Analysis of Volatile Compounds in Noni Fruit (*Morinda citrifolia* L.) Juice by Steam Distillation-Extraction and Solid Phase Microextraction Coupled with GC/AED and GC/MS

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ABSTRACT

Morinda citrifolia (Rubiaceae), also known as noni, is a plant found in the Hawaiian and Tahitian islands. It is considered as one of the most important plants brought to Hawaii by the first Polynesians. The yellow fruits have a distinctive "grenade-like" shape and can grow up to a size of 12 cm. It has a foul taste and a soapy smell when ripe. It was proposed that the fruits of noni might suppress the growth of tumors by stimulating the immune system. In recent years, noni juice has been sold in the US market as a nutraceutical supplement. Here we addressed the volatile compounds in the fresh and ripe noni fruits. Carboxylic acids, aldehydes, ketones, alcohols, esters, terpenes and six organosulfur compounds were isolated by SDE and SPME, and further identified by GC/AED and GC/MS. The sulfur compounds identified may make a major contribution to the characteristic flavor of noni fruit juice. SPME is useful to recover low boiling point and low molecular weigh compounds; in contrast, SDE owns higher extraction capacity and higher recovery for polar compounds. SPME and SDE can provide complementary information; when use together, a deep understanding of aroma profile of food systems can be expected.

Key words: gas chromatography-atomic emission detector (GC/AED), gas chromatography-mass spectrometry (GC/MS), *Morinda citrifolia* (Rubiaceae), organosulfur compounds, solid phase microextraction (SPME)

INTRODUCTION

Morinda citrifolia (Rubiaceae), commonly known as noni, is a plant typically found in the Hawaiian and Tahitian islands. It is believed to be one of the most important plants brought to Hawaii by the first Polynesians[1]. The plant is a small evergreen tree, growing in open coastal regions and forest areas, up to about 1300 feet above sea level. This plant is identifiable by its straight trunk, large green leaves and distinctive "grenade-like" yellow fruit. The fruit can grow to a size of 12 cm due to coalescence of the inferior ovaries of many closely packed flowers. It has a foul taste and a soapy smell when ripe. Noni plant has been used for food, medicinal, and cosmetics purposes for centuries. Native Polynesians use almost every part of this plant, including bark, stem, root, leaf, and fruit, as a folk remedy for many diseases including diabetes, hypertension, and cancer[2,3]. Noni's biological activities have been demonstrated by many studies, such as cardiovascular actions[4], antipanic and sedative activities[5], anti-inflammatory[6], and anticancer activities[7].

Due to its beneficial effects, the sales of noni products increased tremendously through the last two decades in Asia, North American and Europe. Noni juice was registered as novel food in 2003 in the European Union[8]. More then 50 patents have been granted in the United States[9]. Noni fruit juice is the most widely distributed noni product and sold as a nutraceutical and dietary supplement in many countries. Even though three hepatotoxicity cases were reported to be associated with the usage of noni juice[10,11], the consumption of noni juice is considered safe[12].

About 200 phytochemicals have been isolated and identified from noni, including alkaloids, amino

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acids, fatty acids, phenolic compounds and polysaccharides, etc[13]; however, there is only one published paper concerning volatile compounds of noni fruit[14]. A total of 51 detectable volatiles from ripe noni fruit were reported. Twenty acids representing 83% of the total volatiles were identified. Among them, octanoic (58%) and hexanoic acids (19%) dominated the volatile profile.

Several techniques, such as purge and trap technique, steam distillation-extraction (SDE), solvent extraction, and solid-phase microextraction (SPME), have been used for the isolation of volatile compounds in food systems. Among them, SDE and SPME are the most widely used by flavour scientists. SDE combines steam distillation and continuous extraction. It was first developed by Nickerson and Likens for isolating volatiles from beer[15]. In this method, the organic compounds from distillation are almost simultaneously extracted from the water steam by organic solvent vapor. Because only small amount of solvent is used, the volatile compounds can also be concentrated in ten thousand folds[16]. SPME introduced by Belardi and Pawliszyn in 1989 was a solvent-free sampling method[17]. Although it was initially designed as a sampling method for isolating the environmental pollutants from water, this analytical technique was quickly adopted by food scientists for the analysis of volatile components[18,19].

