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Novel UHPLC-(+ESI)MS/MS Method for Determining Amygdalin, Prunasin and Total Cyanide in Almond Kernels and Hulls (*Prunus dulcis*)

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ABSTRACT: Almonds contain cyanogenic glycosides (CNGs), prunasin and amygdalin, which generate hydrogen cyanide upon hydrolysis. Different extraction and analytical methods are currently used to measure CNGs or cyanide (CN), necessitating distinct samples and can lead to inconsistent or incomparable results. To address this, we describe a method that uses ultrasonic-assisted sample extraction. Amygdalin and prunasin are measured directly in the extract, whereas CN is measured in the extract after derivatization with cysteine ethyl ester to form a cyano-*S*-ethyl-*O*-cysteine (CNCysEt) conjugate. The amygdalin, prunasin, and CNCysEt are quantified using the same UHPLC-(+ESI)MS/MS method. This new approach measured total CN in ten common almond kernel and hull varieties. The limit of quantitation ranged from 7.78 μg L^{-1} (amygdalin), 51.36 μg L^{-1} (prunasin), and 7.80 μg L^{-1} (CNCysEt; kernel) and 25.02 μg L^{-1} (CNCysEt; hull). This is the first time CNGs and CN levels are reported for almond hulls. Average total CN levels in hulls (<3 mg kg^{-1}) were significantly lower than levels in kernels (<20 mg kg^{-1}). Based on these findings, the hulls from California sweet almond varieties may be considered for use in human food products without additional processing to reduce CNG levels.

KEYWORDS: *cyanogenic glycosides, cysteine ethyl ester, derivatization, cyanogenesis, UHPLC-(+ESI)QTOF-MS/MS*

INTRODUCTION

Almonds (*Prunus dulcis*) are part of the genus *Prunus* L. within the *Rosaceae* family. They are native to west-central Asia and eastern Europe but are now widely cultivated in regions with a mild Mediterranean climate, including California, Spain, Australia, and Turkey. California plays a significant role in almond production, growing 30 commercial sweet varieties which produced 1.17 billion kilograms of almonds and contributed to 76% of the world's almond supply in 2022−2023.[1] The ten most predominant varieties of almond grown in California are Nonpareil, Monterey, Independence, Butte/Padre, Wood Colony, Aldrich, Carmel, Fritz, Sonora, and Shasta.[1]

Almonds and many food crops in the *Rosaceae*, *Euphorbiaceae*, *Fabaceae*, and *Gramineae* families produce cyanogenic glycosides (CNGs). These compounds are secondary plant defense compounds that hydrolyze to form hydrogen cyanide (HCN) and benzaldehyde in a process termed cyanogenesis.[2,3] CNGs are comprised of an α-hydroxynitrile and one or more sugar moieties, usually glucose. In almonds there are two CNGs, amygdalin ([(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy](phenyl)acetonitrile), a diglucoside, and prunasin ((*R*)-(β-d-glucopyranosyloxy)(phenyl)acetonitrile), a monoglucoside (Figure 1). Although levels of amygdalin are generally higher than prunasin in almond kernels, prunasin is the biosynthetic precursor of amygdalin. Cyanogenesis involves hydrolysis of the glucose moieties from the amygdalin and prunasin and the formation of an intermediate aglycone structure (mandelonitrile), which rapidly hydrolyzes to release HCN and benzaldehyde (Figure 1).[2,4,5]

The primary site of prunasin production within the almond plant and why it is converted to amygdalin are unclear, but both compounds are seen at various levels in the roots, stems, leaves, and kernels.[6] Upon maceration, the catabolic intracellular enzymes β-glucosidase (E.C. 3.2.1.117) hydrolyze amygdalin into prunasin and glucose. Hydrolysis of prunasin by β-glycosidase prunasin hydrolase (EC 3.2.1.21) releases another glucose and mandelonitrile, which is rapidly converted to benzaldehyde and HCN either through a nonenzymatic reaction or one catalyzed by mandelonitrile lyase (EC 4.1.2.10) (Figure 1).[3−5,7,8] Cyanide (CN) is readily absorbed from the gastrointestinal tract and rapidly distributed to all organs. In small doses, humans can detoxify cyanide, but doses between 0.5 and 3.5 mg kg^{-1} body weight (bw) can be acutely toxic and can lead to severe health effects.[9−11] CN inhibits cellular respiration in aerobic organisms by blocking mitochondrial electron transport and preventing oxygen uptake. Symptoms of acute toxicity include nausea, vomiting, dizziness, weakness, falling blood pressure, and convulsions.[10,12] The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) established an acute reference dose (ARfD) of 20 μg CN kg^{-1} bw in foods regardless of dietary

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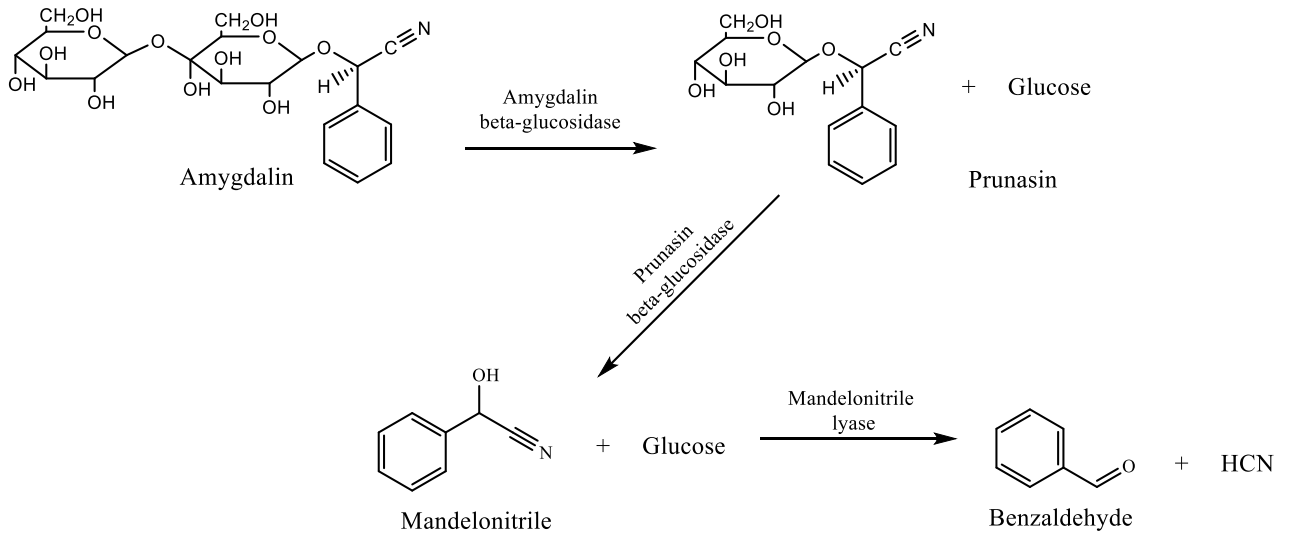

Figure 1. Hydrolysis of amygdalin to form prunasin, mandelonitrile, benzaldehyde and hydrogen cyanide (HCN).

source.[13] Based upon this, EU maximum levels were established for nougat, marzipan, and similar products (50 mg CN kg^{-1}), unprocessed almonds (35 mg CN kg^{-1}), canned pitted fruits (5 mg CN kg^{-1}), apricot kernels (20 mg CN kg^{-1}) and alcohol made from stone fruits (35 mg CN kg^{-1}).[13,14] Most recently, Canadian Health set a total CN limit for apricot kernels as 20 mg kg^{-1} in 2020.[15]

Though all commercially available almonds in California are derived from sweet almond varieties known to produce low levels of cyanogenic glycosides,[6,8] the total CN contribution from amygdalin, prunasin, and CN has not yet been measured in almonds. Levels of CNGs vary in California almonds, with the highest levels of amygdalin (215 mg kg^{-1}) reported in Aldrich.[16] Most analytical methods focus on measuring either amygdalin or CN levels in almond kernels.[6,8,17−20] Estimating the total CN (i.e., contribution from amygdalin, prunasin, and cyanide) can be challenging since different extraction methods are used and values are derived by different analytical methods. A more generalized method that focuses on analysis of the available sources of CN in a single extraction can provide a more robust understanding of the real CN potential of a food.

