# Release of volatile compounds from sliced onion analysed by gas chromatography coupled to mass spectrometry and its antimicrobial activity

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**Summary**

Plants belonging to the genus *Allium* are known for their high content of organosulfur compounds, which give them typical flavour and aroma released during their processing. These plants are also known for various biologically important effects, in particular antimicrobial activity. The volatile compounds are released enzymatically after rupture of the plant cells. The released volatile organosulfur compounds of onion and their monitoring in time was done by using headspace solid phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME/GC-MS). Nineteen organosulfur compounds were identified with different course of release. The main released compounds were thiopropanal S-oxide, methylprop(en)yldisulfide and prop(en)yltrisulfide. Antimicrobial activity of onion juice was tested on 8 microorganisms using disc-diffusion and well-diffusion methods. Completely resistant microorganisms were *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*, the most sensitive was *Candida albicans*.

**Keywords**

onion; organosulfur compounds; headspace solid phase microextraction; antimicrobial activity

Onion (*Allium cepa* L.) is one of the oldest cultivated plants and is widely used throughout the world to prepare food, being popular mainly for its flavour and aroma. Onion is also widely used in the treatment and prevention of many diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, type II diabetes, hypertension and gastrointestinal disorders [1]. Allium plants contain large amounts of organically bound sulfur, which are the highest in nature [2, 3]. The content of sulfur in plants varies according to variety and maturity of plants, and according to cultivation and climatic conditions. Regarding the analysis of organosulfur compounds, their quantity varies according to the method of sample preparation. The studies found the taste, the tear potential and the total sulfur content of the crushed onions to show a significant correlation with the sulfate content in soil [2–5].

Onion is known to be antiseptic, to have pronounced phytoncidic and diurectic effects, to stimulate appetite, increase secretion of gastric juices, formation of bile and pancreatic enzymes, and to reduce the concentration of saccharides in blood [6, 7]. According to Ramos et al. [8], onion has antimicrobial effects against *Helicobacter pylori*. Griffiths et al. [9] suggested that the biological activity and curative effects of onion are mainly due to the high content of organosulfur compounds. Oräşan et al. [10] used the disc-diffusion and well-diffusion methods to test the antimicrobial properties of the liquid extract of onion against *Escherichia coli* and *Staphylococcus aureus*. They reported significant antibacterial efficiency against *Staphylococcus aureus* using the well-diffusion method. A minor antimicrobial effect was observed by using the disc-diffusion method. Santas et al. [11] tested the antimicrobial properties of extracts of lyophilized onion samples against *Bacillus cereus, Staph. aureus*, *E. coli, Pseudomonas aeruginosa* and *Candida albicans*. Kivanç et al. [12] examined the effects of fresh onion juice

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by the well-diffusion method against 10 bacteria and 9 yeasts, and reported significant antibacterial effect agaqinst all tested yeasts. CORZO-MARTINEZ [5] stated that onion, unlike garlic, is not antimicrobially effective against Gram-negative bacteria.

A typical and very pronounced substance in onion is thiopropanal S-oxide (lachrymatory factor), which is formed when onion wall is sliced or disrupted. The main difference between onion and garlic is creation of the lachrymatory factor during processing [13]. *Trans*-S-1-propenyl-L-cysteinesulfoxide (1-PRENSCO), or isoalliin [14], is the dominant precursor of lachrymatory factor. For this precursor, alliinase is activated by the breakdown of the cell wall by the enzyme alliinase, which converts it to propenylsulfenic acid, ammonia and pyruvate, and the lachrymatory factor synthase (LF synthase) causes breakdown of sulfenic acid to thiopropanal S-oxide and thiosulfinates [15].

Thiosulfinates are the most studied and well-known compounds formed in Allium plants. They have been found in all species of this genus, and differences between species are due to the type and relative content of their precursors, S-alk(en)yl-L-cysteine sulfoxides (ACSO). One of the significant thiosulfinates found in onion is isoalliin [2]. Thiosulfinates are unstable, in particular when heated, being decomposed to a complex mixture of compounds in which mono-, di-, tri- and tetrasulfides dominate [16]. Dipropyldisulfide, dipropyltrisulfide and propenyldisulfide are the main volatile substances released from onion, but many other compounds have been identified [1, 17–19].

