ELSEVIER

0308-8146(95)00027-5

Food Chemistry 54 (1995) 183–188

0308-8146/95/$9.50

Pyruvate and flavor development in macerated onions (*Allium cepa* L.) by γ-glutamyl transpeptidase and exogenous C-S lyase

Tirza Hanum,[a] Nirmal K. Sinha,[a] Daniel E. Guyer[b] & Jerry N. Cash[a]*

[a]*Dept of Food Science and Human Nutrition,* [b]*Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA*

(Received 5 November 1994; revised version received and accepted 25 January 1995)

Effect of γ-glutamyl transpeptidase in conjunction with exogenous C-S lyase on pyruvate content in macerated onion and flavor profile was studied. Pyruvate production of 2·5-fold greater than that of the control was obtained in γ-glutamyl transpeptidase and exogenous C-S lyase treated onions held for 20 h at 37°C. Relative abundance of flavor compounds in Spartan Banner, a pungent onion, varied from a yellow sweet salad-type onion. The effect of γ-glutamyl transpeptidase and exogenous C-S lyase on onion flavor profile was shown by a shift of major components from methyl propyl disulfide, methyl propenyl trisulfide, dimethyl tetrasulfide and propyl 1-propenyl trisulfide, into new major components, methyl 1-propenyl disulfide, dipropyl disulfide, propyl 1-propenyl disulfide, methyl 1-propenyl trisulfide, and propyl 1-propenyl trisulfide. This increase in 1-propenyl containing flavor compounds may effect overall flavor of γ-glutamyl transpeptidase and exogenous C-S lyase treated onion extracts.

INTRODUCTION

Onion (*Allium cepa* L.) which belongs to *Allium* genus is prized for its flavor and pungency and is widely used as flavoring agent in food processing. The characteristic onion flavor is developed by the action of the flavor producing enzyme C-S lyase (also known as alliinase or alliin alkyl sulfenate lyase; EC 4.4.1.4), on non volatile, odorless precursors (alk(en)yl-L-cysteine sulfoxide) when onion cells are cut or macerated to produce pyruvate, ammonia and many volatile sulfur compounds associated with flavor and odor (Whitaker, 1976).

A part of the S-substituted amino acids in onion are bound to glutamic acid as γ-glutamyl peptides and are not susceptible to the action of the endogenous alliinase (Matikkala & Virtanen, 1967). Schwimmer (1971) reported that γ-glutamyl transpeptidase (γ-glutamylpeptide : amino acid γ-glutamyl transferase; EC 2.3.2.2) from mammalian kidney and C-S lyase from *Albizzia* acted sequentially in a coupled enzymatic reaction to convert synthetic γ-glutamyl-*S*-methyl-L-cysteine peptides to pyruvate. No pyruvate was produced by transpeptidase in the absence of C-S lyase; however, in the presence of C-S lyase, transpeptidase enhanced pyruvate production beyond that formed in the presence of C-S lyase alone according to the following equations:

$$\gamma\text{-glutamyl-}S\text{-alk(en)yl-L-cysteine sulfoxide} + \text{amino acid} \xrightarrow{\text{transpeptidase}} \gamma\text{-glutamyl-amino acid} + S\text{-alk(en)yl-L-cysteine sulfoxide} \quad (1)$$

$$S\text{-alk(en)yl-L-cysteine sulfoxide} + H_2O \xrightarrow{\text{C-S lyase}} NH_3 + \text{Pyruvate} + \text{S(volatile)} \quad (2)$$

Schwimmer (1971, 1973) and Schwimmer and Austin (1971) reported that transpeptidase enhanced pyruvate formation in dehydrated onion. However, little information about the action of transpeptidase in conjunction with endogenous and exogenous C-S lyase of onion on fresh macerated onion is available. Similarly, little has been published about the flavor profile of onion treated sequentially by transpeptidase and C-S lyase. Flavor compounds in the headspace of chopped onion varieties were reported by Kallio and Salorinne (1990). However, headspace analysis does not give direct information about concentration of the compounds in onion matrix. Recently, using supercritical carbon dioxide (SC-CO_2), fresh onion-like extract has been obtained (Sinha *et al.*, 1992). The SC-CO_2 is a

*To whom correspondence should be addressed.

relatively new extraction technique. In the supercritical phase (pressure 73·9 bar, temperature 31°C) carbon dioxide behaves like organic solvent, it is non-toxic, inexpensive and does not react with product (Rizvi *et al.*, 1986).

The objectives of this study were to determine the efficacy of using γ-glutamyl transpeptidase and exogenous C-S lyase in a sequential reaction for pyruvate and flavor development in macerated onion extracts.