CNGs are generally measured using liquid chromatography coupled with diode array or tandem mass spectrometry (HPLC-ESI-MS/MS) detection.[6,8,17−19,21−23] In almonds, amygdalin is the most studied CNG,[6,8,17,18,21,22,24] with few studies evaluating prunasin.[6,19,23] CN, however, is generally measured through a mixture of direct and indirect analysis, with most studies using indirect methods quantitating the release of CN from amygdalin via acid or enzyme hydrolysis followed by colorimetry, spectrophotometry, or chromatography.[17,25−29] Liquid chromatography-tandem mass spectrometry (LC−MS/MS) and gas chromatography−mass spectrometry are also used for CN analysis after derivatization, offering higher sensitivity and accuracy.[30,31] Regardless, the analysis of the CNGs and CN in a food requires different extraction procedures, samples, and methods of analysis, which can underestimate levels due to the incomplete hydrolysis of amygdalin and prunasin and make comparisons within and between samples difficult.[19,23,28,31]

Most research on the levels of CNGs and CN in almonds focuses on the kernel since, unlike other stone fruits, the kernel is consumed, not the mesocarp. Almond hulls (mesocarp), considered a byproduct of the almond industry, are currently used as livestock feed and are being evaluated for use in human food products as a source of antioxidants and fiber.[32−36] To date, there are no studies measuring levels of CNGs in almond hulls. To ensure hulls are safe for use in human food products, evaluating the levels of CNGs and CN is essential.

As new almond varieties are bred for California's changing climate conditions, and with the new consideration for the use of almond hulls in human foods, a more consistent and comprehensive extraction and analytical method is needed to screen varieties and more accurately quantitate the amount of total CN consumers could be exposed to. In 2019, the EFSA/CONTAM report recommended that new methods need to be developed and validated for the quantitation of CNGs and total CN in different food items.[13] Additionally, this report highlighted the necessity of identifying crop cultivars with relatively low levels of CNGs or hydrolytic enzymes involved in cyanogenesis. Furthermore, it emphasized the requirement for more occurrence data for CN and CNGs in raw and processed food commodities.[13]

To help address this need, we developed a method utilizing ultrasonic-assisted extraction (UAE) to measure CNGs (amygdalin and prunasin) as well as CN, measured as a derivatization of CN. Both CNGs and CN levels were used to measure the total CN potential (i.e., amygdalin, prunasin, and cyanide) in the same sample of almond kernels or hulls, using UHPLC-(+ESI)MS/MS for the analysis of all analytes. This allows for more accurate determination of the total CN content and provides a more robust method of analysis to screen almond kernels and hulls. Additionally, this new method can be adapted to screen other edible CNGs in plants (e.g., elderberry, cassava, lima bean) to determine the total CN potential of these important food crops.

METHODS & MATERIALS

Reagents and Standards. Amygdalin (≥99%), potassium CN (≥98%), and potassium CN ^{13}C were purchased from Sigma-Aldrich (St. Louis, MO). (*R*)-Prunasin (≥95%) was purchased from Cayman Chemical (Ann Arbor, Michigan). Optima LC/MS grade ammonium formate, formic acid, methanol, and acetonitrile were purchased from Fisher Scientific (Ottawa, CAN). Ethanol anhydrous was purchased from Koptec (King of Prussia, PA). A mobile phase conditioning agent (deactivator additive) was purchased from Agilent Technologies

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Table 1. Dynamic Multiple Reaction Monitoring (dMRM) Transitions of Cyano-*S*-ethyl-*O*-cysteine (CNEtCys), ^{13}C Labeled Cyano-*S*-ethyl-*O*-cysteine (^{13}CNEtCys), Amygdalin, and Prunasin

compound	adduct	dMRM transition	retention time (min)	retention window (min)	fragmentor (v)	collision energy (eV)
^{13}CNEtCys	$[H^+]$	176 → 148[a]	1.20	0.7	80	10
		176 → 102	1.20	0.8	90	15
		176 → 59	1.20	0.8	90	45
CNEtCys	$[H^+]$	175 → 101[a]	1.20	0.8	90	15
		175 → 59	1.20	0.8	90	45
amygdalin	$[NH_4^+]$	475 → 325[a]	2.56	0.7	80	5
		475 → 163	2.56	0.7	80	10
prunasin	$[NH_4^+]$	313 → 180[a]	2.89	0.7	80	5
		313 → 163	2.89	0.7	80	5

[a]Denotes quantifying ion transition.

(Santa Clara, CA) to improve sensitivity. L-Cysteine ethyl ester hydrochloride was purchased from United States Biochemical Corp. (Cleveland, OH). Millipore Milli-Q purification system was used to produce type-1 water (18.2 mΩ resistance).

Stock Solutions. Stock mixtures of amygdalin (100 mg L^{-1}) and prunasin (40 mg L^{-1}) were made in 90:10 ethanol/water (v/v) extraction solvent. Cysteine ethyl ester stock (1500 mg L^{-1}), used for CN derivatization, was made fresh in ethanol prior to use. Potassium CN ^{13}C was used as an internal standard (ISTD) for CN analysis. Potassium CN and potassium CN ^{13}C stocks (22.5 mg L^{-1}) were made in type-1 water. All stock solutions except cysteine ethyl ester were stored at 4 °C.

Almond Samples. Ten varieties of almond kernels and hulls (Aldrich, Butte, Carmel, Fritz, Independence, Monterey, Nonpareil, Sonora, Winters, and Wood Colony) were sampled across California from the 2021 and 2022 harvest years. Almonds (∼10 kg) were harvested at commercial maturity and sampled from two to three farms in different California regions. During the 2021 harvest, 52 kernels and 52 hulls were collected, followed by 56 of each in the 2022 harvest, totaling 108 kernels and 108 hulls measured. The hulls were separated from the kernels manually and stored at 4 °C until sample preparation and extraction.

Extraction Optimization. Extraction conditions were optimized for solvent composition, UAE time, and sample mass-to-solvent ratio for amygdalin and prunasin recovery using Nonpareil kernels and hulls. The extraction solvent conditions were 10:90, 50:50, and 90:10 ethanol/water (v/v); the sonication times were 0 to 360 min, with samples taken every 30 min on the highest power setting, and the sample mass to volume ratios include 0.05, 0.1, and 0.2 g mL^{-1} in kernels. In hulls, ratios tested included: 0.1, 0.2 and 0.5 g mL^{-1}. The optimal conditions for the greatest recovery of CNGs were a sonication time of 150 min in 90:10 ethanol/water (v/v) using a ratio of 1 g kernels to 10 mL extraction solvent and 2 g hulls to 10 mL extraction solvent.

