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COMPARITIVE STUDY ON VOLATILE AROMA COMPOUNDS OF TWO DIFFERENT GARLIC TYPES (KASTAMONU AND CHINESE) USING GAS CHROMATOGRAPHY MASS SPECTROMETRY (HS-GC/MS) TECHNIQUE

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Abstract

Backround: The medicinal use of garlic is much older than its usage as a food. The medical importance of garlic comes forward for its sulfur-containing components. In this study, it was aimed to compare Kastamonu garlic type with Chinese garlic type based on their aroma profiles.
Materials and Methods: Fresh Kastamonu garlic samples harvested from Kastamonu region of Turkey and Chinese garlic samples obtained from Turkish market were used as plant material. Volatile aroma compounds were determined using Headspace Gas Chromatography Mass Spectrometry (HS-GC/MS).
Results: Sixteen and twenty aroma components were identified in Kastamonu and Chinese garlic types, respectively. Kastamonu garlic type was found to be richer than Chinese garlic types in terms of sulfur-containing compounds. Diallyl disulphide, which is one of these components, was detected at level of 41.87% and 34.95% in the Kastamonu and Chinese garlic types, respectively. Also di-2-propenyl trisulfide was found only in Kastamonu garlic types. Disulfide, methyl 2-propenyl was determined at similar levels in both garlic types. Conclusion: The majority of garlic grown in Kastamonu region of Turkey is assessed by medical companies.
Conclusion: The results of the current study showed that Kastamonu garlic type has important medical properties. Therefore, this garlic can also be used in the medical field, as well as the consumption as food.

Key words: *Allium sativum*, dially disulfide, di-2-propenyl trisulfide, medicinal plant

Introduction

Garlic (*Allium sativum* L.), belongs to *Alliaceae* (syn. *Amryiladeceae*) family, as one of the important vegetables because of its medicinal properties and nutritional value. It consists 62% moisture, 6.30% protein, 0.10% fat, 4% total sugar, 29% carbohydrate, 0.80% fiber (Puranik et al., 2012), 15 mg/kg Ca, 1 mg/kg Fe, 200 mg/kg P, 6 mg/kg Na, 720 mg/kg K, 9.21 mg/100 g Vit B1, 0.11 mg/100 g Vit B2, 0.9 mg/100 g Niacin and 19 mg/100 g Ascorbic Acid (Artık and Poyrazoğlu, 1994). In addition, garlic has antiviral, antibacterial, anticoagulant, hipotansif, anti-parasitic, anti-micotic, anti-cancerojen, antioxidant, hipolipemic effects. Consumption of garlic as food has begun rather late due to its distinctive smell. Its production began in European countries ago [15-16th centuries]. Garlic production began in Germany in the 15th century and then it was introduced to England in the 16th century (Günay, 2005). However, its medicinal uses has already been known since ancient times. It was used for human health in China in 001/2 BC (Jones and Mann, 1963). According to existing records, soldiers were fed with garlic in Ancient Greece and Roman Empire. Furthermore, garlic cloves were found in ancient Egypt graves (Bayraktar, 1970).

The total garlic production of Turkey was 79 203 tones within 9 694 ha area (Anonymous, 2011), and 18-20%, of this production were provided from Taşköprü districts of Kastamonu province. Kastamonu garlic type is suitable for winter consumption, and thus can be stored for a long period of time. Hence it is suitable for processing because of its dry matter content (Türkeş, 1978; Taşkın et al., 2013). China ranks first among the world garlic-producer countries with 833 400 ha area and a total production yeild of 19 219 939 tones (Anonymous, 2011). Turkish garlic consumers have found two types of garlic in the Turkish market: Kastamonu garlic type and Chinese garlic type. Chinese garlic type is said to be far cheaper than the Kastamonu garlic types in the Turkish market.

Head Space-Gas Chromatography Mass Spectrometry (HS-GC/MS), was used by many researchers for determining volatile aroma compounds of different mushroom. GC/MS gives estimation values of volatile aroma compounds. In addition to, HS/SPME was organic solvent-free, fast, cheap, without risk of contamination. Twenty-one volatile aroma compounds were determined in Korean garlic using a 5% phenylpoly (dimethylsiloxane) column and by GC-MS (Lee et al., 2003). Diallyl disulfide, allyl sulfide and diallyl trisulfide were found to be the main aroma compounds in their studies.

