Modified Method for Rapid Quantitation of
S-Alk(en)yl-L-cysteine Sulfoxide in Yellow Onions
(*Allium cepa* L.)

HUI WANG, JINGMING LI, ZHENGFU WANG, XUE ZHANG, AND YUANYING NI*

College of Food Science & Nutritional Engineering, China Agricultural University,
Beijing 100083, People's Republic of China

In this study, a rapid and sensitive method to determine the flavor precursors was established on the basis of modified Kubec's gas chromatography (GC) method. For the new method, the interference of halogen was removed by adding borohydride that replaced halide as a reducing reagent, and the analysis was completed within 25 min for the determination of *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs). In comparison with the results of high-performance liquid chromatography (HPLC), the modified GC method is much better in the separation and detection of ACSOs in yellow onion bulbs than the original method. A small amount of 2-propenyl-L-cysteine sulfoxides (2-PeCSO) was detected with the modified GC method, and propyl-L-cysteine sulfoxides (PrCSO) were not detected. The results of GC determination indicated that 2-PeCSO probably was the hydrolysate of a byproduct or an isomer of γ-glumtyl-1-propenyl-L-cysteine sulfoxides in biosynthesis process.

INTRODUCTION

Onion is an *Allium* plant cultivated worldwide as a vegetable with distinctive flavor. The flavors in onion are attributed to certain sulfur volatile compounds formed through the hydrolysis of flavor precursors (FPs) under Alliinase catalysis when the tissues are damaged (*1*). At present, four FPs have been found in genus *Allium* in the form of nonprotein sulfur amino acids. They are 1-propenyl-L-cysteine sulfoxides (1-PeCSO) (*2*), 2-propenyl-L-cysteine sulfoxides (2-PeSCO) (*3*), methyl-L-cysteine sulfoxides (MCSO), and propyl-L-cysteine sulfoxides (PrCSO) (*4*). In onion, MCSO, 1-PeCSO, and PrCSO exist, but in several studies PrCSO was reported to be absent (*5*).

Because of the existence of Alliinase in onion tissue and its rapid enzymatic reaction (*6*), accurate quantification seems to be difficult. The indirect or direct quantitative and qualitative analysis method has been mainly used (*7−11*). For the indirect method, the initial concentration and proportion of FPs in the onion are estimated by measuring enzymatic hydrolysates, such as pyruvate, an indicator of enzymatic activity (*12*), and thiosulfinate, an indicator of pungency and part initial FP concentration (*13−16*). However, the two measurements could not provide enough evidence to show the exact stoichiometrical relationship between hydrolysates and FPs. For the direct method, Alliinase must be denatured with heat treatment to remove the interference of multiple enzymatic products before the direct analysis of FPs is used. The direct method shows relatively better results in FPs determination than the indirect method (*17−19*).

* To whom correspondence should be addressed. Phone: (86) 10-62737514. Fax: (86) 10-62737514. E-mail: niyuany@163.com.

Numerous analytical techniques have been applied to the detection and analysis of FPs, such as thin-layer chromatography (TLC) (*7*), amino acid analyzer (*20*), electrophoresis and gel chromatography (*21*), gas−liquid chromatography (GLC) (*22*), high-performance liquid chromatography (HPLC) (*19*), and gas chromatography (GC) (*23*). Different techniques, however, lead to different results and conclusions of the quantitative and qualitative analysis of FPs. TLC is not accurate for the separation and quantification of sample; electrophoresis and gel chromatography require complicated procedures. After extraction by using an ion-exchange column under alkaline condition, there were considerable amount of cyclized 1-PeCSO formed in onion extract when analyzed with amino acid analyzer (*24*). With the technology advancement, GC, GLC, and HPLC are playing the main role in the determination of FPs. The main goal of using these techniques is to determine the initial composition of FPs by measuring the concentration and proportion of the secondary enzymatic products, namely, thiosulfinate. However, volatile sulfides identified in headspace of GLC are dominated by propylated thiosulfinate in crushed onion (*25*), and some studies with HPLC and GC indicated that propylated fragment was not the hydrolysate of endogenous PrCSO but the transformation of 1-propenyl fragment which will be encountered with the increase in temperature during analysis (*15*) or will be induced by the reducing agents in onion extract (*26*). Freeman and Whenhem (*27*) estimated that the original proportion of MCSO, 1-PeCSO, and PrCSO is 6:6:88 in intact onion, and Block et al. (*28*) reported that methyl-1-propenyl-propyl-thiosulfinate is equimolarly distributed in onion maceration extract. However,

