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Journal

Journal of Agricultural and Food Chemistry, 63(37)

ISSN

0021-8561

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Publication Date

2015-09-23

DOI

10.1021/acs.jafc.5b03121

Peer reviewed

Effect of Temperature and Moisture on the Development of Concealed Damage in Raw Almonds (*Prunus dulcis*)

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ABSTRACT: Concealed damage (CD) is a brown discoloration of nutmeat that appears only after kernels are treated with moderate heat (e.g., roasting). Identifying factors that promote CD in almonds is of significant interest to the nut industry. Herein, the effect of temperature (35 and 45 °C) and moisture (<5, 8, and 11%) on the composition of volatiles in raw almonds (*Prunus dulcis* var. Nonpareil) was studied using HS-SPME-GC/MS. A CIE *LCh* colorimetric method was developed to identify raw almonds with CD. A significant increase in CD was demonstrated in almonds exposed to moisture (8% kernel moisture content) at 45 °C as compared to 35 °C. Elevated levels of volatiles related to lipid peroxidation and amino acid degradation were observed in almonds with CD. These results suggest that postharvest moisture exposure resulting in an internal kernel moisture $\geq 8\%$ is a key factor in the development of CD in raw almonds and that CD is accelerated by temperature.

KEYWORDS: *Prunus dulcis*, almond, Nonpareil, concealed damage, volatiles, HS-SPME-GC/MS, gas chromatography, mass spectrometry, color

■ INTRODUCTION

California is the primary producer of almonds (*Prunus dulcis* (Mill.) D.A. Webb), accounting for ~100% of the domestic production and ~80% of world production.¹ A significant problem for the industry is a brown discoloration of the kernel interior (nutmeat) that appears only after moderate to high heat treatment (blanching, drying, roasting, etc.). This defect is called concealed damage and is shown in Figure 1. Almond kernels with CD have no visible defects on the exterior of the raw kernel and no visible signs of CD on the surface of whole roasted kernels.² CD is frequently associated with bitter flavors that can result in immediate consumer rejection.³ Currently there are no screening methods available for detecting CD in raw almonds, and nut processors often do not realize nuts are damaged until after they have been roasted.³

Concealed damage may develop anytime during or after harvest (i.e., in windrows or stockpiles) if kernels are exposed to moisture and heat.^{3,4} Typically, commercial almonds are allowed to dry in windrows to a kernel moisture content of <6% and stockpiled until processed.⁵ Temperatures in windrows can range from ambient to 70 °C.³ Stockpile temperatures can range from 30 to 60 °C, and moisture levels can increase to 30%.^{6,7} During a wet harvest season, almonds may be exposed to rain in windrows and/or stockpiles. When this occurs, almonds often require mechanical drying and may exceed the capacity of the processor. This can create longer periods of time that almond kernels are exposed to moisture.

The development of CD is related, in part, to moisture-induced hydrolysis of sugars, which increases the availability of reducing sugars for Maillard browning reactions. For example, in macadamia nuts exposed to moisture during harvesting, increased levels of reducing sugars are observed and co-locate with internal nut browning.⁸ A similar observation was made in

hazelnuts⁹ and in almonds exposed to simulated rainfall.³ Internal browning of kernel nutmeat also increases in response to temperature.¹⁰ For example, in macadamia nuts exposed to moisture during harvest and dried at ambient temperatures or with heated air at 52 °C, 15 and 17% of the kernels displayed browning, respectively. When these nuts were dried above 60 °C, 100% of the nuts displayed brown centers. Internal browning can be induced in nuts exposed to moisture levels above 20% (wet basis) above 30 °C. At 45 °C and a moisture content >30%, the defect occurs after only 2 days.⁸

There is a corresponding contribution of both lipid oxidation and Maillard reaction products to nonenzymatic browning.¹¹ This may be especially important in almonds and other nuts as they have significant lipid content. For example, 50–60% of the almond kernel weight is derived from lipids, primary oleic acid (70–80%) and linoleic acid (20–30%).^{12,13} Exposure of almonds to high moisture can lead to an increase in the oxidation of lipids and production of a range of compounds such as aldehydes¹⁴ that can act as precursors for the Maillard reaction.^{11,15} For example, 4,5-epoxy-2-alkenals have been shown to react with lysine amino groups to produce N-substituted hydroxyalkylpyrroles. These compounds polymerize spontaneously to form melanoidin-like pigments.¹⁶ During seed aging and storage, lipid peroxidation appears to drive Maillard reactions at water contents around 16% moisture, whereas reducing sugars from sugar hydrolysis dominate at higher moisture contents.^{16,17}

