JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Quantification of Amygdalin in Nonbitter, Semibitter, and Bitter Almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS

Jihyun Lee,[‖] Gong Zhang,[‖] Elizabeth Wood, Cristian Rogel Castillo, and Alyson E. Mitchell*

Department of Food Science and Technology, University of California—Davis, One Shields Avenue, Davis, California 95616, United States

ABSTRACT: Amygdalin is a cynaogenic diglucoside responsible for the bitterness of almonds. Almonds display three flavor phenotypes, nonbitter, semibitter, and bitter. Herein, the amygdalin content of 20 varieties of nonbitter, semibitter, and bitter almonds from four primary growing regions of California was determined using solid-phase extraction and ultrahigh-pressure liquid chromatography electrospray triple-quadrupole mass spectrometry (UHPLC-(ESI)QqQ MS/MS). The detection limit for this method is ≤0.1 ng/mL (3 times the signal-to-noise ratio) and the LOQ is 0.33 ng/mL (10 times the signal-to-noise ratio), allowing for the reliable quantitation of trace levels of amygdalin in nonbitter almonds (0.13 mg/kg almond). Results indicate that amygdalin concentrations for the three flavor phenotypes were significantly different ($p < 0.001$). The mean concentrations of amygdalin in nonbitter, semibitter, and bitter almonds are 63.13 ± 57.54, 992.24 ± 513.04, and 40060.34 ± 7855.26 mg/kg, respectively. Levels of amygdalin ranged from 2.16 to 157.44 mg/kg in nonbitter, from 523.50 to 1772.75 mg/kg in semibitter, and from 33006.60 to 53998.30 mg/kg in bitter almonds. These results suggest that phenotype classification may be achieved on the basis of amygdalin levels. Growing region had a statistically significant effect on the amygdalin concentration in commercial varieties ($p < 0.05$).

KEYWORDS: *almonds, amygdalin, bitterness, flavor, LC-(ESI)MS/MS, UHPLC-(ESI)QqQ MS/MS, Prunus dulcis*

INTRODUCTION

California (USA) is the top producer of almonds (*Prunus dulcis*) worldwide, with an estimated annual production of 1 million tons and accounting for 80% of world almond production in 2012−2013.[1] Almonds are grown in southern (Solano, San Joaquin, Merced, Kern, Kings, Fresno, Stanislaus, Madera, and Tulare counties) and in northern California (Colusa, Solano, Tehama, Glenn, Butte, Yuba, Sutter, and Yolo counties).[1] The top five almond varieties produced in California (2011−2012) are Nonpareil (39%), Monterey (12%), Carmel (9%), Butte (8%), and Fritz (6%).[1] Almond kernels are sold raw or processed and are consumed as snacks (raw and roasted) in a wide variety of foods including cereals and confectionaries.[1]

Almonds are typically characterized into three phenotypes, which include nonbitter (sweet), semibitter, and bitter. Bitterness in almonds is a monogenetic trait, and the inheritance of bitterness is recessive.[2] In general, the bitterness of almonds may be determined by the content of the cyanogenic glycoside amygladin.[3] Amygdalin is a diglucoside found only in the kernels of almonds, whereas the related monoglucoside, prunasin, is found in the roots and leaves and kernels of almonds.[4] Bitter almonds contain high levels of amygdalin (3−5%) and develop a characteristic cyanide aroma with moisture, whereas nonbitter varieties have nutty flavors and contain trace levels of amygdalin.[4] Semibitter almonds have a "marzipan-like" taste. There is no genetic distinction for semibitter varieties.

The disruption of kernel tissue (e.g., chewing) enables amygdalin to come into contact with hydrolytic enzymes to form hydrogen cyanide. This stepwise process involves the initial hydrolysis of amygdalin into prunasin and glucose via the action of β-glucosidase amygdalin hydrolase (EC 3.2.1.117). The prunasin is subsequently hydrolyzed into mandelonitrile by prunasin hydrolase (EC 3.2.1.21) and is finally converted into benzaldehyde and hydrogen cyanide by β-mandelonitrile lyase (EC 4.1.2.10).[5,6] Benzaldehyde is an aromatic aldehyde with a pleasant almond-like aroma. Hydrogen cyanide results in a bitter perception of foods.[7,8] The distinct almond essence of marzipan and almond extract is associated with amygdalin and enzymatic breakdown products (i.e., benzaldehyde and hydrogen cyanide).[9,10] Hydrogen cyanide (prussic acid) can produce nausea, vomiting, and severe abdominal cramps, and at high doses it can lead to death.[11]

Almonds are classified into categories of nonbitter, semibitter, and bitter based generally just on tasting the almond kernels (usually by the grower/breeder). Bitter almonds are easily to distinguish from nonbitter varieties; however, semibitter and nonbitter almonds are often indistinguishable.[4] Classification of almonds based upon amygdalin levels can help breeders develop almond varieties with targeted flavor profiles (i.e., sweet, more marzipan-like, etc.).

