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Thin-Layer Chromatographic Investigation of Color Developer Involved in Pinking of White Onion Purees

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The compounds responsible for pinking of white onion purees were isolated and studied by thin-layer chromatography. At least three compounds were found to have color-developing properties. The major one among these was tentatively characterized by infrared spectrophotometry and gas-liquid chromatography as thiopropanal *S*-oxide, the lachrymatory factor of onion. Preliminary analysis of the other two components indicated that they were also thioalkanal-*S*-oxide types of compounds having lachrymatory properties hitherto unreported.

Pinking of white onion bulb purees was first reported by Joslyn and Peterson (1958). Lukes (1959) investigated the cause of the development of such pink pigment in onion purees and showed that at least two steps were involved in color formation. The first one was enzymic, where an ether-soluble colorless compound named as color developer was rapidly produced, and the second one was nonenzymic, where the color developer reacted with naturally occurring amino acids and carbonyls, resulting in the final pigment. Pigment-forming reactions and precursors, involved in the formation of pink color in onion purees, were elaborated by Shannon *et al.* (1967a,b). They demonstrated that the color developer was produced rapidly by the enzymic action of allinase on *S*-1-propenyl cysteine sulfoxide (PECSO), the primary pigment precursor of onion. However, the nature of color developer was still illusive and to our knowledge no further studies in this regard have been reported. In the present investigation an attempt has been made to elucidate further the nature of color developer compound present in white onion purees.

EXPERIMENTAL SECTION

Fresh white globe onions, stored for about a month after harvest, were purchased from local market and kept at 0° for several hours before extraction.

All solvents and reagents were analytical grade. The solvents were redistilled before use.

Onion Extract. Extraction was carried out at 0° in four batches, each having 500 g of peeled onion. The ether-soluble color developer was obtained by extracting chilled onion juice several times with cold peroxide-free diethyl ether, as described by Lukes (1959). The residual pulp was also repeatedly extracted with the same solvent in a Waring blender. The ether layer from both the pulp and juice was pooled together after cold centrifugation. The combined ether extract was dried over anhydrous sodium sulfate, filtered, and finally concentrated in a rotary evaporator at room temperature. The residue (combined extract) was transferred into a tared flask with a minimum volume of diethyl ether. Ether was removed by blowing a stream of nitrogen and the final residue, a greenish pasty mass, was kept under nitrogen at −40° until use. In a similar way ether extracts from onion juice and the residual pulp were separately prepared from two batches of onions.

Thin-Layer Chromatography (tlc). Glass plates (20 × 20 cm), spread with silica gel G (E. Merck), layers of thickness 400 μ and 0.5 mm (preparative), respectively, were used. Silica gel slurry was prepared in distilled water (1:2 w/v). The plates were dried at room temperature and activated at 120° for 1.5 hr.

The plate was divided into two halves and on both the halves 500 μg of each extract in ether solution was spotted with the help of a micropipette. The plate was developed in a chromatographic tank containing petroleum ether (40°–60°)–diethyl ether–acetic acid (60:40:1 v/v). After development and subsequent removal of the solvent at room temperature, one half of the plate was sprayed with 50% sulfuric acid and the other half with glycine–formaldehyde reagent (4.5 ml of 0.1 *M* glycine and 0.5 ml of 3×10^{-4} *M* formaldehyde) prepared according to the method of Shannon *et al.* (1967a), and this half of the plate was covered with a cleaned glass plate. The chromatograms were visualized within 1 hr by heating the plate at 100°. With glycine–formaldehyde reagent, pink-colored spots were noted only after heating, while with sulfuric acid (Bandyopadhyay *et al.*, 1970) several colored spots were visible even before heating.

Isolation of Color Developer. Color developer compound was isolated from combined onion extract by high vacuum distillation based on the principle of closed system high vacuum transfer (Merritt *et al.*, 1959). The distillation assembly was comprised of a high vacuum pump coupled with a two-stage silicon oil diffusion pump, which was connected to a vacuum manifold. The distillation was accomplished within 6 hr under a pressure of 10^{-3} Torr at 40° in a closed unit consisting of two gas bottles of suitable size fitted with stopcocks and attached to the vacuum manifold by means of glass tubings, according to the procedure described by Merritt *et al.* (1959). The distil-

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late collected at −196° was then transferred to a small glass-stoppered tube with a minimum volume of diethyl ether and stored at −40° under nitrogen.

