Short communication
DOI: /10.5513/JCEA01/21.2.2728
Journal of Central European Agriculture, 2020, 21(2), p.333-337

Impact of harvest date on the content of volatile components in garlic

Iva BAŽON[1,2] (✉), Igor LUKIĆ[1,2], Dean BAN[1,2], Ivana HORVAT[1], Bernard PREKALJ[1,2], Smiljana GORETA BAN[1,2]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia

[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia

✉ Corresponding author: iva@iptpo.hr

Received: 8 January 2020; accepted: 30 March 2020

ABSTRACT

The aim of this study was to define optimal harvesting date of garlic cv. 'Istarski crveni' by comparing the amount of volatile compounds as one of quality indicators. In total 17 volatile compounds were determined in tested garlic samples by headspace solid-phase microextraction coupled to gas chromatography with flame ionization and mass spectrometric detection (HS-SPME-GC-FID-MS) regardless of harvesting date. Major volatiles diallyl trisulfide, diallyl sulfide, methyl allyl disulfide and diallyl disulfide representing 98.5 % of all the determined compounds have shown no significant difference between harvest dates. Significantly higher content of the two minor volatiles, allyl mercaptane and thieno[2,3-b]thiophene, was found in the last and second when compared to the first harvest date. Principal components PC1 and PC2 explained 78% of the total variance and pointed to certain relations between harvest dates and volatiles. The technological maturity had probably been reached at the first harvesting date since similar amounts of volatiles were found during the observed period.

Keywords: agricultural practice, alliin, cultivar, gas chromatography, organosulfur compounds

SAŽETAK

Cilj ovog istraživanja bio je definirati optimalan rok berbe za češnjak cv. Istarski crveni usporedbom sastava hlapljivih spojeva kao jednog od indikatora kvalitete. Pomoću tehnike mikroekstrakcije na čvrstoj fazi iz vršnih para uzorka uz plinsku kromatografiju s detekcijom na osnovi plamene ionizacije i spektrometrije masa (HS-SPME-GC-FID-MS), u ispitivanim uzorcima pronađeno je ukupno 17 hlapljivih spojeva neovisno o roku berbe. Sadržaj važnijih spojeva, dialil trisulfida, dialil sulfida, metil alil disulfida i dialil disulfida koji su predstavljali 98,5% od ukupnog sadržaja pronađenih spojeva, nije se značajno razlikovao obzirom na rok berbe. Značajno viši sadržaj dvaju manje zastupljenih spojeva, alil merkaptana i tieno[2,3-b]tiofena, pronađen je u posljednjem, odnosno u drugom u usporedbi s prvim rokom berbe. Glavne komponente PC1 i PC2 objasnile su 78% ukupne varijance što ukazuje na određene odnose između roka berbe i sadržaja hlapljivih spojeva u istraživanim uzorcima češnjaka. Tehnološka zrelost je vjerojatno već bila postignuta kod prvog roka berbe budući da je tijekom cijelog razmatranog razdoblja utvrđen sličan sadržaj hlapljivih spojeva.

Ključne riječi: poljoprivredna praksa, alin, kultivar, plinska kromatografija, organosumporni spojevi

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the oldest known crops (Bloem et al., 2010) used in both culinary and medicine in many countries. 'Istarski crveni' cv. is an old garlic ecotype grown by local producers and registered on the Variety list of Republic of Croatia. It has red skin colour of the clove and ability to produce scape, mature bulbs weighing 26 g and containing 7 cloves (Prekalj et al., 2019).

The caracteristic alliaceous smell, taste and numerous health beneficial effects of garlic can be mostly attributed to alliin amino acid (Horníčková et al., 2011) and its sulfur by-products (Galoburda et al., 2013), but also to other cysteine sulfoxides such as methiin, ethiin and isoalliin. It is considered that after crushing garlic bulb, enzyme alliinase transforms alliin into unstable compound alliicin, from which sulfur volatile compounds are formed (Grégrová et al., 2013, Galoburda et al., 2013). Consumers' preference might depend upon the intensity of garlic aroma and taste which are crucial for its sensory quality.