Kastamonu garlic clone remains the most cultivated garlic type in Turkey. However, in the recent years, this clone replaces with Chinese garlic clone due to some diseases such as viruses seen in Kastamonu local garlic clone. Our local garlic clone has many positive features and therefore, should be protected. The main objective of this study was to compare aromatic compounds of two different garlic types (Kastamonu and Chinese garlic types), using Headspace Gas Chromatography Mass Spectrometry (HS-GC/MS), technique.

Materials and methods

Fresh garlic samples were used as material in this study. Kastamonu and Chinese garlic samples were provided from Taşköprü-Kastamonu region of Turkey (Figure 1), and from Turkish market, respectively. Kastamonu garlic clone is suitable for winter consumption, can be stored for a long time, and suitable for processing due to its dry matter content (Türkeş, 1978; Taşkın et al., 2013). In a study carried out by Artık and Poyrazoğlu (1994), properties of Kastamonu garlic type were determined as: average bulb weight: 30.85 g (minimum: 12.56 g, maximum: 50.18 g), average clove number: 13 (minimum: 6, maximum: 20), average big clove number: 5 (minimum: 1, maximum: 13), average small clove number: 8 (minimum: 2, maximum: 16), average big clove weight: 2.697 g (minimum: 0.64 g, maximum: 6.7 g), average small clove weight: 1.643 g (minimum: 0.361 g, maximum: 3.759 g), average dry matter: 39.07%, average pH: 6.30, average total sugar: 40.15%. 10 g of

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garlic homogenate was weighed and put on to the magnetic stirrer water bath (Ecorototherm from Dinkelberg analytics GmbH), at 40°C for 20 min. Triplicate analysis was applied. As for thermal effect, the equilibration of the polydimethylsiloxane (PDMS), 100 mm fibers were conditioned for 1 hr at 240°C in the GC injector port before being used, and they were cleaned between analyses to prevent contamination. A standard headspace vial (Supelco 75 mm height x 23 mm opening), was used during the extraction. An SPME device and a fiber coated with polydimethylsiloxane (PDMS), was used for the extraction. The SPME holder, for manual sampling, and fibers used in this study were purchased from Supelco (Bellefonte, PA).

After thermal equilibration, volatiles were extracted at 20 min on the magnetic stirrer. Volatile compounds were analyzed on an automatic HS-40 head space autosampler (Perkin Almer GC with split splitless inlet MSD system). Needle temperature was 120◦C, thermostic time and degree was 30 min and 35◦C during the extraction in the headspace autosampler. HP-5 MS (30 m×0.25 mm×0.25 µm), fused-silica capillary column was used. Helium (1 ml/min), was used as a carrier gas. The injector temperature was 250°C, set for splitless injection. The oven conditions were set to 50°C for 1 min and then the temperature was increased to 200°C at a rate of 4°C/min. Thermal desorption was allowed for 1.5 min. The detector temperature was 280°C. The components were identified by comparison of mass spectra and retention time data with those of authentic samples and complemented with identified by doing a NIST, Wiley, Flavor library search of the acquired mass spectral data.

Figure 1: Kastamonu garlic type harvested from Kastamonu region of Turkey

Results

In this study, sixteen and twenty aroma compounds were determined in the Kastamonu and Chinese garlic types, respectively (Table 1). Diallyl disulphide was detected to be the main aroma component in both types of garlic. However, the amount of this component (41.87%), in Kastamonu garlic type was higher than Chinese garlic type (34.95%). Dially disulfide (DADS), was one of the important organo-sulfur compound derived from garlic and produced by decomposition of allicin (Anonymous, 2013). It has an essential role in medicinal properties of garlic. Inhibition effects of diallyl disulfide against WEH-3 Leukemia cells *in vivo* were reported by Yang et al. (2006). Avato et al. (2000), found that the inhibitory effect of DADS increased with the increasing amount of DADS. Diallyl disulfide was followed by C6 H10 S2 and 3-Vinyl-1,2-dithiacyclohex-4-ene. These two compounds were found to be higher in Chinese garlic type than Kastamonu garlic type. While disulfide, methyl 2-propenyl was determined at similar levels in both the garlic types, di-2-propenyl trisulfide was detected only in Kastamonu garlic type. The other names of di-2-propenyl trisulfide are allyl trisulfide; diallyl trisulfide; diallyl trisulphide. Anticancer effects of diallyl trisulfide were identified by Seki et al. (2008). The effect of diallyl trisulfide on preventing dimethylhydrazine induced colon cancer was studied by Wargovich (1987).

