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Research Note

Change in Organosulfur Compounds in Onion (*Allium cepa* L.) during Heat Treatment

Sunyoung Kim, Sanghee Lee, Dongbin Shin, and Miyoung Yoo*

Food Analysis Center, Korea Food Research Institute, Seongnam, Gyeonggi 13539, Korea

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*Corresponding Author
Tel: +82-31-780-9342
Fax: +82-31-780-9280
E-mail: myyoo@kfri.re.kr

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Abstract Changes in contents of the *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) methiin, isoalliin, propiin, and cycloalliin in onions after boiling, frying, steaming, and microwaving were investigated using Liquid Chromatography Electrospray Ionization-Tandem Mass Spectrometry (LC/ESI-MS/MS). ACSOs contents increased by 34.2-568.0% during frying, steaming and microwaving, whereas ACSOs contents decreased by 32.6-69.4% during boiling. The methiin level in heat-treated onions ranged from 0.18 to 0.47 g/100 g of dry weight (DW), and the cycloalliin concentration in heat-treated onions ranged from 0.31 to 3.50 g/100 g of DW. The amount of isoalliin in processed onions was 0.34-3.32 g/100 g of DW, and propiin was 0.15-1.67 g/100 g of DW. Changes in the ACSO concentrations were dependent on the cooking method. The quality of heat processed onions was evaluated.

Keywords: onion, organosulfur compound, HPLC, LC/ESI-MS/MS

Introduction

Onions (*Allium cepa* L.) have been used as a common food and as a medical plant for heal many diseases, including heart problems, headaches, bites, worms, and tumors (1). Many biological effects of onions are related to phytochemical sulfur-containing compounds and flavonoids that protect cells against damaging effects (2,3). The most important sulfur-containing compounds are *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), which are responsible for the pungent aroma and taste of onions (2). *S*-methyl-cysteine sulfoxide (methiin), *S*-(1-propenyl)-cysteine sulfoxide (isoalliin), *S*-propyl-cysteine sulfoxide (propiin), and (1*S*,3*R*,5*S*)-5-methyl-1,4-thiazane-3-carboxylic acid 1-oxide (cycloalliin) are all present in onions. When an onion is cut or crushed, ACSOs are cleaved by cysteine sulfoxidelyase to produce corresponding alk(en)yl sulfenic acids, which are transformed to thiosulfinates (4) and other volatile compounds that produce characteristic flavors (5). Organosulfur compounds (OSCs) have been reported to be bioactive with an ability to reduce the risk of cancer and cardiovascular and age-related diseases (3).

There are only a few recent studies on the impact of heat-treatment processes on nonvolatile flavor precursors of garlic (6,7). Decomposition of *S*-propyl-L-cysteine sulfoxide (PCSO) was first studied by Nishimura *et al.* (6) in connection with flavor deterioration after γ-irradiation of onions. Regarding organosulfur compounds (OSCs), in particular, common processes such as boiling, can lower the PCSO concentration in garlic by 10-40% at 120°C (7). However, changes in OSC expression have not been studied in relation to effects of blanching, steaming, frying, and microwaving during processing. Sulfur-containing flavor precursors in fresh *Allium* vegetables, which show minimal processing effects on the content of sulfur compounds in onions, have been studied (7,8).

Vegetables frequently undergo heat treatment in commercial processing plants and household kitchen prior to consumption. The degree of phytochemical change during processing depends on the sensitivity of a phytochemical to modification or degradation and the length of exposure to a given processing technique (9). For this reason, attention has recently been focused on phytochemicals that can change during cooking. Lombard *et al*. (10) examined quercetin in onions after cooking by sautéing, baking, and boiling. Lee *et al*. (11) determined the flavonoid contents in raw, home-processed and light-exposed onions and in dehydrated commercial onion products. Analysis of OSCs as major biological compounds in onions is rarely performed during processing of onions. Changes in ACSOs concentrations due to blanching, steaming, frying, and microwaving of onions using HPLC-MS/MS systems were evaluated in this study.

Materials and Methods

Chemicals Methiin was obtained from LKT Laboratories (Saint Paul, MN, USA), and cycloalliin, isoalliin, and propiin were purchased from Medigen (Daejeon, Korea). Water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA, USA). Methanol and acetonitrile were purchased from J.T Baker (Paris, KY, USA) and were used for

sample preparation and as the mobile phase. All other chemicals and solvents were of HPLC grade.