the above results cannot provide any stoichiometrical relationship between thiosulfinates and FPs.

Impact of the interfering factors and complicated relationship between the enzymatic products and FPs led to improper estimate of the initial concentration and proportion of each FP in onion when direct methods with complex procedures and the most indirect methods, as well as simple sample treatments, were used. For HPLC and GC, newly invented derivatization reagents have made an analytical method simple and possible to calculate the amounts of FPs directly. Recently, some derivatization reagents, such as *ortho*-phthaldialdehyde (OPA) (*19*), 9-fluorenylmethyl chloroformate (FMOC) (*5*), Dansyl-Cl (*29*), and phenyl isothiocyanate (PITC) (*30*), have been applied to the direct analysis of FPs with HPLC. However, the disparity of the analytical methods and degree of separation led to different results on yields of FP derivatives. The possible reason may be the different analytical conditions, such as derivatization reagents, elution solutions, solvent gradients, and degradation of derivatives with light-sensitivity and sample preparations. With HPLC derivatization methods, three components of FPs have been detected in most plants of genus *Allium*, but PrCSO had not been detected in the above-mentioned reports except for Randle et al.'s study (*30*). Therefore, GC may be a promising technique because of its excellent resolution and sensitivity. However, the attempts to analyze FPs by GC immediately after derivatization with trifluoroacetic amino acid (TFAA) and (trimethyl silanization) TMS failed because the sulfoxide group in FPs has high thermal instability and polarity (*23*). When the temperature is up to about 160 °C, ruptured chemical bonds lead to FPs decomposing (*21*). Kubec et al. (*23*) developed a new derivatization method allowing a highly sensitive and reproducible determination of FPs with ethyl chloroformate (ECF) reagent by means of GC which, on the other hand, suggests the possibility of developing a more promising method.

The objectives of this research were to establish a set of rapid HPLC and GC analytical methods for quantitative and qualitative analysis of FPs in onion and to study the possible existence of 2-PeCSO and PrCSO as endogenous FPs in onion.

MATERIALS AND METHODS

Reagents and Materials. The derivatization reagents of OPA and methyl chloroformate (MCF) for HPLC and GC analysis were obtained from Sigma-Aldrich Chemical Co. and Fluka Chemie AG, respectively. Acetyl chloride, from Sigma-Aldrich Chemical Co., and *tert*-butylmercaptan, from Fluka Chemie AG., also were used in derivatization reaction. Solvent-grade acetonitrile was obtained from Merck KGaA. All other chemicals used in this experiment were purchased from Beijing Chemical Reagents Company. These were of analytical grade and of the highest purity available. Deionized water was used throughout the study. Fresh onion bulbs with full maturation were purchased from the local wholesale market in Shangyi county of Hebei province, China, without plant diseases and insects, which were harvested in June 2005 and were stored at 4 °C in a refrigerator.

Extraction of FPs. A whole onion with the outer skins or leaves carefully removed was deep frozen at −40 °C for 1 h and then was cut into quarters. About 40 g of plant tissue was placed in 50 mL of 45 °C deionized water for 5 min with continuous stirring and then was crushed with a high-speed tissue homogenizer. Two hundred milliliters of cold acetone (−40 °C) was added to the homogenate, and the mixture was stored for at least 24 h in a refrigerator. With the plant tissue residue precipitated on the bottom of beaker, the mixture was centrifuged with a high-speed refrigerated centrifuge at 5000*g* and 4 °C for 30 min. Thirty milliliters of supernatant was evaporated with a rotary evaporator under vacuum at 30 °C and was lyophilized at −40 °C in vacuo. The yellowish residue obtained was used for both HPLC and GC analysis of the FPs.

