiments: Aliquots (1 ml) of stock solutions of *S*-alk(en)ylcysteine sulfoxides (5 mg/ml)

and glycine (15 mg/ml) in 0.1 M KH$_2$PO$_4$ buffer (pH 5.5) were placed in 13-ml glass vials. A portion of 0.5 ml of an alliinase solution (40 mg/100 ml) was added, the total volume was adjusted to 5 ml with the buffer, and the vials were capped, shaken and immersed in a water bath (40°C) overnight. When studying the role of thiosulfinates and the LF, 2 µl of a thiosulfinate or (Z)-thiopropanal S-oxide were mixed with 3 ml of a glycine solution (10 mg/ml 0.1 M KH$_2$PO$_4$, pH 5.0) in 10-ml glass vials. The vials were capped, briefly sonicated (1 min) and kept overnight at 45°C. After cooling to room temperature, the solutions were filtered (0.45 µm) and their UV/Vis spectra were recorded.

RESULTS AND DISCUSSION

Because the observed discoloration seems to occur only in members of the *Allium* genus, the role of the most typical compounds of these plants,

S-alk(en)ylcysteine sulfoxides, was investigated in the first stage of the study. The four major derivatives occurring in the S-alk(en)ylcysteine sulfoxide pool of alliaceous plants, i.e. S-prop-1-enyl-, S-methyl-, S-propyl- and S-allylcysteine sulfoxides (isoalliin, methiin, propiin and alliin, respectively) (1–4), were prepared by synthesis. Various combinations of the S-alk(en)ylcysteine sulfoxides, glycine and onion alliinase were mixed, incubated overnight at 40°C and the resulting change in color was evaluated. A pigment was formed in only those systems containing both isoalliin and alliinase. Although the addition of glycine significantly enhanced the intensity of the color formed, its presence was not essential for color development (perhaps isoalliin and the other cysteine derivatives were incorporated into the pigments instead of glycine).

Even though (Z)-thiopropanal S-oxide (LF, 5) was reported to be the major color developing compound in onion [10], no pigment was formed
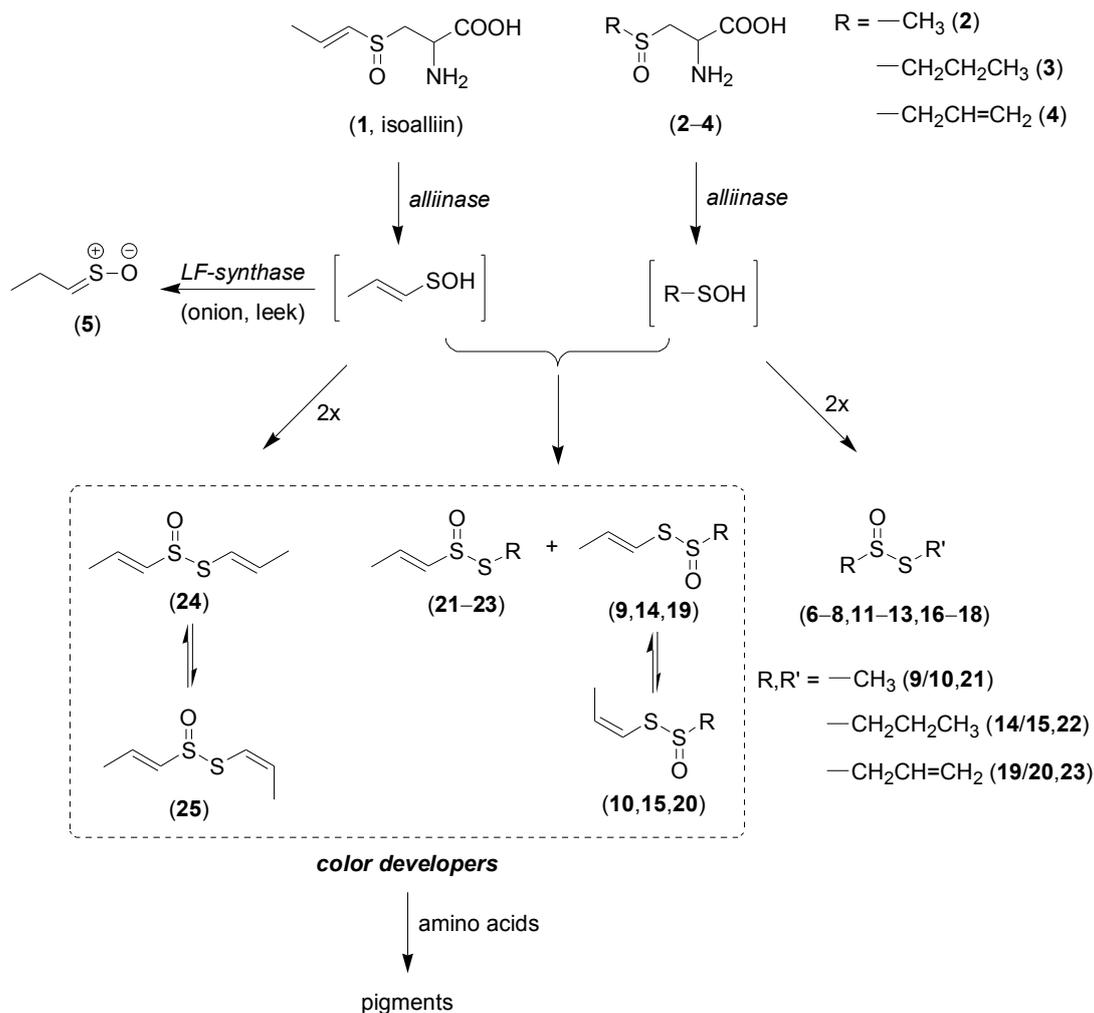