MATERIALS AND METHODS

A yellow sweet onion obtained from a local grocery store and Spartan Banner variety grown and harvested at the Michigan State University Muck Farm in 1993, were used in this study.

Onion C-S lyase prepared according to Hanum *et al.* (1993) with specific activity of 8 unit/mg protein. One unit of C-S lyase activity is defined as the amount of enzyme that will liberate 1 μmol of pyruvate from alk(en)yl-L-cysteine sulfoxide under standard conditions. Kidney transpeptidase with specific activity of 4·9 units/mg protein was obtained from Sigma Chemical Co. (St Louis, MO, USA). One unit of transpeptidase is defined as the amount of enzyme that will liberate 1 μmol of *p*-nitroaniline per min from γ-glutamyl-*p*-nitroanilide under standard conditions (Lancaster & Shaw, 1991).

Trizma base, L-methionine, magnesium chloride, pyridoxal phosphate, ammonium sulfate, methylene chloride, 2,4-dinitrophenyl hydrazine (DPNH) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co.

Preparation of onions for enzyme treatments

Onion bulbs, weighing approximately 750 g were peeled, cut, and immediately processed in an Acme Juicerator (Model 6001, Acme Juicer Manufacturing Co., Sierra Madre, CA, USA). Except for the incubation study, macerated onions was covered and held at room temperature for 2 h to complete endogenous enzymatic C-S lyase action. Standard mixtures for pyruvate formation studies and flavor compound analysis were prepared based on Schwimmer (1971) where each ml of macerated onions contained 100 mM L-methionine, 20 μM pyridoxal phosphate and 1 mM $MgCl_2$.

Effect of incubation time and enzyme concentrations on pyruvate production

The effect of transpeptidase alone or in combination with exogenous C-S lyase on pyruvate production in macerated onion was carried out in triplicate according to a modification of the method described by Schwimmer (1971). Stock enzyme solutions used in this study were 16 units exogenous C-S lyase/ml of 0·05M phosphate buffer pH 7·0 and 4·8 units transpeptidase/ml of 0·05 mM Tris buffer pH 7·5.

The effect of incubation time on pyruvate production in macerated onion treated with exogenous C-S lyase alone, transpeptidase alone and transpeptidase in conjunction with exogenous C-S lyase was determined by adding 8 units exogenous C-S lyase, 2·4 units transpeptidase, and 2·4 units transpeptidase in conjunction with 8 units exogenous C-S lyase to a standard mixture of macerated onions, respectively. Pyruvate was determined every 0, 2, 4, 10, 20 and 36 h. Control containing macerated onion alone was run to show pyruvate production due to endogenous C-S lyase.

To study the effect of enzyme concentrations on pyruvate production 0, 0·48, 0·96, 1·92, 2·40 and 2·88 units transpeptidase and 0, 1·6, 3·2, 6·4, 8·0, and 9·6 units C-S lyase were added to a standard mixture of macerated onions, respectively. Transpeptidase in conjunction with exogenous C-S lyase was prepared by adding 9·6 units C-S lyase to 0·48, 0·96, 1·92, 2·40 and 2·88 units tranpeptidase in the standard mixture, respectively. Pyruvate was measured after 20 h of incubation at 37°C.

Pyruvate determination

Pyruvate concentration in onion standard mixtures was determined in triplicate. Two milliliters of 5% TCA was quickly added to the onion standard mixture to stop the enzymatic reaction. After 1 h the mixture was filtered and diluted to 1:20 ratio with distilled water. Control for background pyruvate measurement was determined by immediately adding 2 ml 5% TCA into 4 ml fresh macerated onion. After 1 h the control was filtered and diluted (1:10) with distilled water. Pyruvate was determined spectrophotometrically at 420 nm according to Wall and Corgan (1992). Results are expressed as μmols per g of fresh weight onion.

Flavor extraction and analysis

Enzyme treated samples were prepared by adding 1000 units kidney γ-glutamyl transpeptidase and 600 units onion C-S lyase into 500 ml macerated onion containing 100 mM L-methionine, 20 μM pyridoxal phosphate and 1 mM $MgCl_2$. Control and enzyme treated macerated onions were held at 37°C in 1 liter glass-stoppered Erlenmeyer flask for 20 h before flavor extraction. Macerated onion was used in this study because it allowed for good mixing with the solvent in the extraction vessel. SC-CO_2 was used to extract flavor compounds from macerated onion. Triplicate extractions were made.