Sample Preparation and Extraction. Approximately 15 g of kernels and 20 g of hulls were separated and cryogenically ground and samples were stored at −20 °C until extraction. The ground hulls (2 ± 0.010 g) and kernels (1 ± 0.010 g) were weighed into 50 mL polypropylene centrifuge tubes, and 10 mL of 90:10 ethanol/water (v/v) extraction solvent were added. Samples were randomized and placed in a sonicator (Fisher brand CPX2800 Ultrasonic Bath) for 150 min, mixing samples every 30 min. The sonicator was set to the highest power setting and the water bath temperature was monitored with temperatures not exceeding 47 °C. After extraction, samples were centrifuged for 10 min at 5000 rpm, and the supernatant was collected. The pellet was rinsed with 3 mL of extraction solvent and centrifuged for 10 min. The supernatant was collected and added to the initial extract. The volume of the sample was calculated via solvent weight. Two 1 ml aliquots of the supernatant were transferred into 2 mL polypropylene tubes, one for CN analysis and one for CNG analysis. Sample supernatant was stored at −20 °C for up to 7 days without degradation.

For amygdalin and prunasin, the sample supernatant was diluted 20-fold into type-1 water and filtered through a 13 mm, 0.2 μm H-PTFE filter directly into 2 mL amber glass autosampler vials for HPLC-ESI(+)-MS/MS analysis.

Derivatization of CN with Cysteine Ethyl Ester (Cyano-*S*-ethyl-*O*-cysteine)[CNCysEt]. A 1 mL aliquot of supernatant obtained from the UAE extraction was spiked with 40 μL of potassium CN ^{13}C stock and stored at −20 °C for up to 7 days without degradation. For HPLC-ESI(+)-MS/MS acquisition, the CN sample was brought to room temperature, after which 160 μL of cysteine ethyl ester stock was added to derivatize CN within the sample. The caps were securely sealed to prevent loss by evaporation and placed into a hot plate at 50 °C for 24 h. The sample was then diluted 20-fold into type-1 water and filtered through a 13 mm, 0.2 μm H-PTFE filter directly into 2 mL amber glass autosampler vials for analysis by HPLC-ESI(+)-MS/MS.

The stability of the CN derivatization reaction was evaluated over 48 h, with samples taken at 2, 4, 6, 8, 10, 12, 24, 36, and 48 h. Potassium CN was spiked into Nonpareil kernel samples to achieve in-sample concentrations of 50, 150, and 450 μg L^{-1}. Potassium CN ^{13}C ISTD and cysteine ethyl ester were added to the samples as previously described. After incubation, the samples were diluted 20-fold into type-1 water and filtered through a 13 mm, 0.2 μm H-PTFE filter for HPLC-ESI(+)-MS/MS analysis.

UHPLC-(+ESI)MS/MS Analysis. CNCysEt, ^{13}CNCysEt, amygdalin, and prunasin were chromatically resolved and identified using an Agilent 1290 Infinity ultrahigh-performance liquid chromatography system (UHPLC) connected to a 6460 triple-quadrupole mass spectrometer (MS/MS) with electrospray ionization (ESI) equipped with Jet Stream Technology (Agilent Technologies; Santa Clara, CA). The UHPLC was equipped with an autosampler and thermostat, a thermostatic column compartment, and a binary pump with an integrated vacuum degasser. A Zorbax Eclipse Plus C18 Column (2.1 × 100 mm, 1.8 μm; Agilent Technologies) with a Zorbax Eclipse Plus C18 UHPLC guard cartridge (2.1 × 5 mm, 1.8 μm; Agilent Technologies) was used to achieve LC separation. The injection volume was 4 μL, and the column compartment was set to 35 °C. Dynamic multiple reaction monitoring (dMRM) was performed in positive mode (+ESI), and the conditions and precursor/product ion transitions monitored are detailed in Table 1. The mobile phases used were 10 mM ammonium formate and water buffered to a pH of 3 with formic acid and 1 mL L^{-1} deactivator additive (A) and 1:1 (v/v) methanol/acetonitrile (B). The flow rate was 0.45 mL min^{-1}. The mobile phase gradient was as follows: 0 min, 8% B; 0.62 min, 20% B; 1.30 min, 20% B; 3.00 min, 50% B; 3.50 min, 95% B; 4.3 min, 95% B; 4.6; 8% B. The AJS ESI optimized source conditions: drying gas temperature, 150 °C; sheath gas temperature, 375 °C; capillary voltage, 3500 V; sheath gas flow, 12 mL/min; nebulizer, 25 psi. The total run time of the method was 6.5 min. Agilent MassHunter Workstation Data Acquisition (Ver.10.0) was used for data acquisition. Agilent MassHunter Qualitative Analysis (Ver.10.0) and MassHunter Quantitative Analysis (Ver.8.0) were used to analyze all data. Compound identification was achieved using the dMRM

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transitions, retention times, and retention time windows specified in Table 1.

Quadrupole Time of Flight (QTOF) Mass Spectral Determination of CNCysEt. The product from the reaction of CN with cysteine ethyl ester was analyzed using UHPLC-(+ESI)QTOF-MS/MS for accurate mass determination (Agilent 1290 Infinity II UHPLC coupled to a G6545A QTOF; Agilent Technologies; Santa Clara, CA). An autosampler and thermostat, thermostatic column compartment, and a binary pump with integrated vacuum degasser were equipped to the UHPLC. The AJS ESI optimized source conditions: drying gas temperature, 200 °C; sheath gas temperature, 350 °C; capillary voltage, 4500 V; drying gas flow, 12 mL min^{-1}; sheath gas flow, 11 mL min^{-1}; nebulizer, 45 psi; fragmentor, 100 v; isolation width was operated in medium resolution. The mobile phases, flow rate, injection volume, and column compartment were the same as reported for the UHPLC-(+ESI)MS/MS method. The mobile phase gradient was as follows: 0 min, 8% B; 0.62 min, 20% B; 1.30 min, 20% B; 3.00 min, 50% B; 3.30 min, 95% B; 4.30 min, 100% B; 4.60 min, 8% B. The total run time of the method was 6.5 min. For LC separation, a Zorbax Eclipse Plus C18 Column (2.1 × 100 mm, 1.8 μm; Agilent Technologies) with a Zorbax Eclipse Plus C18 UHPLC guard cartridge (2.1 × 5 mm, 1.8 μm; Agilent Technologies) was used. The MS parameters utilized three collision energies: the quadrupole time-of-flight was set to alternate between 5, 15, and 30 eV collision energies. The MS experiments utilized a scan range of m/z 100−1000 with a scan rate of 8 spectra/sec. MS/MS experiments had a narrowed scan range of m/z 20−180 with a scan rate of 8 spectra/sec. Fragmentation spectra were processed in Agilent MassHunter Molecular Structure Correlator (Version 8.1), and structural assignments were verified through manual mass spectral interpretation.