In this study, headspace solid phase microextraction (HS-SPME) technique was used for sampling of volatile compounds released from the sliced onion, which were then analysed by gas chromatography coupled to mass spectrometry (GC-MS). In addition, the antimicrobial activity of fresh onion juice was determined against 8 different microorganisms using the disc-diffusion and well-diffusion methods.

**Tab. 1.** Schedule of the extraction process.

| Measurement | Time interval [min] |
|---|---|
| 1 | 0–10 |
| 2 | 30–40 |
| 3 | 60–70 |
| 4 | 90–100 |
| 5 | 120–130 |
| 6 | 150–160 |
| 7 | 180–190 |
| 8 | 210–220 |
| 9 | 240–250 |
| 10 | 270–280 |

Time interval of extraction run after slicing of the onion bulb is given.

## MATERIALS AND METHODS

### Plant material

Onion samples of Czech origin were purchased in a local market in Pardubice, Czech Republic. To assure a representative sample, three different batches from a different place of origin were selected. Sample No. 1 was yellow onion, place of origin Velké Bílovice (supplier Jihomoravská zelenina, Velké Bílovice, Czech Republic, number of batch 4004). Sample No. 2 was yellow onion, place of origin Tábor (supplier Agrico Bohemia, Tábor, Czech Republic, number of batch 0407) and sample No. 3 was red onion, place of origin Semice (supplier Družstvo Bramko, Semice, Czech Republic, number of batch L0807).

### Chemicals and materials

The standard solution of *n*-alkane mixture (C8–C20) dissolved in *n*-hexane (concentrations 40 $mg \cdot l^{-1}$) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Helium 5.0 was purchased from Linde Gas (Prague, Czech Republic). SPME fibre with a mixed sorbent 50/30 $\mu$m divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was purchased from Supelco (Bellefonte, Pennsylvania, USA).

### Sampling by solid phase microextraction

Outer dry layers of onion bulbs were removed and the bulb was cut by a stainless steel knife into small pieces of approximately 3 × 3 × 3 mm. Samples of 2 g ± 0.1 g of individual sliced samples were transferred into a 20 ml headspace vial without a septum, to simulate realistic conditions during preparation of dishes, the saturation of the space resulting eventually in cessation of the enzymatic reaction. SPME was carried out immediately after slicing the onion sample, the extraction time was 10 min at laboratory temperature. Subsequent extractions (10 runs) were repeated with the same sample at same conditions over 4.5 h. For detailed time intervals see Tab. 1.

### Gas chromatography

The gas chromatograph GC-2010 with the mass spectrometer QP 2010 Plus (Shimadzu, Kyoto, Japan) and the PAL Combi sampling device (CTC Analytics, Zwingen, Switzerland) was used to analyse the extracted compounds. The GC-MS system was equipped with a capillary

column ZB-5HT Inferno with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 $\mu$m (Phenomenex, Torrance, California, USA). Helium was used as the carrier gas at a constant linear velocity of 30 cm·s$^{-1}$. The temperature of the injection block was maintained at 200 °C. The temperature gradient was programmed as follows: the initial temperature was 40 °C (3 min) and then increased at 10 °C·min$^{-1}$ to 210 °C. Total analysis time was 20 min. The mass spectrometer was operated in the full scan mode at an *m*/*z* range of 35–500 in the electron ionization mode (70 eV).

Linear retention indices (*RI*) were calculated using homologous series of *n*-alkanes (C8–C20) analysed under the same condition as samples. The compounds were identified by comparing the measured mass spectra with the reference mass spectra from libraries (NIST, National Institute of Standards and Technology, Gaithersburg, Maryland, USA and FFNSC2, Flavour & Fragrance Natural & Synthetic Compounds, Shimadzu, Kyoto, Japan) using the criterion of at least 80% similarity, and on the basis of linear retention indices.