Synthesis of ACSO Reference Compounds. *MCSO Standard.* Diastereomeric MCSO was prepared according to a modified method of Synge and Wood (*32*). A mixture of 1.65 mL of 30% H_2O_2 and 23 mL of 0.6 M *S*-methyl-L-cysteine was kept at 25 °C with continuous stirring for 24 h. Two hundred milliliters of cold (4 °C) ethanol was added, and after 30 min at 4 °C, a white precipitate was recovered by filtration with a typical yield of 73%. The resultant (±) MCSO preparation decomposed at ca. 168 °C. After recrystallization, the purity of this standard was 96%.

2-PeCSO Standard. Diastereomeric 2-PECSO was synthesized according to the procedure of Lancaster and Kelly (*21*). L-Cysteine hydrochloride (4 g) was stirred into 75 mL of ethanol followed by the addition of 5.6 mL of 20 M NaOH. After 5 min, 4.4 mL of 2-propenyl bromide was added, and the mixture was adjusted to pH 5.25 using glacial acetic acid. The mixture was slowly stirred for 24 h at 25 °C. The suspension was cooled at 4 °C for 1 h, and a white precipitate (2-propenyl-L-cysteine) was collected by filtration. The 2-propenyl-L-cysteine was then oxidized to the sulfoxide by treating with H_2O_2 and was recovered from ethanol as described for MCSO. The white, powdery (±) 2-PeCSO preparation decomposed at ca. 166 °C. After recrystallization, the purity of this standard was 94%.

PCSO Standard. The preferred method for preparation of *S*-propyl-L-cysteine was that of Granby and Young (*52*) in which L-cysteine formed in situ by reduction of L-cystine with sodium in liquid ammonia was alkylated by means of 1-bromopropane. The corresponding sulfoxide was prepared in 60% yield by oxidation with H_2O_2. (±) PCSO preparation decomposed at ca. 163−164 °C. After recrystallization, the purity of this standard was 95%.

BCSO Standard. Diastereomeric BCSO was synthesized according to the procedure of Armstrong and Lewis (*31*). The 15.8 g of cysteine hydrochloride was dissolved in 225 mL of 2 M NaOH and 180 mL of ethanol. The stirred solution was cooled to 25 °C, 27.4 g of *n*-butyl bromide was added, and stirring was continued overnight. Concentrated hydrochloric acid was added to adjust the solution to pH 2, it was concentrated in vacuo to dryness, and the product was extracted with two portions of hot absolute ethanol. The combined ethanol extracts were concentrated to dryness, the residue was taken up in 300 mL of water, and the solution was adjusted to pH 5. The product was collected, was rinsed with cold water and then with ethanol, and was dried. The corresponding sulfoxide was prepared by treating with H_2O_2 and was recovered from ethanol as described for MCSO. (±) BCSO preparation decomposed at ca. 160−162 °C. After recrystallization, the purity of this standard was 94%.

1-PeCSO Standard. 1-PeCSO exists naturally in the bulb of onion; *trans-S*-1-propenyl-L-cysteine sulfoxide was isolated from yellow onion according to the procedure described by Carson et al. (*33*), and the white powder of 1-PeCSO preparation decomposed at ca. 153 °C. After recrystallization, the purity of this standard was 91%.

OPA Derivatization for HPLC Analysis. For OPA derivatization reagent preparation, the modified method described by Ziegler and Sticher (*19*) was used as follows: 80 mg of OPA was dissolved in 2.5 mL of anhydrous ethanol. By adding 100 µL of *tert*-butylmercaptan, the mixture was adjusted to 25 mL with 0.05 M solution of sodium phosphate tribasic (pH 9.5). The derivatization solution was placed in darkness for 12 h until use. For OPA derivatization, 10 mg of the extract residue was dissolved in 1 mL of deionized water. One tenth of a milliliter of sample solution was mixed with 0.9 mL OPA derivatization reagent. The centrifuge vials containing OPA−FPs derivatives were allowed to stand at room temperature for 30 min in darkness. The derivative solution was filtrated through 0.22 µm of cellulose acetate filter prior to GC and HPLC analysis.