Currently, there is little information on varietal differences in amygdalin levels in nonbitter and semibitter almonds, and analytical methods may have lacked the sensitivity to measure amygdalin at trace levels found in bitter almonds.[4,5,10,12] For example, in a study of five nonbitter almond varieties of a cross between the cultivars Garrigues and Tuono, three were reported to contain no amygdalin as measured by HPLC UV−vis detection.[4] The limit of detection (LOD) was not reported for this study. Wirthensohn et al. evaluated amygdalin in 26 nonbitter

Received: May 24, 2013
Revised: July 16, 2013
Accepted: July 17, 2013
Published: July 17, 2013

almond genotypes (open-pollinated cross of the cultivar Mission) and reported a range between 0.00 and 87.00 mg/100 g almond with a mean value of 10.01 mg/100 g.[10] No LOD was reported for this method. In a more recent study of amygdalin in bitter almonds, the control sample (a nonbitter almond variety) was reported to contain concentrations of amygdalin lower than LOD (200 mg/kg) as measured by ion-trap LC-MS.[12]

Therefore, a solid-phase extraction method was developed along with an ultrahigh-pressure liquid chromatography triple-quadrupole MS/MS method (UHPLC-(ESI)QqQ MS/MS) to improve sensitivity and selectivity. This methodology allowed for analyzing amygdalin in all types of almonds, including at the trace levels found in nonbitter almond varieties. Amygdalin levels were analyzed in 10 commercially important nonbitter almond varieties grown in four common growing regions of California (i.e., Colusa, Fresno, Kern, and Stanislaus). Levels of amygdalin were also measured in semibitter and bitter almonds obtained from the University of California (UC)—Davis almond-breeding program.

MATERIALS AND METHODS

Chemicals and Reagents. Amygdalin (>99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetic acid, acetonitrile, and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Almond Samples. Raw kernels of 10 commercial nonbitter varieties of almonds (*P. dulcis*) were obtained from the Almond Board of California (Modesto, CA, USA). These varieties included Aldrich, Butte, Carmel, Fritz, Mission, Monterey, Nonpareil, Price, Sonora, and Wood Colony. Each variety was grown in four growing regions (i.e., Colusa, Fresno, Kern, and Stanislaus counties) in the fall of 2010. Exceptions were Butte, which was obtained from Colusa and Fresno counties, and Nonpareil, from Colusa, Fresno, and Stanislaus counties. The almonds (0.5−1 kg) were harvested, bagged, and shipped directly to UC—Davis. Raw kernels of four semibitter (i.e., 98-2-305, D1-25, p63-61, and p63-168) and six bitter almond varieties (i.e., 2100, 542_11, *Prunus webbii*, p63-169, UCD F5C-5-25, and UCD F10CD1-16) were provided by the UC—Davis breeding program. Most commercially important nonbitter almond cultivars were developed from crosses between Nonpareil and Mission varieties.

Approximately 40 g of almond kernel samples was used to make composite samples. The kernels were randomly assigned to one of two composite groups for each sample. Each sample was crushed with a wooden mallet, ground by a mortar and pestle, and passed through a no. 20 sieve.