Figure 1. Formation of the pigments in *Allium* species

in model systems containing LF and glycine under any of the conditions studied (temperatures 40–95°C, various concentrations). Thus, our attention was drawn to another class of compounds arising from enzymatic degradation of isoalliin and its analogues, the thiosulfinates. All the major *Allium*-occurring thiosulfinates [RS(O)SR'; R, R' = CH$_3$-, CH$_3$CH$_2$CH$_2$-, CH$_2$ = CHCH$_2$- and CH$_3$CH = CH-] (**6–25**) were synthesized and allowed to react with glycine at 45°C to test their ability to form the pigments. Indeed, pink, pink-red, magenta and blue compounds were produced overnight in all mixtures consisting of 1-propenyl-containing thiosulfinates. Whereas the onion and leek-occurring thiosulfinates (**9/10**, **14/15**, **21**, **22** and **24/25**) yielded pink, pink-red or magenta compounds, the solutions containing the garlic-related derivatives (**19/20** and **23**) turned blue at pH 5.0.

Our findings indicate that the sequence of reactions leading to pigment formation includes (1) enzymatic cleavage, upon tissue disruption, of isoalliin and other *S*-alk(en)ylcysteine sulfoxides, ultimately yielding prop-1-enyl-containing thiosulfinates, among others; and (2) reaction of the thiosulfinates with amino acids to produce the pigments (Figure 1). The major color-developing compounds formed in onion and leek homogenates are **9/10**, **14/15**, **21**, **22**, **24** and **25**. Their relative proportions would be expected to vary significantly from batch to batch, depending mostly on

the relative ratios of the corresponding amino acid precursors (**1–3**) present in the tissue. Since **9/10** and **21** (and to a very limited extent **24** and **25**) are also present as minor constituents in garlic homogenates, a small amount of the red pigment may be formed in garlic. In fact, some red-colored compounds are perhaps generated even from **19/20** and **23**, as indicates the shoulder at 538 nm in the UV/Vis spectra at pH 5.0. However, the presence of the red pigment in garlic may be masked by the much more abundant blue compounds formed from **19/20** and **23**, which are the key color-developing compounds occurring in crushed garlic.