Atomic emission detector (AED), with the advantages of high sensitivity and elemental specificity, is a powerful tool for gas chromatography. Mass spectrometry (MS) is the only instrument which can provide detail chemical structure information of molecules. In our recent studies, we have demonstrated that, when used together, AED and MS are able to identify compounds containing specific elements in a complex matrix[20].

The objective of this study is thus to identify and compare the constituents of noni fruit volatiles collected by SDE and SPME followed by GC/MS and GC/AED.

MATERIALS AND METHODS

I. *Simultaneous Steam Distillation-Extraction (SDE)*

All noni fruit was from Hawaii, and picked in mature, frozen and shipped to New Jersey. Three hundred and forty g blended noni fruit, 300 mL water, and 150 g NaCl were added to a 1 L round-bottomed flask. Forty mL of hexane in a 100 mL round-bottomed flask was placed in a 70°C water-bath. The sample was extracted for 2 h. After extraction, the collected hexane was dried over anhydrous sodium sulfate and then concentrated to 1 mL by means of a Kuderna-Danish concentrator. This sample solution was ready for GC/AED or GC/MS analysis.

II. *Solid-Phase Microextraction (SPME)*

Fifty grams of blended noni fruit was placed in a 200 mL glass vial and mixed with 50 mL water and 50 g NaCl. The vial was held in a water bath at 50°C. The SPME holder, SPME fibers (75 µm Carboxen/PDMS) and a 0.75 mm i.d. inlet liner were purchased from Supelco, Inc. (Bellefonte, PA, USA). Before sampling, the SPME fiber was conditioned in the gas chromatograph injection port at 275°C for 15 min. The sampling time was 30 min in head space mode. After sampling, SPME fiber was immediately inserted into GC injection port for desorption.

III. *GC/MS Analysis*

GC/MS analysis was performed on an Agilent (Agilent Technologies, Wilmington, DE, USA) 5973 mass spectrometer coupled to a 6890 gas chromatographer. A fused silica capillary column (Supelcowax-10, 30 m × 0.25 mm i.d. × 0.25 µm film thickness, Supelco, Bellefonte, PA, USA) was employed for both SDE and SPME analysis. The injector temperature was 275°C. The GC oven temperature was programmed as follows: 40°C for 10 min, increased to 240°C at a rate of 4°C/min, and held at this final temperature for 10 min. The ion source temperature was 230°C, and the analyzer temperature was 150°C. A 0.75 mm i.d. inlet liner in the GC injection port was used for SPME, instead of a larger volume 2 mm i.d. liner. The injection port was operated in splitless mode. Mass spectra were obtained by EI at 70 eV or NH_3-CI and a mass scan from 40 - 450 amu.

IV. *GC/AED Analysis*

An Agilent 6890 GC coupled with a G2350A (Agilent Technologies, Wilmington, DE, USA) atomic emission detector was employed for GC/AED analysis. Oxygen and hydrogen were used as reagent gas with detection at 179, 196, and 174 nm for carbon, selenium, and nitrogen, respectively. Helium carrier gas was used for all analyses. Both the cavity and transfer line temperatures were 250°C. The hydrogen, oxygen and auxiliary gas pressures were 8.8 psi, 24 psi, and 30.6 psi, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the SPME-GC/AED profiles of noni volatiles and Figure 2 shows the GC/AED profiles of volatiles isolated from noni by SDE. In both figures, the top panel is the profile obtained from the channel C and the bottom panel is from the channel S. AES used here is to identify atoms by measuring the radiation emitted by the electrons of the atom after they are excited to a higher energy level. With the combination of GC-AES, it is possible to identify elements in compounds leaving the column. In the interface, the eluent is atomized and excited by microwave-energized helium plasma that is coupled to a diode-array optical emission spectrometer.