Purification of the Color Developer. The distillate containing the color developer compound was purified by preparative tlc on a thick layer (0.5 mm) of silica gel G plate using petroleum ether (40°–60°)–diethyl ether (60:40 v/v) as developing solvent described above. The plate was prewashed by ascending technique with the above solvent and reactivated for one-half hour before use. The distillate in ether solution was applied in band form on the plate (*ca.* 80 mg/plate). After development and subsequent removal of solvent, both of the edges of the plate (*ca.* 2-cm length) were sprayed with 50% sulfuric acid until pink bands were visible, while covering the remaining silica gel surface with a cleaned glass plate. Two pencil lines just above and below the major pink band (R_f value 0.33) were drawn across the plate; silica gel was carefully scraped off from the unsprayed portion of the plate and transferred into a small glass column (8 mm diameter × 10 cm length) fitted with a grease-free stopcock having a small filter bed (1–1.5 cm) of Celite over a cotton plug. The color developer compound was eluted from the column with *ca.* 20 ml of diethyl ether in a glass-stoppered test tube. Ether solution was concentrated by blowing a stream of nitrogen and the concentrate was kept at −40° under nitrogen.

Analysis of the Distillate and the Purified Product. *Tlc and Absorption Spectra.* A portion of the distillate in ether solution was separated on the tlc plate and the chromatographic pattern was observed as described previously. R_f values of the pink spots, obtained by spraying with glycine–formaldehyde reagent, were calculated. Each of these pink-colored spots was scraped from the plate, transferred into a similar glass column described above, and eluted from the silica gel with a few milliliters of 80% methanol. The absorption spectra of all these spots were determined in visible range by a Beckman DB spectrophotometer.

Similarly, a portion of the purified product was rechromatographed on a tlc plate using varying proportions of petroleum and diethyl ether, as well as chloroform–benzene (1:9 v/v) as solvents, and the chromatographic separation was noted; another portion was incubated with glycine–formaldehyde reagent and the absorption spectra were directly determined (Shannon *et al.*, 1967a).

Infrared (ir) Analysis. The spectra of the distillate and the purified product in thin film on a sodium chloride prism were obtained with the help of a Perkin-Elmer Infracord spectrophotometer, Model 137B.

Lachrymatory Property. Portions of the distillate and purified product were tested for their lachrymatory property. The samples were taken in a small Petri dish and brought near the eyes. Lachrymation was observed by eye irritation when nitrogen was blown in the respective samples.

Derivative Formation. A portion of the distillate in diethyl ether was shaken with an equal volume of 0.1 *M* cysteine for 1 hr (Lukes, 1971) and the ether layer was separated and concentrated. It was then tested for lachrymatory property and color reaction with glycine–formaldehyde reagent on tlc plate.

Gas–Liquid Chromatography (glc). The distillate and the purified product were analyzed under identical conditions in a gas chromatograph (Model BARC) equipped with a flame ionization detector and a glass column (¼ in. o.d. × 6 ft) packed with 10% Carbowax 20M supported on acid-washed Chromosorb W (60–80 mesh). The temperature of the oven and detector was maintained at 70°. The carrier gas was nitrogen, with a flow rate of 25 ml/min.

RESULTS AND DISCUSSION

The yield of onion extract from combined juice and residual pulp is found to be 0.1%, on a wet basis, with ether as solvent. This is comparable to the values obtained by other workers who used dichloromethane (Boelens *et al.*, 1971). However, the yield of the extract from the juice reduces to half of the above value. Since color developer has been reported to be soluble in diethyl ether (Lukes, 1959; Shannon *et al.*, 1967), other solvents have not been tried.