MCF Derivatization for GC. A modified derivatization method of Kubec et al. (*23*) was used as follows: 20 mg of extracted residue was dissolved in 0.3 mL mixture of methanol−water−pyrimidine (30:60:10, v/v/v), and 120 µL of methyl chloroformate (MCF) was added. After briefly shaking, precipitated cloudy MCF disappeared, and the derivatization procedure was completed within 5 s, as the yellow oily drops floated on the surface of the solution. The sulfoxide group of FPs was reduced by adding 0.2 mL of potassium borohydride (1 g/mL), and the speed of the reductive reaction was accelerated by adding 50 µL of acetyl chloride. The reaction mixture was placed at ambient

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temperature for 2 h, the deposit was removed with a high-speed refrigerated centrifuge at 3000*g* and 0 °C for 5 min, and MCF derivatives were extracted with 0.4 mL of trichloromethane. The organic phase (bottom layer) was stored at 0 °C for GC analysis.

Instrumentation and Analytical Conditions. HPLC analysis was performed with Shimadzu LC-10AT liquid chromatography equipped with SIL-10A×L automatic sample injector and SPD-10A variable multiple-wavelength ultraviolet detector. A reversed-phase column Nova-Pak C$_{18}$ (300 mm × 3.9 mm × 5 μm) with a guard column Guard-Pak C$_{18}$ (18 mm × 3.5 mm × 7 μm, Waters, Milford, MA) was used to separate FPs. Solvent A was 0.05 M mixed elution solution (pH 6.5) of phosphate buffer (0.05 M sodium di-hydrogen phosphate and 0.05 M di-sodium hydrogen phosphate), and solvent B was acetonitrile. The deaeration was achieved with Shimadzu DGU-12A degasser prior to elution. Samples (10 μL) were injected into the column, which was maintained at 30 °C in Shimadzu CTO-10A/VP column heater. Eluted components were detected at 335 nm, and all chromatographic data and traces were collected with Shimadzu CBM-10A communication data processor. A flow rate of 1.5 mL/min was used, and the solvent-gradient program was set as follows: starting at 80% A and 20% B, 80% A was maintained for 20 min, 75% A for 20 min, and 72% A and 70% A for 10 min, with a hold of 100% B for 10 min. The column was returned to the initial solvent over 5 min and was re-equilibrated for 10 min before the next injection. A Hewlett-Packard 6890 gas chromatography equipped with an automatic sample injector and a flame ionization detector was used for GC analysis. An HP-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm, Hewlett-Packard) was used to separate the volatile derivatives of FPs. The samples (2 μL) were injected using a split ratio of 1:15. The operating parameters were set as follows: the temperatures of injector and detector were 180 and 250 °C, respectively; the flow rate of nitrogen carrier gas was 2.5 mL/min; and the temperature-gradient program was as follows: 120 °C maintained for 1 min, then increased to 160 °C at 2 °C/min, then to 175 °C at 0.5 °C/min, then to 200 °C at 1.5 °C/min, and then held at the final temperature for 5 min.

Qualitative Analysis of FPs by HPLC. The synthesized *S*-alk(en)-yl-L-cysteine sulfoxides (ACSOs) were mixed with the same weight (5 mg) as a varied aqueous standard according to the derivatization method described above. By comparing with retention times of reference standards, the identification of FPs was performed by HPLC under the same solvent and temperature-gradient program described in the analytical conditions.

Quantitative Analysis of FP Samples. Quantification of each FP component in onion was carried by the peak area of corresponding sulfoxide relative to the internal standard, BCSO. Two individual sample derivatives were analyzed with HPLC and GC, respectively; duplicate analyses of each derivative were performed.