#### Preparation of onion juices for antimicrobial testing

The onion bulb was washed with freshly prepared sterile distilled water. The outer covering of the bulb was manually peeled off. The whole bulb was cut into small pieces and squashed by a stainless steel knife. The juice obtained was immediately applied to the disc or to the well.

#### Antimicrobial testing

The microorganisms used in this study were: *Arcobacter butzleri* CCUG 30484, *Bacillus cereus* CCM 2210, *Bacillus subtilis* CCM 2215, *Enterococcus faecalis*, *Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* CCM 3953, and *Candida albicans* CCM 8215. Strains were obtained from the Czech Collection of Microorganisms, Brno, Czech Republic. Cultures were grown on Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom). Cells were harvested and suspended in physiological saline to approximately $1.5 \times 10^8$ CFU·ml$^{-1}$ (0.5 on McFarland scale).

The agar disc-diffusion and well-diffusion methods were used for antimicrobial testing. Suspensions of microorganisms were spread on the surface of Mueller-Hinton agar. Filter paper discs (6 mm diameter, Oxoid) were impregnated with 8 $\mu$l of the tested onion juice and immediatelly placed onto the inoculated agar surface. In the well-diffusion method, wells of 10 mm in diameter were prepared directly in agar by sterilized stainless steel tubes and filled with the tested onion juice to the brim. Diffusion took place at laboratory temperature for 2 h and subsequently the plates were incubated for 24–48 h at 30–37 °C (depending on strain) under aerobic conditions.

Simultaneously, discs impregnated with various antimicrobial agents (ciprofloxacin, ampicillin, clindamycin, tetracycline, fluconazole; Oxoid) were used as a positive control. The plates were incubated for 24–48 h at 30–37 °C (depending on strain) under aerobic conditions. Inhibition zones were measured using a Bacmed 6iG2 automatized reader (Aspiag, Litomyšl, Czech Republic). Experiments were performed in triplicates and the results were expressed as a mean of inhibition zones (in millimetres) with standard deviation.

## RESULTS AND DISCUSSION

#### Monitoring of volatile substances released from onion

Tab. 2 summarizes the identified compounds. In total, 19 organosulfur compounds were detected with a different course of release. The most interesting sulfur-containing substance for onion consumers is thiopropanal S-oxide, known as lachrymatory factor [20]. Aldehydes and alcohols were also observed among the released volatile compounds.

The areas of peaks obtained by SPME/GC-MS do not necessarily reflect the true proportions of the components in the headspace, because different compounds exhibit different afinity to the SPME fibre [21]. However, trends in the appearance and disappearance of compounds in successive samples could be studied by the used method. The increasing or decreasing amounts of volatile substances were monitored at intervals of time, the measurement starting immediately after slicing of the onion bulb.

The data were evaluated based on the content of the given compound in the first analysis of each sequence being taken as 100 %, and the subsequent increase or decrease was expressed in relative percentages that were calculated from results of further analyses. If appropriate compound was not detected in the first analyse, than first appearing of this compound in next analyses was evaluated as 100 % relative. For each sample, one series of measurements is depicted in Fig. 1–3, time profiles of volatile substances being evaluated where the changes in composition were significant (in case of (Z)-1-(prop-1-en-1-yl)-3-propyltrisulfide, (E)-1-(prop-1-en-1-yl)-3-propyltrisulfide, dipropyldisulfide, dipropyltrisulfide and propanethiol).