Table 1 lists the volatile compounds identified in noni fruit juice. The most abundant compounds are octanoic
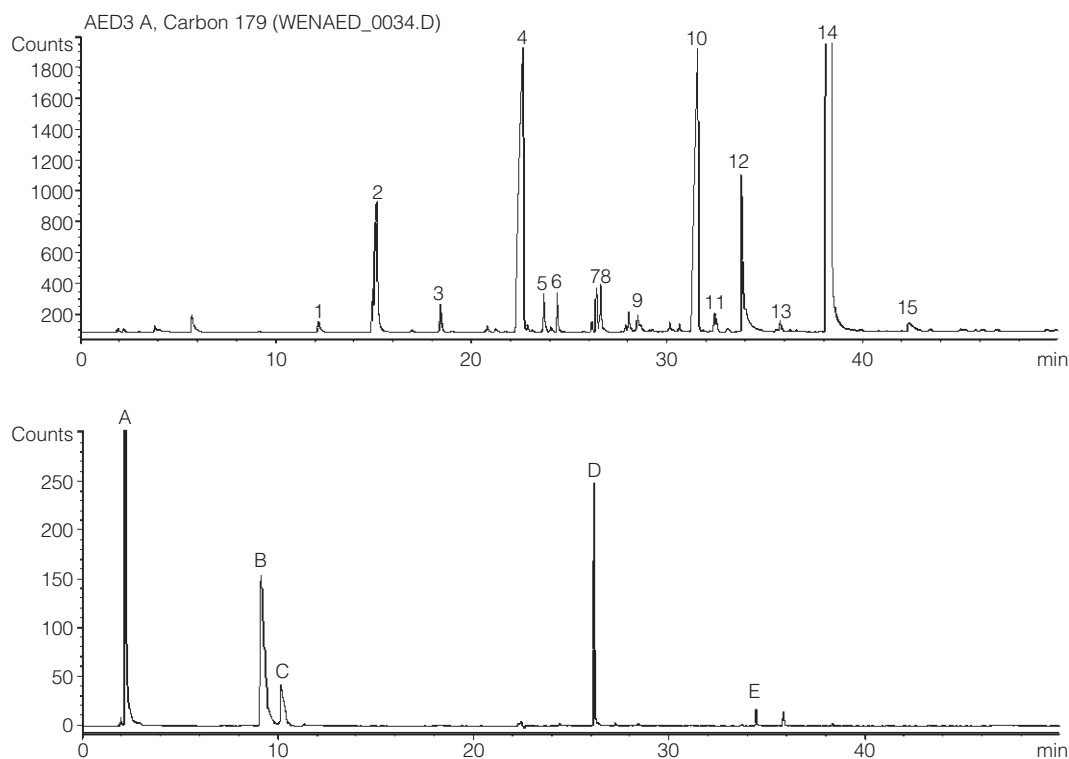
Figure 1. SPME-GC/AED profile of noni: Top C-channel, bottom S-channel. 1. methyl butanoate; 2. methyl hexanoate; 3. ethyl hexanoate; 4.methyl octanoate; 5. ethyl octanoate; 6. 3-methyl-3-butenyl 3-methyl 3-butenoate; 7. methyl decanoate; 8. ethyl decanoate; 9. 