Determination of Amygdalin. The extraction method for amygdalin was modified[4] by increasing the solvent contact-shaking time and by employing a solid-phase extraction (SPE) step. Briefly, a 50 mg almond sample was extracted with 1 mL of methanol and shaken overnight (15−24 h) at room temperature at 250 rpm. The mixture was centrifuged at 4000*g* for 15 min. The supernatant was collected, evaporated under nitrogen gas, and reconstituted in 1 mL of 0.1% acetic acid in water. SPE is necessary to remove interfering ions that can cause ion suppression during the MS analysis, leading to lower intensity of the amygdalin peak and lower recovery. The SPE column (Hypersep C_{18}, 500 mg/3 mL from Thermo Scientific, Waltman, MA, USA) was preconditioned with 2 mL of methanol and 2 mL of water, and the sample was loaded onto the column. An additional 1 mL of 0.1% acetic acid was used to remove remaining residues in the extraction tube. The column was flushed with 2 mL of 0.1% acetic acid in water. Amygdalin was eluted with 4 mL of aqueous methanol (methanol/water, 40:60, v/v). The extract was filtered through a 0.2 μm nylon filter prior to UHPLC-(ESI)QqQ MS/MS analysis.

UHPLC-(ESI)QqQ MS/MS Analysis. Amygdalin analysis was performed on an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography system (UHPLC) interfaced to a 6460 triple-quadrupole mass spectrometer (QqQ MS/MS) with electrospray ionization (ESI) via Jet Stream Technology (Agilent Technologies, Santa Clara, CA, USA). The UHPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A), an autosampler (G4226A) with thermostat (G1330B), and a thermostated column compartment (G1316C). Amygdalin was separated using a Poroshell C_{18} column (2.1 × 150 mm, 2.7 μm, Agilent Technologies). The mobile phase consisted of a linear gradient of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B) as follows: 5% B, 0−3 min; 5−20% B, 3−10 min; 20−60% B, 10−11 min; 60% B, 11−15 min. The column was re-equilibrated between injections for 5 min with initial mobile phase. The flow rate was 0.25 mL/min, and the injection volume was 10 μL.

Negative ESI mode was used. The drying gas temperatures and flow rate were 300 °C and 8.0 L/min, respectively. The sheath gas temperature and flow rate were 350 °C and 11.0 L/min, respectively. The nebulizer gas pressure, capillary voltage, fragmentor voltage, and dwell time were 45 psi, 3.5 kV, 160 V, and 200 ms, respectively. The multiple reaction monitoring (MRM) mode was utilized to analyze amygdalin. Quantification of amygdalin was achieved using an external calibration curve by measuring the area of m/z 456 (precursor ion) to m/z 323 (product ion). Two more transactions were monitored, and the respective m/z values were as follows: m/z 456 (precursor ion) to m/z 179 (product ion), and m/z 456 (precursor ion) to m/z 119 (product ion). Extracts that exceeded the linear range of the standard curve were diluted before injection.

Statistical Analysis. Statistical analysis was performed using IBM SPSS statistics software (v. 20.0, SPSS, Inc., Chicago, IL, USA). Significant differences of amygdalin concentrations among almond varieties and among growing region in the same variety were determined using

Table 1. Almond Varieties

flavor	variety	seed parent	pollen parent
nonbitter	Aldrich	Nonpareil	Mission
	Butte	Nonpareil	Mission
	Carmel	Nonpareil	Mission
	Fritz	Mission	Drake
	Mission	old variety	
	Monterey	Nonpareil	Mission
	Nonpareil	old variety	
	Price	Nonpareil	Mission
	Sonora	Nonpareil	Nonpareil × Eureka
	Wood Colony	Nonpareil	Mission
semibitter	98-2-305	F10D,3-11	F7,1-1
	D1-25	SB20,1-23	Sonora
	p63-61	*Prunus webbii*	Butte
	p63-168	*Prunus webbii*	Padre
bitter	2100	rootstock	
	542_11	rootstock	
	Prunus webbii	*Prunus webbii*	wild species
	p63-169	*Prunus webbii*	Padre
	UCD F5C-5-25	Nonpareil	open pollinated
	UCD F10CD1-16	D3-19 (Mission by *Prunus fenzliana*)	Solano

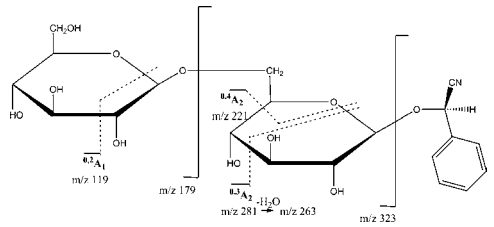

Figure 1. Proposed fragmentation pathway of amygdalin in ESI negative mode MS/MS.