Received: June 25, 2015

Revised: August 24, 2015

Accepted: August 29, 2015

Published: August 29, 2015

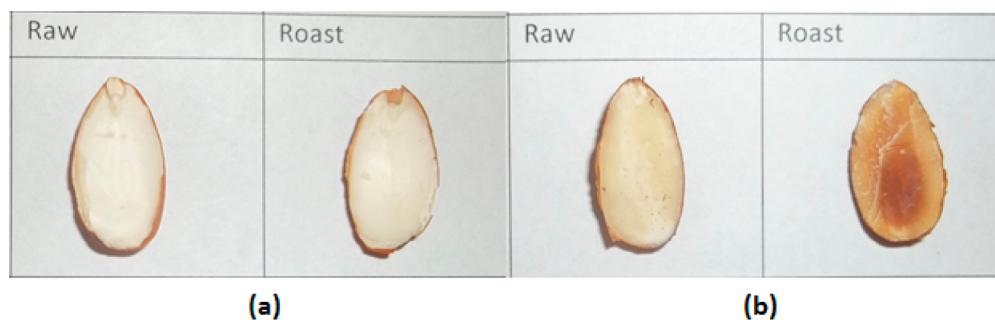


Figure 1. Effect of moisture on color development before and after roasting at 120 °C for 90 min in (a) raw and roasted almonds exposed to 5% moisture (control) and (b) raw and roasted almonds exposed to 11% moisture.

Little information is available regarding the chemical changes that occur in raw almonds with CD as most experimental data are derived on roasted nuts where the defect can be seen. However, information on the chemical changes that occur in the raw almonds is critical for developing screening methods and for optimizing drying, roasting, and storage conditions to decrease product loss related to CD. The specific objectives of this study were to (1) develop a colorimetric method based upon CIE *LCh* color values to identify CD in raw almonds; (2) evaluate the influence of temperature and moisture on the development of CD in raw almonds; and (3) evaluate the volatile profiles of raw almonds with CD to further identify the chemical changes that occur with the development of CD.

MATERIALS AND METHODS

Chemicals and Reagents. Reagents were purchased from either Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA) and included C_7 – C_{40} saturated alkanes standard (1000 $\mu\text{g}/\text{mL}$ in hexane), ethanol (HPLC/spectrophotometric grade), and 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, heptanol, 1-octanol, 1-nonanol, benzyl alcohol, phenylethyl alcohol, hexanal, nonanal, and benzaldehyde. Octanal- d_{16} , 2-methylpyrazine- d_6 , and *n*-hexyl- d_{13} alcohol were used as stable isotope internal standards for three major categories of identified compounds (i.e., aldehydes, pyrazines, and alcohols). Stable isotopes were purchased from C/D/N Isotopes Inc. (Quebec, Canada).

Experimental Design. Dehulled raw kernels (100 lb, var. Nonpareil) were supplied by the Nickels Soil Laboratory (Arbuckle, CA, USA) in September 2012. Twenty-seven vessels each containing 100 almond kernels were exposed to conditions that produced an internal kernel moisture content of 5% (actual $4.75 \pm 0.4\%$), 8% (actual $8.4 \pm 0.35\%$), or 11% (actual $11.55 \pm 0.35\%$) moisture in a controlled-atmosphere incubator (Thermo Scientific, Marietta, OH, USA) at 35 ± 2 °C. Three individual vessels were removed every other day, and samples were evaluated for moisture, CIE *LCh* color, and volatile analysis by HS-SPME-GC/MS. Almonds treated at 5 and 8% moisture were monitored for 18 days. Almonds treated with 11% moisture were monitored for 7 days. The same procedure was repeated at 45 ± 2 °C.

Moisture Determination. The moisture content of the almonds was determined gravimetrically by drying homogenized samples (~ 1 g) at 95–105 °C under vacuum for 48 h. Moisture was determined in triplicate, and the results were averaged.

Colorimetric Measurements. Raw almond kernels (420) were split in half along the natural seam, and each half was placed into one of two identical racks with individual identifying slots. One rack of the split kernels was roasted at 120 °C for 90 min, whereas the other rack was not roasted. Almonds with visual apparent darkening after roasting (i.e., those with CD) were grouped separately from the nondarkened kernels (i.e., those without CD), and the surface color of each individual kernel was measured using a LabScan XE spectrophotometer (HunterLab, Reston, VA, USA). The color values L^*

(lightness), C (chroma), and h (hue), according to the CIE *LCh* color scale, were recorded using a port size of 0.4 in. with a D65 optical sensor, 0° geometry, and 10° angle of vision.

As CD is apparent only after heat treatment, raw almond half-kernels with CD were identified by matching the half-kernels that developed CD upon roasting with the corresponding raw half-kernels. Color measurement of raw kernels was made as described above. Evaluations were performed in triplicate and the results averaged.