2-methylbutanoic acid; 10. 3-methyl-3-butenyl octanoate ; 11. 5-methyl-5-hexenoic acid; 12. hexanoic acid; 13. heptanoic acid; 14. octanoic acid; 15. decanoic acid; A. methanethiol; B. S-methyl thioacetate; C. dimethyl disulfide; D. methyl 3-methylthiopropanoate; E. 3-methylthiopropanoic.
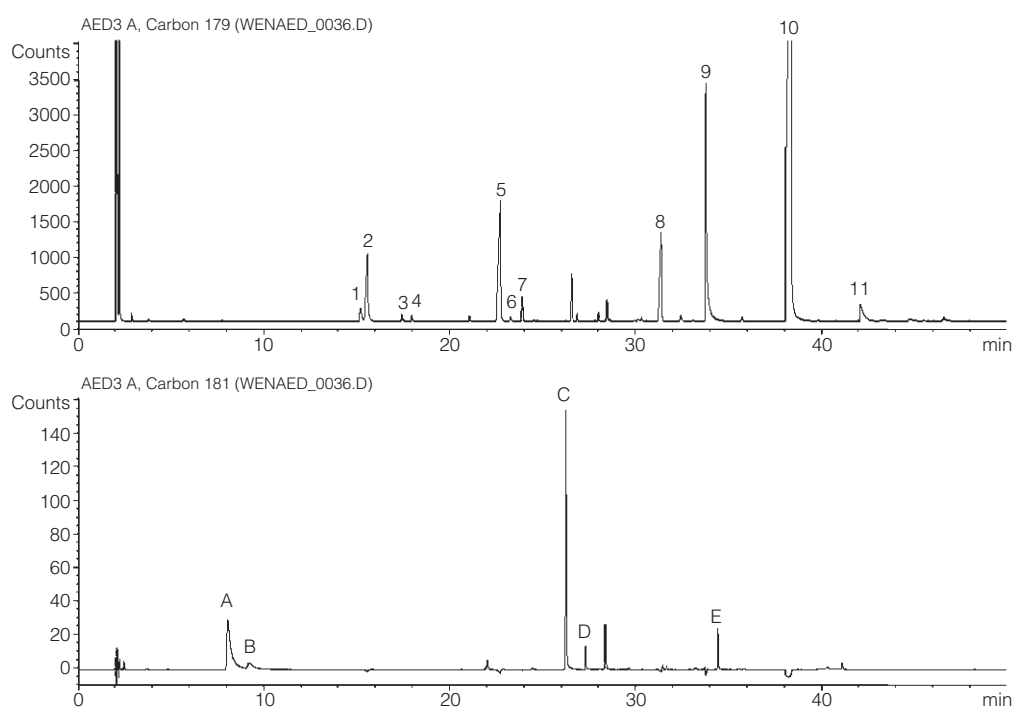