dx.doi.org/10.1021/jf402295u | J. Agric. Food Chem. 2013, 61, 7754−7759

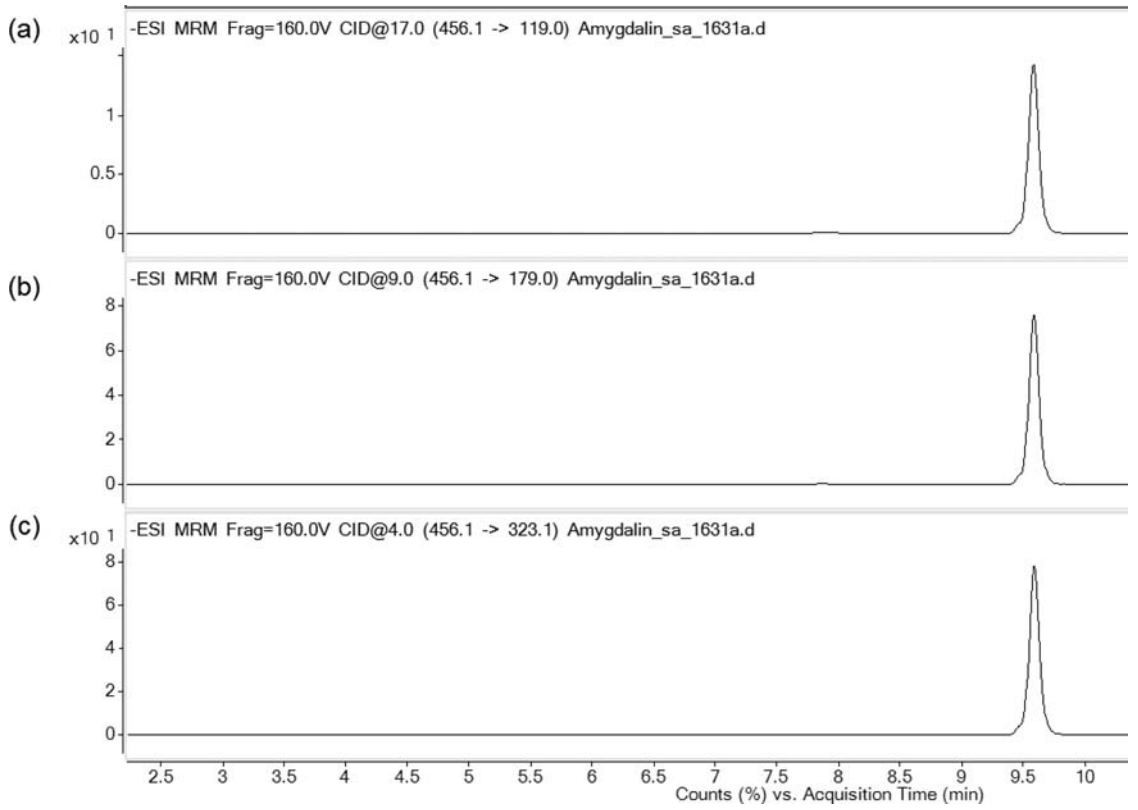

Figure 2. Negative mode ESI multiple reaction monitoring (MRM) chromatograms of amygdalin (12.5 mg/kg) in nonbitter almonds: (a) 456 → 119 (qualifier ion); (b) 456 → 179 (qualifier ion); (c) 456 → 323 (quantifier ion).

one-way ANOVA followed by the Duncan's multiple-range test at $p < 0.05$. Growing region differences of Butte almonds were evaluated with an independent t test at $p < 0.05$.

RESULTS AND DISCUSSION

Breeding parents for nonbitter, semibitter, and bitter almond varieties are given in Table 1.

Amygdalin is typically extracted from almond kernels using polar extraction solvents.[4,10,13] Herein we found that polar extraction solvents result in low recoveries of amygdalin and that recovery could be improved with the addition of acid to the extraction solvent and by employing SPE to assist in sample cleanup. Amygdalin converts to neoamygdalin (an amygdalin epimer) in aqueous solvents.[14,15] Approximately 35% of the 200 ppb amygdalin standard in methanol/water, 40:60 v/v, was converted to neoamygdalin during the 24 h extraction process. This conversion could be prevented with the addition of acetic acid to the almond extract prior to SPE.[14] Under these solvent conditions, amygdalin was stable for a week at room temperature.