HS-SPME-GC/MS Analysis. Volatiles were analyzed using a previously reported method, with a smaller sample size (200 mg versus 5 g).¹⁶ Briefly, ground almonds (200 mg) were added to a 2 mL crimp-top vial with 5 μL of mixture of internal standard (10 $\mu\text{g}/\text{mL}$, octanal- d_{16} , 2-methylpyrazine- d_6 , and *n*-hexyl- d_{13} alcohol) and sealed with an aluminum seal (PTFE/silicone liner, Fisherbrand, Fisher Scientific, USA). The samples were incubated for 60 min at room temperature. Samples were exposed to a previously conditioned 1 cm 50/30 μm DVB/CAR/PDMS solid phase microextraction (SPME) fiber (Supelco, Inc., Bellefonte, PA, USA) over the headspace (HS) of the sample for 60 min at ambient temperature. The SPME fiber was immediately injected for 10 min into a Hewlett-Packard 6890 series gas chromatography (GC) system coupled with a HP 5973 mass selective detector (MS; Agilent Technologies, Palo Alto, CA, USA). An Agilent DB-Wax column (30 m length, 0.25 mm i.d., 0.25 μm film) was used to separate compounds. The oven temperature program started with an initial setting of 40 °C for 1 min, followed by a ramp of 5 °C/min to 180 °C, then 10 °C/min to 210 °C with a hold time of 3 min. The injector temperature was set at 240 °C. Helium was used as the carrier gas at a flow at 0.7 mL/min. MS transfer line temperature was set at 250 °C. The temperatures of MS quadrupole and MS source were 150 and 230 °C, respectively. Total ion chromatograms (TICs) were collected by scanning from m/z 30 to 180 at a rate of 2.48 scans/s.

Identification and Relative Quantitation. Volatile compounds were identified by comparison of their MS spectra and retention times with those of authentic standards. Volatile compounds without authentic standards were tentatively identified by comparing the Kovats' retention indices (KI) and/or mass spectrum with those reported in the NIST Mass Spectral Search Program (version 2.0 a) with >80% as a cutoff to match compounds. The KIs were calculated from the retention times of C_7 – C_{40} *n*-alkanes.

Relative quantitation of each volatile compound was performed by comparing the total peak area at of each compound to the total peak area of one of three internal standards (IS) (i.e., octanal- d_{16} , 2-methylpyrazine- d_6 , and *n*-hexyl- d_{13} alcohol, for aldehydes, pyrazines, and alcohols, respectively). Relative concentration was determined using eq 1 according to Baek et al.¹⁷ and Hopfer et al.¹⁸

$$\text{rel concn} \left(\frac{\text{ng}}{\text{g}} \right) = \frac{\left(\frac{\text{peak area}}{\text{IS peak area}} \right)}{\text{sample wt}} \times \text{IS added} \quad (1)$$

Statistical Analysis. All statistical analyses were performed using Graphpad Pism version 6.01 (GraphPad Software, Inc.). All data sets were tested for significance (p value < 0.05) using a t test for

Table 1. CIE *LCh* (Lightness, Chroma, and Hue) Color Values for Raw and Roasted Almonds with Concealed Damage (CD) and without Concealed Damage (NCD)^a

	raw almonds		roasted almonds	
	NCD	CD	NCD	CD
lightness (<i>L</i>)	79.46 ± 2.97	70.62 ± 6.40	78.71 ± 3.69	58.82 ± 8.22
chroma (<i>C</i>)	16.65 ± 2.26	20.69 ± 2.32	20.55 ± 3.32	31.82 ± 4.48
hue (<i>h</i>)	87.24 ± 1.15	86.24 ± 1.52	82.73 ± 12.45	71.20 ± 4.11

^aAll values are significant at the $p < 0.05$ level.

comparing temperature (35 and 45 °C), moisture content (5, 8, and 11%), and group (NCD and CD).

Values given in Table 3 were calculated as follows. The mean (18 day average) was calculated for each volatile at 5 and 8% moisture, in each group (i.e., CD and NCD) at 35 °C (data not shown). The difference between the means was determined (CD – NCD) and is given in column (a) (5% moisture) or column (b) (8% moisture). A positive value indicates that the volatile is higher in the CD group as compared to the NCD group. The value reported in column (c) (% relative change) was obtained using eq 2.

$$\text{rel \% change} = \frac{(\text{mean at 8\%} - \text{mean at 5\%})}{\text{mean at 5\%}} \times 100 \quad (2)$$

A positive value indicates that there was an increase in this volatile in the 8% moisture group relative to the 5% moisture group. Values for Table 4 were calculated as above but using data obtained at 45 °C.