VALIDATION OF UHPLC-(ESI+)-MS/MS METHOD

The method was validated independently in almond kernels and hulls by establishing the limit of detection (LOD), the limit of quantitation (LOQ), method LOQ, analyte recovery, and accuracy in each matrix. Separate calibration curves were constructed for each analyte. Fifteen standard solutions ranging from 10 to 20,000 μg L^{-1} were used for the prunasin standard curve. The amygdalin calibration curve ranged from 10 to 35,000 μg L^{-1} with 16 calibration points. Each standard was prepared in 90:10 ethanol/water (v/v), same as used for sample extraction, and diluted 20-fold for acquisition on UHPLC-(+ESI)MS/MS. The concentration versus analyte response was plotted, and quadratic regression was applied using the equation $y = ax^2 + bx + c$. The CNCysEt calibration curve included eight calibration points between 7.81 and 1000 μg L^{-1} in a matrix-based background. Following the previously stated sample extraction, Nonpareil kernels and hulls were extracted to create a matrix-based solvent for CNCysEt calibration standards. The endogenous CN content was subtracted from the CN measured in the calibration standards to construct the calibration curve. The relative concentration versus analyte relative response was plotted, and a linear regression was applied using the equation $y = ax + b$.

Calibration curves were evaluated based on their correlation coefficients (R^2). The LOD and LOQ were calculated as 3 times and 10 times the signal-to-noise ratio, respectively. The method's accuracy and matrix effects were evaluated by the recovery of each analyte after spiking low and high concentrations of authentic standards into Nonpareil kernel or hull matrix. The accuracy of the spike and recovery experiments was assessed by calculating the percent recovery (%R) using eq 1:

$$\%R = \frac{C_{spiked} - C_{control}}{C_{spiked}} \times 100\% \quad (1)$$

where C_{spiked} and $C_{control}$ are the concentrations in the spiked and control samples, respectively. The method LOQ was calculated following eq 2:

$$\text{method LOQ} = \frac{\left(\text{LOQ} \times \frac{1}{\%R} \times \text{DF}\right)}{1000} \quad (2)$$

where DF is the dilution factor.

Total CN. Given that amygdalin and prunasin undergo hydrolysis, a total CN value was calculated to estimate the potential CN contribution from their hydrolysis, in addition to the CN concentrations directly measured. The theoretical CN values of amygdalin and prunasin were calculated based on the assumption that the complete hydrolysis of 1 mg of amygdalin (MW 457.43 g mol^{-1}) produces 0.06 mg of CN (MW 26.03 g mol^{-1}), while the complete hydrolysis of 1 mg of prunasin (MW 295.29 g mol^{-1}) produces 0.09 mg of CN (MW 26.03 g mol^{-1}).

Statistical Analysis. Statistics were done using JMP (Version 18.0.1). One-way ANOVA was used to evaluate the difference between each variety across harvest seasons for amygdalin, prunasin, cyanide, and total CN content, as well as evaluating difference between levels of each compound in kernels and hulls.

RESULTS & DISCUSSION

This method was developed to provide quantitation of total CN derived from both the CNGs and CN within the same sample. The optimal conditions for the recovery of CNGs included a sonication time of 150 min using a solvent of 90:10 ethanol/water (v/v) at a ratio of 1 g kernels to 10 mL extraction solvent, and 2 g hulls to 10 mL extraction solvent. The extract was then aliquoted for analysis of CNGs or derivatization of CN. The CNGs were measured in the extract after a 20-fold dilution and filtration with no further sample preparation. To measure the CN, an aliquot of the extract was reacted with cysteine ethyl ester for 24 h to form a CNCysEt conjugate. This extract was then diluted by 20-fold, filtrated and analyzed with no further sample preparation. The CNGs and the CNCysEt conjugate were quantified using the same 6.5 min UHPLC-(+ESI)MS/MS method (Figure S1, Supplemental). By using the same sample for both analyses, the amounts of CNGs and CN can be more directly compared. CN analysis by other methods such as the picrate method, acid or enzymatic hydrolysis, and titration require extensive sample preparation and frequently underestimate the levels of CN present in samples due to incomplete hydrolysis of amygdalin or prunasin.[11]

CN Derivatization with Cysteine Ethyl Ester. A derivatization reaction based on the reaction of cysteine ethyl ester with CN was developed to analyze the CN content in kernels and hulls. In previous studies, Kang and Shin (2015) used cysteine and hypochlorite to measure CN in drinking water.[30] This reaction produced cyanogenic chloride, which was reacted with cysteinyl chloride to produce β-thiocyanoalanine which was then measured using LC−MS/MS and related to HCN concentration.[30] The method reported herein avoids creating cyanogenic chloride, a highly toxic byproduct, by using ethanol as an extraction solvent. Cysteine was first evaluated as a derivatizing agent for CN, but it resulted in a

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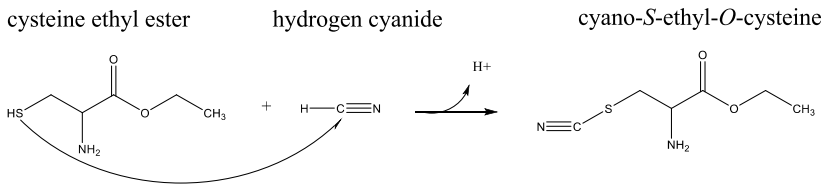

Figure 2. Nucleophilic addition of cysteine ethyl ester with CN to form cyano-*S*-ethyl-*O*-cysteine.

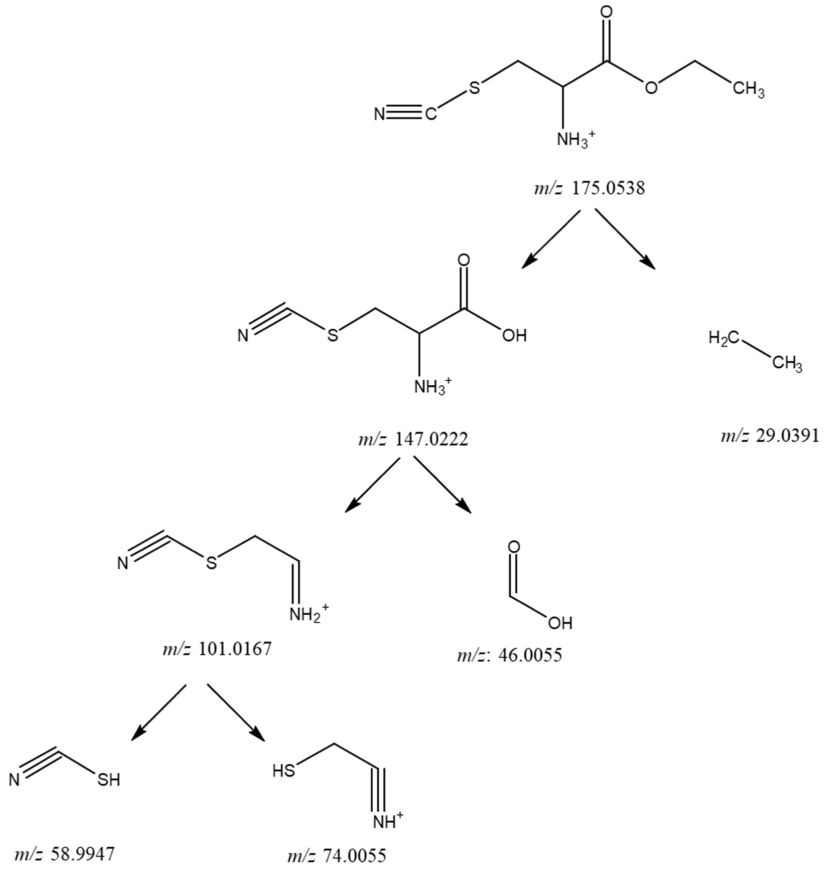

Figure 3. Mass spectral fragmentation of cyano-*S*-ethyl-*O*-cysteine (CNCysEt). Mass errors are 1.29 mg kg^{-1} for $C_{63}H_{11}N_2O_2S$, −0.51 mg kg^{-1} for $C_4H_7N_2O_2S$, −0.76 mg kg^{-1} for $C_3H_5N_2S$, −4.09 mg kg^{-1} for C_2H_4NS, and −5.26 mg kg^{-1} for CHNS.