Martins et al. (2016) have reported that sulfur-containing compounds can be affected by cultivation practices or genotype, as well as, by post-harvest conditions including storage and processing. Influence of agricultural practices on garlic quality was investigated scarcely and is not well understood. According to De La Cruz Medina and García (2007), harvest may initiate when lower third to half of the leaves have turned brown or when hardneck scapes are still straight but before the umbel containing bulbils gets opened. Garlic cv. 'Istarski crveni' is usually harvested in the second half of June when approximately 50% of aerial plant parts die off.

The aim of this research was to compare the amount of volatile compounds at different harvesting dates as a possible quality parameter that may indicate the harvest date and possibly adjust it according to market preferences.

MATERIALS AND METHODS

Garlic cv. 'Istarski crveni' was grown at the Institute of Agriculture and Tourism (Poreč, Croatia) trial field. Garlic was planted in October 2017 at the density of 38 plants/m^2. Cultivation practices were applied according to Lešić et al. (2002). Field experiment was conducted as a randomized complete block design with four replicates for each harvesting date. Garlic was harvested every two weeks during 2018, precisely at 5th of June (bottom leaves died off, other leaves were turning yellow), 21st of June (90% of plants foliage had died off, scapes were still green) and 5th of July (scapes turned yellow) and stored at an average temperature of 24°C during three months. Representative bulbs with no visible symptoms of plant diseases or physiological damage were sampled from each harvesting date for a composite sample.

Volatile compounds were isolated by headspace solid-phase microextraction (HS-SPME). A number of uniform garlic bulbs were selected for each harvest date, representative cloves of similar size were peeled, crushed and homogenized, and the amount of 0.3 grams was weighted into a 10-ml vial containing a stirring bar which was then sealed with a PTFE/silicone septum. Four ml of distillated water were added. Before extraction, the headspace in the vial was stabilised by equilibration at 40°C for 5 min with stirring at 800 rpm. The extraction was carried out at 40°C for 5 min. SPME fibre used was coated with divinylbenzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS), 1 cm length and 50/30 µm film thickness (Supelco, Bellefonte, PA). All the samples were analyzed in duplicates.

For the analysis of volatile compounds Varian 3350 gas chromatograph with a flame ionization detector (GC-FID; Varian Inc., Harbour City, CA, USA) was used. The column used was an Rtx-WAX 60 m × 0.25 mm i.d. × 0.25 µm df (Restek, Belafonte, PA, USA). Oven temperature was increased at 6°C/min from 40°C to 245°C and then kept for 10 min. Thermal desorption was achieved by inserting the SPME fibre into the injection port for 10 min (splitless mode). Injector and detector temperatures were 245°C and 248°C, respectively.

The carrier gas was helium at 17.5 psi at the column head, while detector gases were hydrogen and air. Blank runs were injected daily.

Volatile compounds were identified by comparing their retention times to those of the available standards, allyl mercaptane (≥90%), allyl sulfide (≥97%), allyl disulfide (80%), and by comparing the calculated linear retention indices relative to n-alkanes (C9 to C18) to those from the literature. Volatile compounds were additionally identified by comparison of their mass spectra to those from the NIST05 mass spectral library after GC-MS anaylsis using a Varian 3900 GC coupled to a Varian Saturn 2100T mass spectrometer (Varian Inc.), with the same column and analysis parameters as described above. The amounts were presented as peak area counts obtained in GC-FID analysis.

One-way analysis of variance (ANOVA) and principal component analysis (PCA) were carried out using Statistica 13.4 software (TIBCO Software Inc., 2018).