The SC-CO_2 extraction apparatus consisted of industrial grade CO_2 gas, 99·5% purity from a gas cylinder compressed with a gas booster. A pressure regulator positioned between the reservoir and the extraction vessel controlled the extraction pressure. The total volume of the stainless steel extraction vessel was 750 ml. The temperature of the vessel was maintained at 37°C. Macerated onion in the volume of 500 ml was poured into the vessel. The extraction process was started by

slowly raising the pressure in the extraction vessel while the system outlet was closed. Carbon dioxide passed through the contents at a flow rate of about 1 liter/min. (STP) and was monitored with a flow meter and a wet test meter. After the extraction vessel pressure reached 204 atm, a heated (40°C) micrometering outlet was opened. Onion extract was collected in an armed trap tube. As CO_2 solvent and the onion solute separated, the solvent returned to atmospheric pressure. The extraction was performed for about 24 h or until 800–1000 liters CO_2 had passed through the extraction vessel.

For the purpose of flavor analysis, 0·1 mg of onion extract was diluted with 100 μl methylene chloride and injected into a gas chromatography–mass spectrophotometer (GC–MS). The GC–MS system consisted of JEOL AX 505H double focusing mass spectrometer coupled with a Hewlett-Packard HP 5890J gas chromatograph. The column was a fused silica DB-1 capillary column (30 m × 0·25 mm × 0·25 mm). The operating conditions were as follows: injector, 150°C; detector, 150°C; oven, 35–150°C at 5°C/min linear; and GC–MS transfer line 150°C. Helium carrier gas flow rate was 1 ml/min. The mass spectra were obtained by electron ionization at 70 eV. Kovat retention indices (I_k) were calculated against the retention time of normal hydrocarbon of C_7–C_{16} coinjected with the sample (Ettre, 1964).

RESULTS AND DISCUSSION

Effect of incubation time and enzyme concentrations on pyruvate production

Spartan Banner variety was selected for the pyruvate production study. Maximum pyruvate production of 8·5 μmols/g fresh wt, catalyzed by endogenous C-S lyase (control) in macerated onion was achieved 2 h after maceration (Fig. 1). Adding the exogenous onion C-S

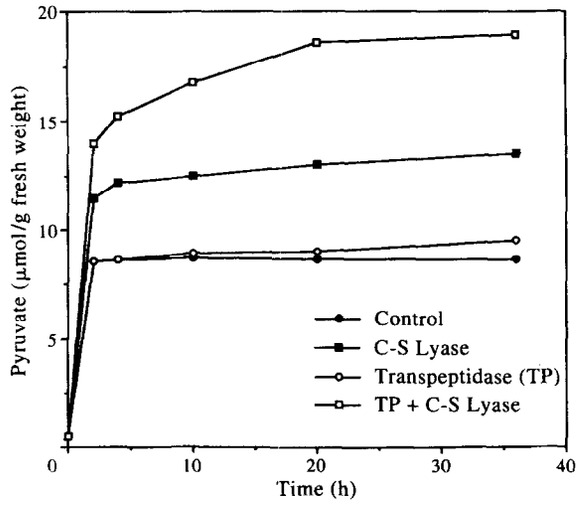

Fig. 1. Effect of incubation time at 37°C on pyruvate production in control, exogenous C-S lyase, transpeptidase and transpeptidase in conjunction with exogenous C-S lyase treated onion macerates.

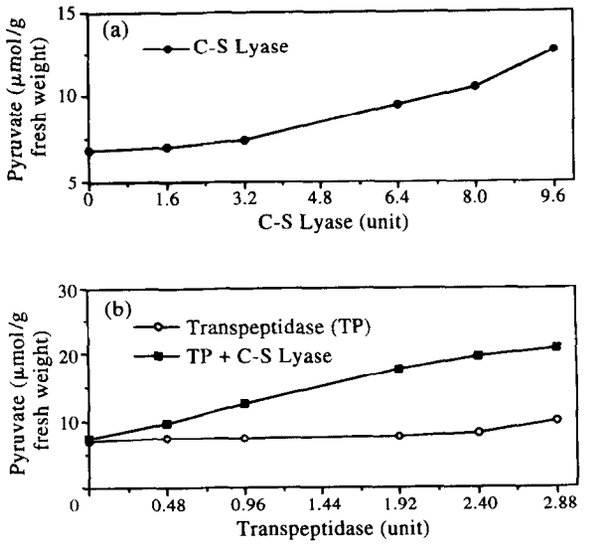

Fig. 2. Effect of enzyme concentration on pyruvate production in (a) exogenous C-S lyase added onion macerates, and (b) transpeptidase and transpeptidase in conjunction with exogenous C-S lyase added onion macerates.

lyase delayed the maximum production of pyruvate to 4 h, while transpeptidase alone, and in conjunction with exogenous C-S lyase, continuously increased the production of pyruvate until 20 h of incubation. Exogenous onion C-S lyase was added to ensure that total endogenous and exogenous C-S lyase was enough to convert the available flavor precursors. Prolonged incubation up to 36 h showed little noticeable increase in pyruvate production.