Figure 2. GC/AED profile of volatiles from noni by steam distillation: Top C-channel, bottom S-channel. 1. 2-heptanone; 2. methyl hexanoate; 3. ethyl hexanoate; 4. methyl-3-buten-1-ol; 5. methyl octanoate; 6. butyl hexanoate; 7. ethyl octanoate; 8. 3-methyl-3-butenyl octanoate; 9. hexanoic acid; 10. octanoic acid; 11. decanoic acid; A. S-methyl thioacetate; B. dimethyl disulfide; C. methyl 3-methylthiopropanoate; D. ethyl 3-methylthiopropanoate; E. 3-methylthiopropanoic.

Table 1. Volatile compounds identified from ripe fruit of *Morinda citrifolia*

Compounds	Steam distillation	SPME	Compounds	Steam distillation	SPME
Acids			3-Methyl-3-buten-1-yl 3-methylbutanoate	+	+
Formic acid	trace	+	Methyl 2-methylbutanoate	+	+
Acetic acid	trace	+	Methyl hexanoate	++	+++
Butanoic acid	+	+	Ethyl hexanoate	++	++
Hexanoic acid	+++	++++	Butyl hexanoate	+	+
Heptanoic acid	+	+	4-Pentenyl hexanoate	+	+
Octanoic acid	++++	++++	3-Methyl-3-buten-1-yl hexanoate	+	+
2-Octenoic acid	+	+	Hexyl isovalerate	+	+
Nonanoic acid	+	+	Methyl heptanoate	+	+
Decanoic acid	+	+	Methyl ocatanoate	++	++++
Aldehydes and Ketones			Ethyl octanoate	+	+
Acetaldehyde	n.d.	+	Butyl octanoate	+	+
2-Methylbutanal	+	+	3-Methyl-3-buten-1-yl octanoate	+	+
3-Methylbutanal	+	+	Methyl 2-octenoate	+	+
2-Pentanone	+	+	Methyl 3-octenoate	+	+
3-Methyl-2-butanone	+	+	Methyl 6-octenoate	+	+
2-Hexanone	+	+	Methyl nonanate	+	+
Hexanal	+	+	Methyl 5-nonenoate	+	+
2-Heptanone	+	+	Methyl decanoate	++	++
2-Hexenal	+	+	Ethyl decanoate	+	+
Furfural	+	+	Methyl 4-decenoate	+	+
Benzaldehyde	+	+	Ethyl 4-decenoate	+	+
Alcohols			Methyl salicylate	+	+
Ethanol	+	+	Methyl hexadecanoate	+	+
2-Methyl-3-buten-1-ol	+	+	**Terpenes**		
1-Butanol	+	+	Linalool oxide	+	trace
3-Methyl-3-buten-1-ol	+++	++	(*Z*)-3,7-Dimethyl-1,3,6-octatriene	+	trace
3-Methyl-2-buten-1-ol	+	+	(+)-4-Carene	+	trace
Benzyl alcohol	n.d.	trace	D-Limonene	+	trace
Esters			Ocimenol	+	trace
Ethyl acetate	+	+	Terpineol	+	trace
Butyl acetate	+	n.d.	**Sulfur Compounds**		
Methyl 2-methylpropanoate	+	+	Methanethiol	n.d.	+
Methyl butanoate	+	+	*S*-Methyl thioacetate	+	+
Ethyl butanoate	+	+	Dimethyl disulfide	+	+
Butyl butanoate	+	+	Methyl 3-methylthiopropanoate	+	+
4-Pentenyl butanoate	+	+	**Ethyl 3-methylthiopropanoate**	+	n.d.
Methyl 3-methylbutanoate	+	+	3-methylthiopropanoic acid	+	trace

n.d. = not detectable

acid and hexanoic acid, as well as their corresponding methyl and ethyl esters. Several glycosides were isolated and identified from Hawaii by Wang *et al*.[21] It is interesting to note that several glycosides identified contain hydrolyzable fatty acids, such as octanoic acid and hexanoic acid and alcohol, such as 3-methyl-3-buten-1-ol. These glycosides isolated from noni include a trisaccharide fatty acid ester, 2,6-di-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranoside and two disaccharide fatty acid esters, 6-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranoside and 6-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranoside. It is obvious that the saccharide esters of fatty acids are the precursors of these acids and esters in noni fruit. They are also the major contributors to the soapy aroma of the ripe noni fruit. In addition to hexanoic acid and octanoic acid, a series of medium chain fatty acids such as heptanoic, nonanoic and decanoic acid were also characterized from ripe noni fruit by both the SDE and SPME methods. In addition, glycoside of 3-methyl-3-buten-1-ol has also been identified in the nonvolatile extract of noni fruits[22]. In the present study, we observed a relatively large quantity of 3-methyl-3-buten-1-ol in the volatile components of noni fruit juice.

Thirty two esters were identified in noni juice. It is not surprising that the esters of hexanoic acid and octanoic acid are the major esters identified. Two new esters were tentatively characterized. The mass spectral data of both compounds are very similar. They have a base peak at *m/z* 68, followed by *m/z* 57, 127 and 41 fragments. CI/MS data show that these compounds have molecular weight of 182 and 212, respectively. Figure 3 shows the proposed fragmentation pathway for the generation of *m/z* 41, 57, 68 and 127 ions. They are also derived from 3-methyl-3-buten-1-ol which exists as one of the major glycosides in noni fruit. The molecular weight of these two compounds have also been reported by Farine[14] as two unknown compounds representing 0.57 and 1.65% of the total volatiles in ripe noni fruit. These two compounds were tentatively identified as 3-methyl-3-buten-1-yl esters of hexanoic acid and octanoic acid.