Propylthioacetate always began to appear in the

**Tab. 2.** Identified compounds.

| Compound name | Calculated retention index | CAS number |
|---|---|---|
| Acetaldehyde | < 800 | 75-07-0 |
| Methylmercaptan | < 800 | 74-93-1 |
| Propanal | < 800 | 123-38-6 |
| 1-Propanol | < 800 | 71-23-8 |
| Propanethiol | < 800 | 107-03-9 |
| Thiopropanal S-oxide | < 800 | 32157-29-2 |
| 2-Methyl-2-pentenal | 830 | 623-36-9 |
| Propylthioacetate | 867 | 2307-10-0 |
| 3,4-Dimethylthiophene | 901 | 632-15-5 |
| Methylpropyldisulfide | 930 | 2179-60-4 |
| (E)-1-Methyl-2-(prop-1-en-1-yl)disulfide | 937 | 23838-19-9 |
| 1-Allyl-2-isopropyldisulfide | 1 091 | 67421-85-6 |
| (Z)-1-Allyl-2-(prop-1-en-1-yl) disulfide | 1 099 | 2179-57-9 |
| Dipropyldisulfide | 1 107 | 629-19-6 |
| (E)-1-(Prop-1-en-1-yl)-2-propyldisulfide | 1 116 | 23838-21-3 |
| 1-(E)-Prop-1-en-1-yl-2-(Z)-prop-1-en-1-yldisulfide | 1 125 | 121609-82-3 |
| 2-Merkapto-3,4-dimethyl-2,3-dihydrothiophene | 1 135 | 137363-86-1 |
| Methylpropyltrisulfide | 1 156 | 17619-36-2 |
| Allylpropyltrisulfide | 1 317 | 33922-73-5 |
| Dipropyltrisulfide | 1 330 | 6028-61-1 |
| (Z)-1-(Prop-1-en-1-yl)-3-propyltrisulfide | 1 336 | 23838-26-8 |
| (E)-1-(Prop-1-en-1-yl)-3-propyltrisulfide | 1 344 | 23838-27-9 |
| 1,3-Di((E)-prop-1-en-1-yl) trisulfide | 1 354 | 115321-81-8 |

second measurement (Tab. 1), its concentration had first a rising character, after 1.5 h gradually dropping to below detectability. 3,4-Dimethylthiophene had its maximum content in the first measurement and its content decreased over time. One hour and half after cutting of the onion bulb, 3,4-dimethylthiophene was no more detected. Methylpropyldisulfide was already present in the first measurement, the maximum content being detected in the second run, then it decreased and, in eighth run, its content dropped below its detectability. (E)-1-Methyl-2-(propenenyl)disulfide was present only in the first 4 measurements with maximum concentration in the second run. The organosulfur compound 1-allyl-2-isopropyldisulfide was only present in the second measurement. (E)-1-(Prop-1-en-1-yl)-2-propyldisulfide was detected from the first measurement and the maximum concentration was reached in the second measurement with a gradual decrease to below the detectable amount. 1,3-Di-((E)-prop-1-en-1-yl)trisulfide was detected only in the first two measurments.

Colina-Coca et al. [22] analysed sliced onion, in a way similar to this study, by using dynamic headspace analysis in conjunction with GC-MS, but allylmethylsulfide was added to the sample as an internal standard and the treated samples were heated to 80 °C for 40 min, during which the volatile substances were captured and concentrated in a purge and trap system and subsequently desorbed for gas chromatography analysis. Despite the different assay conditions, the results were consistent regarding several identified compounds including propanal, propanethiol, 2-methylpentene, propylthioacetate, 3,4-dimethylthiophene, methylpropyldisulfide, (E)-1-methyl-2-(prop-1-en-1-yl)disulfide, 1-allyl-2-isopropyldisulfide, dipropyldisulfide, (E)-1-(propen-1-yl)-2-propyldisulfide and methylpropyltrisulfide. The study also stated that the main volatile components of onion are disulfides, trisulfides and aldehydes.

Boelens et al. [23] studied headspace over sliced onions and then analysed the compounds using GC-MS. Results of our study coincide with theirs in some identified compounds, namely, propanal, 2-methyl-2-pentenal, 3,4-dimethylthiophene, methylpropyldisulfide, (E)-1-(prop-1-enyl)-2-propyldisulfide, dipropyldisulfide and (E)-1-(prop-1-en-1-yl)-2-propyldisulfide. In that study, unlike in our work, no trisulfides were identified.