The effect of enzyme concentrations on pyruvate production was analyzed (Fig. 2). Maximal pyruvate produced by endogenous C-S lyase or control (shown in Fig. 1), exogenous C-S lyase, transpeptidase alone, and transpeptidase in conjunction with exogenous C-S lyase in macerated onions was 8·5, 12·7, 10·0 and 20·8 μmols/g fresh wt, respectively. The increase in pyruvate production by exogenous C-S lyase (4·2 μmols/g fresh wt) may indicate that in macerated onions the enzyme reaction does not go to completion. The increase in pyruvate due to transpeptidase in conjunction with C-S lyase from that of control was 12·3 μmols/g fresh wt or 1·5-fold of control. Hence, maximal pyruvate formed was 2·5-fold greater than that of control.

Schwimmer and Gudagni (1968) found that lack of pungency in some onion preparations is due to lack of C-S lyase. The increase in pyruvate due to transpeptidase alone (1·5 μmols/g fresh wt) or in conjunction with exogenous C-S yase (12·3 μmols/g fresh wt) may be a result of the action of transpeptidase on γ-glutamyl peptides releasing *S*-alk(en)yl-L-cysteine sulfoxide which becomes available for C-S lyase to produce pyruvate, ammonia and sulfur compounds. More pyruvate was produced by transpeptidase in conjunction with exogenous C-S lyase, suggesting that adding exogenous C-S lyase in addition to transpeptidase was necessary to enhance pyruvate production in macerated onions.

Table 1. Flavor compounds in transpeptidase and exogenous C-S lyase added onion extracts