Taking advantage of the superior sensitivity of AED detector used, we were able to identify six sulfur-containing compounds. The major mass fragments are listed in Table 2. There are methanethiol (I), S-methyl thioacetate (II), dimethyl disulfide (III), methyl 3-methylthiopropanoate (IV), ethyl 3-methylthiopropanoate (V), and 3-methylthiopropanoic acid (VI) as shown in Figure 4. Among them, 3-methylthiopropanoic and methyl 3-methylthiopropanoate had been reported by Farine in noni fruit[14]. As shown in Figure 5, methionine may undergo a transamination to form a keto acid. Subsequent decarboxylatiom leads to the formation of a CoA ester. Incorporation of water, methanol or ethanol to the CoA ester may form 3-methylthiopropanoic, methyl 3-methylthiopropanoate and ethyl 3-methylthiopropanoate, respectively.

The sulfur-containing compounds profiles generated by SPME and SDE have significant difference. SPME method showed that methanethiol is the most abundant sulfur-containing volatile compound in noni fruit juice, but not detectable in SDE; it possibly exists in trace

Table 2. The mass list of sulfur-containing compounds identified

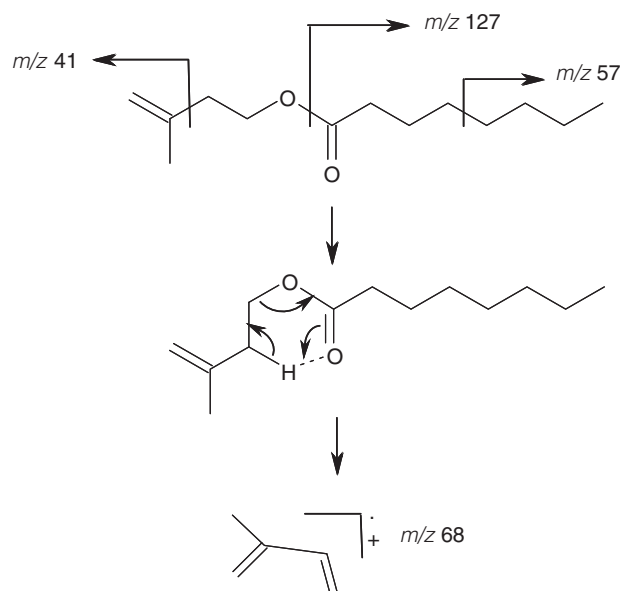| Sulfur Compounds	m/z (%)
Methanethiol	45(46), 46(11), 47(100), 48(92)
S-Methyl thioacetate	43(100), 45(8), 47(7), 90(20),
Dimethyl disulfide	45(50), 46(24), 47(20), 61(15), 64(12), 79(60), 94(100), 96(10)
Methyl 3-methylthiopropanoate	41(36), 45(40), 47(28), 59(26), 61(70), 74(90), 75(30), 103(34), 134(100),160(30)
Ethyl 3-methylthiopropanoate	45(20), 47(18), 61(88), 74(100), 75(42), 103(28), 148(70)
3-methylthiopropanoic	45(14), 61(100), 74(12), 120(30), 147(10)

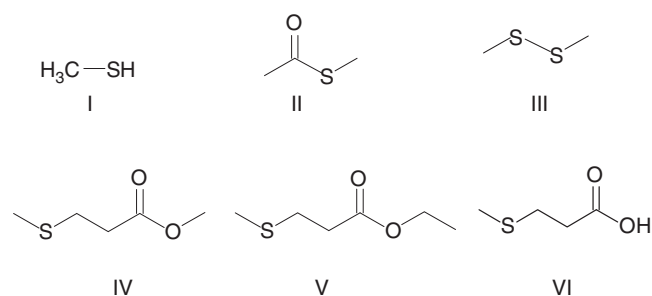**Figure 3.** Mass spectral fragmentation pathway for 3-methyl-3-buten-1-yl octanoate.