RESULTS

HPLC Analysis of FPs. The HPLC chromatogram of onion extract is presented in **Figure 1**. By using the solvent-gradient program described, MCSO was eluted first (retention time of 16.902 min), followed by 1-PeCSO (29.054 min). 2-PeCSO was detected at 27.753 min with low concentration. PrCSO was not detected in this study. The degradation of OPA−ACSO peaks reported by Thomas and Parkin (*5*) was not observed after the derivatized samples were placed at room temperature for 24 h in darkness.

Extraction and Pretreatment of FPs. Yoo and Pike (*29*), as well as Lancaster and Kelly (*21*), extracted the FPs from onion with 80% ethanol and MCF, respectively. However, there was no significant difference in quantities of ACSO between the two methods, and FPs extract contained two solvents or more and was separated into two phases immediately with the water disrupted in onion tissue. Thomas and Parkin (*5*) and Yoo and Pike (*29*) used higher temperature and longer time for solvent evaporation treatment. However, as an extracting solvent, the cold acetone (−40 °C) was faster and easier to evaporate at
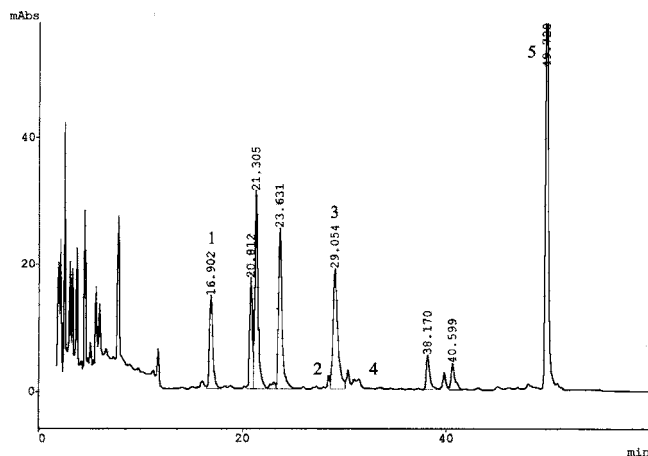

Figure 1. HPLC chromatogram of onion extract containing *S*-methyl-L-cysteine sulfoxide (peak 1); *S*-2-propenyl-L-cysteine sulfoxide (peak 2); *S*-1-propenyl-L-cysteine sulfoxide (peak 3); *S*-propyl-L-cysteine sulfoxide (peak 4); and S-butyl-L-cysteine sulfoxide (peak 5) as internal standard. *S*-Propyl-L-cysteine sulfoxide (peak 4) was undetected in this variety of onion.
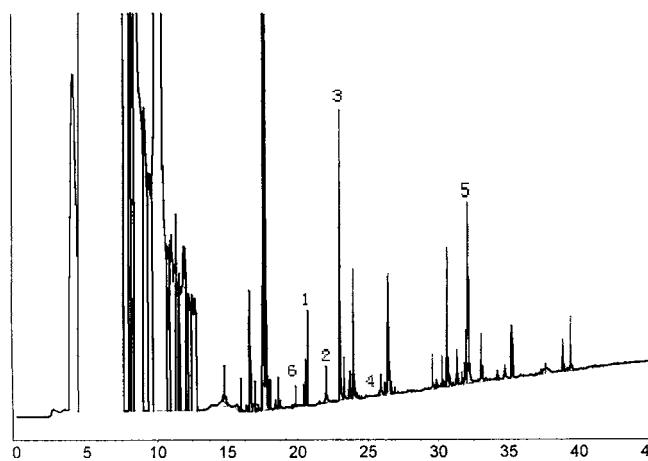

Figure 2. GC chromatogram of onion extract containing *S*-methyl-L-cysteine sulfoxide (peak 1); *S*-2-propenyl-L-cysteine sulfoxide (peak 2); *S*-1-propenyl-L-cysteine sulfoxide (peak 3); *S*-propyl-L-cysteine sulfoxide (peak 4); cycloalliin (peak 6); and *S*-butyl-L-cysteine sulfoxide (peak 5) as internal standard. *S*-Propyl-L-cysteine sulfoxide (peak 4) was undetected in this variety of onion.

room temperature than ethanol. In addition, the influence of higher processing temperature on FPs was avoided.