Number	I_k[a]	Compound	Mass weight	Mass spectral data[b]	Refs
1	760	Thiopropanal S-oxide	90	92(2), 91(1), 90(55), 41(100), 42(30), 45(20), 48(18), 73(16), 43(10), 44(6), 72(5), 47(4), 75(2), 74(2).	Brodnitz *et al.* (1971), Oshumi *et al.* (1993), Block *et al.* (1992).
2	768	Dimethyl disulfide	94	96(8), 95(1), 94(90), 45(100), 79(60), 46(52), 47(38), 65(32).	Heller & Milne (1980), Vernin *et al.* (1986), Sinha *et al.* (1992), Oshumi *et al.* (1993).
3	777	2-Methyl pentanal	100	100(40), 43(100), 58(75), 85(45), 57(35), 41(22), 55(15), 71(11).	Boelens *et al.* (1971), Schereyen *et al.* (1976).
4	867	3,4-Dimethyl thiophene	112	114(10), 113(8), 112(76), 111(100), 97(50), 45(32), 69(22), 59(22), 39(22), 77(12), 71(10), 67(9).	Boelens *et al.* (1971), Schereyen *et al.* (1976), Weast & Grasselli (1989), Sinha *et al.* (1992).
5	872	Diallyl sulfide (di-1-propenyl sulfide)	114	116(3), 115(2), 114(60), 99(100), 113(41), 41(40), 45(30), 39(22), 71(18), 59(18), 84(10), 65(5).	Yu *et al.* (1989), Sinha *et al.* (1992)
6	895	Methyl propyl disulfide	122	124(7), 123(2), 122(70), 80(100), 43(70), 41(50), 39(40), 45(38), 47(22), 64(22), 61(17), 105(8).	Weast & Grasselli (1989), Sinha *et al.* (1992), Oshumi *et al.* (1993).
7a	902	Methyl 1-propenyl disulfide*	120	122(20), 121(2), 120(87), 45(100), 39(40), 75(40), 47(15), 80(13), 46(12.5), 105(8).	Brodnitz *et al.* (1969), Kallio & Salorinne (1990), Sinha *et al.* (1992).
7b	910	Methyl 1-propenyl disulfide[c]	120	122(9), 121(2), 120(100), 45(90), 105(76), 39(70), 75(38), 47(25).	Brodnitz *et al.* (1969), Vernin *et al.* (1986), Kallio & Salorinne (1990).
8	931	Dimethyl trisulfide	126	128(18), 127(10), 126(100), 45(61), 79(59), 47(30), 46(27), 111(22), 64(18), 84(7).	Weast & Grasselli (1989), Sinha *et al.* (1992)
9	1010	Methyl methane thiosulfonate	126	128(25), 127(101), 126(75), 47(100), 45(95), 81(75), 63(65), 79(55), 64(30).	Boelens *et al.* (1971), Sinha *et al.* (1992)
10	1023	Dipropyl disulfide	150	152(5), 151(2), 150(45), 43(100), 45(80), 75(52), 73(47), 47(41), 59(41), 108(22), 39(18), 66(15).	Schereyen *et al.* (1976), Wu *et al.* (1982), Sinha *et al.* (1992), Oshumi *et al.* (1993).
11a	1099	Propyl 1-propenyl disulfide[c]	148	150(9), 149(3), 148(100), 41(100), 42(25), 44(60), 106(57), 72(18), 73(35), 74(20), 64(20), 38(13).	Kallio & Salorinne (1990), Sinha *et al.* (1992), Oshumi *et al.* (1993).
11b	1050	Propyl 1-propenyl disulfide[c]	148	150(10), 149(7), 148(100), 43(100), 42(80), 44(62), 106(54), 73(35), 47(35), 72(40), 64(38), 37(13).	Kallio & Salorinne (1990), Sinha *et al.* (1992), Oshumi *et al.* (1993).
12a	1113	3-Ethyl-1,2-dithi-4-ene	146	148(11), 147(7), 146(62·5), 45(100), 39(55), 113(42·5), 73(32), 82(30), 101(17.5), 59(12), 147(8).	Kallio & Salorinne (1990), Sinha *et al.* (1992).
12b	1117	3-Ethyl-1,2-dithi-5-ene	146	148(11), 147(7), 146(72), 45(100), 39(55), 113(37·5), 73(32), 41(32), 82(26), 101(21), 59(21), 131(13).	Yu *et al.* (1989), Kallio & Salorinne (1990), Sinha *et al.* (1992).
13	1143	Methyl propyl trisulfide	154	156(22), 155(10), 154(100), 41(85), 43(77), 47(54), 112(45), 138(21), 45(18), 79(18), 64(17).	Schereyen *et al.* (1976), Wu *et al.* (1982), Sinha *et al.* (1992).
14a	1153	Methyl 1-propenyl trisulfide[c]	152	154(18), 153(10), 152(100), 45(95), 88(48), 3(42), 79(33), 47(27), 39(27), 41(18), 41(18), 103(10).	Wu *et al.* (1982), Sinha *et al.* (1992).
14b	1158	Methyl 1-propenyl trisulfide[c]	152	154(9), 153(6), 152(65), 45(100), 88(43), 73(42), 39(30), 79(23), 47(18), 41(15), 64(13), 154(12), 105(10).	Wu *et al.* (1982), Yu *et al.* (1989), Sinha *et al.* (1992).
15	1205	Dimethyl tetrasulfide	158	160(20), 159(9), 158(100), 45(100), 79(80), 64(38), 47(22·5), 46(21), 94(18), 111(12.5), 61(6), 48(6).	Boelens *et al.* (1971), Wu *et al.* (1982), Sinha *et al.* (1992).
16	1213	3,4-Dimethyl-2,5-dihidrothiophene	128	130(9), 129(8), 128(53), 45(100), 43(82), 41(62), 39(58), 85(30), 73(28), 99(25), 55(23), 112(20), 81(20), 67(17).	Boelens *et al.* (1971).
17a	1325	Propyl-1-propenyl trisulfide[c]	180	182(22), 181(15), 180(100), 45(100), 115(60), 106(46), 74(38), 73(37.5), 39(37.5), 83(33), 116(30), 151(22), 59(7).	Schereyen *et al.* (1976), Wu *et al.* (1982), Sinha *et al.* (1992).
18	1342	Dipropyl trisulfide	182	184(8), 183(5), 182(37), 43(100), 75(68), 45(23), 39(22), 98(10), 131(8), 117(5).	Beolens *et al.* (1971), Wu *et al.* (1982), Sinha *et al.* (1992).
17b	1356	Propyl 1-propenyl trisulfide[c]	180	182(20), 181(18), 180(100), 45(100), 73(78), 43(55), 41(48), 115(42), 116(39), 74(30·5), 75(30), 47(26), 181(18), 87(12), 138(7).	Wu *et al.* (1982), Sinha *et al.* (1992)
19	1364	Diallyl trisulfide	178	180(10), 179(8), 178(72), 113(100), 41(100), 45(90), 73(58), 58(48), 79(34), 99(28), 61(28), 52(22), 133(20).	Vernin *et al.* (1986), Yu *et al.* (1989), Sinha *et al.* (1992)
20	1505	Methyl 3,4-dimethyl-2-thienyl disulfide	190	192(14), 191(10), 190(78), 143(100), 59(81), 41(47), 45(45), 99(33), 111(20), 67(20), 65(18).	Kuo *et al.* (1990), Sinha *et al.* (1992).

