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SHORT COMMUNICATION

Volatile organic compounds released by enzymatic reactions in raw nonpareil almond kernel

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Abstract Benzaldehyde is well recognized as the predominant aroma in bitter almond (*Prunus dulcis* var amara) and is released from amygdalin upon enzymatic hydrolysis followed by a loss of hydrogen cyanide. Sweet almond (*Prunus dulcis* Mill. D.A. Webb) has a sweeter, nuttier aroma than the bitter variety. While benzaldehyde is detected in raw sweet almond, it is not the predominant compound contributing to aroma due to the lower level of amygdalin. Although a variety of volatile organic compounds (VOCs) in sweet almond have been identified, the identity of VOCs due to enzymatic reactions in sweet almond has not been well documented. In this study, we investigated the VOCs released by enzymatic reactions in raw nonpareil (sweet) almond kernel samples and identified several alcohols such as isobutanol, 2-pentanol, 3-methylbutanol, 3-methyl-3-buten-1-ol, and 3-methyl-2-buten-1-ol as the major enzyme-released VOCs. Their released amounts were greater in the sweet almond kernels than in the bitter ones analyzed, suggesting that these alcohols may contribute to the characteristic aroma in the raw sweet almond.

Keywords Nonpareil almond · Volatile organic compounds (VOCs) · Enzymatic reactions · Solid-phase microextraction (SPME) · Gas chromatography/mass spectrometry (GC/MS)

Introduction

Benzaldehyde is well recognized as the predominant aroma in bitter almond (*Prunus dulcis* var amara; sin. *Prunus amygdalus* Batsch. var amara; wild *Amygdalus* spp.). Upon maceration of almond, which liberates substrates and enzymes, benzaldehyde is released from amygdalin by enzymatic hydrolysis followed by a loss of hydrogen cyanide [1]. Water and/or hydrolytic enzymes are added to increase the level of benzaldehyde during the process of almond oils [2].

Sweet almond (*Prunus dulcis* Mill. D.A. Webb; syn. *Prunus amygdalus* Batsch.; *Amygdalus communis* L.; *Amygdalus dulcis* Mill.) has a sweet, nutty aroma that differs from that of bitter almond. Whereas benzaldehyde is the predominant volatile organic compound (VOC) in raw bitter almond [2], it is not the predominant compound contributing to aroma in raw sweet almond although it is present in sweet almond [3, 4]. This difference is attributed to the much lower level of amygdalin in the sweet almond kernel, compared with the bitter almond [4–6].

Our sensory panels (co-authors JK, AF, and GP, all of whom had some experience with sensory evaluation) observed that the characteristic sweet aroma was enhanced when water was added to ground, raw nonpareil (sweet)

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almond kernel samples, which was not reminiscent of benzaldehyde. We hypothesized that the characteristic aroma components may be formed by almond kernel enzymes released by water. While studies have been conducted to investigate volatile profiles in unprocessed sweet almond [3, 4, 7–12], the identity of VOCs due to enzymatic reactions in sweet almond has not been well documented. Therefore, in this study, we investigated the VOCs released by enzymatic reactions in raw nonpareil (sweet) almond kernel samples.

Materials and methods

Chemicals

Hexanal, isobutanol, 2-pentanol, 3-methylbutanol, pentanol, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, hexanol, nonanal, benzaldehyde, benzyl alcohol, and phenylethyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO).

Almond

Nonpareil almond samples (*Prunus dulcis* Mill. D.A. Webb, variety Nonpareil) were obtained from the Almond Board of California and bitter almond samples (breeding selection D3-50 ["Mission" × *Prunus fenzliana* × "Solano"]; harvested in 2007 and stored at 40 °F) were from Thomas Gradziel in the Department of Plant Science, University of California, Davis. Six different individual kernels from both types of almonds were extracted and analyzed for their VOCs. Each individual almond kernel was ground using a mortar and pestle, and three 100 mg aliquots were prepared. Each aliquot underwent different extraction procedures. One aliquot was immediately extracted without any treatment. Another aliquot was extracted after 400 µl water was added to it, and the other was extracted after addition of 400 µl of 8 M guanidine hydrochloride (GdmCl), a protein denaturant.

Collection of almond volatiles by solid-phase microextraction (SPME)

One hundred milligram of each almond sample was placed in a 4-ml glass vial, and a 2-cm, three-component SPME fiber (30 µm carboxen, 50 µm divinylbenzene, polydimethyl siloxane, Supelco Corp, Bellefonte, PA) was used for collection of the headspace volatiles in the vial. The vial was submerged in a water bath at 37 °C and was equilibrated for 10 min. Then, the headspace volatiles were extracted by the SPME fiber for 10 min. The almond sample in the vial was agitated using a magnetic stirrer during the entire extraction period.