Values given in Table 5 were calculated as follows. The mean (18 day average) was calculated for each volatile at 35 and 45 °C, at 5 or 8% moisture (data not shown). The difference between the group means (45 – 35 °C) for each volatile was calculated, and data are given in column (a) for 5% moisture or in column (b) for 8% moisture. A positive value indicates that there was an increase in the volatile at 45 °C relative to that at 35 °C. The value reported in column (c) (% relative change) was obtained using eq 3.

$$\text{rel \% change} = \frac{(\text{mean at 45 } ^\circ\text{C} - \text{mean at 35 } ^\circ\text{C})}{\text{mean at 35 } ^\circ\text{C}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Although CD is defined by the almond industry as a “dark” brown color covering ~50% of the kernel after heat treatment, this definition varies among processors and identification relies on a subjective visual inspection of the roasted kernels.¹⁹ To date, processors have tried several methods to identify the extent of CD before roasting, including blanching raw almonds and splitting open the kernels and visually determining the number of kernels with CD. Currently, there are no reliable methods for measuring CD in raw almonds, and a lack of screening methods can result in considerable production losses. Herein the CIE lightness (*L*), chroma (*C*), and hue (*h*) color values were measured in raw and roasted almonds with the goal of identifying a numerical color value corresponding to CD in both raw and roasted almonds. As CD is not visible in raw almonds, kernels were first split in half along the natural seam, and half of the kernel was roasted at 120 °C for 90 min, whereas the other half was not. CIE color values were measured in the roasted kernel halves with visual discoloration over ≥50% of the kernel (i.e., those with visible CD; Figure 1b) and in roasted kernels without visual discoloration (those with no visible CD (NCD); Figure 1a), and the results are given in Table 1. The means of the lightness (*L*) color value had the greatest difference (78.71 ± 3.69 for NCD and 58.82 ± 8.22 for CD) as compared to hue (*h*) and chroma (*C*) and allowed for the separation of CD and NCD almonds using an *L* color value cutoff of 70.00 (Table 1). More than 400 almonds were

evaluated in this way to establish this relationship. Therefore, for the purpose of this study, roasted almonds with CD are defined as those with an *L* color value of ≤70.0, whereas those with an *L* color value of ≥70.0 are defined as having no concealed damage (NCD).

Using this definition, the roasted kernel halves were matched to their corresponding raw kernel halves, and the CIE *LCh* color values were measured in the raw kernels. In the raw kernels, the mean *L* color value was significantly lower in almonds with CD (70.62 ± 6.40) as compared to almonds with NCD (79.46 ± 2.97). The mean *C* color value was significantly higher in almonds with CD (20.69 ± 2.32) as compared to the NCD almonds (16.65 ± 2.26), and the mean *h* color value was significantly higher in NCD almonds (87.24 ± 1.15) as compared to the almonds with CD (86.98 ± 1.52). Although color values were significantly different at the $p < 0.05$ level in the raw almonds, there is not enough difference (±2 SD) for these values to be used as a robust screening tool for detecting CD in raw almonds by colorimetry. Therefore, all studies investigating the influence of moisture and temperature on volatiles relied on splitting almond kernels in half, roasting half, and identifying the roasted half kernels with CD using an *L* color value of ≤70.0. These were then matched with the corresponding raw kernel half to identify the raw kernels with CD.

In raw almonds exposed to 5% moisture and 45 °C, only 2–3% of the kernels formed CD over the 18 days of the study (Figure 2). These almonds were considered the control group for all comparisons. An approximately 20% increase in CD was observed in almonds exposed to 8% moisture at 35 °C. These levels remained consistent at ~25% over the 18 days. At 45 °C, the trend was similar and approximately 20% of the almonds

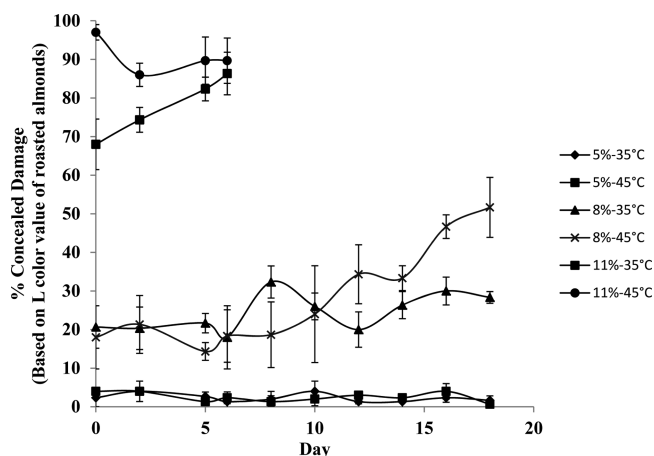


Figure 2. Percent concealed damage (CD) as measured by the CIE *L* color value in raw almonds held at 5, 8, and 11% moisture and stored at 35 and 45 °C for 18 days.

