Chemical composition of garlic fermented in red grape vinegar and kombucha

Ali Ebrahimi Pure, Seyyed Mofid Ghods Mofidi, Freshteh Keyghobadi, Monir Ebrahimi Pure

Abstract

Garlic (Allium sativum), was fermented by red grape vinegar and Kombucha vinegar, following which organosulfurs, non-sulfur compounds and fatty acids of each sample was analyzed using GC-MS. The chemical composition of fresh and fermented garlcs was significantly different with each other; Organosulfurs, which most biological properties of garlic are attributed to them, constitute 54.92% of chemical composition of fresh garlic, 24.1% of kombucha fermented garlic and 7.65% of vinegar fermented garlic. Low molecular compounds were found to be more in kombucha fermented garlic than two other samples. Linoleic acid and alpha linoleic acid have only been detected in kombucha fermented garlic. Both fermented garlcs have their own special characteristics but kombucha vinegar showed to be a more suitable fermenting medium than red grape vinegar, giving a fine fermented garlic product.

1. Introduction

Several reports have introduced garlic (Allium sativum) as a widely well-known food ingredient as well as a medicinal herb, with a long history of thousand years being used almost all over the world (Corzo-Martínez, Corzo, & Villamil, 2007; Ebrahimi Pure, Daraei Garmakhany, & Ebrahimi Pure, 2016; Lanzotti, 2006). Due to many beneficial health effects and uses for garlic, it was regarded as “holy plant” among ancient Egyptians (Ebrahimi Pure et al., 2016).

Garlic has been and still is being used in forms of fresh vegetable or processed. Different type of garlic products are traditionally or industrially produced and available in markets (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Corzo-Martínez et al., 2007). Some types of these products, like garlic powder, are widely common while some types like milk extracted garlic or garlic soaked in wine are less widely known but still common in some cultures (Amagase, 2006; Ebrahimi Pure & Ebrahimi Pure, 2016a). Processing methods of garlic products are generally differed into heating, drying, fermentation, aging, extraction or a combination of these methods. Most of these treatments are applied to eliminate the strong odor of raw garlic and increase its palatability; however, various changes occur in chemical composition affecting flavor, color and nutritious content of garlic (Bae, Cho, Won, Lee, & Park, 2014).

There are different types of fermented garlic products, also garlic is a common ingredient in many fermented foods like kimchi or fermented meat products (Farnworth, 2008); Even already-processed products like black garlic, which is a heat treated garlic, can be fermented by Saccharomyces cerevisiae to produce a two-step processed garlic product (Jung et al., 2011).

A very popular type of fermented garlic in Iran is called “seven years old garlic” which is garlic bulbs soaked under red grape wine or vinegar, kept in sealed jars for seven years before consumption (Ebrahimi Pure & Ebrahimi Pure, 2016a). During this long-term fermentation, the color of garlic changes to brown and the caustic taste and strong odor of raw garlic eliminate. The original “seven years old garlic” which was traditionally prepared, is a highly valued seasoning for its special taste and odor. Although the pickled garlic in vinegar, with most of the properties of “seven years old garlic”, takes few weeks to get prepared, but there are still slight changes happening as fermentation takes longer and longer. Nowadays, pickled garlic in red grape vinegar is widely produced in different cities of Iran, in household or industrial scales.
It is not only Iranians who use vinegar to make fermented garlic. “Laba Suan” is a Chinese type of fermented garlic which is made by soaking peeled garlic cloves in rice vinegar for few weeks. The final product is a green colored garlic with special taste and flavor, very popular between Chinese people (Bai et al., 2005).