A proposed fragmentation pathway for amygdalin is shown in Figure 1. MRM chromatograms indicate that amygdalin elutes at 9.6 min (Figure 2). Gradient HPLC elution improved the reproducibility and LOD of amygdalin measurement. Negative ionization resulted in better sensitivity as compared to positive ionization. UHPLC (ESI)QqQ MS/MS conditions were optimized on the major precursor (m/z 456) and product ions (m/z 323, 179, and 119) for amygdalin (Figure 3). Loss of the disaccharide (m/z 456 → 323) resulted in the highest ion abundance (Figure 3). Transitions were also observed at m/z 456 → 179 corresponding to glycosidic bond cleavage and the loss of the A_1 glucose and at m/z 456 → 119 corresponding to

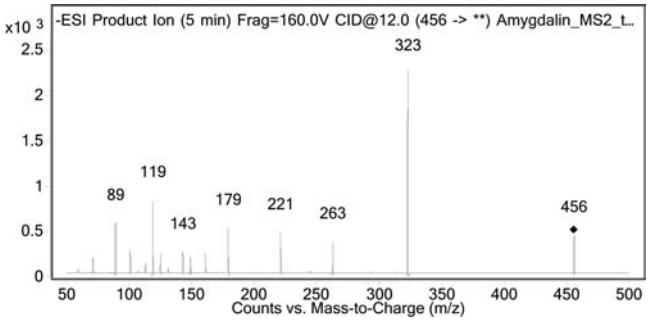

Figure 3. Negative mode product ion spectrum of amygdalin (465 m/z, precursor) at collision energy of 12 ev.

the cross-ring bond cleavage of the A_1 glucose (Figures 1 and 3).[16] Quantification of amygdalin was achieved using the MRM mode transition of 456 → 323. The transitions observed at m/z 456 → 179 and 456 → 119 were used to increase analytical fidelity (qualifier transitions). Fragment ions of m/z 221 and 263 were also observed at nearly equal intensities as m/z 179. These ions were identified previously by Neilson et al. and correspond to the cross-ring bond cleavage of glucose A_2.[16]

The limit of detection (3 times the signal-to-noise ratio) for amygdalin is 0.1 ng/mL of extract (i.e., 0.04 mg/kg almond), corresponding to 1 pg mass on column, the LOQ is 0.33 ng/mL (0.13 mg/kg almond; 10 times the signal-to-noise ratio), and the linear dynamic range of the method is 2.5−200 ng/mL of extract with an R value of 0.998−1.000. The LOD is about 400 times lower than the lowest mean level of amygdalin observed in these samples (Table 2). Recovery was measured after the addition of two different concentrations of an

Table 2. Variety and Growing Region Comparisons of Amygdalin in 10 Commercial Varieties of Nonbitter (Sweet) Almonds

		amygdalin[a] (mg/kg)				
		growing region				
flavor	variety	Colusa	Fresno	Kern	Stanislaus	mean concn
nonbitter	Butte	3.47 ± 0.17	0.85 ± 0.65			2.16 ± 1.25
	Price	7.49 ± 0.06 d	2.49 ± 0.30 b	1.43 ± 0.05 a	5.85 ± 0.38 c	4.32 ± 2.45
	Sonora	1.83 ± 0.18 a	7.08 ± 1.26 b	5.17 ± 0.51 b	16.95 ± 0.37 c	7.76 ± 6.04
	Nonpareil	7.05 ± 0.56 a	12.92 ± 0.57 b		16.72 ± 1.26 c	12.23 ± 4.41
	Monterey	108.75 ± 1.20 c	44.87 ± 1.12 a	62.17 ± 6.55 b	34.08 ± 10.13 a	62.47 ± 27.19
	Wood Colony	78.25 ± 8.70 c	81.20 ± 3.71 c	63.68 ± 1.22 a	76.99 ± 1.46 ab	75.03 ± 8.07
	Carmel	75.04 ± 5.89 ab	94.72 ± 5.32 b	74.19 ± 19.52 ab	63.94 ± 5.06 a	76.97 ± 15.22
	Mission	72.47 ± 8.84 a	138.11 ± 6.06 b	68.75 ± 26.97 a	79.07 ± 6.11 a	89.60 ± 32.34
	Fritz	133.62 ± 8.37 a	130.05 ± 3.38 a	114.91 ± 16.67 a	200.90 ± 28.82 b	144.87 ± 36.44
	Aldrich	90.06 ± 5.01 a	214.87 ± 11.65 c	194.49 ± 1.55 c	130.34 ± 19.25 b	157.44 ± 54.01
	all varieties					63.13 ± 57.54

[a]Mean values followed by different letters indicate significant growing region differences for the same variety at $p < 0.05$. Significant difference (t test) in the levels of amygdalin was noted at $p < 0.05$. t tests were run for Butte as only two regions were available for comparisons. ANOVAs were run on all other varieties.

