S-Substituted Cysteine Derivatives in Production of Flavour and Colour

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Abstract: A brief review of our continuous investigations on *S*-substituted cysteine derivatives is presented herein. It comprises a summary on nonenzymatic (thermal) decomposition of these amino acids as well as on their role in the formation of blue and pink pigments during processing of garlic and onion, respectively. The emphasis is put on four most common derivatives, namely *S*-methyl-, *S*-allyl-, *S*-1-propenyl- and *S*-propylcysteine sulfoxides (methiin, alliin, isoalliin and propiin, respectively). Our results demonstrate that these sulfur-containing amino acids are the key primary precursors in the formation of both flavour and colour of alliaceous plants.

Keywords: *Allium*; garlic; onion; flavour precursor; pigment; cysteine sulfoxide; isoalliin; alliin

INTRODUCTION

S-Alk(en)ylcysteines and their sulfoxides belong to the most common secondary metabolites. Although their occurrence is typically associated almost exclusively with *Allium* species, their distribution in the plant kingdom appears to be much broader. They commonly occur in many other plants (e.g. genera *Brassica, Vigna, Petiveria, Tulbaghia, Scorodocarpus* and *Acacia,* among many others) as well as in several mushrooms (e.g. genera *Marasmius, Collybia, Lentinus*) and marine algae (e.g. genera *Chondria* and *Undaria*).

Garlic (*Allium sativum* L.), onion (*A. cepa* L.) and other members of the genus *Allium* contain typically 1–5% dry weight of non-protein sulfur amino acids. Generally, the pool consists of varying relative proportions of four major derivatives: *S*-allyl-, *S*-methyl-, *S*-propyl- and (*E*)-*S*-(propen-1-yl)cysteine sulfoxides (alliin, methiin, propiin and isoalliin, respectively). The presence of two more derivatives, namely *S*-ethyl- and *S*-butylcysteine sulfoxides, has recently been reported [1, 2]. However, due to their very low abundance, their contribution to the aroma formation is likely to be rather limited in the most common edible *Allium* species.

The enzymatic formation of flavour in *Allium* vegetables has been thoroughly studied and the substrate–enzyme systems well characterized. The typical flavour of these vegetables is formed by the enzymatic cleavage of *S*-alk(en)ylcysteine sulfoxides when the cellular tissue is disrupted. In the intact tissue, alliin and the other *S*-alk(en)ylcysteine sulfoxides are located in the cytoplasm and the C–S lyase enzyme (alliinase, EC 4.4.1.4) in the vacuole. A disruption of the plant tissue (by cutting, slicing, chopping etc.) results in the release of alliinase and subsequent α,β-elimination of *S*-alk(en)ylcysteine sulfoxides, affording the corresponding alk(en)ylsulfenic acids and α-aminoacrylate. The latter compound spontaneously decomposes to ammonia and pyruvic acid (*via* α-iminoacrylic acid). Condensation of the arising alk(en)ylsulfenic acids leads to the formation of thiosulfinates, the flavour principles of freshly disrupted *Allium* vegetables (Figure 1).

The distinctive flavours of different *Allium* species reflect varying amounts of the flavour precursors, particularly of isoalliin, the precursor of the onion lachrymator: (*Z*)-propanethial *S*-oxide, and of alliin which is the precursor of the characteristic allylic compounds of garlic.
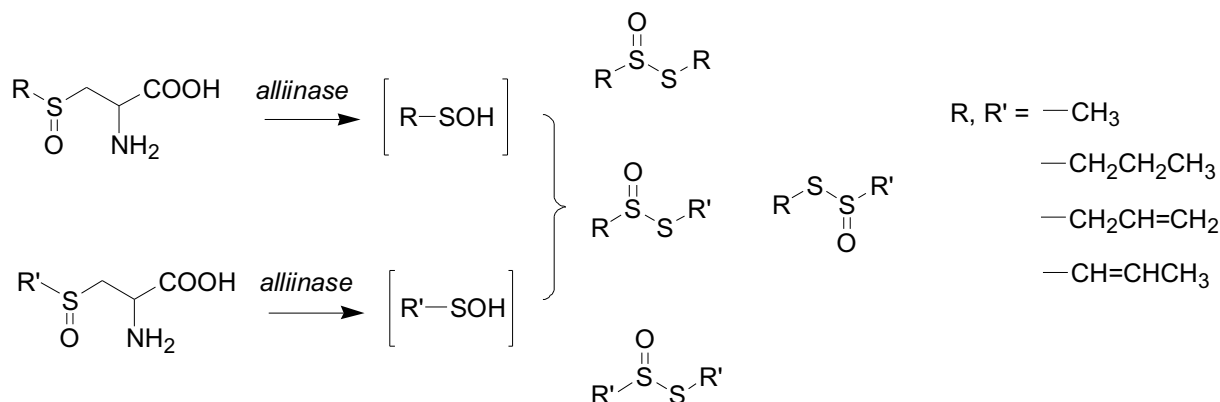
Figure 1. Enzymatic formation of thiosulfinates in *Allium* species