[a] Kovat index.
[b] *m/z* with intensity in parentheses.
[c] May be isomers.

Pyruvate which has been shown to have a high correlation with flavor perception is used as a measure of flavor strength of onions (Wall & Corgan, 1992). It provides a measurement of total flavor, but does not provide any information about relative amount of individual flavor volatiles. Analysis of SC-CO_2 extract of macerated onion may provide the effect of γ-glutamyl transpeptidase and exogenous C-S lyase on individual flavor volatiles.

Flavor profile of SC-CO_2 onion extracts

Flavor compounds in onion extracts were identified by comparing their mass spectral data with published works. Spartan Banner and yellow sweet which had pyruvate level of 8·60 ± 1·10 and 5·70 ± 0·78 μmols/g fresh wt were chosen because they represented a pungent or cooking type onion and a sweet or salad-type onion, respectively. Table 1 shows the flavor compounds identified in onion extracts of both varieties along with their mass spectral data (including the isotope distribution pattern of the molecular ion region). A relatively constant retention time of each compound analyzed, made their quantitation possible. From 20 compounds (including the isomers) identified in SC-CO_2 extract of both onions, only 15 compounds (including the isomers) could be measured accurately and another five compounds (dimethyl disulfide, 3,4-dimethylthiophene, diallyl sulfide, methyl methane thiosulfonate, methyl 3,4-dimethyl-2-thienyl disulfide) were present in trace amounts so they were excluded from the calculation. There was a similarity in flavor constituents of all the onions analyzed; however, their relative abundance differed.

Addition of kidney transpeptidase in conjunction with exogenous onion C-S lyase prior to extraction affected the relative proportion of flavor compounds in onion extract. Total peak area of 15 flavor compounds in transpeptidase and exogenous C-S lyase treated Spartan Banner onion extract increased by about 60% from that of control. The flavor profile showed an increase in relative abundance of some compounds; thiopropanal S-oxide(1), and methyl 1-propenyl disulfide (4) dipropyl disulfide (6) propyl 1-propenyl disulfide (7), methyl 1-propenyl trisulfide (10), and propyl 1-propenyl trisulfide (13). The increase in relative abundance of compounds listed above in enzyme treated onion extract from control was 2·7–4·3%, 5·8–11·1%, 6·7–9·5%, 4·3–9·0%; 9·5–16·3% and 13·1–22·0%, respectively (Fig. 3). The increase in *trans*-1-propenyl containing flavor compounds in transpeptidase and exogenous C-S lyase treated onion extract compared to control were higher than that of the remaining flavor compounds.

Total peak area of flavor compounds in transpeptidase and exogenous C-S lyase-treated yellow sweet onion was only 20% higher than the control. Like Spartan Banner variety, increase in relative abundance of dipropyl disulfide (6) methyl propyl trisulfides (9) and flavor constituents containing *trans*-1-propenyl

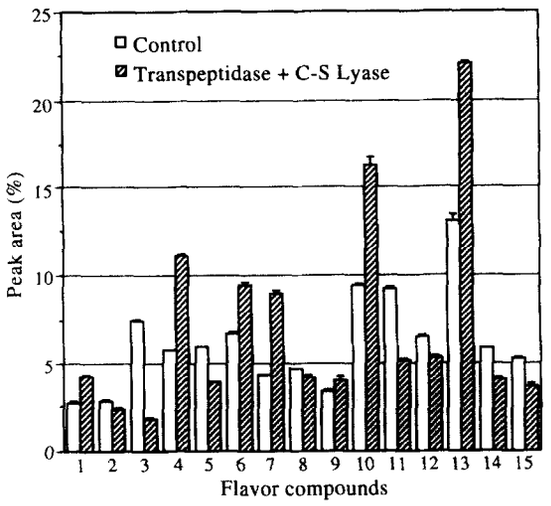

Fig. 3. Effect of transpeptidase in conjunction with exogenous C-S lyase on flavor profile of Spartan Banner onion. (1) Thiopropanal S-oxide, (2) 2-methyl pentanal, (3) methyl propyl disulfide, (4) methyl 1-propenyl disulfide, (5) dimethyl trisulfide, (6) dipropyl disulfide, (7) propyl 1-propenyl disulfide, (8) 3-ethyl-1,2-dithi-5-ene, (9) methyl propyl trisulfide, (10) methyl 1-propenyl trisulfide, (11) dimethyl tetrasulfide, (12) 3,4-dimethyl-2,5-dihidrothiophene, (13) propyl 1-propenyl trisulfide, (14) dipropyl trisulfide, and (15) diallyl trisulfide.

containing sulfur compound (4, 7, 10, 13) were observed in transpeptidase and exogenous C-S lyase treated yellow sweet onion (Fig. 4).