Liguori et al. [4] used for the analysis of volatile components of the mixed onion an extract obtained by steam distillation and subsequent extraction with dichloromethane, which was analysed by GC-MS. Those authors identified in total 22 compounds from different chemical classes, such as organosulfur compounds, aldehydes and ketones. In that study, several same compounds were detected as in our present work, namely, propanal, propanethiol, 2-methyl-2-pentenal, 3,4-dimethylthiophene, methylpropyldisulfide, (E)-1-(prop-1-enyl)-2-propyldisulfide, dipropyldisulfide, (E)-1-(propen-1-yl)-2-propyldisulfide, methylpropyltrisulfide and dipropyltrisulfide. Despite the very different conditions of sample preparation, many identified compounds are consistent.

Kremr et al. [24] studied organosulfur compounds in *Allium* plants. They analysed onion using SPME-GC-MS and detected 18 organosulfur compounds, out of which only 4 compounds were found also in our study, namely, (E)-1-methyl-2-(prop-1-en-1yl)disulfide, dipropyldisulfide, (E)-1-(prop-1-en-1-yl)-2-propyldisulfide and dipropyltrisulfide. The differences might have been caused by different times between the first distur-

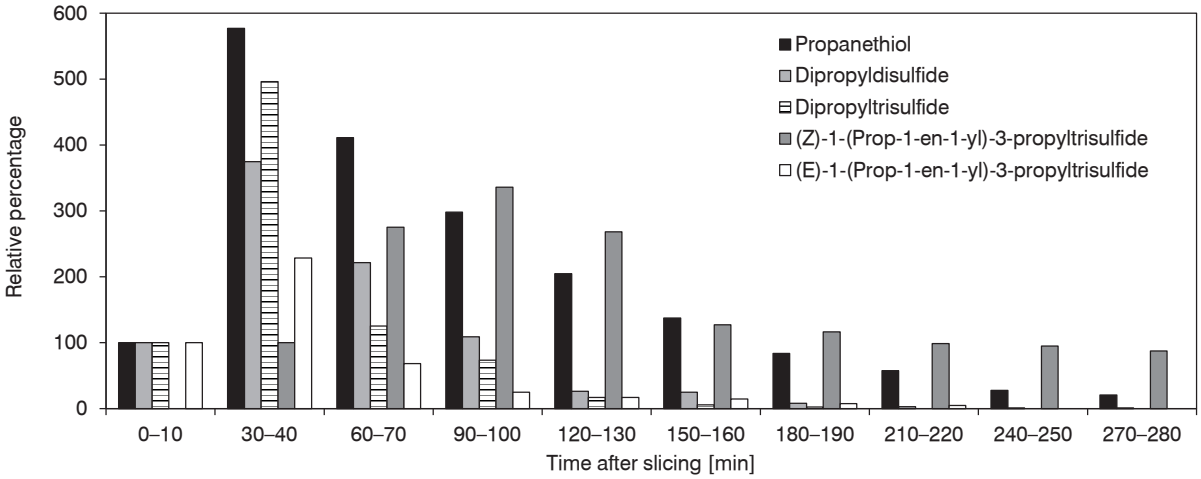


**Fig. 1.** Relative contents of selected compounds in various times after slicing of yellow onion sample No. 1.

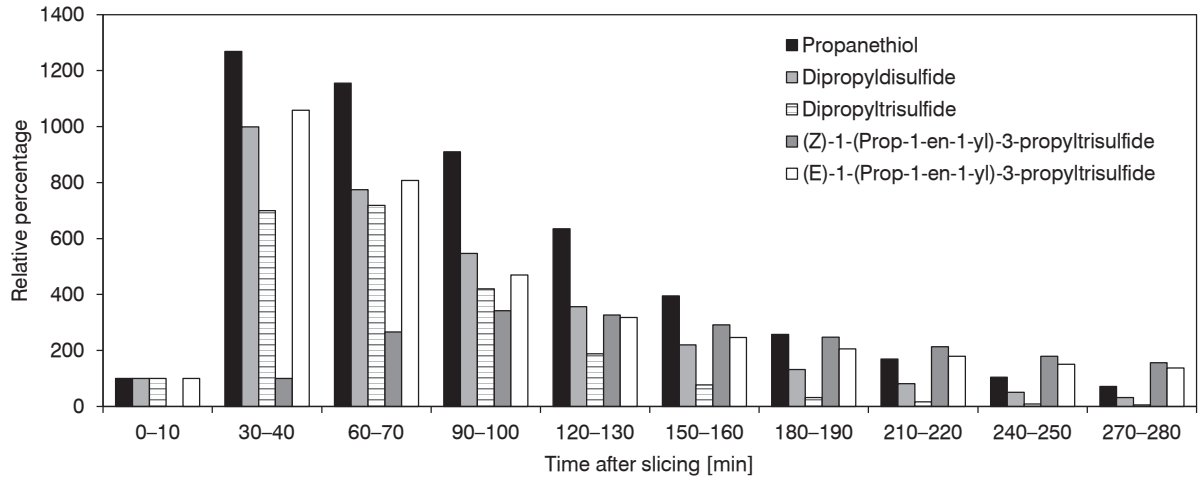


**Fig. 2.** Relative contents of selected compounds in various times after slicing of yellow onion sample No. 2.

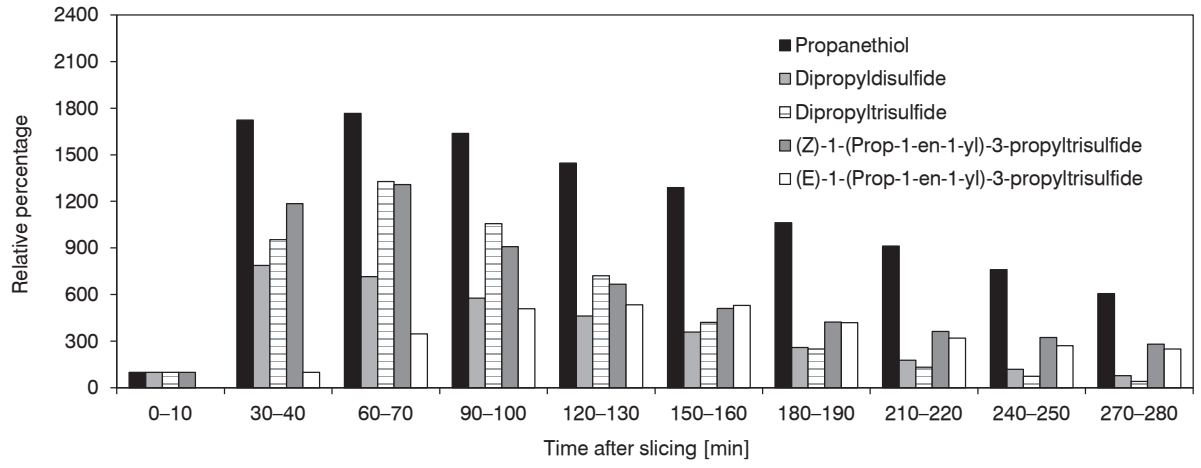