There are not many reports on fermented garlic, but as far as author’s knowledge, all reports indicate the bioactive sulfur content of garlic being affected by fermentation. Fermentation of garlic with *Lactobacillus pentosus* have been reported to lower the organosulfur (OS) content of garlic (Beato, Sánchez, de Castro, & Montaño, 2012) while fermentation with *S. cerevisiae* increased the reducing power, thus antioxidant activity of garlic (Jung et al., 2011). Kim et al., also reported increases in OS content of garlic fermented by three different cultures, *S. cerevisiae, Mimulus pilosus*, and *Lactobacillus plantarum*. (Kim et al., 2016). Therefore, it is very expected that chemical composition of fermented garlics differ from that of raw garlic.

Actually, the fundamental study on the chemistry of garlic have been investigated by Professor Eric Block (Block, 1985; Block, 1992; Block, Naganathan, Putman, & Zhao, 1993; Block et al., 2001), where they introduced OS compounds as the main active components, providing variety of health benefits (Amagase, 2006; Amagase et al., 2001).

There are many fermented foods made by molds, yeast or bacteria cultures (Farnworth, 2008); while there are also some foods like Jiang (Ebrahimian Pure, 2016), soy sauce (Sulaiman, Gan, Yin, & Chan, 2014; Yong & Wood, 1976) or Kombucha (Teoh, Heard, & Cox, 2004) which are fermented by a mixed culture. Particularly about kombucha, it is typically sweetened black tea infusion, fermented with a symbiosis mixture of acetic bacteria (*Acetobacter xylinum, Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti* and *Acetobacter pasteurianus*) and yeasts (*Schizosaccharomyces pombe, Saccharomyces cerevisiae, Zygossaccharomyces bailii, Torulaspora delbrueckii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii* and *Candida stellata*). The final product is an acidified beverage/vinegar (pH about 2) were acetic acid is the dominant acid, rich in phenolic compounds, showing high antioxidant activity (Battikh, Bakhrouf, & Ammar, 2012; Ebrahimian Pure & Ebrahimian Pure, 2016b).

Following our previous studies on kombucha (Ebrahimian Pure & Ebrahimian Pure, 2016b) and fermented garlics (Ebrahimian Pure & Ebrahimian Pure, 2016a), the aim of the current work was to study and compare the chemical composition of garcils fermented in red grape vinegar, kombucha vinegar and fresh garlic. The use of kombucha vinegar as fermenting liquid for garlic is a novelty of the current work. We also couldn’t find report on chemical analysis of garlic fermented in red grape vinegar, which is considered in current work.

### 2. Material and method

#### 2.1. Preparation of kombucha vinegar

The method used by Battikh et al., with slight modification, which was also used in our previous study on kombucha, was followed. Dry black tea was bought from a local market in Gorgan city-Iran. 10 g of dry tea leaves with 20 g of sucrose were moved into a glass jar. 1 L of 5 min boiled distilled-water was also poured into the jar, steeped for 15 min. The mixture was left to cool down at room temperature and then was filtered to remove leaves. The resulting infusion was incubated with 10 g L\(^{-1}\) of actively growing kombucha culture from our previous work. 50 mL of previously fermented kombucha vinegar was added to medium to stimulate the fermentation process. A piece of cotton cloths was used to cover the jar lid. The jar was kept at room temperature for 1 month while the external body of container was covered with papers to prevent photo oxidation. The fermented liquid (kombucha vinegar) was then passed through sterile cotton clothes, ready to be used in further processing (Battikh et al., 2012; Ebrahimian Pure & Ebrahimian Pure, 2016b). Although pH of both final kombucha vinegar and red grape vinegar was 2, but acidity of red grape vinegar has been reported to be 10 times higher than kombucha vinegar (Ebrahimian Pure & Ebrahimian Pure, 2016a).

#### 2.2. Preparation of fermented garlic

Fresh white garlic (FG) was bought from daily vegetable market (bazar) of Gorgan city, Iran. 150 g of not peeled cloves were weighted and moved into 500 mL sterile glass jars. For preparation of kombucha fermented garlic (KFG), 300 mL of kombucha vinegar (pH = 2) was added to garlic cloves; and for preparation of vinegar fermented garlic (VFG) 300 mL of red grape vinegar (pH = 2) (5% acetic acid containing -Taksa industry, Mashhad-Iran) was added to garlic jar. The lids of the jars were closed. The jars were kept at room temperature for one month while outside body of containers were covered with papers.