RESULTS AND DISCUSSION

Garlic cv. 'Istarski crveni' was harvested at three harvesting dates to assess the influence on the volatile compunds that may change its market and/or medical value. Seventeen volatile compounds were determined and quantified, namely: allyl mercaptan (AM), methyl tiirane (MTII), methyl allyl sulfide (MAS), dimethyl disulfide (DMDS), hexanal (HEX), diallyl sulfide (DAS), methyl allyl disulfide (MADS), 1-propenyl methyl disulfide (1-PMD), dimethyl trisulfide (DMTS), diallyl disulfide (DADS), metoxymethyl isothiocyanate (MMIC), methyl allyl trisulfide (MATS), 3-vinyl-1,2-dithiocyclohex-4-ene (3V4), diallyl trisulfide (DATS), 3-vinyl-1,2-dithiocyclohex-5-ene (3V5), (methylsulfinnyl) (methylthylthio) methane (MSMTTM), thieno[2,3-b]thiophene (TT).

Only the amounts of DATS, AM, DAS, MADS, DADS and TT were reported since they represented 99% of all the volatiles determined (Figure 1). DADS, followed by MADS, were the dominant compounds (Figure 1) at all the three harvest dates, which corresponded to the results obtained by Mondy et al. (2001).

The percentage in total peak area for DATS (0.08-0.09%), DAS (1.48-1.51%) and DADS (92-94%) was higher in cv. 'Istarski crveni', while AM (0.4-0.9%) and MADS (3.7-4.9%) was lower than those reported by Galoburda et al. (2013) for Latvian garlic detected after 5 months of storage (DATS (0.04%), DAS (0.51%), DADS (90%), AM (1%) and MADS (7,3%)).

The results of one-way ANOVA have shown significant differences between harvesting dates for AM and TT (Figure 1) which were further confirmed by Tukey's comparison of means. No significant differences were found for other volatiles having similar average peak area at all the three harvesting dates.

PCA was applied to better visualize the relations between the harvest dates (Figure 2a) and volatile compounds (Figure 2b). The first two principal components (PCs) summed up a contribution of 78%, among which the first principal component had a contribution of 44% of total variance. Certain relations were observed and harvest dates were relatively distinguished. The compounds with positive PC1 coordinate values could be related to the samples collected at the 1st harvest date, while those with negative PC1 values were linked to the samples collected at the 2nd harvest date. The samples from the 3rd harvest date were in between and gravitated towards the interception of the two axes.

Origin of the flavour precursors in the garlic clove has to be considered in the context of the development of the whole plant (Jones et al., 2007). According to Bloem et al. (2010) late harvest could be used to enhance alliin content for improving bioactive potency of garlic since alliin accumulated in leaves translocates to bulbs with an increasing trend during vegetation (Martins et al., 2016; Bloem et al., 2010). Similar conclusion was made by Horníčková et al. (2011) who found the highest amount of alliin concentration in bulbs just before harvest, while in the green parts the amount was decreasing during whole vegetation period. In our research, no such clear relationship was found maybe because the investigated garlic samples already reached the physiological stage after which the content of volatile compounds is stable.