Table 3. Amygdalin Levels in Semibitter and Bitter Almonds

flavor	variety	amygdalin[a] (mg/kg)	mean concn (mg/kg)
semibitter	p63-61	523.50 ± 26.73 a	
	D1-25	711.80 ± 5.66 a	
	98-2-305	960.95 ± 75.31 b	
	p63-168	1772.75 ± 137.67 c	
			992.24 ± 513.04
bitter	542_11	33006.60 ± 669.63 a	
	2100	34043.65 ± 515.27 a	
	UCD F10CD1-16	35026.40 ± 82.45 ab	
	Prunus webbii	39736.90 ± 3582.91 bc	
	p63-169	44550.20 ± 3490.84 c	
	UCD F5C-5-25	53998.30 ± 1166.02 d	
			40060.34 ± 7855.26

[a]Mean values followed by different letters are significantly different at $p < 0.001$ within each flavor phenotype.

amygdalin standard into the almond samples (final concentrations of 1.6 and 16 mg/kg). The average recoveries ($n = 5$) ranged from 92.4 to 103.2% with an RSD below 6.2%.

Amygdalin concentrations were determined in 10 varieties of commercial nonbitter (sweet) almonds (Table 2). The mean concentration of amygdalin in nonbitter almonds was 63.13 ± 57.54 mg/kg, with concentrations ranging between 2.16 and 157.44 mg/kg. Levels of amygdalin in semibitter and bitter almonds can be found in Table 3. The mean concentration of amygdalin for semibitter almonds was 992.24 ± 513.04 mg/kg and that in bitter almonds was 40060.34 ± 7855.26 mg/kg. The levels ranged between 523.50 and 1772.75 mg/kg (semibitter) and 33006.60−53998.30 mg/kg (bitter) (Table 3). The mean amygdalin concentration in semibitter almonds was ∼16-fold greater than that for nonbitter almonds, indicating that amygdalin may be a useful marker for distinguishing between nonbitter and semibitter almonds.

The amygdalin concentration varied significantly within each flavor phenotype, ranging up to a 73-fold difference in nonbitter almonds (Figure 4). The Aldrich and Fritz varieties contained significantly ($p < 0.001$) higher amygdalin concentrations (157.44 ± 54.01 and 144.87 ± 36.44 mg/kg, respectively) than other nonbitter varieties (Figure 4). Amygdalin concentrations in Butte, Price, Sonora, and Nonpareil varieties were significantly

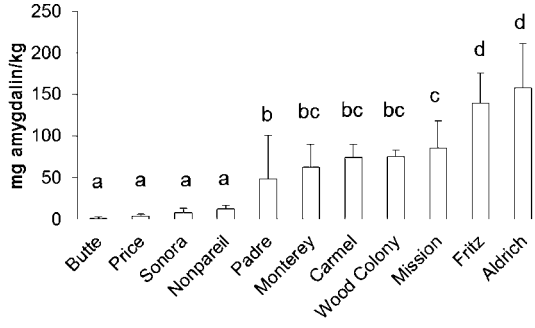

Figure 4. Comparisons of amygdalin from (a) nonbitter, (b) semibitter, and (c) bitter almond varieties. The same letters are not significantly different at $p < 0.001$.

($p < 0.001$) lower than in other varieties, ranging between 2.16 and 12.23 mg/kg. The Monterey, Wood Colony, Carmel, and Mission varieties had intermediate amygdalin concentrations among the 10 nonbitter almond varieties. The leading nonbitter almond variety in Spain, Marcona, contains a mean value of 30 mg amygdalin/kg,[5] and Italian cultivars vary from 73 to 195 mg/kg.[17] Different ratios of biosynthetic and catabolic enzymes may explain the varietal differences in amygdalin concentrations.[18]