JOSLYN and PETERSON [3] were the first to report on the significant influence of carbonyl compounds on the rate of pigment formation. Their findings were later confirmed by YAMAGUCHI *et al.* [6] and SHANNON *et al.* [8] who demonstrated that some carbonyl compounds were apparently involved in the discoloration process. We have made very similar observations. Addition of a carbonyl compound (e.g. formaldehyde, acetaldehyde, propionaldehyde and acrolein) to model solutions had a significant effect on the intensity of the resulting color. The rate of pigment formation in the carbonyl compound-enriched samples was somewhat higher than in those with no aldehyde added. However, as we have demonstrated, the pigments are also formed in solutions consisting only of prop-1-enyl-containing thiosulfinates and glycine. This indicates that
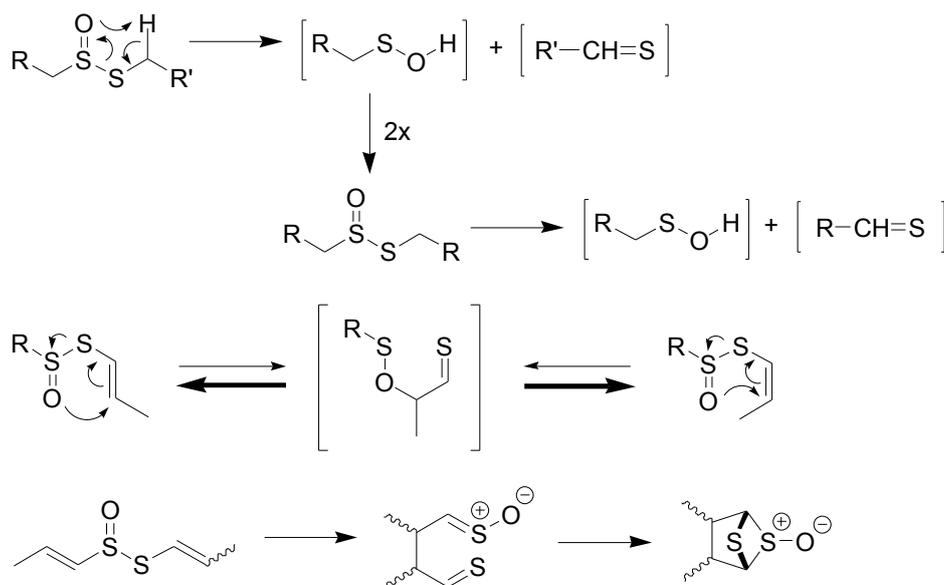

Figure 2. Formation of various thioaldehydes from thiosulfinates

some carbonyl or even thiocarbonyl compounds formed *via* decomposition of thiosulfinates are intermediates in pigment formation. Several very reactive thiocarbonyl species can be generated from prop-1-enyl-containing thiosulfinates [11] (Figure 2), and it is conceivable that some of them may be involved in pigment formation. Out of these thiosulfinate decomposition/rearrangement pathways, the *E/Z* isomerization *via* the thialsulfenate intermediate is the most prominent one for prop-1-enyl-containing thiosulfinates and may play the pivotal role. The apparent involvement of both amino and (thio)carbonyl compounds is reminiscent of Maillard-type reaction chemistry.

CONCLUSIONS

Apparently, discoloration can occur upon tissue disruption of any *Allium* species that contains at least traces of isoalliin. In many cases however, pigment formation would be expected to be masked by chlorophyll (e.g. in chive, scallion or Chinese chive) or other natural pigments (e.g. anthocyanins in red varieties of onion). Further research is ongoing to determine the structure of the pigments and clarify the detailed reaction pathways leading to their formation. Preliminary HPLC results indicate that the discoloration process is very complex, yielding several colored products even when only one pair of thiosulfinate regiomers and a single amino acid are allowed to react. Apparently, the natural pigment is a mixture of structurally-related compounds whose differences are correlated with the structures of the amino acid(s) and thiosulfinates which serve as the reactants.

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