**Fig. 3.** Relative contents of selected compounds in various times after slicing of red onion sample No. 3.

bance of the onion tissue and by the analysis itself.

The only available study experimentally consistent with our study is the work by JÄRVENPÄÄ et al. [20]. Authors analysed the headspace over sliced onion in time intervals using SPME-GC-MS, using poly(dimethyl)siloxane (PDMS) fibre with a 100 $\mu$m film thickness (Supelco) for SPME. The authors mentioned presence of thiopropanal S-oxide and also small amounts of diprop(en)yldisulfides in the first minute after cutting of the onion. One hour after cutting, thiopropanal S-oxide degradation products were detected, such as 2-methyl-2-pentenal and various (di)sulfides, which is consistent with our results. They stated that, after 90 min, the heaspace over the onion sample was richest for dipropyldisulfide, which is in good agreement with our results. Methylprop(en)yldisulfides were detected in this study occurring after a large amount of diprop(en)yldisulfides was present in the headspace above the sample. This was confirmed in our study, where methylpropyldisulfide and (E)-1-methyl-2-(prop-1-enyl) disulfide were detected in the largest amount in the second measurement and in the third measurement. Results of the study of JÄRVENPÄÄ et al. [20] differed from ours in the detection of tetrasulfides, which were not found in our experiments in any measurement.