Gas chromatography/mass spectrometry (GC/MS)

A Thermo-Finnigan Trace GC/MS (Thermo Electron, San Jose, CA) system was used for all analyses. The SPME fiber containing the extracted almond VOCs was inserted into the injection port of the GC/MS and desorbed for 5 min at 230 °C. A Stabilwax column (30 m × 0.32 mm with 1.0 µ coating; Restek, Bellefonte, PA) was used for separation of the desorbed VOCs. We employed the following chromatographic protocol for separation before MS analyses: 60 °C for 4 min and then programmed at 6 °C/min to 210 °C with a 20-min hold at this final temperature. Column flow was constant with 1.5 ml/min of helium. The injection port was held at 230 °C. Operating parameters for the mass spectrometer were as follows: ion source temperature at 200 °C; ionizing energy at 70 eV; and scanning frequency with four times per second from *m/z* 41 to *m/z* 300. Compound identification was accomplished through manual interpretation of spectra as well as matching against the NIST'02 library in the GC system and comparison with standard samples purchased from Sigma-Aldrich (St. Louis, MO).

Results

Typical chromatograms of the VOCs extracted from bitter and sweet almond kernels are shown in Fig. 1. The VOC profiles of the dried, ground bitter and sweet almond kernels were similar (Fig. 1). In both types of dried almonds, benzaldehyde was barely detected, while hexanol (compound # 8 in Fig. 1) was the major volatile compound. When water was spiked into the ground samples, the volatile profiles of both types of almonds were substantially changed. A large amount of benzaldehyde (10) was released from bitter almond upon addition of water, and its volatile profile became distinct from that of the sweet almond (Figs. 1b, e, 2). In addition, the levels of benzyl alcohol (11) and phenylethyl alcohol (12) were increased (Fig. 2). These three compounds were the major VOCs in the water-spiked bitter almond kernels.

On the other hand, 3-methylbutanol (4) became the predominant VOC in sweet almond upon addition of water (Fig. 1e). As observed in the bitter almond samples, there was an increased release of benzaldehyde (10), benzyl alcohol (11), and phenylethyl alcohol (12) in the sweet almond samples when water was added (Fig. 1e). However, the increases of benzaldehyde (10) and benzyl alcohol (11) in the sweet almond samples were much less than those in the bitter ones (Fig. 2). The levels of other VOCs in the sweet almond samples were also increased upon addition of water (Fig. 1e). Notably, isobutanol (2), 3-methyl-3-buten-1-ol (6), and 3-methyl-2-buten-1-ol (7) were not detected in