#### 2.3. Extract preparation

To prepare extracts from garlics, 100 g of KFG and VFG clove were separated and rains washed with distilled water. The water drops were then dried using oil-free Whatman\(^{\circledR}\) papers. Cloves were grating using stainless steel grater, following by homogenization with hand-mixer utensil. Mixing was done very gently to prevent temperature raise up. The mixture was filtered using sterile cotton cloths and exuded liquid was then passed through 0.22 \(\mu\)m sterile syringes filter (Biofill – China). The resulting liquid was considered as total extract (juice) of garlics, poured in sterile glass jars. Same procedure was followed for fresh garlic clove extracts except rains washing (Ebrahimian Pure & Ebrahimian Pure, 2016a; Ebrahimian Pure et al., 2016). To prevent further chemical reactions, extracts were prepared just before injection to GC-MS. All jars and tools have been washed with ethanol and deionized water and then dried before use.

#### 2.4. GC-MS analyzing

GC-MS analysis was performed in chemistry laboratory of Food and Drug Organization (FDO) of Golestan province, Gorgan – Iran. An Agilent GC system (Agilent Technology, model 7890B, USA) with HP-5 ms column (30 m \(\times\) 0.25 mm i.d. \(\times\) 0.25 \(\mu\)m) followed by Mass detector (Agilent Technology, model 5977A MSD, USA) was used. Reading different reports on identification of chemical composition of garlic with GC (Fei, Tong, Wei, & De Yang, 2015; Gupta, Sharma, Maina, & Shulka, 2014; Mnayer et al., 2014; Nyaitondi, 2013; Shan, Wang, Liu, & Wu, 2013; Wang, Liu, Yang, & Zhang, 2015), the following method was found to be the suitable apparatus condition which is almost similar to reports, with slight modification. 0.1 \(\mu\)L of samples was injected while the initial temperature of oven was set to 50 °C. 1 min stay at 50 °C and then raised to 300 °C at the rate of 20 °C/min and stayed at 300 °C for 2 min. He2 was the carrier gas, flow rate of 0.6 mL/min. The mass range between 30 and 300 was scanned. Identification of...
compounds was done on the basis of NIST 11 library mass data base and comparing with other reports.

2.5. Statistical analysis

SPSS software ver. 20 (IBM Inc. USA) was used for analysis of variances followed by Duncan multiple range test at the significance level of 0.05. Microsoft Excel 2013 (Microsoft Inc. – USA) was used to draw chart.

3. Results and discussions

3.1. Chemical composition of garlics

Obtained reports from GC-MS analysis are summarized in Fig. 1 (TIC), Table 1 (organosulfurs) and Table 2 (non-sulfur compounds). TIC of samples clearly showed differences between KFG, VFG and FG chemical composition. It can be seen that major peaks are raised between RT 6 and 8 (Fig. 1). 20 min runs have also been experimented but there was no significant peak after 15 min.

3.1.1. Organosulfurs (OS)

OS compounds could be found in all samples, while there are significant differences between FG, KFG and VFG as term of quantity and type of OS (Table 1).

54.92% of compounds in FG were detected as 9 OS which means 86% more than VFG (7.65% OS, 5 compounds) and 56% more than KFG (24.1% OS, 7 compounds). Also KFG contained 68.2% more OS than VFG; so it can clearly be concluded that KFG has significantly higher amount of OS compounds than VFG has.

As mentioned before, most biological properties and characteristics of garlic are reported to be due to OS compounds (Lanzotti, 2006), therefore it can be expected that KFG provide higher biological properties than VFG. Antibacterial and antioxidant activity of KFG have been indicated being higher than VFG (Ebrahimi Pure & Ebrahimi Pure, 2016a) which supports this expectation.