The complexity of the flavour components of *Allium* vegetables is chiefly due to two reasons. The first is the multiplicity of the precursors present together in the tissue (usually two or three). Therefore, up to nine thiosulfinates can be formed as the primary enzymatic products. The second reason is the unbelievable variability of the secondary reactions. The arising *S*-alk(en)yl alkanethiosulfinates can participate in an astonishing variety of subsequent reactions which strongly depend on the conditions (particularly on the polarity of medium and temperature) and which afford miscellaneous types of organosulfur volatile compounds, such as sulfides, vinyldithiins, ajoenes, thiophenes, etc. [3].

However, alliinase is a rather thermolabile and pH-sensitive enzyme. Therefore, its activity can be largely affected by culinary and industrial processing such as chopping, crushing, heating, smoking, pickling, canning, boiling, drying, frying, roasting, irradiation, etc. For example, the activities of alliinase in some industrially processed onions were found as follows (fresh onion alliinase 100%): freeze-dried 45%, laboratory-frozen 18%, hot-air dried 8.8–10%, commercially-frozen 6.6%, boiled 5.2%, and pickled 0.01% [4]. If alliinase is inactivated, a substantial amount of the aroma precursors will remain unreleased. Being by far the most abundant free amino acids present in the tissue, they can subsequently participate in non-enzymatic (thermal) developing of the characteristic flavour of processed vegetables. Moreover, along with free *S*-alk(en)ylcysteine sulfoxides, considerable amounts of both γ-glutamyl-*S*-alk(en)ylcysteines and their sulfoxides are also present in the tissue.

Since these dipeptides are not directly cleaved by alliinase, it can be assumed that they also significantly participate in thermal generation of flavour.

During the past several years, we have thoroughly studied thermal decomposition of *Allium*-occurring *S*-alk(en)ylcysteines and the corresponding sulfoxides [5–10]. We focused mainly on the effect of the water content and the temperature and time of heating on the stability of these amino acids. The arising volatile products were identified, quantified and their sensory properties determined by AEDA. Formation pathways leading to the formation of the major volatile products were proposed. Recently, our attention has also been drawn to investigations of the formation of blue and pink pigments during processing of garlic and onion, respectively [11]. A brief overview of our results on these topics is given here.

EXPERIMENTAL

The role of S-alk(en)ylcysteine derivatives in flavour formation. Amino acids were heated in sealed glass tubes at temperatures ranging 80–200°C in the presence of a variable water amount (0–98%) for 1–60 min. The arising volatile compounds were extracted with diethyl ether and analyzed by GC/FID and GC/MS. For more details see references [5–10].

The role of S-alk(en)ylcysteine derivatives in colour formation. Various combinations of aqueous solutions of *S*-alk(en)ylcysteine sulfoxides, glycine and alliinase were mixed and incubated overnight (40°C). After cooling to room temperature, the model solutions were filtered and their UV/Vis spectra recorded [11].

RESULTS AND DISCUSSION

The role of *S*-substituted cysteine derivatives in flavour formation

S-Alk(en)ylcysteine sulfoxides are very thermolabile compounds compared to the protein-occurring amino acids. Naturally, the stability of all the *S*-alk(en)ylcysteine sulfoxides considerably decreases with the time of heating (at 120°C, 10% water). The two derivatives containing an unsaturated side chain (alliin and isoalliin) are significantly less stable upon heating than those containing a saturated chain (methiin and propiin). Both alliin and isoalliin decompose nearly quantitatively within 15 min, whereas propiin and methiin break down almost completely during heating for 1 h. In the case of alliin and propiin, the total amounts of volatiles initially increase with time, reach the maximum in 15 min, and decrease with a prolonged time of heating. It can be explained by the thermal instability of the primary breakdown products which can subsequently form various nonvolatile pigments and other macromolecules (in part by self-condensation, in part by reactions with other low-molecular weight compounds, e.g. ammonia, acetaldehyde, and hydrogen sulfide).