Onion preparation Onions (*Allium cepa* L.) harvested in 2013 Seosan province, Korea were commercial cultivars. Onion peels were removed and onions were divided into quarters using experimental knife before heat treatment. A fresh onion was used as the control sample. Heat treated onions were stored at −20°C prior to being freeze dried. Samples were freeze dried using laboratory freeze dryer (TFD 8505; Ilshin Lab. Co. Ltd., Dongducheon, Korea). The temperature was −80 °C and the vacuum was 50 mTorr. The weight of the dried samples was measured, and then, they were blended to a find powder using a DH 850 laboratory blender (Oscar, Seoul, Korea) and stored at −80°C (MDF-U54V ULT Freezer; Panasonic Corporation, Tokyo, Japan) prior to extraction and analysis. Onion samples were treated as follows:

***Blanching*:** Onions were treated in a stainless steel pot (WPCT-14C; Poongnyun Co. Ltd., Ansan, Korea) containing 1.5 L of distilled water covered with a lid. Onions were blanched for 5, 10, 15, and 20 min.

***Steaming*:** Onions were steamed for 5, 10, 15, and 20 min in a pressure cooker (HFPC-06; Poongnyun Co. Ltd.) filled with 150 mL of distilled water.

***Frying*:** Onions were cut into small pieces, approximately 5.0 cm length, for frying in an open frying fan and fried with 200 mL of soybean oil (Sajo Co., Seoul, Korea) at 120°C for 1, 2, 3, and 4 min.

***Microwaving*:** Onions were placed on a plate and cooked at 700 W for 1, 2, 3, and 4 min using a microwave oven (RE-C20DV; Samsung, Suwon, Korea).

Extraction for *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) analysis The extraction process for ACSOs from processed onions was performed according to the method described by Yoo *et al*. (12). Five g of a sample was weighted and added to 70 mL of water in a 100 mL volumetric flask. The mixture was vortexed for 1 min using a vortex mixer (G-560; Thermo Scientific, Waltham, MA, USA) and sonicated (5510E-DTH; Branson Ultrasonics Corporation, Danbury, CT, USA) for 10 min at room temperature. Additional water was added to the mixture to bring the final volume to 100 mL. The extract was filtered with a 0.2 μm membrane syringe filter (Sartorious Stedium Biotech, Geottingen, Germany) and analyzed by Liquid Chromatography Electrospray Ionization-Tandem Mass Spectrometry (LC/ESI-MS/MS) system.

LC/ESI-MS/MS Analysis HPLC-MS/MS analyses were performed on an Agilent LC 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled to a 4000 QTRAP mass spectrometer equipped with a Turbo Ion Spray Ionization source (Applied Biosystems, Waltham, MA, USA). The HPLC-MS/MS system was controlled using both Quant software and Analyst 1.5 software (Applied Biosystems). For HPLC-MS/MS analysis of ACSOs in heat-treated onions, separation was carried out on a C18 column (2.1×100 mm, 1.7 μm) (Fortis Technologies, Cheshire, UK). Chromatographic elution was performed with a mobile phase consisting of 0.1% formic acid in distilled water (A) and 0.1% formic acid in acetonitrile (B). The gradient system was as follows: 100% A (0 min), 10% A (12-12.5 min), and 100% A (13-19 min). The column flow rate was 100 μL/min, and the injection volume was 5 μL. The mass spectrometer was operated using a Turbo Ion Spray Ionization source configured for electro spray ionization (ESI) in positive ion mode, and acquisition was conducted using multiple reaction monitoring (MRM). Positive ionization was performed with an ion spray voltage of 5,000 V, a curtain gas of nitrogen at 20 psi, an ion source gas 1 and 2 at 50 psi and a temperature of 550°C. Mass parameters of precursor ion, product ion, declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were optimized using infusing standard stock solutions (1 μg/mL). The precursor ion and product ion for each compound are shown in Fig. 1. Other mass parameters were DP=41.0 V, EP=3.0 V, CE=12.0 V, CXP=15.0 V for methiin, DP=53.0 V, EP=10.0 V, CE=23.0 V, CXP=10.0 V for cycloallin, DP=35.0 V, EP=10.0 V, CE=14.0 V, CXP=5.0 V for isoalliin, DP=21.8 V, EP=3.9 V, CE=8.6 V, and CXP=7.7 V for propiin.

Statistical analysis Each experiment was performed in triplicate. Statistical significance tests were performed using SAS software (version 8.2; SAS Institute, Inc., Cary, NC, USA). Analysis of variance (ANOVA) was used in multiple group comparisons.