Krest et al. (*34*) applied the method described by Ziegler and Sticher (*19*) to analyze FPs in *Allium* plants, and the time used for analyzing one sample was nearly 50 min. With the rapid elution method established in this study, a complete test of one sample was completed within 35 min, and the peaks of sulfoxide components of FPs showed better separations than that reported by Thomas and Parkin (*5*), in which FP peaks were coeluted with other amino acids and showed low resolution. Cycloalliin, occurring in alkaline condition, was not detected in derivatized sample (about pH 9.0) throughout the analytical procedure of HPLC.

GC Analysis of FPs. With the temperature-gradient program described, cycloalliin was separated first with retention time 19.864 min followed by MCSO at 20.617 min, 2-PeCSO at 21.745 min, and 1-PeCSO at 23.169 min by comparing with retention time and relative abundance ratio of reference standards by GC-MS, as shown in **Figure 2**. 2-PeCSO showed a relatively larger peak in GC than that in HPLC, and PrCSO was still

Table 1. Concentration (Milligrams per Gram of Fresh Weight) and Relative Proportion (Percentage) of FPs Determined by HPLC and GC of Improved Method and Kubec's Method[a]

Peak No.	Compound	Structure	Improved method		Kubec's method (23)	
			HPLC	GC	HPLC	GC
1	S-methyl-L-cysteine sulfoxide	CH₃-S-CH₂-CH(NH₂)COOH	0.22a (14.5)	0.23a (14.3)	0.31	0.57
2	S-2-propenyl-L-cysteine sulfoxide	CH₂=CH-CH₂-S-CH₂-CH(NH₂)COOH	Trc	0.04a (2.5)	7.97	8.47
3	S-1-propenyl-L-cysteine sulfoxide	CH₃-CH=CH-S-CH₂-CH(NH₂)COOH	1.30a (85.5)	1.32a (82.0)	nd	Trc
4	S-propyl-L-cysteine sulfoxide	CH₃-CH₂-CH₂-S-CH₂-CH(NH₂)COOH	nd	nd	nd	nd
6	Cycloalliin		ndb	0.02a (1.2)	1.61	Trc
	Total ACSOs		1.52	1.61	8.28	9.03

[a] "a" indicates the replicate data determined. "b" indicates component was not detected with analytical method. "c" indicates the concentration of this component was at trace level and under the limit of detection.

undetected in GC analysis. The cycloalliin observed may have been formed by adding slightly basic potassium borohydride.

Choice of Derivatization Reagent for GC Analysis. Four FPs in *Allium* plants were gradually decomposed when the temperature was above 150 °C (*3*, *33*). Similar problems have been described in GC determination of the glucoseinolates with a sulfoxide moiety in the side chain, such as glucoiberin, glucoraphanin, and glucoallysin (*23*). The presence of highly polarized and labile sulfoxide group led to the decomposition of FPs with the raising temperature gradient in GC analysis. The key treatment was to keep the sulfoxide group stable by transformation, and there were two methods used for the transformation of the unsaturated S=O band, namely, oxidizing or reducing. The first method is to change the sulfoxide group into a more stable state of sulfone group by adding hydrogen peroxide; the second method is to change the S=O band into the −S− band with reducing reagents such as the iodides. However, in the oxidation procedure of sulfoxide group, FPs containing the unsaturated propenyl fragment would be transformed into some other substance if the addition of hydrogen peroxide was used. This matter would underestimate the concentration and proportion of 1-PeCSO or 2-PeCSO. Kubec et al. (*23*) used sodium iodide as a reducing reagent to deoxygenate sulfoxides into the corresponding sulfides. It is desirable to perform this fast reduction reaction under mild conditions, but the production of free iodide would complicate the subsequent analysis if it is not completely removed. In this study, potassium borohydride, which was first used by Freeman and Whenham (*27*) as a reducing reagent for deoxygenation of the S=O bond, was applied to convert sulfoxides to thioether; therefore, the interference of halogens formed by the addition of halides was avoided. At the injector and column temperature used, no conversion of *S*-alkenyl-L-cysteine sulfoxides into *S*-propyl-L-cysteine sulfoxide was observed.