Pentanoic acid and N,N'-dimethyl thiourea were found at levels of 5.11% and 4.26% in the Kastamonu garlic types. This component was not identified in Chinese garlic types. While benzene and allyl mercaptan were detected at levels of 4.93% and 3.13% in Chinese garlic type and these two compounds were not determined in Kastamonu garlic types. Trimethylsilyldiazomethane, 2,6-bis (1,1-dimethylethyl)-4-methyl phenol and 5, 7-dimethyl-2-methylthio-1-azacyclo [3.2.2] azine were only found in the Kastamonu garlic types. Dimethyl silanediol, 2,4-dimethyl thiophene, isophorone, isothiazole, 3,4-dimethyl, furan, methylthio, butylated hydroxytoluene 3,4-dimethylthiophene and butylated hydroxytoluene were only in the Chinese garlic type.

Methyl-d3 3-Methyl-3-butenyl Ether Hydrazinecarbodithioic acid; 2-Ethylidene [3.3.1] dithiane crotonic acid; Methane; Propanoic acid, 3-(2,3,4-trithia-6-heptenyl)-1-thia-cyclohex-5-ene; 2-Thiophenecarboxylic acid, 5-methyl; 1,3-Benzenedithiol; Dihydrothienothiophene; Thiazolidin-4-one were determined to be other compounds in Kastamonu garlic types. Benzonitrile; 1,4-Dithiacyclohept-2-ene; 1,3-Dithiolane-2-thione; 3H-1,2-Dithiole-3-thione; 2-Aminomethyl-1-methylbicyclo [2.2.1] hexan-2-ol; N-Methoxy-N-methylaminodifluoro phosphine; 3-(2,3,4-trithia-6-heptenyl)-1-thia cyclohex-5-ene; 2-Thiopenecarboxylic acid, 5-methyl were identified to be other compounds in Chinese garlic types.

Discussion

Taşköprü-Kastamonu garlic type is a local garlic clone of Turkey and quite famous for its strong aroma, large cloves, dry matter and

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storability for long time. Pests and diseases noticed within the garlic growing areas cause yield and quality loss. In Turkey, same areas are used both for garlic and seedling production. Therefore, pests and diseases can be carried via contaminated materials. Garlic is sexually sterile and therefore, is propagated vegetatively (Taşkın et al., 2013). In recent years, Chinese garlic clone was seen as an alternative to Kastamonu garlic clone both in Turkish markets and production areas. In this study, we tried to figure out volatile aroma compositions of these two garlic types and compared them with each other.

Table 1: Volatile composition of Kastamonu (KG) and Chinese (CG) garlic types

Compound name	KG RT	KG Area%	CG RT	CG Area%
Methyl 2-propenyl disulfide	5.862	3.89	6.229	3.35
Pentanoic acid	6.807	5.11	ND	ND
N,N'-dimethyl thiourea	6.773	4.26	ND	ND
Diallyl disulphide	10.277	41.87	10.172	34.95
3, 3' thiobis 1-Propene	10.459	2.11	17.146	1.69
C6 H10 S2	10.748	15.55	10.725	22.23
3-Vinyl-1,2-dithiacyclohex-4-ene	17.53	14.89	13.252	15.17
3-Vinyl-1,2-dithiacyclohex-5-ene	13.872	1.31	13.870	0.82
2-Vinyl-1,3-dithiane	14.063	0.25	14.055	0.60
4-(Methylthio) butyric acid	11.714	0.70	11.718	0.87
Di-2-propenyl trisulfide	16.399	0.99	ND	ND
Trimethylsilyldiazomethane	17.149	1.04	ND	ND
2,6-bis (1,1-dimethylethyl)-4-methyl Phenol	22.101	0.77	ND	ND
1,5-Dimethyl-3,7-dithiabicyclo [3.3.1] nonan-9-one Hydrazone	32.721	4.77	32.723	5.57
5, 7-dimethyl-2-methylthio-1-azacyclo [3.2.2] azine	32.483	0.46	ND	ND
1,3-Benzenedithiol	29.017	2.00	29.018	1.96
Benzene	ND	ND	6.762	4.93
Allyl mercaptan	ND	ND	2.435	3.13
Dimethyl Silanediol	ND	ND	3.034	0.69
2,4-dimethyl Thiophene	ND	ND	12.764	0.30
Isophorone	ND	ND	11.212	0.60
Isothiazole, 3,4-dimethyl	ND	ND	16.391	0.88
Furan, methylthio	ND	ND	17.051	0.72
Butylated Hydroxytoluene	ND	ND	21.009	0.32
3,4-Dimethylthiophene	ND	ND	25.730	0.87
Butylated Hydroxytoluene	ND	ND	21.009	0.32
Other compounds		0.03		0.03