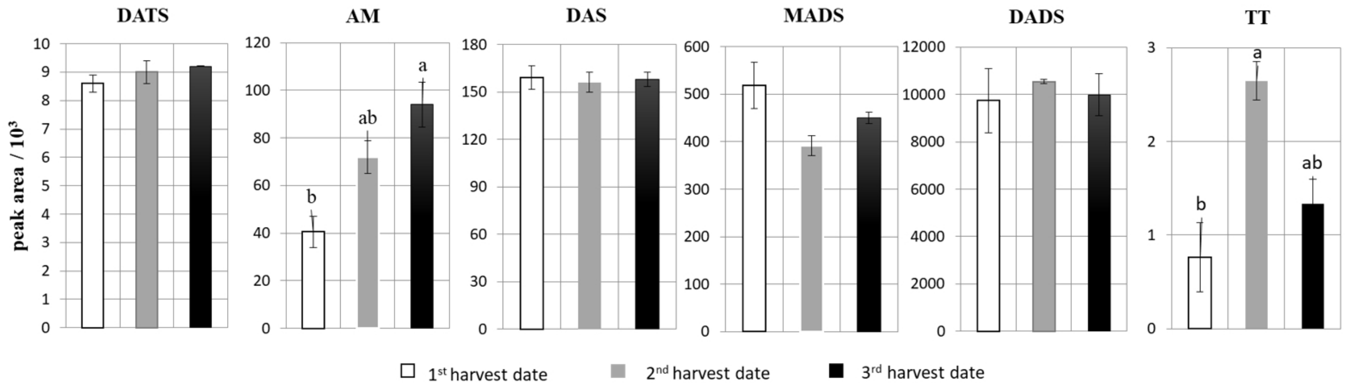

Figure 1. Amounts (peak area counts / 1000) of DATS, AM, DAS, MADS, DADS, TT at different harvesting dates of garlic cv. 'Istarski crveni' (Abbreviations: DATS - diallyl trisulfide, AM - allyl mercaptan, DAS - diallyl sulfide, MADS - methyl allyl disulfide, DADS - diallyl disulfide, TT - thieno[2,3-b]thiophene)

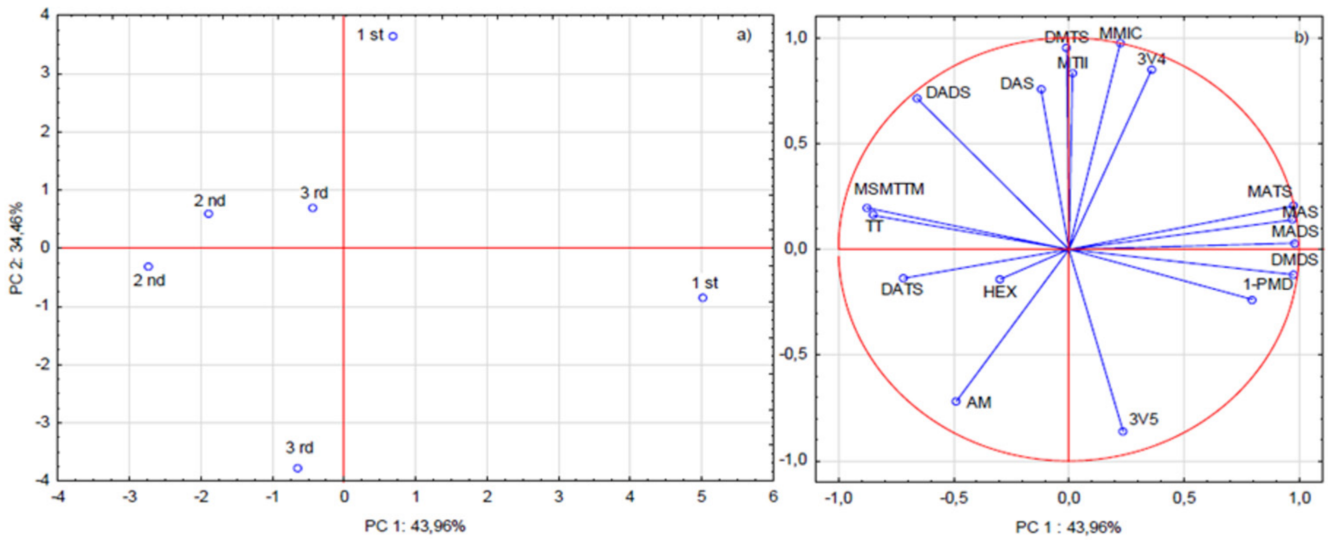

Figure 2. Principal component analysis (PCA) of garlic cv. 'Istarski crveni': (a) samples (cases) at 3 harvesting dates; (b) 17 volatile compounds (variables) (Abbreviations: 1^{st} – first harvest date, 2^{nd} - second harvest date, 3^{rd} - third harvest date, AM - allyl mercaptan, MTII - methyl tiirane, MAS - methyl allyl sulfide, DMDS - dimethyl disulfide, HEX - hexanal, DAS - diallyl sulfide, MADS - methyl allyl disulfide, 1-PMD - 1-propenyl methyl disulfide, DMTS - dimethyl trisulfide, DADS - diallyl disulfide, MMIC - metoxymethyl isothiocyanate, MATS - methyl allyl trisulfide, 3V4 - 3-vinyl-1,2-dithiocyclohex-4-ene, DATS - diallyl trisulfide, 3V5 - 3-vinyl-1,2-dithiocyclohex-5-ene, MSMTTM - (methylsulfinnyl) (methylthylthio) methane, TT - thieno[2,3-b]thiophene)