had CD until day 10. After the 10th day, CD increased, reaching 52% on the 18th day of the study. In comparison, almonds exposed to 11% moisture displayed a significant level of CD beginning the first day of the experiment. For example, $68 \pm 7\%$ of the almonds stored at $35\text{ }^{\circ}\text{C}$ displayed CD on the first day of this study and levels increased to $\sim 100\%$ by day 5, whereas $97 \pm 2\%$ of the almonds stored at $45\text{ }^{\circ}\text{C}$ displayed CD on the first day of the study (Figure 2). As so many of the almond kernels exposed to 11% moisture developed CD immediately, we were unable to make reliable comparisons between the CD and NCD almonds in this group. Walton et al.⁸ demonstrated a similar result in raw macadamia nuts exposed to a moisture content $>20\%$ for 5 days at temperatures $\geq 30\text{ }^{\circ}\text{C}$; after 5 days, discoloration affected 90% of the kernels.

The profile of volatile compounds was measured in raw almond kernels using HS-SPME-GC/MS.¹⁶ The main volatiles identified include 19 alcohols, 3 aldehydes, 3 lactones, and 3 organic acids (Table 2). Volatiles identified herein were similar to volatiles reported in Monterey and a Greek variety of almond (name not provided)^{20–22} and include 3-methyl-butanol, 3-methyl-3-buten-1-ol, 1-pentanol, 1-hexanol, benzyl alcohol, phenylethyl alcohol, hexanal, nonanal, benzaldehyde, butyrolactone, γ -hexalactone, and acetic acid. Herein, 1-nonanol was identified in almonds for the first time, whereas the following compounds were tentatively identified: 1-propanol, 2-methyl-2,3-pentanediol, 3-methyl-2-butanol, 2-methyl-3-pentanol, 2-methyl-2-buten-1-ol, γ -pentalactone, pentanoic acid, and hexanoic acid.

Almonds exposed to 5% moisture and stored at $35\text{ }^{\circ}\text{C}$ showed little significant difference in the volatile composition between the CD and NCD groups (Table 3, column a). More significant differences were observed when CD and NCD almonds from the 8% moisture group (Table 3, column (b)) were compared. In general, increasing the moisture content from 5 to 8% significantly increased levels of most volatiles (Table 3, column (c)) with the exceptions of hexanal and 3-methyl-1-butanol, which decreased. A decrease in hexanal could be due to increased dehydrogenase activity at the higher moisture level.

In contrast, at $45\text{ }^{\circ}\text{C}$ and either 5 or 8% moisture, significant differences were observed for numerous volatile compounds (Table 4, columns (a) and (b)). Significant increases ($p < 0.05$) were observed in the aldehydes (i.e., hexanal, nonanal, and benzaldehyde) and lactones (i.e., γ -pentalactone, butyrolactone, and γ -hexalactone) at $45\text{ }^{\circ}\text{C}$ as compared to $35\text{ }^{\circ}\text{C}$. In general, increases in temperature enhance the rate at which many reactions (chemical, enzymatic, and metabolic) occur,²³ which could explain the higher levels of volatile found in both the 5 and 8% moisture groups at $45\text{ }^{\circ}\text{C}$ as compared to $35\text{ }^{\circ}\text{C}$. Increasing moisture from 5 to 8% at $45\text{ }^{\circ}\text{C}$ also produced a significant increase in the relative concentrations of almost all volatiles (Table 4, column (c)). Volatiles that increased can be broadly categorized as byproducts of lipid oxidation (3–9 carbon alcohols, aldehydes, and organic acids), lipid metabolism (lactones), and compounds resulting from increases in enzymatic activities (benzaldehyde, phenylethyl alcohol, benzyl alcohol). These findings agree with results reported by Mira et al.,²⁴ who characterized the volatiles in lettuce seeds stored at $35\text{ }^{\circ}\text{C}$ with water contents ranging from 3 to 9% moisture. Seeds stored at a water content $>6\%$ moisture showed an increase in volatiles related with lipid peroxidation (4–7 carbon alkanes, alcohols, aldehydes, and ketones). When the effect of just temperature (Table 5) is considered, it is apparent that

Table 2. HS-SPME-GC/MS Identification of Volatiles in Raw Almonds (var. Nonpareil)