Results and Discussion

Validation of the analytical method for *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) For analysis of ACSOs with an HPLC-photodiode Array (PDA) system, the analytical method for processed onions was performed following Yoo *et al*. (12). However, complex onion matrix effects resulted in overestimation and baseline shifting. Therefore, *S*-alk(en)yl-L-cysteine sulfoxides in processed onions were identified and quantified based on LC/ESI-MS/MS (Fig. 1).

The proposed analytical method for ACSOs in processed onions was validated based on linearity, limit of detection (LOD), limit of quantification (LOQ), and accuracy in accordance with Food and Drug Administration (FDA) guidelines (13). LOD, LOQ, and accuracy values for ACSOs are shown in Table 1. Correlation coefficients (*R*) of all of compounds were >0.997. The LOD ranged from 0.02 to 0.11 μg/mL, and the LOQ ranged from 0.05 to 0.34 μg/mL for ACSOs. Additionally, accuracy of the proposed analytical method was evaluated based on recovery testing. Recovery values were satisfactory, ranging from 89.60 to 92.68% for ACSOs. Therefore, the proposed analytical method was adequate for detection and quantification of ACSOs.

ACSOs Changes in processed onions ACSOs changes in heat treated onions and the influence of heat treatments are shown in Fig. 2. Fresh onions used as a control exhibited ACSOs contents of