CONCLUSIONS

In this research 17 volatile compounds were identified and quantified in garlic cv. 'Istarski crveni'. Considering relatively small changes of their amounts during the investigated period, it was concluded that cv. 'Istarski crveni' could be harvested at any of three tested harvest dates. It was assumed that at the first harvest date the technological maturity of bulbs had already been reached and for this reason a further increase, which was expected according to the literature data, was not observed. However, other parameters should be analysed and taken into account, including total phenols, yield or color, to better assess the effect of harvest date on garlic quality. As well, volatile compounds should be investigated in the following seasons to evaluate the effect of harvest year.

ACKNOWLEDGEMENTS

This research has been supported by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia. The work of PhD student Iva Bažon has been supported by Croatian Science Foundation ESF DOK-2018-01/1.

REFERENCES

Bloem, E., Haneklaus, S., Schung, E. (2010) Influence of fertilizer practices on S-containing metabolites in garlic (*Allium sativum* L.) under field conditions. Journal of Agricultural and Food Chemistry, 58 (19), 10690-10696.
DOI: https://pubs.acs.org/doi/10.1021/jf102009j

De La Cruz Medina, J., García, H.S. (2007) Garlic post-harvest operations, Post-production operations. Food and Agriculture Organization of the United Nations. [Online] Available at: http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_Garlic.pdf [Accessed 16 November 2019]

Galoburda, R., Bodniece, K., Talou, T. (2013) *Allium sativum* flavour compounds as an indicator for garlic identity and quality determination. Journal of Food Science and Engineering, 3, 226-234.

Grégrová, A., Čížková, H., Bulantová, I., Rajchl, A., Voldřich, M. (2013) Characteristics of garlic of the Czech origin. Czech Journal of Food Sciences, 31 (6), 581–588.
DOI: https://doi.org/10.17221/539/2012-CJFS

Horníčková, J., Kubec, R., Velíšek, J., Cejpek, K., Ovesná, J., Stavělíková H. (2011) Changes of S-alk(en)ylcysteine sulfoxide levels during the growth of different garlic morphotypes. Czech Journal of Food Sciences, 29 (4), 373–381.
DOI: https://doi.org/10.17221/3/2011-CJFS

Jones, M.G., Collin, H.A., Tregova, A., Trueman, L., Brown, L., Cosstick, R., Hughes, J., Milne, J., Wilkinson, M.C., Tomsett, A.B., Thomas, B. (2007) The biochemical and physiological genesis of alliin in garlic. Medicinal and Aromatic Plant Science and Biotechnology, 1 (1), 21-24. [Online] Available at: http://www.globalsciencebooks.info/Online/GSBOnline/OnlineMAPSB_1_1.html [Accessed 8 November 2019]

Lešić, R., Borošić, J., Buturac, I., Herak Ćustić, M., Poljak, M., Romić, D. (2002) Povrćarstvo. Čakovec: Zrinski d.d.

Martins, N., Petropoulos, S., Ferreira, I.C. (2016) Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chemistry, 211, 41-50. DOI: https://doi.org/10.1016/j.foodchem.2016.05.029

Mondy, N., Naudin, A., Christides, J.P., Mandon, N., Auger, J. (2001) Comparison of GC-MS and HPLC for the analysis of *Allium* volatiles. Chromatographia Supplement, 53 (S1), 356-360.
DOI: https://doi.org/10.1007/BF02490356

Prekalj, B., Franić, M., Ban, D., Bažon, I., Cvitan, D., Goreta Ban, S. (2019) Morfološka svojstva lukovice ekotipova češnjaka u Istri. In: Mioč, B., Širić, I., eds. Proceedings from 54th Croatian & 14th International Symposium on Agriculture. Vodice, Croatia, 17-22 February 2019, University of Zagreb, Faculty of Agriculture, pp.291-295.

TIBCO Software Inc. (2018) TIBCO Statistica, Version 13.4.0. Palo Alto, California.