Of the semibitter varieties tested, p63-168 contained statistically greater levels of amygdalin (1772.75 ± 137.67 mg/kg) as shown in Table 3. In general, semibitter flavor almonds contained higher concentrations of amygdalin than nonbitter flavor almonds as noted previously.[4] When chewing a bitter almond, an initial slight bitterness is detected (i.e., amygdalin) within a few seconds, and a strong bitter/amaretto taste is perceived as the result of the enzymatic breakdown of amygdalin into benzaldehyde and cyanide. Therefore, both the concentration of amygdalin and enzymatic hydrolysis rates will affect the perception of bitterness.[8] This may be the reason that the perception of bitterness can vary in almonds with differing levels of amygdalin. Thus, sensitive and reliable quantitative determination of amygdalin by UHPLC-(ESI)QqQMS/MS offers a more reliable measure by which to categorize almond flavor.

Bitter almond kernels contained significant concentrations of amygdalin ranging between 33006.60 and 53998.30 mg/kg as shown in Table 3. These values are similar to values, ∼50000 mg/kg[19] and ∼41000 mg/kg,[5] reported by others. Of the bitter almond varieties tested, UCD F5C-5-25 contained the highest amount of amygdalin (53998.30 ± 1166.02 mg/kg). Although two semibitter almond varieties (p63-61 and p63-168) and two bitter almond varieties (*Prunus webbii* and p63-169) had the same seed parent, the amygdalin contents were significantly different ($p < 0.001$).

Growing region comparisons of amygdalin in the 10 nonbitter commercial almond varieties are shown in Table 2. Growing region significantly affected the amygdalin content. For example, Fritz grown in Stanislaus (200.90 ± 28.82 mg/kg) contained significantly higher amygdalin than Fritz grown in other regions (114.91−133.62 mg/kg) ($p < 0.01$). Also, Sonora grown in Stanislaus had significantly higher amygdalin (16.95 ± 0.37 mg/kg) than that grown in other growing regions (1.83−7.08 mg/kg) ($p < 0.001$). The amygdalin concentration of Monterey from Colusa was significantly higher (108.75 ± 1.20 mg/kg) than that from other growing regions (34.08−62.17 mg/kg) ($p < 0.001$). Mission variety from Fresno contained significantly higher amygdalin concentration (138.11 ± 6.06 mg/kg) than that from other regions (68.75−79.07 mg/kg) ($p < 0.05$). Aldrich grown in Fresno and Kern contained higher amygdalin (194.49−214.87 mg/kg) than Aldrich grown in Colusa (90.06 mg/kg) and Stanislaus (130.34 mg/kg) ($p < 0.001$).

In summary, an UHPLC-(ESI)QqQ MS/MS method was developed for measuring amygdalin in almonds with high sensitivity (<0.1 ng/mL) and high selectivity. The Aldrich and Fritz varieties had statistically higher amygdalin concentrations among nonbitter commercial almond varieties. Amygdalin concentrations were significantly different in the nonbitter and semibitter phenotypes. Our results indicate that the combination of SPE and UHPLC-(ESI)QqQ MS/MS can be used to reliably quantify amygdalin in nonbitter almonds and may be a useful tool for distinguishing between nonbitter and bitter varieties.

AUTHOR INFORMATION

Corresponding Author

*(A.E.M.) Phone: (530) 304-6618. Fax (530) 752-4759. E-mail: aemitchell@ucdavis.edu.

Author Contributions

‖J.L. and G.Z. contributed equally to this work.

Funding

We thank the Almond Board of California for providing financial support for this study.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Guangwei Huang from the Almond Board of California for numerous thoughtful discussions and for providing almond samples. We also thank Dr. Thomas Gradziel (Plant Science, UC Davis) for many thoughtful conversations on almond flavor and breeding and for providing almond samples. Additionally, we thank Dr. Susan Ebeler and Carolyn Doyle from the UC Davis Food Safety and Measurement Facility and Dr. Jerry Zweigenbaum from Agilent Technologies for technical support.

ABBREVIATIONS USED

ESI, electrospray ionization; QqQ, triple quadruple; SPE, solid phase extraction; UHPLC, ultrahigh-pressure liquid chromatography

REFERENCES

(1) California Almond Board. *2012 Almond Almanac*; http://almondboard.com/AboutTheAlmondBoard/Documents/2012%20Almond%20Almanac_FINAL.pdf.

(2) Dicenta, F.; Garcia, J. E. Inheritance of the kernel flavour in almond. *Heredity* **1993**, *70*, 308−312.