volatile compound	KI	standard KI	literature KI ^a	internal standard ^b
1-propanol ^c	1037		1037/1038	hexyl- <i>d</i> ₁₃ alcohol
hexanal ^d	1078	1070	1084/1067	octanal- <i>d</i> ₁₆
2-methyl-1-propanol ^d	1098	1093	–/1085	hexyl- <i>d</i> ₁₃ alcohol
2-methyl-2,3-pentanediol ^c	1114			hexyl- <i>d</i> ₁₃ alcohol
3-methyl-2-butanol ^c	1125			hexyl- <i>d</i> ₁₃ alcohol
1-butanol ^d	1149	1147	1145/1138	hexyl- <i>d</i> ₁₃ alcohol
2-methyl-3-pentanol ^c	1161			hexyl- <i>d</i> ₁₃ alcohol
3-methyl-1-butanol ^d	1214	1207	1205/1206	hexyl- <i>d</i> ₁₃ alcohol
3-methyl-3-buten-1-ol ^c	1254		–/1240	hexyl- <i>d</i> ₁₃ alcohol
1-pentanol ^d	1257	1251	1255/1244	hexyl- <i>d</i> ₁₃ alcohol
2-methyl-2-buten-1-ol ^c	1329		–/1315	hexyl- <i>d</i> ₁₃ alcohol
1-hexanol ^d	1362	1354	1360/1360	hexyl- <i>d</i> ₁₃ alcohol
nonanal ^c	1399	1393	1385/1402	octanal- <i>d</i> ₁₆
acetic acid ^c	1454		1450/1452	hexyl- <i>d</i> ₁₃ alcohol
heptanol ^d	1464	1456	1467/–	hexyl- <i>d</i> ₁₃ alcohol
2-ethyl-1-hexanol ^c	1498		–/1492	hexyl- <i>d</i> ₁₃ alcohol
benzaldehyde ^d	1527	1519	1495/1525	octanal- <i>d</i> ₁₆
1-octanol ^d	1567	1559	1553/1566	hexyl- <i>d</i> ₁₃ alcohol
1,2-propanediol ^c	1599		–/1603	hexyl- <i>d</i> ₁₃ alcohol
γ -pentalactone ^c	1614		–/1600	octanal- <i>d</i> ₁₆
butyrolactone ^c	1631		1647/1640	octanal- <i>d</i> ₁₆
1-nonanol ^d	1670	1661		hexyl- <i>d</i> ₁₃ alcohol
γ -hexalactone ^c	1707		–/1694	octanal- <i>d</i> ₁₆
pentanoic acid ^c	1746			hexyl- <i>d</i> ₁₃ alcohol
hexanoic acid ^c	1855		–/1847	hexyl- <i>d</i> ₁₃ alcohol
benzyl alcohol ^d	1882	1872	1865/–	hexyl- <i>d</i> ₁₃ alcohol
phenylethyl alcohol ^d	1919	1908		hexyl- <i>d</i> ₁₃ alcohol
total 2,3-butanediol ^{c,e}				hexyl- <i>d</i> ₁₃ alcohol

^aKI, Kovats' indices. Values were obtained from Flavornet, http://www.flavornet.org/f_kovats.html/Pherobase, <http://www.pherobase.com/database/kovats/kovats-index.php> ^bInternal standard used for relative quantitation. ^cCompounds "tentatively identified" on the basis of their MS spectra and MS fragmentation pattern. ^dCompounds verified with authentic standards. ^eCorresponds to the sum of the two isomers.

increases in temperature enhanced the levels of most volatiles measured. Greater than 90% increases were observed with compounds primarily related with lipid oxidation and include hexanal, nonanal, acetic acid, 2-ethyl-1-hexanol, γ -pentalactone, and pentanoic and hexanoic acid.

Although benzaldehyde and benzyl alcohol are important flavor components in bitter almonds, the levels appear to be

Table 3. Effect of Increasing Moisture from 5 to 8% at 35 °C on the Relative Concentrations of Volatiles in Raw Almonds^a

	(a) 5% moisture, difference between means (CD – NCD) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	(b) 8% moisture, difference between means (CD – NCD) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	(c) rel % change
1-propanol	-1.04 ± 2.24	-4.07 ± 2.69	5
hexanal	6.19 ± 4.26	ND	-100*
2-methyl-1-propanol	-2.06 ± 3.62	-3.31 ± 6.83	70*
2-methyl-2,3-pentanediol	ND	-0.13 ± 0.36	1267*
3-methyl-2-butanol	-0.08 ± 0.60	0.13 ± 2.16	169*
1-butanol	$-1.75 \pm 0.97^*$	0.03 ± 0.77	-15*
2-methyl-3-pentanol	0.07 ± 0.89	-0.50 ± 0.53	42*
3-methyl-1-butanol	15.10 ± 8.98	24.30 ± 47.90	139*
3-methyl-3-buten-1-ol	-0.80 ± 1.35	$1.56 \pm 0.74^*$	-7
1-pentanol	-6.99 ± 5.31	$40.60 \pm 15.20^*$	9
2-methyl-2-buten-1-ol	-0.61 ± 0.89	-1.09 ± 1.14	47*
1-hexanol	5.11 ± 11.90	$181 \pm 100^*$	152*
nonanal	0.95 ± 2.11	$16.10 \pm 2.52^*$	51*
acetic acid	12.50 ± 7.56	12.20 ± 20.90	153*
1-heptanol	-0.17 ± 1.27	7.15 ± 11.20	108*
2-ethyl-1-hexanol	-0.24 ± 0.59	-2.43 ± 5.52	609*
benzaldehyde	-0.17 ± 0.51	$3.96 \pm 1.28^*$	40*
1-octanol	-1.02 ± 1.09	3.84 ± 7.12	168*
1,2-propanediol	0.17 ± 0.48	-0.60 ± 0.51	18*
γ -pentalactone	ND	$3.62 \pm 0.78^*$	100*
butyrolactone	4.05 ± 3.11	$19.50 \pm 2.24^*$	87*
1-nonanol	$1.32 \pm 0.76^*$	-1.55 ± 1.24	215*
γ -hexalactone	ND	$2.90 \pm 0.51^*$	100*
pentanoic acid	0.42 ± 0.42	-0.34 ± 0.96	8
hexanoic acid	-0.26 ± 1.78	$-9.17 \pm 3.60^*$	125*
benzyl alcohol	-0.84 ± 0.59	$-3.66 \pm 1.33^*$	235*
phenylethyl alcohol	-0.19 ± 0.49	$-2.40 \pm 0.66^*$	678*
total 2,3-butanediol	0.60 ± 3.72	12.10 ± 12.00	240*