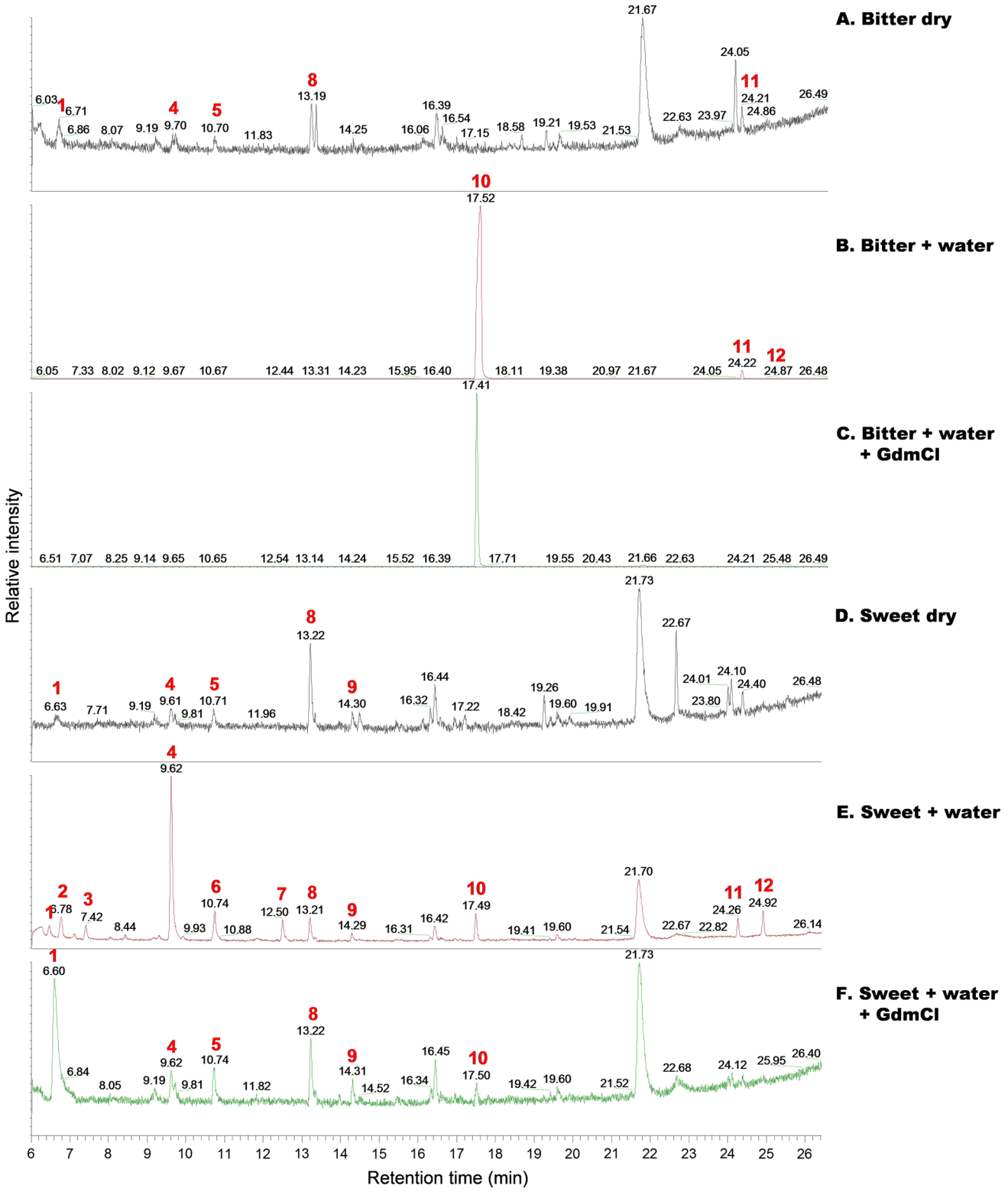

Fig. 1 Total ion chromatograms of volatile organic compounds extracted from bitter and sweet (nonpareil) almond kernels. Prominent compound peaks include hexanal (*1*), isobutanol (*2*), 2-pentanol (*3*), 3-methylbutanol (*4*), pentanol (*5*), 3-methyl-3-buten-1-ol (*6*), 3-methyl-2-buten-1-ol (*7*), hexanol (*8*), nonanal (*9*), benzaldehyde (*10*), benzyl alcohol (*11*), and phenylethyl alcohol (*12*)