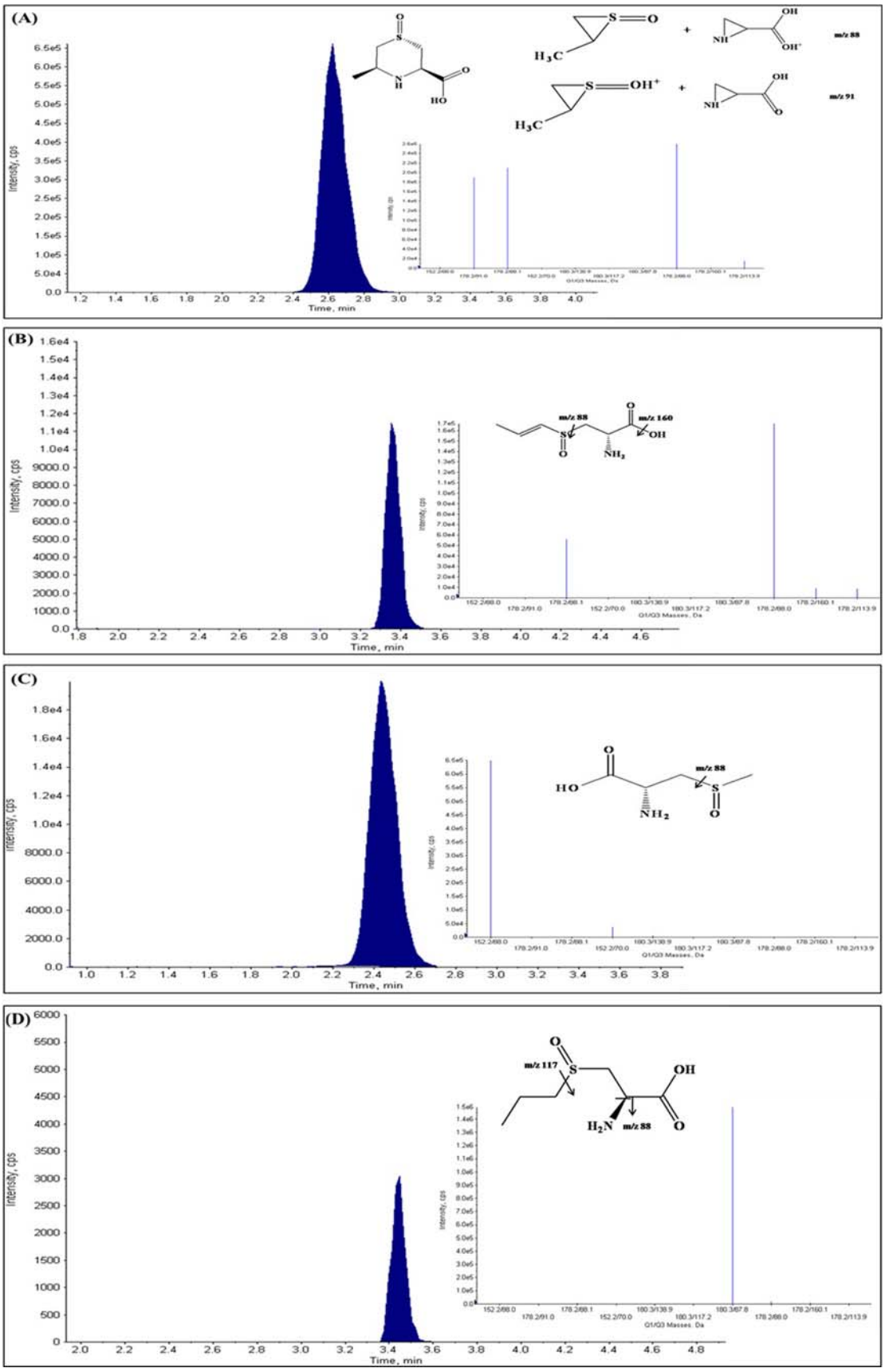

Fig. 1. *S*-Alk(en)yl-L-cysteine sulfoxides structure and LC/ESI-MS/MS results for chromatographic peaks in processed onions. Cycloalliin (A), isoalliin (B), methiin (C), and propiin (D) in positive mode.

Table 1. Abbreviations and validation data on *S*-alk(en)yl-L-cysteine sulfoxides

S-Alk(en)yl-L-cysteine sulfoxides (ACSOs)	Abbreviations	Linearity range (μg/mL)	Correlation coefficient (R)	LOD (μg/mL)	LOQ (μg/mL)	Recovery (%)
(+)-*S*-Methyl-L-cysteine sulfoxide	Methiin	0.10-4.00	1.000	0.02	0.05	90.21±1.69
(*1S,3R,5S*)-5-Methyl-1,4-thiazane-3-carboxylic acid 1-oxide	Cycloalliin	0.05-2.00	0.999	0.11	0.34	92.68±3.39
(+)-*S*-(Trans-1-propenyl)-L-cysteine sulfoxide	Isoalliin	0.05-2.01	0.999	0.03	0.08	91.45±2.14
S-Propyl-cysteine sulfoxide	Propiin	0.06-2.21	0.997	0.04	0.12	89.60±3.29