As expected, the stability of all the *S*-alk(en)ylcysteine sulfoxides also greatly decreases with temperature of heating. Generally, the total amounts of the arising volatiles somewhat decrease at temperatures beyond 180°C, likely due to miscellaneous subsequent reactions of the primary volatile breakdown products. Also, the sensory properties of the model mixtures heated at 180°C are much less acceptable, exhibiting strong ammonia, pyridine, sulfury, biting and burnt off-notes. On the other hand, those heated at 120°C possess quite pleasant aroma of thermally processed vegetables. It could be described as garlic-like, cabbage-like and onion-like in the case of alliin, methiin, isoalliin and propiin, respectively.
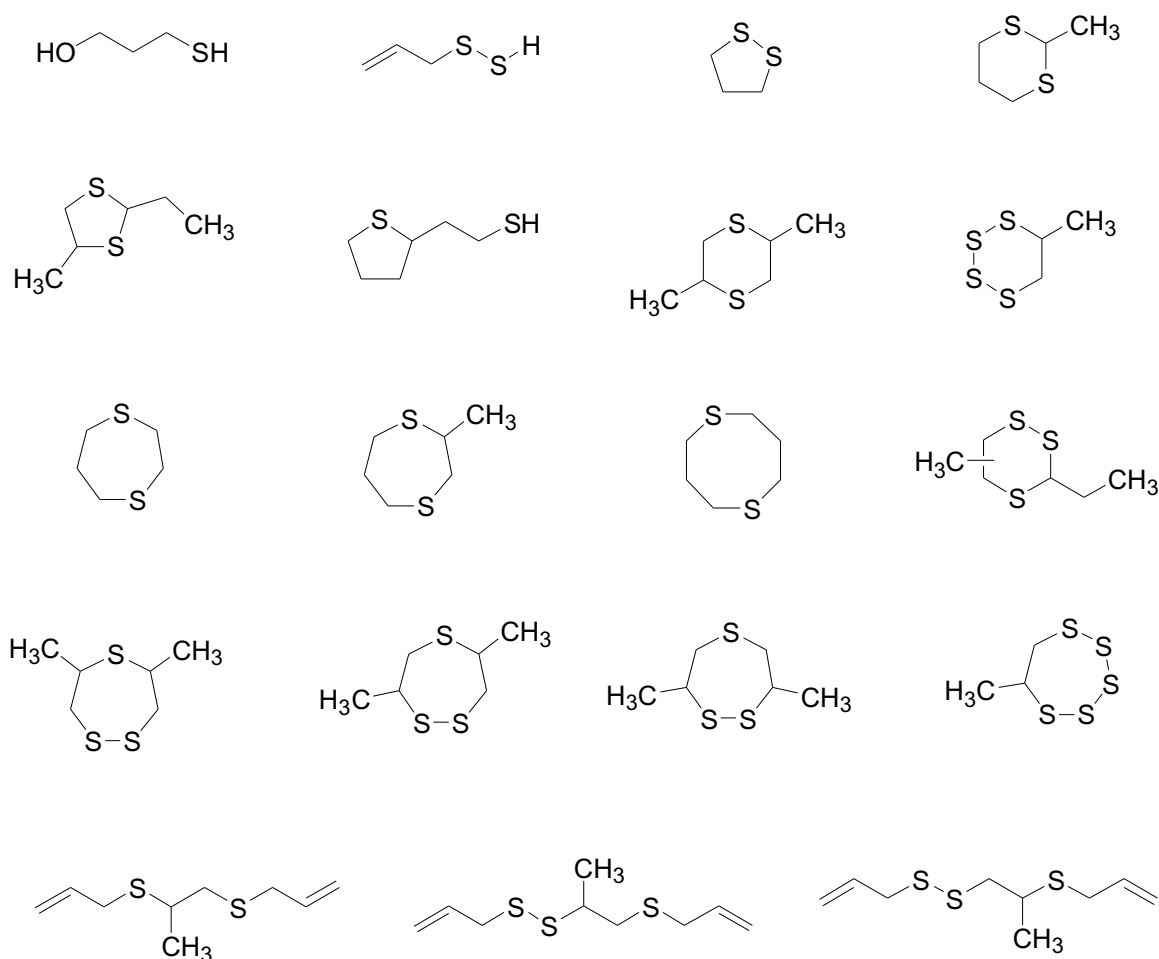

Figure 2. Some of the sulfur-containing volatiles thermally generated from alliin

The generally ignored reaction parameter, namely the content of water in the system, has been found to considerably influence not only the extent of the degradation but also the main degradation pathways. Generally, the formation of volatiles is maximized in the presence of 10–80% water and decreases greatly both at higher and lower water amounts. Such conditions can be expected during common culinary processing. However, on frying, roasting, baking, or during a commercial hot-air dehydration the content of water greatly decreases (particularly in the outer layers of the food) and the formation of some unpleasantly smelling compounds (e.g. pyridines and thiazoles) can become much more significant. Conversely, generation of some volatile compounds is favoured in the presence of higher water amounts, e.g. thiosulfinates and thiosulfonates from methiin and propiin, allyl alcohol from alliin, and 2-methylalk-2-enals from isoalliin.