**Antimicrobial activity of onion juices**

Antibacterial activity of onion juices was tested using disc-diffusion method with antibiotic discs used as a positive control (Tab. 3), results are shown in Tab. 4. Using the disc-diffusion method in our experiments, onion juice demonstrated no inhibitory effect on most microorganisms. Inhibition of growth appeared only in the case of the yeast *Candida albicans* (inhibition zone of yellow onion juice was 11.0 ± 0.8 mm, of red onion juice 14.3 ± 1.0 mm). Antimicrobial effects of red onion juice were evaluated as stronger than the antimicrobial effect of yellow onion juice. These results were in accord with data previously presented in literature [6, 25]. Despite of the different experimental conditions, our results were the same as those of ORĂŞAN et al. [10], i. e. no inhibition in case of *E. coli* and *Staph. aureus*. Absence of the inhibitory effect against *E. coli* and *Ps. aeruginosa* in our work coincided with the results of the study by SANTAS et al. [11].

**Tab. 3.** Antimicrobial effect of the positive controls in disc-diffusion tests.

| Microorganism | Mean of inhibition zones [mm] | | | | |
|---|---|---|---|---|---|
| | Ciprofloxacin | Ampicillin | Clindamycin | Tetracycline | Fluconazole |
| *Arcobacter butzleri* CCUG 30484 | × | × | 11.5 | 20.5 | × |
| *Bacillus cereus* CCM 2210 | 35.0 | 20.0 | × | × | × |
| *Bacillus subtilis* CCM 2215 | 26.0 | 7.0 | × | × | × |
| *Candida albicans* CCM 8215 | × | × | × | × | 18.0 |
| *Enterococcus faecalis* NPK101 | 24.0 | 21.0 | × | × | × |
| *Escherichia coli* CCM 3954 | 36.0 | 0.0 | × | × | × |
| *Pseudomonas aeruginosa* CCM 3955 | 36.0 | × | × | 19.0 | × |
| *Staphylococcus aureus* CCM 4223 | 34.0 | × | × | 23.0 | × |

The diameter of the discs was 6 mm. × – test was not performed.

**Tab. 4.** Antimicrobial effect of onion juices samples.

| Microorganism | Inhibition zone [mm] | | | |
|---|---|---|---|---|
| | Disc-diffusion method | | Well-diffusion method | |
| | Yellow onion juice (sample No. 1) | Red onion juice (sample No. 3) | Yellow onion juice (sample No. 1) | Red onion juice (sample No. 3) |
| *Arcobacter butzleri* CCUG 30484 | 6.0 ± 0.0 | 6.0 ± 0.0 | 18.0 ± 2.0 | 22.5 ± 2.4 |
| *Bacillus cereus* CCM 2210 | 6.0 ± 0.0 | 6.0 ± 0.0 | 18.5 ± 0.6 | 24.0 ± 2.5 |
| *Bacillus subtilis* CCM 2215 | 6.0 ± 0.0 | 6.0 ± 0.0 | 10.0 ± 0.0 | 10.0 ± 0.0 |
| *Candida albicans* CCM 8215 | 11.0 ± 0.8 | 14.3 ± 1.0 | 34.8 ± 1.3 | 36.8 ± 2.4 |
| *Enterococcus feacalis* NPK101 | 6.0 ± 0.0 | 6.0 ± 0.0 | 18.3 ± 1.0 | 20.3 ± 0.5 |
| *Escherichia coli*CCM 3954 | 6.0 ± 0.0 | 6.0 ± 0.0 | 10.0 ± 0.0 | 10.0 ± 0.0 |
| *Pseudomonas aeruginosa* CCM 3955 | 6.0 ± 0.0 | 6.0 ± 0.0 | 10.0 ± 0.0 | 10.0 ± 0.0 |
| *Staphylococcus aureus* CCM 4223 | 6.0 ± 0.0 | 6.0 ± 0.0 | 19.0 ± 0.8 | 22.0 ± 0.8 |

Discs of 6 mm in diameter were used in the disc-diffusion tests and wells of 10 mm in diameter were used in the well-diffusion tests. Mean values ± standard deviation are given, $n = 3$.