Table 2. Regression, Correlation Coefficient (R^2), Limit of Detection (LOD), Limit of Quantitation (LOQ), and Method Limit of Quantitation (MLOQ) for Kernels and Hulls[a]

compound	matrix	calibration curve	R^2	LOD	LOQ	kernel MLOQ	hull MLOQ
amygdalin	solvent	$y = 5.248 \times 10^{-5}x^2 + 1.138x + 21.927$	0.998	2.34	7.78	1.70	1.10
prunasin	solvent	$y = 7.705 \times 10^{-6}x^2 + 0.234x + 0.931$	0.999	15.41	51.36	9.00	4.29
CNCysEt	kernel	$y = 4.376x + 0.002$	0.998	2.34	7.80	1.87	—
	hull	$y = 4.416x + 0.004$	0.999	7.51	25.02	—	2.21

[a]LOD an LOQ units are μg L^{-1}, and MLOQ units are in mg kg^{-1}.

product with poor chromatographic retention. To obtain better retention and separation from the solvent front, cysteine ethyl ester was used instead. In this reaction, the sulfur atom of the cysteine ethyl ester acts as a soft nucleophile, adding to the partially positively charged carbon atom of HCN and forming cyano-*S*-ethyl-*O*-cysteine (CNCysEt; Figure 2).

The reaction of cysteine ethyl ester with CN resulted in only one product with a measured mass of 175.0538 Da corresponding to CNCysEt (Figure 2S, Supplemental). Fragmentation of the CNCysEt adduct results in the formation of product ions at 147.0222 Da (loss of CH_2−CH_3); 101.01067 (loss of CO_2); and 58.9947 Da (loss of NCSH) and 74.0055 (loss of CN). A fragmentation scheme is presented in Figure 3.

The optimal reaction time for this reaction was evaluated over 48 h at low (50 μg L^{-1}), medium (150 μg L^{-1}), and high (450 μg L^{-1}) concentrations, with samples taken at 2, 4, 6, 8, 10, 12, 24, 36, and 48 h. Moderate to high variability between samples was observed between 2 and 8 h, especially at high concentrations; however, the reaction was stable at 12 h and remained stable for up to 48 h. The reaction was allowed to

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Table 3. Nonpareil Almond Kernel and Hull Matrix Recoveries for Amygdalin, Prunasin, and Cysteine Ethyl Ester Derivatized Cyanide (CNCysEt)

	low-spiked recovery		high-spiked recovery	
compound	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)
		Kernels		
amygdalin[a]	13.70 ± 0.73	91.34 ± 4.84	48.05 ± 0.61	96.10 ± 1.21
prunasin[b]	1.14 ± 0.17	114.07 ± 17.30	3.32 ± 0.17	110.74 ± 5.61
CNCysEt[c]	0.84 ± 0.02	83.51 ± 2.07	2.57 ± 0.17	85.80 ± 5.76
		Hulls		
amygdalin[a]	0.71 ± 0.05	71.05 ± 2.52	3.05 ± 0.31	61.02 ± 3.14
prunasin[b]	0.60 ± 0.17	119.75 ± 16.73	2.54 ± 0.16	127.12 ± 3.99
CNCysEt[c]	0.06 ± 0.01	112.99 ± 20.78	0.89 ± 0.02	104.93 ± 2.86

[a]Amygdalin was spiked at 15 and 50 mg kg^{-1} for kernels and 1 and 5 mg kg^{-1} for hulls. [b]Prunasin was spiked at 1 and 3 mg kg^{-1} for kernels and 0.5 and 2 mg kg^{-1} for hulls. [c]CNCysEt was spiked at 1 and 3 mg kg^{-1}for kernels and 0.05 and 0.85 mg kg^{-1} for hulls.

react for a minimum of 12 h for an optimal and consistent response.

Calibration Curves. Calibration curves for each compound were individually determined using quadratic or linear regression models and evaluated based on their correlation coefficients (R^2). The R^2 values ranged from 0.998 to 0.999 for all compounds, indicating a good fit (Table 2). The LOD and LOQ were calculated as three times the signal-to-noise ratio and ten times the signal-to-noise ratio, respectively. The high correlation coefficients, along with the calculated LOD and LOQ values, demonstrate the high sensitivity and accuracy of the analytical method (Table 2). In the literature, the limits of quantitation for amygdalin and prunasin are reported between 1 and 2000 μg L^{-1} amygdalin[8,18,20,23,37] and 0.048 to 10 μg L^{-1} prunasin.[20,23] Most studies on almond CNGs primarily focus on reporting amygdalin values, with relatively few evaluating prunasin.[6,19] Cyanide analysis is more prevalent in the literature and employs methods that include picrate paper, titrations, and acid hydrolysis.[20,23,25−27,31] However, similar to prunasin, information on limits of detection and quantitation in almond matrices is sparse, with one reference reporting a LOQ of 13 mg kg^{-1}.[17,28,31]

Although mandelonitrile was initially included and evaluated as part of our method for this study, it hydrolyzes rapidly, and subsequently all analyzed samples were below the LOD of 1300 μg L^{-1}. However, in samples containing higher concentrations of CNGs (e.g., alcohol made from stone fruits) it is possible to detect measurable levels of mandelonitrile.

Qualitative and Quantitative dMRM Transitions and Analytical Selectivity. Amygdalin, prunasin, CNCysEt and ^{13}CNCysEt were measured using UHPLC-ESI(+)-MS/MS and dynamic multiple reaction monitoring (dMRM). The most abundant fragment ion produced was used for quantitation and included the following m/z transitions: amygdalin (475 → 325), prunasin (313 → 180), CNCysEt (175 → 101), and ^{13}CNCysEt (176 → 148) (Table 1). One or two additional dMRM transitions were used as qualifier ions to increase fidelity and include the following transitions: amygdalin (475 → 163), prunasin (313 → 163), CNCysEt (145 → 59), and ^{13}CNCysEt (176 → 102, 176 → 59) (Table 1).

Optimizing the mobile phases was essential to achieve resolution and improve peak shape for UHPLC-ESI(+)-MS/MS analysis. Mobile phase A was formulated with 10 mM ammonium formate adjusted to a pH of 3 with formic acid. Acidification of the mobile phase protonated the silanol groups in the column's stationary phase, enhancing peak shape and reducing tailing. A mobile phase deactivator additive was used to decrease the interactions between analytes and trace metals in the system, reducing metal chelation. These modifications significantly improved the peak shape, retention of analytes, resolution and reproducibility of results.

Extraction of Cyanogenic Glycosides. The solvent composition, extraction time, and sample mass were individually evaluated and optimized to ensure consistent extraction of amygdalin and prunasin from kernels and hulls. The ideal conditions were 90:10 (v/v) ethanol/water extraction solvent, 150 min of UAE, and sample masses of 1 g for kernels and 2 g for hulls per 10 mL of extraction solvent. As the proportion of ethanol in the solvent increased (10%, 50%, and 90%), the signals for amygdalin and prunasin also increased.