The increase in relative abundance of flavor compounds in transpeptidase and exogenous C-S lyase treated macerated onions from that of control may be due to the increase of availability of flavor precursors.

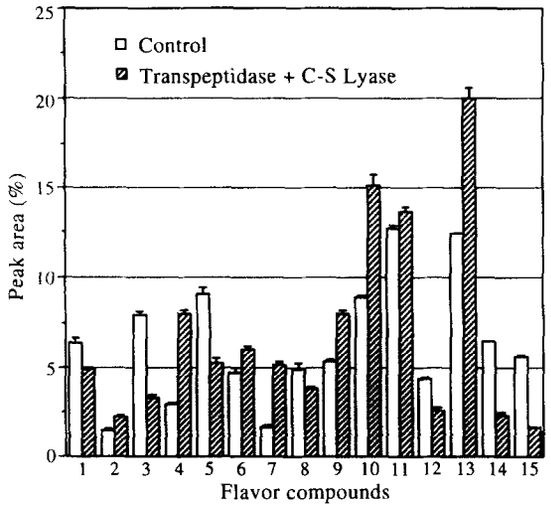

Fig. 4. Effect of transpeptidase in conjunction with exogenous C-S lyase on flavor profile of yellow sweet onion. (1) Thiopropanal S-oxide, (2) 2-methyl pentanal, (3) methyl propyl disulfide, (4) methyl 1-propenyl disulfide, (5) dimethyl trisulfide, (6) dipropyl disulfide, (7)propyl 1-propenyl disulfide, (8) 3-ethyl-1,2-dithi-5-ene, (9) methyl propyl trisulfide, (10) methyl 1-propenyl trisulfide, (11) dimethyl tetrasulfide, (12) 3,4-dimethyl-2,5-dihidrothiophene, (13) propyl 1-propenyl trisulfide, (14) dipropyl trisulfide, and (15) diallyl trisulfide.

Most prominently, the increase of *trans*-1-propenyl containing sulfur compounds in transpeptidase and exogenous C-S lyase treated samples compared to other compounds may be due to a relative higher level of γ-glutamyl-*trans*-*S*-1-propenyl-L-cysteine sulfoxide in onion sample. Shaw *et al.* (1989) showed that of total γ-glutamyl peptides present in onion, the major proportion is γ-glutamyl *trans*-1-propenyl-L-cysteine sulfoxide at levels between 1·24 and 2·18 mg/g fresh wt, equivalent to 5–6 μmol/g fresh wt or about 50–60% of total peptide compounds in fresh onion (Matikkala & Virtanen, 1967).

The effect of transpeptidase in conjunction with exogenous C-S lyase on onion flavor profile was shown by a shift of major components from methyl propyl disulfides (3), dipropyl disulfides (6), methyl 1-propenyl trisulfide (10), dimethyl tetrasulfide (11) and propyl 1-propenyl trisulfide (13), into new major components of methyl-1-propenyl disulfide (4), dipropyl disulfide (6), propyl-1-propenyl disulfide (7), methyl-1-propenyl trisulfide (10) and propyl-1-propenyl trisulfide (13). This increase in 1-propenyl containing flavor compounds may contribute to the alteration of flavor composition of enzyme treated sample and their overall aroma.

In conclusion, adding kidney γ-glutamyl transpeptidase in conjunction with exogenous C-S lyase enhanced pyruvate formation in macerated onions and effected a change in overall flavor profile of SC-CO_2 onion extracts.

ACKNOWLEDGMENTS

Acknowledgment is made to the Michigan Agricultural Experiment Station for its support of this research and to Drs D. D. Warncke, Department of Crop & Soil Science; M. J. Zabik, Pesticide Research Center; and D. A. Gage, Mass Spectrometry Facility Michigan State University.

REFERENCES

Block, E., Putman, D. & Zao, S. H. (1992). Allium chemistry: GC–MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive, and Chinese chive. *J. Agric. Food Chem.*, **40**, 2431–8.

Boelens, M., de Valois, P., Wobben, H. J. & van der Gen, A. (1971). A volatile flavor compounds from onion. *J. Agric. Food Chem.*, **19**, 984–90.

Brodnitz, M. H., Pollock, C. L. & Vallon, P. P. (1969). Flavor components of onion oil. *J. Agric. Food Chem.*, **17**, 760–3.

Brodnitz, M. H., Pascale, J. V. & Van Derslice, L. (1971). Flavor components of garlic extract. *J. Agric. Food Chem.*, **19**, 273–5.