2-Methyl-3-(methylthio)-1-propene is the OS which was only found in VFG but absent in FG and KFG. In a previous study, VFG has been observed to inhibit Pseudomonas aeruginosa more than KFG and FG did (Ebrahimi Pure & Ebrahimi Pure, 2016a), therefore, probably Methyl-3-(methylthio)-1-propene has anti- Pseudomonas activity. The formation pathway for this compound in fermented garlic is unknown. Regarding the chemical structures, the probable precursor for the formation of Methyl-3-(methylthio)-1-propene might be Thiophene, 2-chloro- which was only found in FG. Swarm of atomic radiuses around Carbon No. 2 propel the breakage of its bound and makes the compound less stable. As far as authors knowledge it is the first report of attribution of this properties to the mentioned compounds. More kinetically investigations are needed.
A notable point about investigated OS was regarding to diallyl trisulfide (DATS) that its content decreased by fermentation in red grape vinegar but significantly increased (%243) in KFG. The synthesis of DATS might be due to propylation reaction where two propene, a byproduct of fermentation, are introduced to dimethyl trisulfide, an OS compound of FG, thus the resulting will be Trisulfide, di-2-propenyl also known as diallyl trisulfide. DATS is a stable final transformation product from allicin being used as treatment for bacterial, fungal and parasitic infections such as giardiasis, *E. histolytica* and *Trichomonas vaginalis* (Lun, Burri, Menzinger, & Kaminsky, 1994). Strong antiviral activity of DADS and DATS have also been reported (Weber et al., 1992). They have been reported to be effective against *Helicobacter pylori* thus, reduces the risk of gastric neoplasia (You et al., 1998).

### Table 1

Organosulfurs detected in samples by GC-MS analysis.

<table>
<thead>
<tr>
<th>RT (int)</th>
<th>Component</th>
<th>FG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
<th>VFG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
<th>KFG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
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<td>4</td>
<td>Diallyl sulfide, methyl 2-propenyl</td>
<td>0.699</td>
<td>6.14</td>
<td>58</td>
<td>0.682</td>
<td>6.9</td>
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<td>0.130</td>
<td>1.02</td>
<td>94</td>
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<tr>
<td>5</td>
<td>Dimethyl trisulfide</td>
<td>0.218</td>
<td>1.58</td>
<td>96</td>
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<td>0.101</td>
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<td>1-Oxa-4,6-diazacyclooctane-5-thione</td>
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<td></td>
<td>0.267</td>
<td>1.95</td>
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<td>8</td>
<td>Diallyl sulfide</td>
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<td>0.023</td>
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<td>54.92</td>
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<td>7.65</td>
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<td>24.1</td>
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</table>

(int) = integer; (frc) = fraction; (As%) = percent of assurance.

### Table 2

Non-sulfur compounds detected in samples by GC-MS analysis.

<table>
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<tr>
<th>RT (int)</th>
<th>Component</th>
<th>FG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
<th>VFG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
<th>KFG (RT frc)</th>
<th>Area%</th>
<th>As%</th>
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<tr>
<td>3</td>
<td>Acetic acid</td>
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<td>86</td>
<td>0.283</td>
<td>16.17</td>
<td>91</td>
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<tr>
<td>4</td>
<td>2-Propanone, 1-hydroxy-acetoin</td>
<td>0.313</td>
<td>3.84</td>
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<td>0.447</td>
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<td>Propargyl alcohol</td>
<td>0.496</td>
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<td>6</td>
<td>Diazene, dimethyl-glyoxal</td>
<td>0.711</td>
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<td>0.620</td>
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<td>8</td>
<td>Propargyl alcohol</td>
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<td>Diazene, dimethyl-propanoic acid, 2-oxo-, methyl ester</td>
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(int) = integer; (frc) = fraction; (As%) = percent of assurance.
and anticancer properties of these sulfur compounds have also been investigated. They have shown to inhibit both early and late stages of carcinogenesis (Sigounas, Hooker, Li, Anagnostou, & Steiner, 1997). Considering several investigations indicating biological properties of DADS and DATS, also OS composition of FG, KFG and VFG, it can be concluded that KFG can potentially express those properties related to DATS more than FG and VFG can.