Quantitative Determination of FPs with HPLC and GC. In this case, 1-PeCSO 1.30 mg/g fw (fresh weight) was found as the major FP present in onion, and MCSO (0.22 mg/g fw) was found at considerably lower concentration by HPLC. By GC, cycloalliin (0.02 mg/g fw) was detected to be the minor component of FPs. Our result was comparable with previous reports on total FP amounts. For example, onion was reported to contain 2.4 mg/g fw (*20*), 3.6−4.6 mg/g fw (*21*), 1.12−1.54 mg/g fw (*5*), or 0.92−2.50 mg/g fw (*29*) of total FPs. Because of different cultivars, environments, ontogenies, fertilizer treatments, and analytical methods, there must exist some discrepancies among analytical results from various studies, even from bulk of the same yellow onion. The relative proportion of 1-PeCSO was 82∼85.5% of total FPs determined with HPLC and GC. The values of 1-PeCSO and MCSO were somewhat different from the 3.85 and 0.35 mg/g fw, respectively, as reported by Matikkala and Virtanen (*20*), and the 0.60 and 0.90 mg/g fw, respectively, as reported by Lancaster and Kelly (*21*). However, the contents of 1.31 and 0.24 mg/g fw of 1-PeCSO and MCSO in white onion (*5*) were similar to our data obtained from the analysis of yellow onion, and the relative proportions of 1-PeCSO (84.6%) and MCSO (15.4%) in TG 1015Y onion (*29*) were similar to those of 83.6% and 14.4% in our experiment. 2-PeCSO (0.04 mg/g fw) was not detected with HPLC, but it was with GC, probably because of the difference of detection sensitivity (*23*) of the two methods. The detection limit of GC used in this experiment was 0.0001%, and HPLC was 0.001%. As seen in **Table 1**, GC data are slightly higher than that of HPLC. 2-PeCSO in samples showed a slight peak in HPLC and a visible small peak in GC. Obviously, GC had a better detection of some trace substances than HPLC did. Besides, from **Table 1**, cycloalliin can be detected well in low concentration by the improved GC method, while both 1-PECSO and cycloalliin cannot be detected by the original method (*23*).

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Validation of 2-PeCSO in Onion by GC. A trace amount of 2-PeCSO was found in this study by GC, but most research reports did not consider 2-PeCSO the component of FPs in onion (*27*, *29*, *35*−*37*). If this were true, the enzymatic products containing 2-propenyl fragment should not be found in the form of thioethers and thiosulfinates with low concentrations in the analysis of sulfur compounds in the ether extract and essential oil of onion (*38*−*42*). There may be three explanations: first, 2-PeCSO exists as a trace isomer of 1-PeCSO, which is formed by the transformation of double bond (C1−C2 vs C2−C3) (*33*) under certain unknown catalytic conditions; second, 4-PeCSO is formed directly under the hydrolysis of γ-L-glutamyl-2-PeCSO, which exists as an endogenous byproduct with low concentration in the biosynthetic pathway of γ-L-glutamyl-1-PeCSO formation with the decarboxylation and oxidation of γ-L-glutamyl-*S*-2-carboxy-propyl-L-cysteine (*43*); last, the thioethers and thiosulfinates with 2-propenyl fragment are formed by the transformation of 1-propenyl fragment into 2-propenyl fragment. If the alkenyl group in 1-PeCSO could perform the transformation of double bond, it would need more activation energy, but so far no such research has been published. Lancaster and Boland (*44*) speculated that the reason why γ-L-glutamyl peptides of 2-PeCSO were not found was their low concentration rather than their absence from the plant tissues. By our calculation, the concentration of 2-PeCSO obtained from GC could account for nearly all volatile sulfur compounds with 2-propenyl fragment in onion (*40*). Thus, the comparison between the results of HPLC and GC indicated that the second hypothesis is more possible.