(3) Haque, M. R.; Bradbury, J. H. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chem.* **2002**, *77*, 107−114.

(4) Dicenta, F.; Martinez-Gomez, P.; Grane, N.; Martin, M. L.; Leon, A.; Canovas, J. A.; Berenguer, V. Relationship between cyanogenic compounds in kernels, leaves, and roots of non-bitter and bitter kernelled almonds. *J. Agric. Food Chem.* **2002**, *50*, 2149−2152.

(5) Sanchez-Perez, R.; Jorgensen, K.; Olsen, C. E.; Dicenta, F.; Møller, B. L. Bitterness in almonds. *Plant Physiol.* **2008**, *146*, 1040−1052.

(6) Sánchez-Pérez, R.; Howad, W.; Garcia-Mas, J.; Arús, P.; Martínez-Gómez, P.; Dicenta, F. Molecular markers for kernel bitterness in almond. *Tree Genet. Genomes* **2010**, *6*, 237−245.

(7) Socias, R.; Kodad, O.; Alonso, J. M.; Gradziel, T. M. Almond quality: a breeding perspective. *Hortic. Rev.* **2008**, *20*, 197−238.

(8) Gradziel, T. Almond (*Prunus dulcis*) breeding. In *Breeding Plantation Tree Crops: Temperate Species*; Springer: New York, 2009; pp 1−31.

(9) Conn, E. E. Cyanogenic compounds. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1980**, *31*, 433−451.

(10) Wirthensohn, M. G.; Chin, W. L.; Franks, T. K.; Baldock, G.; Ford, C. M.; Sedgley, M. Characterising the flavour phenotypes of almond (*Prunus dulcis* Mill.) kernels. *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 462−468.

(11) Shragg, T. A.; Albertson, T. E.; Fisher, C. J., Jr. Cyanide poisoning after bitter almond ingestion. *West. J. Med.* **1982**, *136*, 65−69.

(12) Toomey, V. M.; Nickum, E. A.; Flurer, C. L. Cyanide and amygdalin as indicators of the presence of bitter almonds in imported raw almonds. *J. Forensic Sci.* **2012**, *57*, 1313−1317.

(13) Berenguer-Navarro, V.; Giner-Galvan, R. M.; Grane-Teruel, N.; Arrazola-Paternina, G. Chromatographic determination of cyanoglycosides prunasin and amygdalin in plant extracts using a porous graphitic carbon column. *J. Agric. Food Chem.* **2002**, *50*, 6960−6963.

(14) Hwang, E. Y.; Lee, J. H.; Lee, Y. M.; Hong, S. P. Reverse-phase HPLC separation of D-amygdalin and neoamygdalin and optimum conditions for inhibition of racemization of amygdalin. *Chem. Pharm. Bull.* **2002**, *50*, 1373−1375.

(15) Nahrstedt, A. Isomerization of amygdalin and its homologues. *Arch. Pharm.* **1975**, *308*, 903−910.
(16) Neilson, E. H.; Goodger, J. Q.; Motawia, M. S.; Bjarnholt, N.; Frisch, T.; Olsen, C. E.; Møller, B. L.; Woodrow, I. E. Phenylalanine derived cyanogenic diglucosides from *Eucalyptus camphora* and their abundances in relation to ontogeny and tissue type. *Phytochemistry* **2011**, *72*, 2325−2334.
(17) Barbera, G.; Di Marco, L.; Fatta Del Bosco, G.; Inglese, P. Behaviour of 26 almond cultivars growing under rainfed and semiarid conditions in Sicily. *7^e Colloque du Grempa Groupe de Recherche et d'étude Méditeranéean pour le Pistachier et l'Amandier*; Commission des Communautés Européennes: Brussles, Belgium, 1988; catalog no. CD-NA-11557-4F-C, pp 17−32.
(18) Sanchez-Perez, R. L.; Møller, B. L.; Olsen, C. E.; Dicenta, F. Cyanogenic glucoside patterns in non-bitter and bitter almonds. *Acta Hortic. (ISHS)* **2009**, *814*, 481−486.
(19) Micklander, E.; Brimer, L.; Engelsen, S. B. Noninvasive assay for cyanogenic constituents in plants by raman spectroscopy: content and distribution of amygdalin in bitter almond (*Prunus amygdalus*). *Appl. Spectrosc.* **2002**, *56*, 1139−1146.