Another notable point about OS compounds of garlic was regarding Disulfide, methyl 2-propenyl (Fig. 2) and 1,3-Dithiane (Fig. 3). Regarding the reports on the chemistry of garlic OS compounds, most disulfide and trisulfide compounds are formed from allicin. Allicin is an unstable compound known as the primary OS in garlic but other OS compounds formed from allicin have S-S and S-S-S bounds that makes them more stable (Borlinghaus, Albrecht, Gruhlke, Nwachukwu, & Slusarenko, 2014; Corzo-Martínez et al., 2007). GC-MS analysis of VFG suggested the presence of 1,3-Dithiane by 38% assurance; this compound have also been reported in other investigations (Gîtin, Dinică, Neagu, & Dumitrascu, 2014; Shan et al., 2013); spectrums have showed peaks near 45,46 and 120 m/z which matches with standard spectrum of 1,3-Dithiane but there is also small peaks near 55 m/z. As main peaks in standard spectrum of 1,2-Dithiane appears in 45,55 and 120 m/z, also comparing the structure and sulfide bounds of 1,3-Dithiane with other OS in garlic, we are doubtful on detections and presence of 1,3-Dithiane. It is more expectable to see disulfides and trisulfides containing S-S and S-S-S bounds than an S-C-S bound-containing cyclic OS. Omar and Al-Wabel have described the formation of dithiins as decomposition of one allicin to 2-propenesulfenic acid and thioacrolein, where two 2-propenesulfenic acid regenerate an Allicin and two thioacrolein form dithiins (Omar & Al-Wabel, 2010). In our assumption there might be a more energy-optimized pathway which is suggested in Fig. 4 and produces a more acceptable form of C₄H₈S₂ which is Disulfide, methyl 2-propenyl.

### 3.1.2. Non-sulfur compounds

Table 2 presents GC-MS analysis of non-sulfur compounds found in FG, VFG, and KFG.

#### 3.1.2.1. Fatty acids

9,12-Octadecadienoic acid also known as linoleic acid (C₁₈H₃₂O₂; MW: 280.4455 g/mol; PubChem CID: 3931) and 9,12,15-Octadecatrienoic acid also known as alpha linoleic
acid (C18H30O2; MW: 278.436 g/mol; PubChem CID: 98073) fermentation of garlic in kombucha increased the content of this compound by 93.44% more than FG. Methanal (C2H5O; MW: 30.04 g/mol; PubChem CID: 98073) is another hydrocarbon which could only be detected in FG. It could also be found in Piper longum (long pepper).

3.1.2.2. Identical compounds found in all samples. The following 4 compounds have been found in all garlic samples; while the quantity of each on in VFG, KFG and FG was different.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone also known as disulfide, methyl 2-propenyl and diallyl trisulphide in VFG. (C6H11NO2; MW: 129.112 g/mol; PubChem CID: 12097) is a flavoring ingredient found in pepper; a secondary product from saccharides (HMDB, 2016d). VFG contains 57.1% more of it than FG do.

3.1.2.4. Found in both FG and KFG. 2,4-Dimethyl-2-oxazoline-4-methano also known as 5-methyl furfural (C6H6O2; MW: 110.112 g/mol; PubChem CID: 12097) is a flavoring ingredient found in pepper; a secondary product from saccharides (HMDB, 2016d). VFG contains 57.1% more of it than FG do.

3.1.2.5. Found in both VFG and KFG. The following 4 compounds have been detected in both fermented garlics but absent in FG. Acetic acid (C2H4O2 or CH3COOH; MW: 60.052 g/mol; PubChem CID: 176) the most dominant acid in both fermenting liquids (Ebrahimi Pure & Ebrahimi Pure, 2016b). VFG contains 36.3% more acetic acid than KFG.