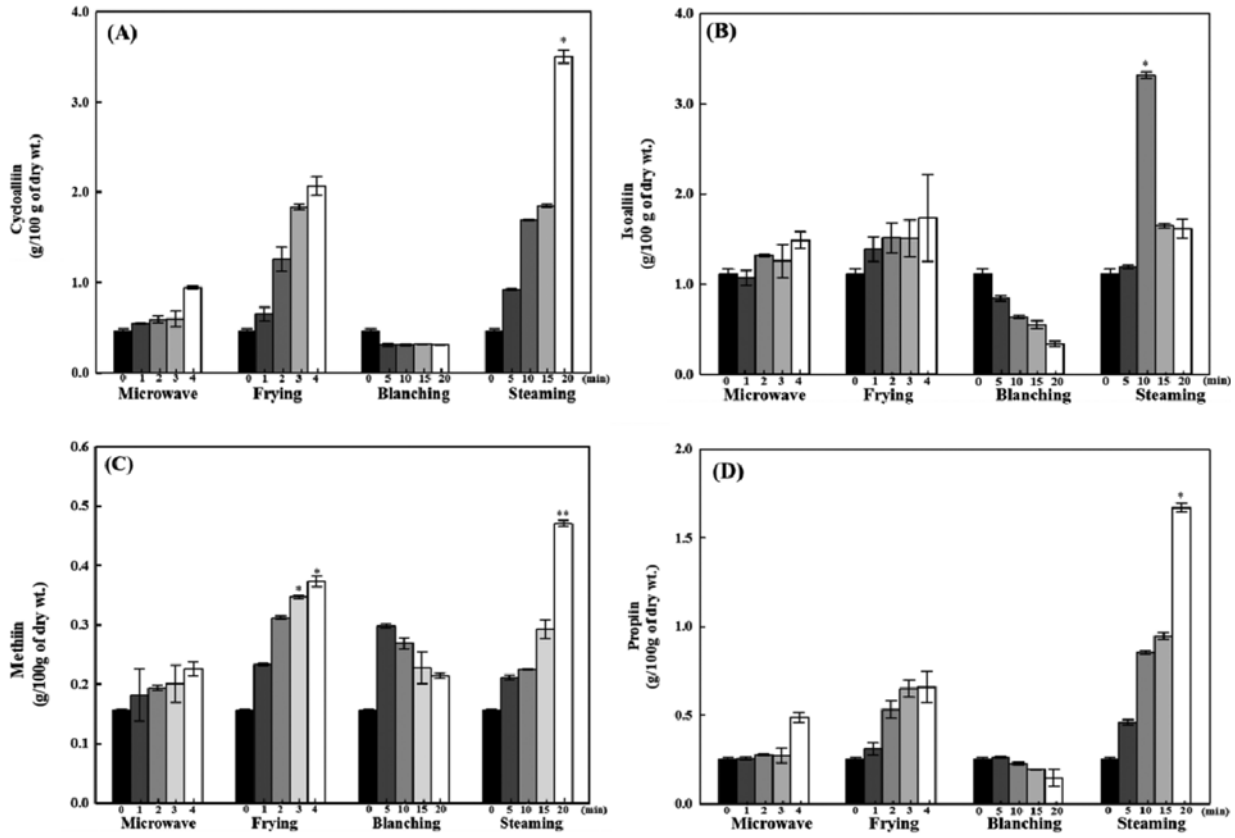

Fig. 2. Changes in *S*-alk(en)yl-L-cysteine sulfoxides in processed onions for cycloalliin (A), isoalliin (B), methiin (C), and propiin (D). Results represent a mean±standard deviation (SD). Significant differences were determined through using ANOVA (**p<0.01 and *p<0.05 vs. a control).

methiin=0.16 g/100 of g of dry weight (DW), cycloalliin=0.46 g/100 g of DW, isoalliin=1.11 g/100 g of DW, and propiin=0.25 g/100 g of DW. Contents of methiin, isoalliin, and cycloalliin in onion powder from Japan were previously reported as 0.35, 2.11, and 0.31 g/100 g of DW, respectively (8). Kubec and Dadàkovà (7) measured *S*-substituted cysteine derivatives in *Allium* plants with methiin and isoalliin contents of 0.12 and 0.44 mg/g of fresh weight (FW), respectively. Ueda *et al*. (14) determined the amount of sulfur-containing components from onions of different geographic sites using ethanol extracts and found that amounts of ACSOs differed significantly among diverse cultivar areas and among species.

Onions are generally consumed after processing or cooking rather than raw. Depending on the cooking method, ACSOs contents increased, compared with raw onions. The methiin content in heat treated onions ranged from 0.18 to 0.47 g/100 g of DW and the cycloalliin content ranged from 0.31 to 3.50 g/100 g of DW. The amount of isoalliin in heat treated onions was 0.34 to 3.32 g/100 g of DW, and propiin was 0.15 to 1.67 g/100 g of DW. With the exception of boiling, amounts of ACSOs dramatically increased with p<0.05 and p<0.01 in heat-treated onions. In particular, cycloalliin, isoalliin, and propiin were significantly difference with p<0.05, and methiin was statistical significant with p<0.01 in steaming onion.

The ACSOs precursors *S*-alk(en)yl-L-cysteines are produced from γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteines by γ-glutamyl peptidase, and an oxidase chemically converts *S*-alk(en)yl-L-cysteines to ACSOs (15,16). Although ACSOs are found at low levels in raw onions, the level was increased during domestic processing of steaming, frying, and microwave cooking. Therefore, formation of the ACSOs precursors *S*-alk(en)yl-L-cysteines catalyzed by γ-glutamyl peptidase and oxidase were activated sequentially by heat. In this study, enhancement of ACSOs contents occurred based on the heat treatment method and treatment time. In particular, the amount of cycloalliin increased 7.6x

after steam preparation. Cycloalliin is considered to be a beneficial bioactive compound for human health with a lipid level-lowering effect (16-18). After heat treatment, steamed onions exhibited increased amounts of ACSOs except for the isoalliin content, which was decreased after 15 min. It assumed that a reduction in the amount of isoalliin as a cycloalliin precursor would cause the amount of cycloalliin to increase at the same time. Also, the isoalliin may have been pyrolyzed by high temperatures over time.

Ueda *et al*. (14) reported that isoalliin degradation is complicated due to co-existence of other amino acids and sugars. In the case of boiling, ACSOs leaches from onions into cooking water. Therefore, boiled onion exhibited only decreased ACSOs contents, compared with steaming and microwaving. Therefore, degradation of *S*-alk(en)yl-L-cysteines in raw onions was due to heating, and subsequent conversion to ACSOs was verified (19). Increased level of ACSOs indicated that γ-glutamyl peptides were converted into corresponding sulfoxides by γ-glutamyl peptidase and oxidase (15). Changes in ACSOs compositions were different in heat-treated onions.

In this study, changes in ACSOs expressions in onions during heat-treatments were investigated using LC/ESI-MS/MS. Amounts of cycloalliin and isoalliin were increased during steaming, but amounts of all compounds were decreased during blanching. Development and quality evaluation of onions and the way that onions are processed may benefit from this study. Consequently, research into processes of changes in organosulfur compounds (OSCs) during heat treatment will continue.

Disclosure The authors declare no conflict of interest.

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