Ettre, L. S. (1964). The Kovats retention index system. *Rep. Anal. Chem.*, **36**(8), 31–7.

Hanum, T., Sinha, N. K., Cash, J. N. & Guyer, D. E. (1993). Characterization of alliinase from a selected pungent onion (*Alliium cepa* L.) from Michigan. Presented at the 55th meeting of the Institute of Food Technologists, Chicago, USA.

Heller, S. R. & Milne, G. W. A. (1980). *WPA/NIH Mass Spectral Data base* (Vol. II). National Bureau of Standards, Washington, DC, USA.

Kallio, H. & Salorinne, L. (1990). Comparison of onion varieties by headspace gas chromatography-mass spectrometry. *J. Agric. Food Chem.*, **38**, 1560–4.

Kuo, M. C., Chien, M. & Ho, C. T. (1990). Novel polysulfides identified in volatile components from Welsh onion (*Alium fistulosun* L. var. *maichuon*) and Scallions (*Allium fistulosun* L. var. *caespitosum*). *J. Agric. Food Chem.*, **38**, 1378–81.

Lancaster, J. E. & Shaw, M. L. (1991). Metabolism of γ-glutamyl peptides during development, storage, and sprouting of onion bulbs. *Phytochemistry*, **30**, 2857–9.

Matikkala, E. J. & Virtanen, A. I. (1967). On the quantitative determination of amino acid and γ-glutamyl peptides in onion. *Acta Chem.*, **21**, 2891–4.

Oshumi, C., Hayashi, T., Kubota, K. & Kobayashi, A. (1993). Volatile flavor compounds formed in interspecies hybrid between onion and garlic. *J. Agric. Food Chem.*, **41**, 1808–10.

Rizvi, S. S. H., Benado, A. L., Zollweg, J. A. & Daniels, J. A. (1986). Supercritical fluid extraction; Fundamental principles and modeling methods. *Food Technol.*, **40**, 55–64.

Schereyen, L., Dirinck, P., Van Wassenhove, F. & Schamp, N. (1976). Volatile flavor components of leek. *J. Agric. Food Chem.*, **24**, 336–41.

Schwimmer, S. (1971). Enzymatic conversion of γ-L-glutamyl cysteine peptides to pyruvic acid, a couple reaction for enhancement of onion flavor. *J. Agric. Food Chem.*, **19**, 980–3.

Schwimmer, S. (1973). Flavor enhancement of *Allium* products. US Patent 3,275,085.

Schwimmer, S. & Austin, S. J. (1971). Enhancement of pyruvic acid release and flavor in dehydrated allium powders by gamma glutamyl transpeptidases. *J. Food Sci.*, **36**, 1081–5.

Schwimmer, S. & Guadagni, E. V. (1968). Kinetics of the enzymatic development of pyruvic acid and odor in frozen onion treated with cysteine C-S lyase. *J. Food Sci.*, **33**, 193–6.

Shaw, M. L., Lancaster, L. E. & Lane, G. (1989). Quantitative analysis of the major γ-L-glutamyl peptides in onion bulbs (*Allium cepa* L.). *J. Sci. Food Agric.*, **48**, 459–67.

Sinha, N. K., Guyer, D. E., Gage, D. A. & Lira, C. T. (1992). Supercritical carbon dioxide extraction of onion flavor and their analysis by gas chromatography-mass spectrometry. *J. Agric. Food Chem.*, **40**, 842–5.

Vernin, G., Metzger, J., Fraisse, D. & Scharff, C. (1986). GC–MS (EI, PCI, NCI) computer analysis of volatile sulfur compounds in garlic essential oils. Application of the mass fragmentometry SIM technique. *Planta Med.*, **32**, 96–101.

Wall, M. M. & Corgan, J. N. (1992). Relationship between pyruvate analysis and flavor perception for onion pungency determination. *Hort. Sci.*, **27**, 1029–30.

Weast, R. C. & Grasselli, R. G. (1989). *CRC Handbook of Data on Organic Compounds* (Vols III, IV and VI). CRC Press, Boca Raton, FL, USA.

Whitaker, J. R. (1976). Development of flavor, odor, and pungency in onion and garlic. *Adv. Food Res.*, **22**, 73–133.

Wu, J. L., Chou, C. C., Chen. M. H. & Wu, C. M. (1982). Volatile flavor compounds from shallots. *J. Food Sci.*, **47**, 606–8.

Yu, T. H., Wu, C. M. & Liou, Y. C. (1989). Volatile compounds from garlic. *J. Agric. Food Chem.*, **37**, 725–30.