Tlc study of the onion extracts, obtained separately from the juice and the residual pulp, reveals that both the extracts contain varying proportions of at least three different compounds which are capable of forming pink color with glycine–formaldehyde reagent. The major one, which is the subject of the present investigation, is predominant in the juice, and the remaining two are largely present in the residual pulp. However, for a better understanding of the chromatographic behavior of the color-developer compounds present in white onion purees as a whole, the combined extract has been studied by tlc. The chromatogram of the combined extract on tlc plate indicates that several other compounds soluble in ether (Bandyopadhyay *et al.*, 1970) are also being extracted from onion purees along with the color-developer compounds. These are inactive to glycine–formaldehyde reagent, but can be detected with sulfuric acid.

High vacuum distillation of the combined onion extract, employed in the present study, avoids most of the other contaminants mentioned above and gives rise to a representative sample consisting mainly of the volatile color-developer compounds. The yield of distillate is found to be 0.4 g/kg of fresh onion. Separation of the distillate on tlc plate shows three components identified as pink spots by glycine–formaldehyde reagent. Their R_f values are 0.11, 0.33, and 0.50, respectively. From the nature and intensity of the spots on the chromatogram, it appears that the spot having an R_f value of 0.33 is the major component of the distillate. Further, all these pink-colored compounds show an absorption maximum at 520 mμ. Since glycine–formaldehyde reagent reacts with the color-developer compound present in onion juice to give a pink color having the same absorption maximum (Shannon *et al.*, 1967a), these three components of the distillate are thus considered to be color-developer compounds.

Purification of color-developer compound present in ether extract of onion juice was first attempted by Shannon *et al.* (1967b) by means of paper chromatography. Using a highly polar solvent system, *e.g.*, 1-butanol–acetic acid–water (4:1:5 v/v), they were able to isolate the fast migrating color-developer compound. However, its identification was obscured possibly due to the presence of other concurrently migrating contaminants. The present method of isolation of color-developer compounds and purification of the major one by preparative tlc proves to be a simple technique not reported previously. The purity of the major color developer compound has been tested by tlc and glc. On tlc plate it gives a single spot irrespective of various solvent systems and spray reagents. However, on increasing the proportion of diethyl ether to petroleum ether, a corresponding increase in the R_f value of the major spot is observed. Figure 1 shows the glc separation of the distillate and the purified major color developer compound. From Figure 1B it appears that the present tlc method of purification does not alter the retention time of the major color-developer compound, and that it is substantially pure and retains its entity.

Ir spectra of the distillate and the purified major color-developer compound are presented in Figure 2. In the distillate (Figure 2A) the absorptions at 5.83 and at 5.92 μ followed by 6.1 μ indicate the presence of carbonyl compounds having -CHO and -C=CCHO functional groups. The strong absorption at 8.86 and 9.15 μ concludes the presence of the C=S=O functional group in compounds like thioalkanal *S*-oxide having lachrymatory properties (Brodnitz and Pascale, 1971). In the purified sample (Fig-

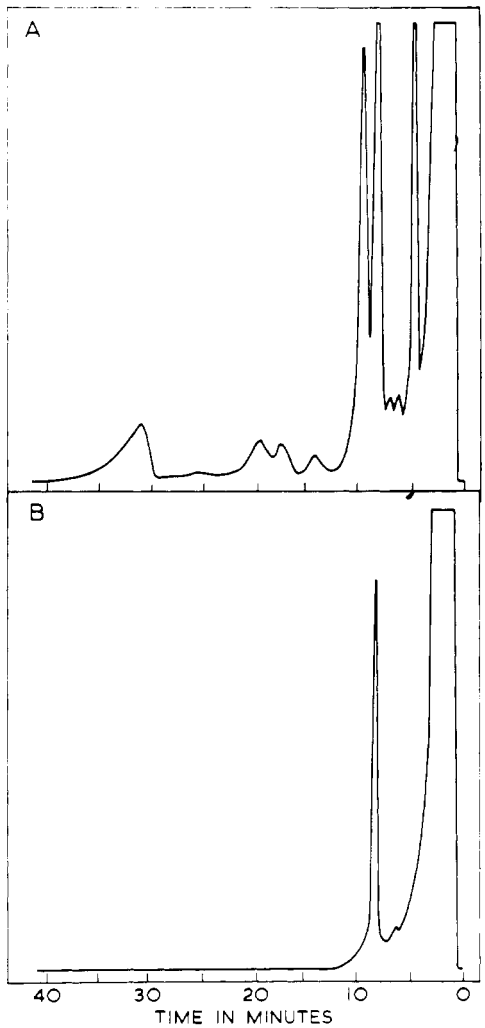

Figure 1. Gas chromatogram of the distillate (A) and purified color-developer compound (B) obtained from onion extract. (See Experimental Section for details.)