^aColumns (a) and (b) represent the difference between CD and NCD group means at 5 and 8% moisture, respectively. Column (c) represents the relative % change induced by increasing moisture from 5 to 8%. *, values significant at $p < 0.05$. ND, not detected; SEM, standard error of the mean.

relatively low in sweet almond varieties.^{16,18} The opposite appears to be true with 2,3-butanediol, for which levels are relatively higher in some sweet varieties as compared to bitter varieties.²⁵ To date, little information is available with respect to the content and relationships of these compounds in different varieties of sweet almonds. Herein, we show that benzyl alcohol and 2,3-butanediol are influenced by moisture but not temperature and that levels of benzaldehyde are influenced by temperature at low moisture levels (<5%).

In conclusion, we demonstrate that CIE *LCh* colorimetric measurement of raw almond kernels, for routine screening for CD, is not possible using the conditions described herein. However, CIE *LCh* colorimetric measurements of roasted almonds can be used to identify CD in roasted almonds and

Table 4. Effect of Increasing Moisture from 5 to 8% at 45 °C on the Relative Concentrations ($\mu\text{g}/\text{kg}$) of Volatiles in Raw Almonds^a

	(a) 5% moisture, difference between means (CD – NCD) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	(b) 8% moisture, difference between means (CD – NCD) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	(c) rel % change
1-propanol	$-12.00 \pm 1.40^*$	$-14.40 \pm 6.71^*$	90*
hexanal	$26.40 \pm 5.87^*$	$15.60 \pm 1.62^*$	-74*
2-methyl-1-propanol	$-14.60 \pm 3.62^*$	$-26.20 \pm 10.50^*$	87*
2-methyl-2,3-pentanediol	ND	$-2.32 \pm 0.55^*$	100*
3-methyl-2-butanol	$-2.87 \pm 0.47^*$	$-7.90 \pm 3.28^*$	224*
1-butanol	$-16.00 \pm 2.58^*$	$-15.70 \pm 3.73^*$	40*
2-methyl-3-pentanol	$-6.16 \pm 0.64^*$	$7.20 \pm 2.21^*$	61*
3-methyl-1-butanol	-22.70 ± 16.40	$-207 \pm 103^*$	264*
3-methyl-3-buten-1-ol	$-2.94 \pm 0.67^*$	$-1.93 \pm 0.74^*$	-17*
1-pentanol	$-57.60 \pm 22.00^*$	18.70 ± 23.30	-8
2-methyl-2-buten-1-ol	$-2.87 \pm 0.59^*$	1.43 ± 1.90	31*
1-hexanol	-55.40 ± 39.00	$287 \pm 113^*$	120*
nonanal	$20.60 \pm 4.90^*$	$30.70 \pm 3.17^*$	-10
acetic acid	$-15.70 \pm 3.84^*$	-66.20 ± 100.00	733*
1-heptanol	$-6.97 \pm 3.43^*$	$28.90 \pm 15.80^*$	188*
2-ethyl-1-hexanol	-0.33 ± 1.89	-0.96 ± 3.48	151*
benzaldehyde	$2.76 \pm 0.69^*$	$10.10 \pm 1.62^*$	-18
1-octanol	-3.78 ± 2.63	$19.20 \pm 9.45^*$	213*
1,2-propanediol	$-2.75 \pm 0.23^*$	-2.63 ± 1.89	165*
γ -pentalactone	$2.25 \pm 0.34^*$	$10.50 \pm 2.31^*$	945*
butyrolactone	$-3.91 \pm 0.92^*$	$32.77 \pm 7.15^*$	102*
1-nonanol	1.95 ± 1.14	-0.13 ± 2.53	117*
γ -hexalactone	$1.42 \pm 0.25^*$	$7.54 \pm 1.55^*$	1000*
pentanoic acid	0.45 ± 0.79	0.81 ± 1.72	92*
hexanoic acid	-0.41 ± 3.53	-1.17 ± 16.50	217*
benzyl alcohol	-0.64 ± 1.17	-1.33 ± 1.39	50*
phenylethyl alcohol	-0.59 ± 0.52	-1.94 ± 2.41	386*
total 2,3-butanediol	3.67 ± 4.84	-13.1 ± 11.15	78*