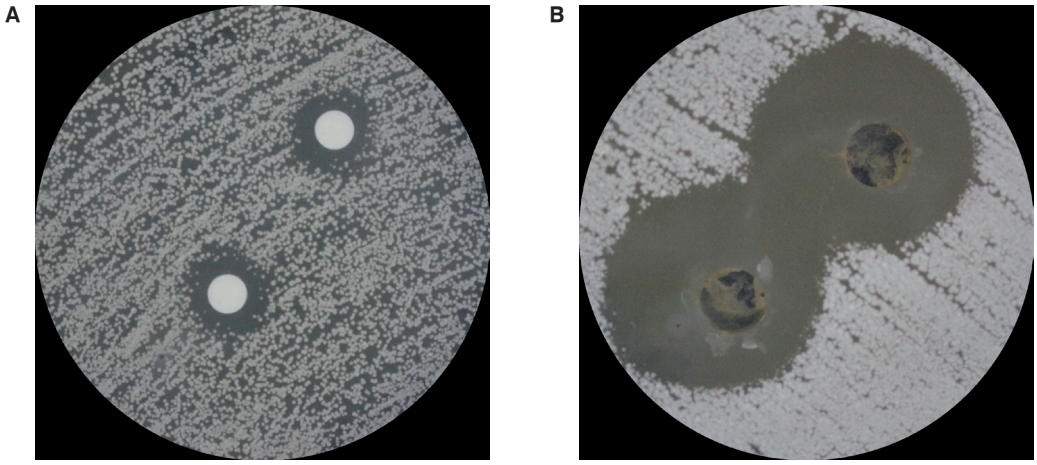


**Fig. 4.** Antimicrobial effect of the yellow onion juice against *Candida albicans* CCM 8215.
A – disc-diffusion method, B – well-diffusion method.

The results on the antibacterial activity of onion juices determined using well-diffusion method are shown in Tab. 4. The obtained results were different from those obtained by the disc-diffusion method. In case of *B. subtilis*, *E. coli* and *Ps. aeruginosa*, no inhibition zones were observed for both red and yellow onion juices. However, the juices had inhibitory effects on other microorganisms. This method also confirmed that red onion juice had a higher antibacterial activity than that from the yellow onion. The strongest antimicrobial effect of onion juice was clearly against *C. albicans* (Fig. 4).

In the study of Oräşan et al. [10], onion juice showed antimicrobial effects against *E. coli*, which does not match our results. The tested microorganisms in the study of Kivanc et al. [12] were consistent with our study, but the results are different. They described antimicrobial effects of onion juice just against *B.cereus*, *B. subtilis* and *E. faecalis*. In our study, using the well-diffusion method, significant inhibitory effect of onion juice was observed against Gram-negative *Arcobacter butzleri* CCUG 30484 (inhibition zones 18.0 mm and 22.5 mm, respectively). This is a different result compared to the study of Corzo-Martínez et al. [5].

Of course, it cannot be expected that all volatiles are effective as antimicrobial agents. Most of the active compounds can remain in the liquid phase. The study of Ahiabor et al. [25] stated that the volatile allicin is an antimicrobial compound in the crushed onions. According to other studies [11, 25], quercetin is a non-volatile substance with strong antimicrobial effects, followed by kaempferol as an antimicrobially active compound of a lower effectiveness.

## CONCLUSIONS

By monitoring the profile of volatile substances released from onion, primarily disulfides and trisulfides were detected. The relative contents of these substances mostly had an increasing trend during the first hour after cutting and their content began to decrease over time, sometimes even to below the detectable level. In the second part of the study, antimicrobial effects of the juice from freshly grated onions were investigated with 8 microorganisms using disc-diffusion and well-diffusion methods. *E. coli*, *Ps. aeruginosa* and *B. subtilis* were found to be completely resistant to onion juice, while other microorganisms were inhibited in well-diffusion tests and *C. albicans* was the only microorganism inhibited alse in the disc-diffusion test.

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Received 29 August 2019; 1st revised 1 November 2019; accepted 19 November 2019; published online 26 November 2019.