Extraction time was evaluated over 0−360 min, with samples taken every 30 min. The yields of amygdalin and prunasin were highest at 120 and 180 min, so an intermediate time of 150 min was determined to be optimal. Above 180 min, amygdalin levels decreased while prunasin levels increased, suggesting that the hydrolysis of amygdalin to prunasin occurs over extended extraction periods.

The extraction efficiency for amygdalin and prunasin was evaluated based on the yield of amygdalin in three sequential extractions of the kernels and hulls using the optimized conditions. The average recovery across the three varieties was 94.42% in the first extraction, 5.18% in the second extraction, and less than 1% in the third extraction, with standard deviations (SD) at or below 0.70%. These results indicate that a single extraction is sufficient for achieving high recovery and is optimal for analysis.

Recovery and Matrix Effect. A spike and recovery method was used to evaluate the accuracy and precision of the analytical method for amygdalin, prunasin, and CNCysEt (Table 3). Samples were spiked with low and high concentrations of each analyte, followed by extraction and evaluation. In kernels, amygdalin and prunasin recoveries ranged from 91.34 to 96.10% and 110.74 to 114.07%, respectively. Other studies report similar recoveries of amygdalin in kernels, which range between 90 and 126%.[8,18,20] In hulls, amygdalin and prunasin recoveries ranged from 61.02% to 71.05% and 119.75% to 127.12%, respectively. Currently, no studies have evaluated CNGs in almond hulls, which limits our ability to contextualize whether these observed values are acceptable. However, we investigated whether a matrix effect could be influencing these values by comparing a matrix-based calibration curve with a solvent-

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Table 4. Total Cyanide Levels Measured in Almond Kernels and Hulls, Contributed from Amygdalin, Prunasin, and Cyanide

variety	n 2021	avg. ± SD	range	n 2022	avg. ± SD	range
			Kernel Total Cyanide (mg kg^{-1})			
aldrich	6	14.14 ± 3.32	11.79−18.87	6	16.27 ± 3.41	11.74−19.60
butte	4	0.37 ± 0.27	0.10−0.73	6	0.46 ± 0.54	0.02−1.32
carmel	6	5.92 ± 2.19	3.79−8.91	4	8.81 ± 1.33	7.49−10.80
fritz	4	8.68 ± 2.04	6.37−11.25	4	7.35 ± 1.71	4.65−9.33
independence	4	0.10 ± 0.07	0.03−0.19	6	0.13 ± 0.06	0.03−0.20
monterey	4	5.89 ± 2.00	3.73−7.86	6	5.01 ± 0.88	4.26−6.54
nonpareil	6	0.95 ± 0.15	0.82−1.14	6	1.40 ± 0.30	0.95−1.85
sonora	6	0.67 ± 0.23	0.47−1.12	6	0.63 ± 0.19	0.40−0.95
winters	6	0.14 ± 0.07	0.06−0.26	6	0.35 ± 0.18	0.12−0.67
wood colony	6	7.06 ± 1.68	5.49−9.42	4	6.26 ± 1.86	3.84−8.37
			Hull Total Cyanide (mg kg^{-1})			
aldrich	6	0.49 ± 0.26	0.30−0.93	6	0.20 ± 0.02	0.18−0.22
butte	4	0.27 ± 0.13	0.15−0.45	6	0.24 ± 0.05	0.18−0.31
carmel	6	0.38 ± 0.21	0.21−0.72	4	0.26 ± 0.09	0.17−0.35
fritz	4	0.97 ± 0.21	0.79−1.22	6	0.89 ± 0.56	0.19−1.65
independence	4	0.68 ± 0.15	0.50−0.86	6	1.35 ± 0.74	0.40−2.18
monterey	4	0.37 ± 0.20	0.15−0.56	6	0.14 ± 0.07	0.08−0.23
nonpareil	6	0.20 ± 0.09	0.12−0.37	6	0.11 ± 0.07	n.d.−0.19
sonora	6	0.23 ± 0.08	0.10−0.33	6	0.45 ± 0.12	0.33−0.61
winters	6	1.33 ± 0.85	0.61−2.50	6	1.18 ± 0.47	0.67−1.92
wood Colony	6	0.31 ± 0.15	0.18−0.55	4	0.38 ± 0.14	0.21−0.51

Table 5. Amygdalin, Prunasin, and Cyanide Levels Measured in Almond Kernels[a]

variety	n 2021	avg. ± SD	range	n 2022	avg. ± SD	range
			Amygdalin (mg kg^{-1})			
aldrich	6	158.32 ± 42.83	120.83−225.56	6	193.09 ± 35.90	154.84−234.00
butte	4	4.78 ± 2.24	0.27−6.13	6	5.59 ± 6.03	0.26−14.03
carmel	6	63.99 ± 27.54	29.23−98.99	4	100.63 ± 18.46	82.90−124.00
fritz	4	105.91 ± 31.16	70.81−117.64	6	91.03 ± 21.73	55.57−103.08
independence	4	1.76 ± 1.16	0.53−3.24	6	2.18 ± 1.09	0.46−3.44
monterey	4	60.82 ± 13.08	47.08−78.02	6	69.34 ± 14.20	45.57−72.94
nonpareil	6	9.85 ± 2.67	6.64−14.50	6	18.27 ± 4.06	11.82−23.57
sonora	6	7.99 ± 3.01	4.13−11.46	6	9.15 ± 2.38	6.73−13.26
winters	6	2.36 ± 1.10	0.94−4.33	6	5.05 ± 2.01	2.07−8.18
wood colony	6	79.14 ± 19.33	54.78−99.31	4	78.40 ± 25.04	42.93−113.50
			Prunasin (mg kg^{-1})			
aldrich	6	19.07 ± 7.70	9.37−30.96	6	19.24 ± 6.06	10.81−28.91
butte	4	0.24 ± 0.48	n.d.	6	0.71 ± 1.11	n.d.−2.86
carmel	6	7.25 ± 3.66	4.40−12.26	4	10.48 ± 1.14	9.39−12.67
fritz	4	8.00 ± 1.78	5.69−9.67	4	7.94 ± 4.10	4.62−15.82
independence	4	n.d.	n.d.	6	n.d.	n.d.
monterey	4	7.87 ± 3.58	3.81−11.02	6	4.15 ± 1.85	0.69−7.29
nonpareil	6	0.82 ± 0.46	n.d.−1.74	6	1.11 ± 0.39	0.63−1.53
sonora	6	0.48 ± 0.54	n.d.−1.22	6	n.d.	n.d.
winters	6	n.d.	n.d.	6	n.d.	n.d.
wood colony	6	7.64 ± 3.64	3.83−13.52	4	6.34 ± 2.33	3.12−10.26
			Cyanide (mg kg^{-1})			
aldrich	6	3.66 ± 0.76	2.37−4.76	6	3.72 ± 1.26	1.96−4.94
butte	4	0.07 ± 0.14	n.d.−0.28	6	0.08 ± 0.14	n.d.−0.32
carmel	6	1.71 ± 0.59	0.91−2.61	4	2.25 ± 0.32	1.84−2.73
fritz	4	1.95 ± 0.31	1.56−2.24	4	1.50 ± 0.46	0.96−2.06
independence	4	n.d.	n.d.	6	n.d.	n.d.
monterey	4	1.83 ± 1.28	0.60−3.54	6	0.67 ± 0.25	0.36−1.00
nonpareil	6	0.32 ± 0.10	0.20−0.48	6	0.26 ± 0.07	0.17−0.37
sonora	6	0.17 ± 0.15	n.d.−0.37	6	0.09 ± 0.07	n.d.−0.15
winters	6	n.d.	n.d.	6	0.05 ± 0.08	n.d.−0.19
wood colony	6	1.93 ± 0.96	0.94−3.63	4	1.25 ± 0.56	0.51−2.20