Acetic acid (C2H4O2 or CH3COOH; MW: 60.052 g/mol; PubChem CID: 176) the most dominant acid in both fermenting liquids (Ebrahimi Pure & Ebrahimi Pure, 2016b). VFG contains 36.3% more acetic acid than KFG.

2-Propanone, 1-hydroxy- also known as hydroxycetone (C3H6O; MW: 74.079 g/mol; PubChem CID: 8299) is the simplest hydroxy ketone structure, a Maillard reaction product which can be produced by degradation of various sugar and can react further to form various aromatic compounds (Nursten, 2005). KFG contains 28.8% more hydroxycetone than VFG do.

2-Furanmethanol also known as furfural alcohol (C5H6O; MW: 98.101 g/mol; PubChem CID: 7361) is also a flavoring ingredient found in frans; being an endogenous compound made from gamma-aminobutyrate might be the reason of its higher content in KFG than VFG and FG. It is also used as a pharmacological agent and solvent (MeSH, 1991). It is a colorless oily liquid with a pleasant odor (NTP, 1992). Although the presence of this compound has been reported in many vinegars, red wines, white wines and other alcoholic beverages, but there is awareness noticed about consumption of approximately >2 g of it (Elliott & Burgess, 2005).

3.1.2.3. Found in both FG and VFG. 2-Furancarboxaldehyde, 5-methyl- also known as 5-methyl furfural (C6H6O2; MW: 110.112 g/mol; PubChem CID: 12097) is a flavoring ingredient found in pepper; a secondary product from saccharides (HMDB, 2016d). VFG contains 57.1% more of it than FG do.

3.1.2.7. Found in both VFG and KFG. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone also known as DMHF (C6H8O3; MW: 128.127 g/mol; PubChem CID: 19309) is an important aromatic compound in many fruits, inducing strawberry-like flavor in dilute and caramel-like flavor in concentrate. Its flavor-enhancing properties and a low flavor threshold, increased interests on using the compound as flavoring in foods. Zygosaccharomyces rouxii have been reported to produce DMHF from D-Fuctose-1,6-Diphosphate (Dahlen, Hauck, Wein, & Schwab, 2001) and glycosides have been suggested to be the probable precursors in biosynthesis of DMHF (Zabetakis, Gramshaw, & Robinson, 1999). The higher DMHF content of KFG in comparison with VFG and FG could be attributed to fermentation of glycosides in garlic (Corzo-Martinez et al., 2007) by Zygosaccharomyces genus in kombucha culture (Ebrahimi Pure & Ebrahimi Pure, 2016b).

Butyrolactone (C4H6O2; MW: 86.09 g/mol; PubChem CID: 7302) is a cyclic lactone from furans; being an endogenous compound made from gamma-aminobutyrate might be the reason of its higher content in KFG than VFG and FG. It is also used as a pharmacological agent and solvent (MeSH, 1991). It is a colorless oily liquid with a pleasant odor (NTP, 1992). Although the presence of this compound has been reported in many vinegars, red wines, white wines and other alcoholic beverages, but there is awareness noticed about consumption of approximately >2 g of it (Elliott & Burgess, 2005).
tomato, garden onion and many plants (HMDB, 2016c). VFG contains 82.6% more 5-Hydroxymethylfurfural than KFG does.

3.1.2.6. Found only in FG. **5-Methyl-2-pyrazinylmethanol** (C6H8N2O; MW: 94.117 g/mol; PubChem CID: 7976) is a flavoring agent found in many foods e.g. bakery products, dairy products, meats, baked potato or French fries, roasted barley, cocoa, coffee, tea, roasted filbert, roasted pecan, peanut, soy products and alcoholic beverages (Asikin et al., 2016).

3.1.2.7. Found only in KFG. **Methylpyrazine** (C5H6N2; MW: 96.908 g/mol; PubChem CID: 558521) regarding its low molecular weight and cyclic structure, it might be an odorant compound.