In total, 34, 53 and 57 volatile compounds were detected in the model systems containing methiin, propiin and alliin, respectively. The corresponding mono-, di-, tri- and tetrasulfides are typically formed as the major volatile decomposition products. Other volatiles formed in significant quantities are dimethyl thiosulfinate and thiosulfonate (from methiin), propanethiol and dipropyl thiosulfonate (from propiin), allyl alcohol and various dithiianes, trithiolanes, dithiepanes and trithiepanes (from alliin) (Figure 2). Significant amounts of various pyridines (both alkyl- and alkylthio-substituted) were also identified in the model systems, in particular at higher temperatures. These compounds are proposed to be mainly generated from the interactions of ammonia, one of the major thermal degradation product, and aldehydes. On the other hand, no pyrazines were detected under any conditions studied. However, their formation would be expected to greatly increase in the presence of other food components, especially sugars.

In contrast to the very rich chemistry of the compounds arising enzymatically from isoalliin, thermal degradation of this major amino acid of onion leads to formation of only a very limited number of volatile products. Only 4 volatile compounds are generated at significant levels under the conditions studied, namely 2,4- and 3,4-dimethylthiophenes and two unsaturated aldehydes, 2-methylbuten-2-al and 2-methylpent-2-enal. An explanation of this odd behaviour can be found in the extremely facile self-cyclization of isoalliin into cycloalliin (3-carboxy-5-methyl-1,4-thiazane S-oxide) (Figure 3). Cycloalliin is likely to be quite a thermostable compound, not generating significant amounts of volatiles upon heating.

The role of *S*-substituted cysteine derivatives in colour formation

Intensely colourful pigments are often formed during processing of garlic, onion and leek. In the case of garlic, green, blue-green or blue pigments are generated, whereas onion and leek homogenates may turn pink or red within several hours. The pigments are, however, quite unstable and eventually turn into yellow or brown compounds which significantly lower the organoleptic quality of the product. Effective controlling of this discoloration process is apparently of great economic importance for the food industry.

Several explanations of this puzzling phenomenon have been suggested. The pinking of onion was once believed to be caused by the growth of some fungi or bacteria [12] or by formation of a betanine-type pigment [13]. Some researchers suggested that pinking of leek was a result of enzymatic oxidation of phenolic substances to quinones, which may in turn have reacted with amino acids [14]. On the other hand, others concluded that greening of garlic was a physiological disorder occurring temporarily as a consequence of the external stress the garlic had been exposed to [15]. In the non-scientific literature, this phenomenon is popularly explained by the reaction of sulfur-containing compounds of garlic with copper to yield blue copper sulfate.

Recently, we have shown that the discoloration of both onion and garlic is of a very similar nature,

