

Title Identification of candidate amino acids involved in the formation of blue pigments in crushed garlic cloves (*Allium sativum* L.)

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Abstract

The color-forming ability of amino acids with thiosulfinate in crushed garlic was investigated. We developed reaction systems, for generating pure blue pigments using extracted thiosulfinate from crushed garlic and onion and all 22 amino acids. Each amino acid was reacted with thiosulfinate solution and was then incubated at 60°C for 3 hours to generate pigments. Unknown blue pigments, responsible for discoloration in crushed garlic cloves (*Allium sativum* L.), were separated and tentatively characterized using high performance liquid chromatography (HPLC) and a diode array detector ranging between 200-700 nm. Blue pigment solutions exhibited two maximal absorbance peaks at 440 nm and 580 nm, corresponding to yellow and blue, respectively. With different retention times. Our findings indicated that green discoloration is created by the combination of yellow and blue pigments. Eight naturally occurring blue pigments were separated from iscolored garlic extracts using HPLC at 580 nm. This suggests that garlic discoloration is not caused by only one blue pigment, as reported earlier, but by as many as 8 pigments. Overall free amino acids that formed blue pigment when reacted with thiosulfinate were glycine, arginine, lysine, serine, alanine, aspartic acid, asparagine, glutamic acid and tyrosine. Arginine, asparagine and glutamine had spectra that were more similar to naturally-greened garlic extract.