Figure 4. The structures of organosulfur compounds identified.
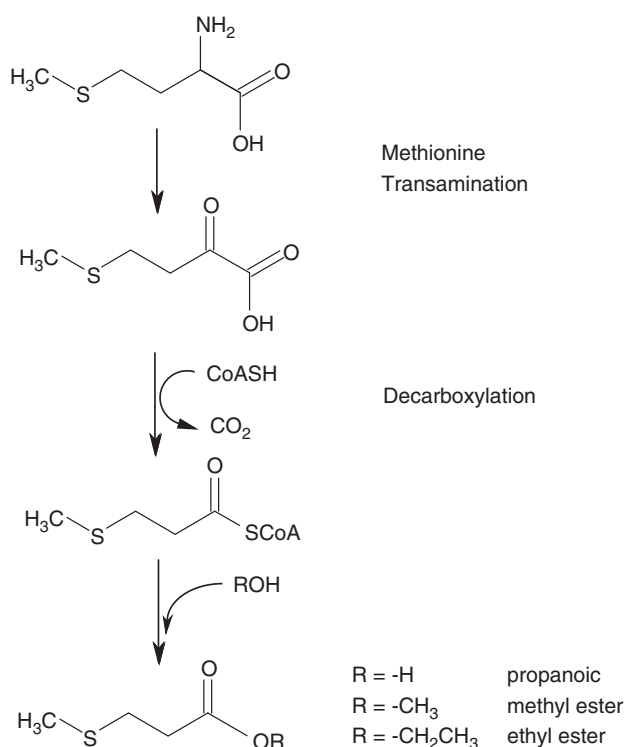
Figure 5. Proposed biosynthetic pathway for methyl and ethyl esters of 3-methylthiopropanoate.

amount and co-elutes with hexane. Ethyl 3-methylthiopropanoate was detected in SDE but SPME. The presence of ethyl 3-methylthiopropanoate could be artifact generated by high temperature during extraction, because it was not detected in SPME[23,24]. Compared to SPME, SDE is an extraction method with higher extraction capacity especially for polar compounds. Even though, almost all volatiles in food can be extracted by SDE, with generally lower recoveries of compounds of low molecular weight and low boiling point[25].

SPME is a simple extraction technique for isolating volatile compounds in food systems. It is not only a combination of extraction and concentration technique but also a sample introduction technique for GC and HPLC. During sampling, volatiles diffuse out of food matrix to fiber and are adsorbed. When the partition equilibrium is reached, the concentration of volatile adsorbed by fiber, C_f, is determined by K_{fh}, the equilibrium constant for volatile between fiber and headspace, and C_h, the concentration of volatile in headspace. The relationship cab be expressed as $C_f = K_{fh} \times C_h$. The equilibrium constant K_{fh}, is fiber materials and volatile components dependant[26]. This can explain how the bias is generated in SPME. SPME is considered an artifact-free technique, because usually no solvents and less heating are involved. In contrast, modifications of volatile compounds by heat or solvent effect are observed in SDE[27].

SPME method is sensitive for sulfur-containing compounds in noni fruit juice. However, Madruga *et al.* used SPME to study the volatile flavour in goat meat. They observed that SPME was not an appropriate method for extracting sulfur-containing compounds in cooked goat meat[19]. Because the same SPME fibers were used, different food matrix may make significant contribution to the results.

CONCLUSIONS

Taking advantage of the high sensitivity of the atomic emission detector, we were able to identify several minute sulfur-containing compounds, which were previously not found in noni fruit. The sulfur compounds identified may have important contribution to the flavor characteristics of noni fruit juice.

SPME is a convenient, efficient and artifact-free technique. In addition, it is suitable to recover compounds of low boiling point and low molecular weight. SDE is noted for its high extraction capacity and high recovery of polar compounds. It is obvious that both SPME and SDE methods introduce significant bias. The true aroma profiling of foods is always a challenge to flavour scientists. No single extraction method can really display the true aroma profile of food systems. However, SPME and SDE can provide complementary information; when use together, a better understanding of aroma profile of food systems could be expected.

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