RT: Retention time ND: Not detected

Garlic is consumed for both culinary and medical purposes. In a review study conducted by Lanzotti (2006), medicinal properties of garlic were indicated. Antioxidant (Lee et al., 2005), antibacterial (Pasteur, 1858), antimicrobial (Whitmore and Naidu, 2000), antitumor and cancer preventing effects of garlic (Block, 1994; Kamel and Saleh, 2000; Miron et al., 2003), were determined by different researchers. Additionally, its effects against certain important diseases such as epidemic diseases (typhus, cholera, diphtheria, and tuberculosis), cancer, hypercholesterolemia (Ernst et al., 2003), ulcer (Elsom et al., 2000; Canizares et al., 2004), were studied. *Heliobacter pylori* caused gastric cancer and ulcer is reduced with the consumption of Allium species (O'Gara et al., 2000).

In previous studies carried out by different researchers, 1-Propanethiol, Dipropyl disulfide, Methyl (E)-propenyl disulfide, Methyl 2-propenyl disulfide, Propyl (E)- propenyl disulfide, Di-2-propenyl disulfide, methyl propyl trisulfide, Dimethyl trisulfide, Dipropyl trisulfide, Di-2-propenyl trisulfide, Methyl 2-propenyl trisulfide, Allicin, Propyl methanethiosulfonate, Propyl propane thiosulfonate, 3,4-Dimethyl-2,5-dihydrothiophene, 3-Mercapto-2-methylpentan-1-ol, Thiopropanal-S-oxide, 2-Propen-1-ol, 1-Octen-3-ol, Pentanal, Hexanal and Decanal were detected to be key flavor compounds in *Allium* species (Berger, 2007). In a study conducted by Lee et al., (2003), different extraction techniques (steam distillation (SD), simultaneous distillation and solvent extraction (SDE), solid-phase trapping solvent extraction (SPTE), and headspace solid-phase micro-extraction (HS-SPME)), were compared with each other. Diallyl disulfide, allyl sulfide and diallyl trisulfide were identified at levels of 97.85%, 0.01%, and 0.01% by HS-SPME, respectively. Additionally Propylene sulfide, Allyl methyl sulfide, Dimethyl disulfide, 4-Heptenal, 1-(1-Propenylthio) propane, 1,3-Dithiane, Methyl propyl disulfide, 5-Methyl 1,2,3-thiadiazole, 2-Vinyl-1,3-dithiine, 1,2-Dithiolane, Methyl 2-propynyl sulfide, 1,3-Dithiolane, Propenyl 1-propynyl sulfide, Thiirane, 3-Vinyl-1,2-dithiocyclohex-5-ene, Diallyl tetrasulfide, 2-Methyl-3-pentanol were detected.

The results obtained from this study showed the differences besides similarities in terms of volatile aroma compositions in garlic clones of Kastamonu and Chinese. In terms of its medical properties, the aromatic composition of Kastamonu garlic type comes to the fore a bit more. In recent years, in the Turkey due to the virus problem, Chinese garlic takes the place of Kastamonu garlic. By taking into consideration all the positive characteristics of Kastamonu garlic type; production needed to be continued on the increase. Problems should be solved in production region and caution taken to increase its quality and efficiency. This presented study has contributed highlighting the importance of Kastamonu garlic type significantly.

Conclusion

In this study, aromatic compositions of Kastamonu and Chinese garlic types were compared with each other. Sixteen and twenty aroma compounds were identified in Kastamonu and Chinese garlic types, respectively. Ten aromatic compounds were found in both the garlic types. Six and ten components were identified only in Kastamonu and Chinese garlic types, respectively. Diallyl disulphide as one of the most important aroma compounds in garlic. This component was found to be higher in the Kastamonu garlic type than Chinese garlic type. Also di-2-propenyl trisulfide was not detected in the Chinese garlic types. Results of our experiment clearly showed that Kastamonu garlic type was found as being richer than Chinese garlic type in terms of sulfur compounds. Sulfur components play an essential role in medicinal properties of garlic.

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