ure 2B), on the other hand, the carbonyl absorptions are minimized to a great extent without impairing the strong absorption at 8.86 and 9.15 μ and its ir spectrum closely resembles the spectral data of thiopropanal *S*-oxide, the lachrymatory factor of onion, reported recently by Brodnitz and Pascale (1971). The purified color-developer compound having a strong lachrymatory property has a retention time of 8 min (Figure 1B) in the Carbowax 20M column. Brodnitz and Pascale (1971) have reported the retention time of thiopropanal *S*-oxide in the SE 30 column to be 9 min. This difference in retention time could be attributed to the variation in polarity of the respective liquid phases and also to the change in conditions of the chromatographic separation. The presence of volatile carbonyl components, *e.g.*, propanal, 2-methyl-2-pentenal, etc., other than the color-developer compounds in the distillate can be accounted for from the rapid biogenesis of PECSO (Virtanen, 1967) schematically represented by Boelens *et al.* (1971). The removal of these carbonyl compounds and the separation of the color-developer compounds from the distillate are successfully carried out by the method described in the present study.

Shannon *et al.* (1967b) suggested that the color-developer compound might be a propenyl thiosulfinate, a secondary product, derived from the enzymic reaction on the primary precursor, PECSO. However, propenyl thiosulfinate has never been found to occur in onion (Boelens *et al.*, 1971; Carson, 1967; Virtanen, 1967). From the present study, on the other hand, it is suggested that the major color-developer compound is a thioalkanal *S*-oxide type of compound and probably thiopropanal *S*-oxide, the lachrymatory factor of onion, is derived solely from PECSO (Brodnitz and Pascale, 1971; Virtanen, 1967). The other two color-developer compounds present in the distillate of the combined onion extract are presumed to be volatile thioalkanal *S*-oxide type of compounds, as they also have lachrymatory property. These color-developer compounds along with the major one, after reacting with cysteine, fail to respond to the pink color test with glycine–formaldehyde reagent and completely lose their lachrymatory property. Recently, Lukes (1971) has separated on a cellulose tlc plate the cysteine derivatives of lachrymator and other flavor compounds isolated from onion juice and studied the flavor strength of onion. Although the occurrence of more than one lachrymator, or in other words, the color-developer compounds has not been reported, the evidences presented in this study for such occurrence are circumstantial in nature, since related synthetic compounds are not available.

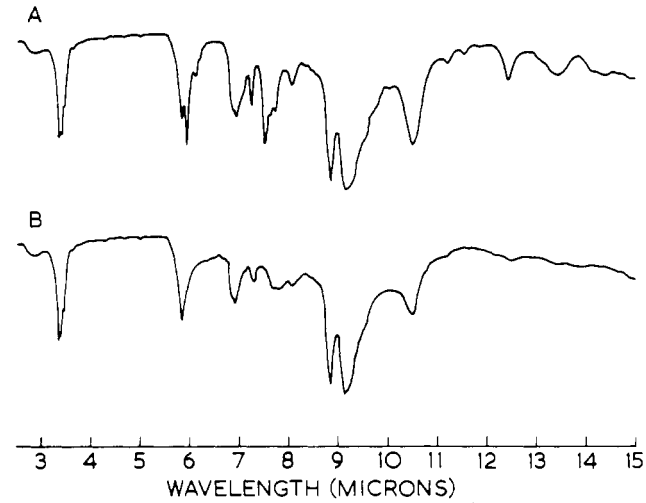

Figure 2. Infrared spectra of the distillate (A) and purified color-developer compound (B).

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