3.1.2.8. Found only in VFG. **Furfural** (C5H4O2 or C4H3OCHO; MW: 90.085 g/mol; PubChem CID: 7362) is a flavoring ingredient which can be found in many foods like coffee, pumpkin, malt, and various fruits, e.g. apple, apricot, sweet cherry, orange, grapefruit and more (HMDB, 2016a).

9H-Pyrido[3,4-b]indole, 1-methyl- also known as harmane (C12H10N2; MW: 182.226 g/mol; PubChem CID: 5281404) is an alkaloid found in chicory and the may pop (Passiflora incarnata), has been shown to exhibit anti-depressant function (Abramets & Dolzhenko, 1986).

**Pyrazine, 2,6-dimethyl-** (C6H8N2; MW: 108.144 g/mol; PubChem CID: 7938) is a flavoring additive and odorant in foods and can be found in raw asparagus, baked potato, French fries, wheat bread, crisp bread, black or green tea, roasted barley, roasted filberts or pecans, malt, wild rice (Zizania aquatica) and squid (PubChem, 2017a).

**Bis(2-ethylhexyl) phthalate** also known as DEHP (C24H38O4 or C6H4(COOCH17H17)2; MW: 390.564 g/mol; PubChem CID: 8343) is an ester of phthalic acid and is light-colored. It shows low toxicity from short-term and long-term exposures. Acute exposure to large oral doses of DEHP can cause gastrointestinal distress in humans (PubChem, 2017b). A very low amount have been detected in VFG.

**Acetoin** (C4H8O2; MW: 88.106 g/mol; PubChem CID: 179) is a product of fermentation inducing pleasant buttery odor (Gossauer, 2006), However, the detected amount of acetoin in VFG is very low.

**Propanoic acid, 2-oxo-, methyl ester** also known as methyl pyruvate (C4H6O3; MW: 102.089 g/mol; PubChem CID: 11748).

**2-Amino-oxazole** (C3H4N2O; MW: 84.078 g/mol; PubChem CID: 558521) regarding its low molecular weight and cyclic structure, it might be an odorant compound.

**Eicosane** (C20H42; MW: 282.556 g/mol; PubChem CID: 8222) is an acyclic alkane.

3.2. Molecular weight of compounds

Mean and standard deviation of MW of 18 compounds in FG was 137.7 ± 35.1 (min 86, max 254.4); for 25 compound of VFG 148.3 ± 82.3 (min 60, max 390.5), and for 25 compounds of KFG it was 147.2 ± 69.4 (min 60, max 284.4). Mean of MW of FG compounds was significantly smaller than those of VFG and KFG, but statistically there was no significant differences between mean of MW of VFG and KFG compounds ($P \leq 0.01$).

According to Fig. 5, showing the frequency histogram of compounds by their molecular weight, it could be seen that although there was no significant difference between mean of MW in KFG and VFG, but both low weight and high weight compounds were more abundant in KFG than in FG or VFG. Compounds with lower MW are more volatile, so KFG could be expected to have more odorant compounds than two other samples.

4. Final conclusion

The chemical composition of fresh and fermented garlics were significantly different with each other, so it can be expected that they also provide different biological properties.

While acetic acid was the dominant acid in both fermenting liquids, the differences in chemical composition of final product is assumed to be due to (1) Acidity of liquids: pH of both liquids were almost similar but the acidity of red grape vinegar was about 10 times higher than kombucha vinegar, this fact could have significant effects on chemical pathways. (2) Biochemical and enzymatic activities: kombucha is an actively growing medium which means there are a wide range of biochemical and enzymatic reactions continuously taking place, so it was well expected these reactions affect the chemical changes in substrate.
Both fermented garlies had their own special characteristics but kombucha vinegar showed to be a more suitable fermenting medium than red grape vinegar, giving a fine fermented garlic product.

Declaration of interest

All authors declare that they have no conflict of interest on this report.

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