Presence of PrCSO in Onion. PrCSO peak was not observed as a major FP peak as previously reported (*21*, *30*, *45*, *46*) but was observed as a minor component (*20*, *47*) in both HPLC and GC chromatograms. The reports with mild analytical conditions showed that the ratio of MCSO:PrCSO:PeCSO in onions was 15:2:83 (*20*), 20:65:15 (*21*), or 10:80:10 (*44*). Saghir et al. (*35*), Freeman and Whemham (*27*), and Mazza et al. (*48*) analyzed the enzymatic products in onion with GC method and demonstrated that propyl disulfides were the major volatile sulfur compounds. Two reasons could be used to explain these different results: first, a reducing system may be present in onion tissue which is saturating the double bond of 1(2)-propenyl fragment (*49*); second, at a higher temperature gradient, 1(2)-propenyl compounds may be transformed into a more stable saturated state, that is, propyl groups. According to the second hypothesis, PrCSO and its derivatives were considered to be artificial because of the temperature increase during sample treatments and analysis (*15*). Whether PrCSO exists as an endogenous FP or as artifact in onion has been discussed for a long time. The γ-L-glutamyl-*S*-propyl-cysteine, which was found in garlic bulbs (*50*) but not in onion bulbs, was considered to be derived from propenyl fragment via the saturation of double bond (*26*) induced by self-reduction in onion. Otherwise, according to the biosynthesis pathway of FPs (*51*), γ-L-glutamyl peptide of PrCSO should be in onion as the precursor of PrCSO, but it has not been found in any plants of *Allium*. Presumably, this compound is in the same situation as γ-L-glutamyl-2-PeCSO (*44*).

DISCUSSION

In this study, with the multiple-solvent extraction and gradient elution for the separation of FPs, and with the highly sensitive GC, no PrCSO was detected. It is possible that PrCSO is an endogenous trace FP that is below the detection limit, and it is not produced under the normal growth condition of *Allium*

plants. However, under some given conditions, such as the planting environment change, the gene variation, the high S fertilizer used, the different cultivars (*30*), and the understress conditions, the activation of certain enzymes in onions would induce the increasing concentration of γ-L-glutamyl-PrCSO as a stable byproduct of biosynthesis of FPs, and the reserves of sulfur and nitrogen in the natural growth of plants would be deserved.

With the modified HPLC and GC methods, a set of rapid and highly sensitive analytical methods is established for the quantitative and qualitative determination of FPs in fresh onion. 2-PeCSO was successfully detected in low concentration with GC method, but PrCSO was not detected. However, because of many variables, such as different onion varieties, sample treatments, analytical methods, planting environments, and so on, the relativity and comparability among the various studies are generally impossible. The emphasis of additional studies should be placed on the biosynthesis pathways of FPs and their hydrolysis pathways as well as on the stoichiometric relations between FPs and their hydrolysates under the different conditions. With the great needs of characterization and standardization of flavor and therapeutic compounds, the understanding of the complicated interaction between the external and internal factors influencing FPs may become increasingly important.

ABBREVIATIONS USED

1-PeCSO, 1-propenyl-L-cysteine sulfoxides; 2-PeSCO, 2-propenyl-L-cysteine sulfoxides; ACSO, *S*-alk(en)yl-L-cysteine sulfoxide; BCSO, *S*-butyl-L-cysteine sulfoxide; ECF, ethyl chloroformate; FMOC, 9-fluorenylmethyl chloroformate; FP, flavor precursor; fw, fresh weight; GC, gas chromatography; GLC, gas−liquid chromatography; HPLC, high-performance liquid chromatography; MCF, methyl chloroformate; MCSO, methyl-L-cysteine sulfoxides; OPA, *ortho*-phthaldialdehyde; PrCSO, propyl-L-cysteine sulfoxides; TFAA, trifluoroacetic amino acid; TLC, thin-layer chromatography; TMS, trimethyl silanization.

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JF070298D