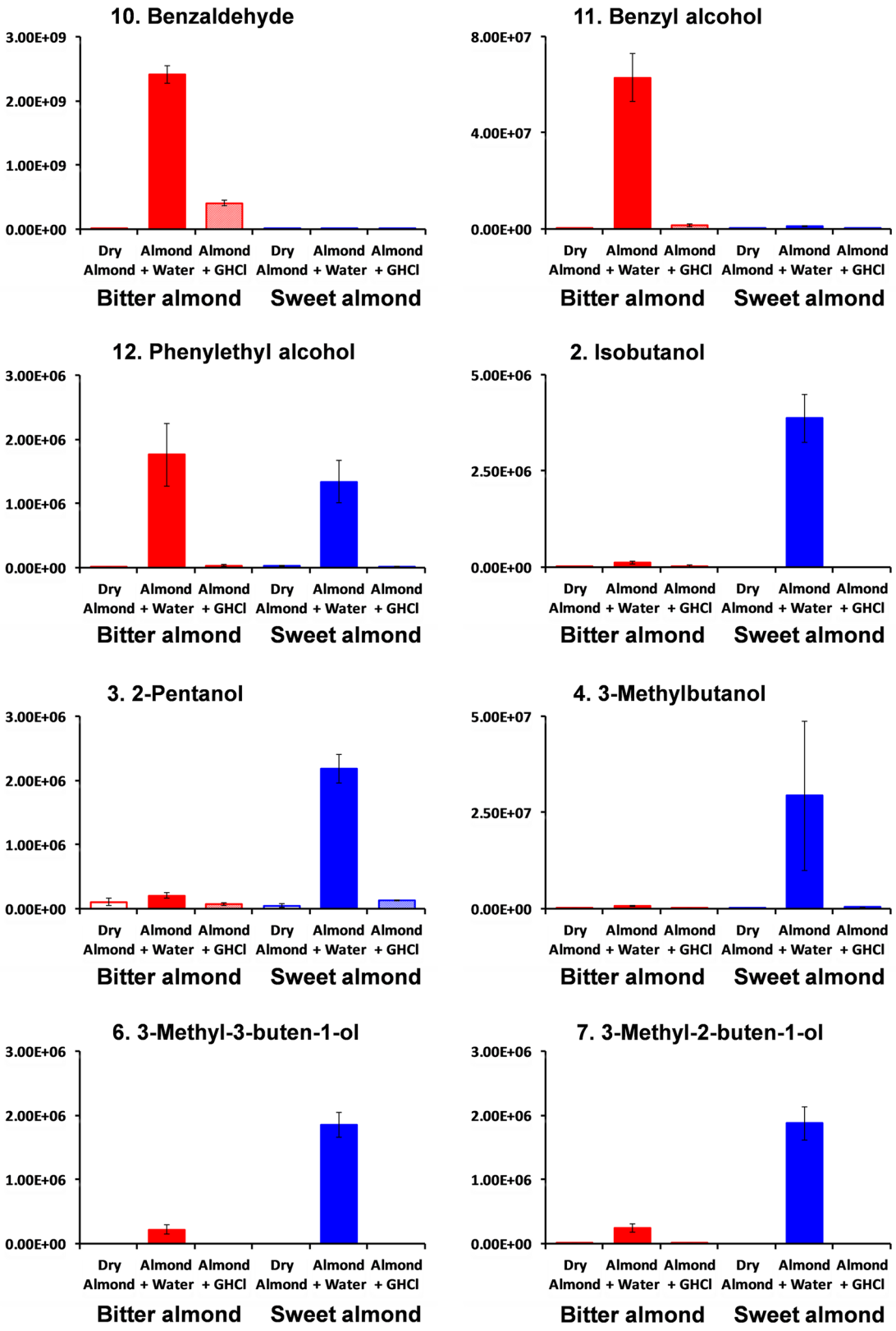

Fig. 2 Levels of volatile organic compounds collected from ground, water-spiked, and guanidine hydrochloride-spiked bitter and sweet almond kernels ($N = 6$ each sample)

the dried, ground sweet almond samples and released only after water was added (Fig. 2).

We hypothesized that the release of these alcohols upon addition of water to the samples may be facilitated by enzymatic reactions. To test this hypothesis, 8 M GdmCl, a protein denaturant, was added to both sweet and bitter almond kernels, and then, their volatile profiles were examined. In the enzyme-denatured sweet almond samples, isobutanol (2), 3-methyl-3-buten-1-ol (6), and 3-methyl-2-buten-1-ol (7) were not detected (Figs. 1f, 2). In addition, other alcohols as well as benzaldehyde were barely detected or greatly reduced in amount in the GdmCl-spiked samples (Fig. 1f), demonstrating that these alcohols are released by enzymatic reactions in the almond kernel.

Discussion

Our results showed that several alcohols such as isobutanol (2), 2-pentanol (3), 3-methylbutanol (4), 3-methyl-3-buten-1-ol (6), and 3-methyl-2-buten-1-ol (7) were released upon addition of water to the raw sweat almond kernels and became the major volatile compounds. In particular, the latter two alcohols in almond were demonstrated only in the study conducted by Mexis et al. [9] where the extraction of VOCs was preceded by the maceration of the raw almond samples. These alcohols were not detected in the dried samples and were released only after water was added in our study (Fig. 2). This led us to perform the subsequent experiment with GdmCl. The compounds were not detected in the enzyme-denatured samples, suggesting that they were enzymatic products. Indeed, Fan et al. [13] observed that 3-methyl-2-buten-1-ol was released upon addition of β-glucosidase in the peel of Jincheng orange. A glycoside of 3-methyl-2-buten-1-ol was isolated from passion fruit [14]. Monitoring the VOCs released by enzymes may be used to estimate water content in raw sweet almond kernels, which may be used for setting drying conditions for almond kernels.

We suggest that these alcohols may contribute to the characteristic sweet aroma. The presence of 3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol has been reported in a variety of fruit. For example, they were the major volatile constituents in pepino [*Solanum muricatum*] [15], and 3-methyl-2-buten-1-ol was a major volatile compound in lychee (*Litchi chinensis* Sonn.) [16]. The odor quality of 3-methyl-2-buten-1-ol was described as cooked marzipan [17]. In addition, these alcohols were detected in honey [18], roasted Arabica coffee [19], cheese [20], and fresh beef [21]. We propose that 3-methyl-2-buten-1-ol may be synthesized via the mevalonate pathway. Mevalonate is a precursor of dimethylallyl pyrophosphate (DMAPP) which is a diphosphate ester of 3-methyl-2-buten-1-ol [22], and DMAPP can be hydrolyzed to 3-methyl-2-buten-1-ol in acidic conditions [23]. Further studies remain to be performed to elucidate their biochemical pathway.

In summary, we identified some alcohols released by enzymatic reactions in raw sweet (nonpareil) almond kernel samples, suggesting that these alcohols may contribute to the characteristic aroma in the raw sweet almond and enrichment with these may improve consumer acceptance.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any experiments with human or animal subjects.

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