[a]n.d., not detected

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Table 6. Amygdalin, Prunasin, and Cyanide Levels Measured in Almond Hulls[a]

variety	n 2021	avg. ± SD	range	n 2022	avg. ± SD	range
			Amygdalin (mg kg^{-1})			
aldrich	6	2.46 ± 2.01	0.79−5.77	6	0.56 ± 0.27	0.29−1.09
butte	4	0.83 ± 0.91	0.10−1.99	6	1.05 ± 0.49	0.19−1.22
carmel	6	0.87 ± 0.65	0.29−1.69	4	0.28 ± 0.11	0.19−0.44
fritz	4	3.85 ± 1.62	0.87−5.72	6	4.78 ± 3.36	0.71−8.99
independence	4	5.47 ± 2.68	2.97−8.63	6	11.58 ± 7.30	2.47−22.01
monterey	4	1.10 ± 1.02	0.22−1.99	6	0.30 ± 0.19	0.10−0.60
nonpareil	6	0.19 ± 0.10	0.08−0.33	6	0.28 ± 0.20	n.d.−0.46
sonora	6	0.55 ± 0.37	0.19−0.93	6	0.71 ± 0.68	0.11−1.73
winters	6	4.94 ± 3.89	1.50−8.13	6	6.67 ± 3.78	2.70−12.60
wood colony	6	0.85 ± 0.36	0.61−1.47	4	0.74 ± 0.22	0.51−1.03
			Prunasin (mg kg^{-1})			
aldrich	6	2.08 ± 1.72	0.73−4.98	6	0.56 ± 0.45	n.d.−1.27
butte	4	1.04 ± 1.36	n.d.−2.87	6	1.15 ± 0.80	n.d.−2.16
carmel	6	0.98 ± 0.93	n.d.−2.87	4	0.40 ± 0.05	0.35−0.48
fritz	4	10.07 ± 2.54	7.94−13.04	6	7.68 ± 5.45	1.28−16.03
independence	4	2.52 ± 0.68	1.89−3.49	6	6.59 ± 3.43	1.85−10.63
monterey	4	2.39 ± 2.77	n.d.−4.98	6	0.33 ± 0.18	n.d.−0.53
nonpareil	6	0.13 ± 0.21	n.d.−0.44	6	0.52 ± 0.30	n.d.−0.79
sonora	6	0.62 ± 0.52	n.d.−1.22	6	1.63 ± 2.01	0.29−5.29
winters	6	13.48 ± 12.02	n.d.−28.87	6	11.10 ± 4.46	5.59−17.43
wood colony	6	0.47 ± 0.30	n.d.−0.79	4	0.47 ± 0.22	0.28−0.73
			Cyanide (mg kg^{-1})			
aldrich	6	0.22 ± 0.07	0.14−0.31	6	0.13 ± 0.04	0.08−0.17
butte	4	0.16 ± 0.02	0.14−0.19	6	0.11 ± 0.04	0.06−0.17
carmel	6	0.27 ± 0.12	0.17−0.49	4	0.22 ± 0.09	0.13−0.32
fritz	4	0.15 ± 0.05	0.11−0.21	6	0.15 ± 0.07	0.07−0.25
independence	4	0.21 ± 0.10	0.14−0.35	6	0.28 ± 0.16	0.14−0.57
monterey	4	0.17 ± 0.06	0.11−0.25	6	0.11 ± 0.05	0.07−0.18
nonpareil	6	0.18 ± 0.09	0.12−0.34	6	0.07 ± 0.06	n.d.−0.16
sonora	6	0.16 ± 0.05	0.11−0.22	6	0.32 ± 0.12	0.17−0.49
winters	6	0.24 ± 0.10	0.14−0.37	6	0.13 ± 0.02	0.10−0.16
wood colony	6	0.23 ± 0.18	0.09−0.52	4	0.31 ± 0.16	0.13−0.46

[a]n.d., not detected.

based calibration curve, using eq 3. We observed an error of 17.82% for amygdalin and 19.57% for prunasin in almond hulls. An error blow 20% suggests a low to moderate matrix effect, as reported in Shin et al. (2018) and Ferre et al. (2011), and we deemed the solvent-based calibration curve acceptable.[38,39]

For CNCysEt, recoveries ranged from 83.51% to 85.80% in kernels and 104.93% to 112.99% in hulls. Recoveries in kernels are comparable to other studies, which ranged from 86% to 102%; however, these studies evaluate CN content through colorimetric methods utilizing picrate paper and acid hydrolysis.[27,31] A calculated matrix effect of 0.07% for kernels and 0.98% for hulls was measured for CNCysEt, indicting little to no matrix effect. However, separate calibration curves prepared using Nonpareil kernel and hull extracts were used to better account for free CN and CN generated during extraction and derivatization steps. The average endogenous CN levels, measured as CNCysEt, in unspiked samples were subsequently subtracted from CNCysEt levels measured in spiked samples to account for matrix contributions:

$$\text{matrix effect} = \left(\frac{m_{\text{matrix}}}{m_{\text{calibration}}} - 1\right) \times 100\% \quad (3)$$

where m_{matrix} is the slope of the matrix and $m_{\text{calibration}}$ is the slope of the solvent calibration curve.

Cyanogenic Glycoside and Hydrogen Cyanide Levels in California Almond Varieties. The combined levels of amygdalin, prunasin, and cyanide (total cyanide content) measured in all almond varieties, in both kernels (<20 mg kg^{-1}) and hulls (<3 mg kg^{-1}), were well under the EU maximum level of 35 mg kg^{-1} equivalents of CN in both the 2021 and 2022 harvest years (Table 4).[14] At the highest measured levels (20 mg CN kg^{-1} almond kernel), a 28 g serving of almond kernels would result in only 7.18 μg CN kg^{-1} bw for an adult with an average body weight of 78 kg. In general, almond hulls have significantly lower levels of CN as compared to almond kernels. In kernels, Aldrich, Carmel, Fritz, and Wood Colony varieties show the highest total cyanide content across all measured varieties (Table 5), similar to previous studies.[8,22,24,40,41] The higher levels of amygdalin generally corresponded with higher levels of prunasin and CN in the kernels. This trend was not apparent in the hulls as prunasin levels were frequently higher than amygdalin levels and were variety dependent (Table 6, Figure 4). The low levels of cyanide-producing compounds found in hulls indicated that they may be considered for use in human food products without additional processing or fermentation (as with cassava).