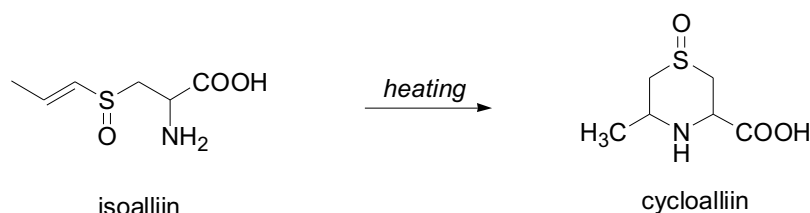isoalliin   *heating* →   cycloalliin

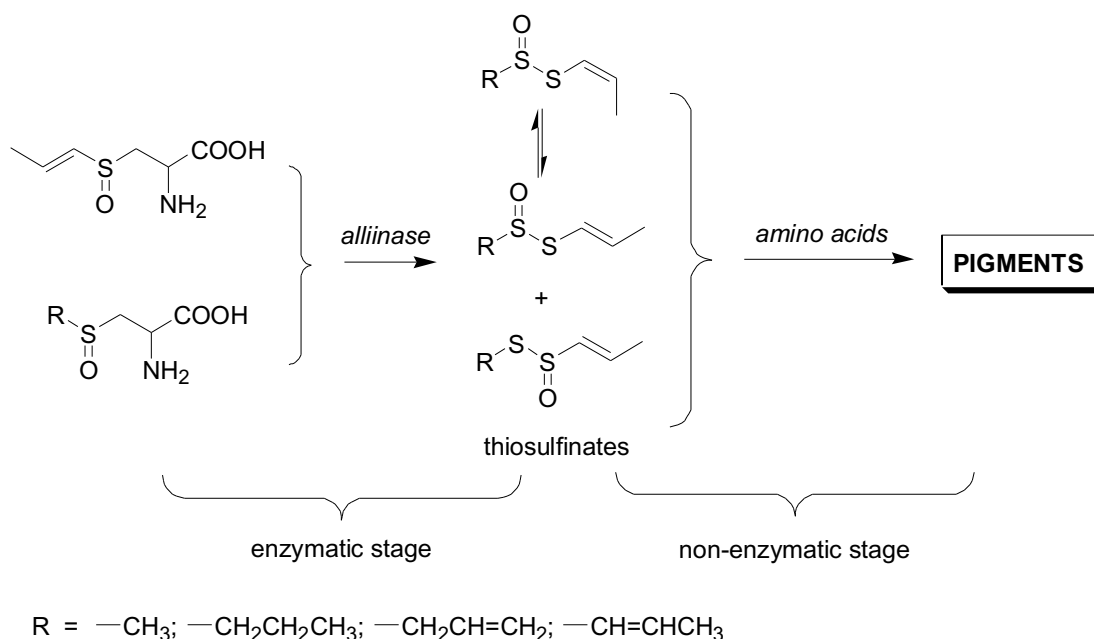Figure 3. Formation of cycloalliin from isoalliin

Figure 4. Formation of the pigments in *Allium* species

with isoalliin being the primary precursor [11]. The process is quite complex, consisting of enzymatic and non-enzymatic stages. In the first stage, isoalliin, together with other *S*-alk(en)ylcysteine sulfoxides (mainly methiin, alliin and propiin), is enzymatically cleaved upon disruption of the tissue, forming propen-1-yl-containing thiosulfinates [$CH_3CH=CHS(O)SR$; R = methyl, allyl, propyl, propen-1-yl], among others. The thiosulfinates subsequently react with various amino acids to produce the pigments (Figure 4). Whereas the propyl-, propen-1-yl and methyl-derivatives form pink, pink-red and magenta compounds, those containing the allyl group give rise to blue products after reacting with glycine at pH 5.5. The chemical structure of the pigments as well as the detail reaction pathways involved in the discoloration process still remain to be determined. However, our results indicate that some (thio)carbonyl compounds formed *via* decomposition of the thiosulfinates are intermediates in the pigment formation. The apparent involvement of both amino and (thio)carbonyl compounds in the pigment formation is reminiscent of Maillard-type reaction chemistry.

CONCLUSIONS

Based on our results, it can be concluded that *S*-alk(en)ylcysteine sulfoxides represent a class of very reactive and highly thermally unstable compounds. Apparently, they belong to the most labile amino acids naturally occurring in foods. This thermal instability, together with their exceptionally high contents in alliaceous plants, makes them very important nonvolatile flavour precursors. The extent of their decomposition and the formation of volatiles can be strongly influenced by many factors, such as the time and temperature of heating, and the water content in the reaction system. The importance of nonenzymatic degradation of *S*-alk(en)ylcysteine sulfoxides becomes apparent especially at higher temperatures when the water content has decreased, for example, during onion dehydration or baking. Naturally, many factors other than those investigated in our work are involved in real food systems. Their decomposition would be considerably more complex in the presence of other food components, sugars and lipids in particular.

On the other hand, *S*-alk(en)ylcysteines seem to be significantly less important nonvolatile flavour precursors. Not only they occur in considerably lower quantities in *Allium* species, they also generate very limited amounts of volatile products upon heating. This is mainly due to the absence of the very thermolabile sulfoxide moiety. Thus, decarboxylation and deamination are the most important degradation pathways of these amino acids.

S-Alk(en)ylcysteine sulfoxides, namely isoalliin, also play a pivotal role in the formation of blue and pink compounds during processing of garlic and onion, respectively. Such a type of discoloration can occur upon tissue disruption of any *Allium* species that contains isoalliin, the precursor of 1-propenyl-containing thiosulfinates. In many cases however, the pigment formed is likely 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 coloured 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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