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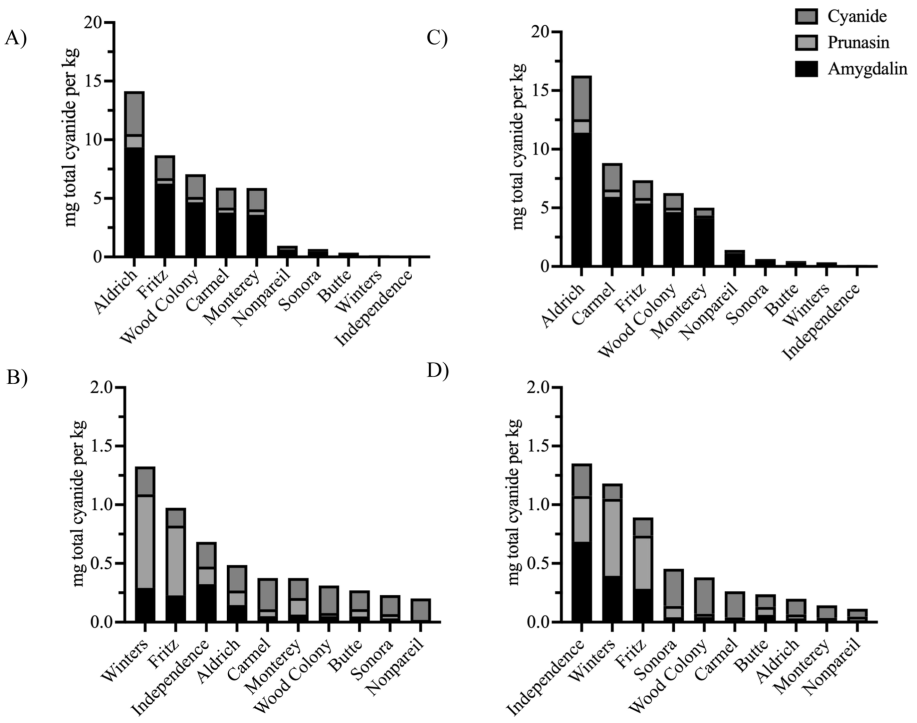

Figure 4. Levels of amygdalin, prunasin, and hydrogen cyanide (HCN) expressed as total HCN equivalent amount for (A) 2021 kernels, (B) 2021 hulls, (C) 2022 kernels, and (D) 2022 hulls.

The highest levels of average total cyanide content were found in Aldrich kernels with 14.14 and 16.27 mg kg^{-1} in the 2021 and 2022 harvest season, respectively. In hulls, Winters was the only variety to have a higher total cyanide content in the hulls (1.33 and 1.18 mg kg^{-1}; 2021 and 2022 respectively) as compared to kernels (0.14 and 0.35 mg kg^{-1}; 2021 and 2022 respectively). For all other varieties the levels of total cyanide were significantly lower in hulls as compared to kernels, with all varieties producing less than 3 mg kg^{-1}. However, the total cyanide contribution from amygdalin, prunasin, and CN differs between kernels, hulls and variety. Amygdalin is the main cyanide contributor for kernels (Figure 4); however, in hulls, prunasin and CN contribute more depending on the variety (Figure 4).

When looking at each analyte by their own mass contribution to CN, amygdalin is significantly higher ($P < 0.001$) than both prunasin and CN in almond kernels. However, in the hulls only a few varieties showed differences across compounds. Amygdalin levels were significantly higher ($p < 0.05$) than CN in Aldrich, Butte, Independence, and Wood Colony. Fritz and Winters had significant ($p < 0.05$) differences between all compounds where prunasin had the highest levels, followed by amygdalin, and then CN. There was no obvious trend in the levels of amygdalin, prunasin, and CN in almond hulls across all varieties, as compared to the kernels, where amygdalin was the significant cyanide contributing compound.

Herein, amygdalin and prunasin were measured in both the kernels and hulls. The higher levels of amygdalin found in mature kernels and the presence of prunasin in both the kernels and hulls provides some support to a theory proposed by Dicenta et al. (2002) that amygdalin is used for nitrogen storage in kernels, while prunasin is primarily transported through the plant.[6] Similarly, Sánchez-Pérez et al. (2008) and Thodberg et al. (2018) reported the presence of both prunasin and amygdalin in almond kernels with the intact seed coat (tegument), finding that amygdalin levels were higher in kernels, while prunasin levels were higher in the seed coat.[7,42] Thodberg et al. (2018) connected these higher levels of prunasin in the seed coat to the presence of PdCYP79D16, PdCYP71AN24, and PdUGT85A19/PdUGT94AF3 genes found there, which primarily synthesize prunasin; however, the PdUGT94F1 and PdUGT94F2 genes found in the kernels primarily convert prunasin to amygdalin, resulting in higher levels of amygdalin.[42] Furthermore, Sánchez-Pérez et al. (2008) reported prunasin was undetected in the mesocarp (hull) in the bitter, semibitter, and sweet almond varieties, with measurable levels of prunasin primarily found in the seed coat of the fruiting body.[7] However, California almonds reported in our paper had detectable levels of prunasin (and amygdalin) in the hulls and mature kernels, slightly differing from the findings of Dicenta et al. (2002) and Sánchez-Pérez (2008) where no prunasin was detected. It is likely that the methods used by Dicenta et al. (2002) and Sánchez-Pérez (2008) had LODs (unreported) below the levels of prunasin found in mature kernels and hulls, and consequently did not detect it in their studies.[6,7] However, our study only focused on mature kernels with intact seed coats, not allowing differentiation between levels of prunasin in the seed coat compared to the kernel tissue. Future studies analyzing the seed coat separately from kernel tissue using the method presented in this paper could provide more insight on the levels of CNGs, especially prunasin, in different components of the almond. Overall, the general trends and presence of amygdalin and prunasin in California almond kernels and hulls in our study provide support to the theories of Sánchez-Pérez et al. (2008) and Thodberg et al. (2018) that prunasin, present in the seed coat, is transferred to the kernel where it is glycosylated to form amygdalin.[7,42,43]

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Overall, cyanogenic glycosides and cyanide levels in California sweet almonds, for both kernels and hulls, are well below the EU maximum of 35 mg CN kg^{-1} almond.[14] This new method provides comprehensive analysis of the cyanide forming compounds in almonds and is highly sensitive and accurate. If hulls are considered for food products, methods measuring only amygdalin and/or CN may not accurately predict total CN levels since prunasin is a major contributor to CN content for some varieties. However, levels of CNGs and CN reported from this method show that hulls, which accumulate considerably less CNGs and CN than kernels, are likely safe for use in human food products, potentially providing a new fiber and antioxidant source.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c08437.

UHPLC-(+ESI)QTOF-MS/MS spectra for the reaction of cysteine ethyl ester with CN as well as UHPLC-(ESI+)-MS/MS chromatograms of all compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HCN, hydrogen cyanide; ^{13}CNCysEt, ^{13}C labeled cyano-*S*-ethyl-*O*-cysteine; CN, cyanide; CNGs, cyanogenic glycosides; CNCysEt, cyano-*S*-ethyl-*O*-cysteine; UAE, ultrasonic-assisted extraction; LOD, limit of detection; LOQ, limit of quantitation; MLOD, method limit of quantitation; UHPLC-(+ESI)-MS/MS, ultrahigh performance liquid chromatograph-electrospray ionization tandem mass spectrometer; dMRM, dynamic multiple reaction monitoring; SD, standard deviation; UHPLC-(+ESI)QTOF- MS/MS, ultrahigh performance liquid chromatograph-electrospray ionization quadrupole time-of-flight mass spectrometer; ISTD, internal standard

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