^aColumns (a) and (b) represent the difference between CD and NCD group means at 5 and 8% moisture, respectively. Column (c) represents the relative % change induced by increasing moisture from 5 to 8%. *, values significant at $p < 0.05$. ND, not detected; SEM, standard error of the mean.

have potential as a post-roasting screening tool. Studies of volatiles indicate that although the profiles are similar between CD and NCD almonds, the levels of volatiles related to lipid peroxidation and amino acid degradation are higher in the CD group. These studies suggest that postharvest moisture exposure resulting in a kernel moisture content $\geq 8\%$ is a key factor in the development of CD in almonds and that increases in temperature will accelerate this process. Future studies should focus on investigating if CD can be reduced by drying almonds with moisture levels $\geq 8\%$ to $< 6\%$ prior to roasting, evaluating the impact of CD on storage and shelf life of almonds, and alternative screening methods (e.g., near infrared reflectance spectroscopy, NIR) for detecting CD in raw almonds.

Table 5. Effect of Temperature at a Moisture Content of either 5 or 8% on the Relative Concentration of Volatiles in Raw Almonds^a

	(a)	(b)	(c)
	5% moisture, difference between means (45 – 35 °C) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	8% moisture, difference between means (45 – 35 °C) ($\mu\text{g}/\text{kg} \pm \text{SEM}$)	rel % change
1-propanol	-3.45 ± 1.88*	12.10 ± 3.92*	19*
hexanal	17.70 ± 4.70*	12.20 ± 1.95*	103*
2-methyl-1-propanol	1.94 ± 3.01	9.61 ± 6.80	12
2-methyl-2,3-pentanediol	ND	-1.21 ± 0.42*	-16
3-methyl-2-butanol	-0.08 ± 0.50	3.42 ± 2.12	13
1-butanol	3.31 ± 2.28	11.60 ± 2.58*	61*
2-methyl-3-pentanol	1.72 ± 0.91*	4.38 ± 1.38*	37*
3-methyl-1-butanol	28.50 ± 9.62*	280.00 ± 60.10*	65*
3-methyl-3-buten-1-ol	-1.90 ± 0.81*	-3.04 ± 0.58*	19*
1-pentanol	40.00 ± 13.1*	33.50 ± 13.80*	48*
2-methyl-2-buten-1-ol	1.61 ± 0.62*	0.26 ± 1.10*	7
1-hexanol	60.80 ± 20.80*	91.10 ± 83.00	32
nonanal	23.30 ± 3.51*	11.7 ± 4.44*	90*
acetic acid	1.12 ± 4.72	208.00 ± 50.50*	173*
1-heptanol	-2.07 ± 1.95	7.47 ± 10.00	11
2-ethyl-1-hexanol	1.73 ± 0.96*	-4.74 ± 3.19	310*
benzaldehyde	3.44 ± 0.52*	-1.83 ± 1.60	8
1-octanol	0.72 ± 1.45	5.81 ± 6.18	23
1,2-propanediol	-1.42 ± 0.41*	3.44 ± 1.00*	19
γ -pentalactone	1.13 ± 0.31*	5.11 ± 1.74*	94*
butyrolactone	2.37 ± 1.71	7.22 ± 5.70	21
1-nonanol	1.45 ± 0.72*	-1.16 ± 1.3* ⁸	2
γ -hexalactone	0.71 ± 0.20*	2.46 ± 1.22*	59*
pentanoic acid	2.35 ± 0.44	6.68 ± 0.96*	168*
hexanoic acid	5.49 ± 1.92*	27.0 ± 8.36*	103*
benzyl alcohol	2.53 ± 0.65*	-2.58 ± 1.04*	-0.3
phenylethyl alcohol	1.36 ± 0.35*	3.57 ± 1.27*	54*
total 2,3-butanediol	17.8 ± 3.00*	-20.4 ± 8.24*	-2

^aColumns (a) and (b) represent the difference between means at 35 and 45 °C. Column (c) represents the relative % change due to increasing temperature from 35 to 45 °C. *, values significant at $p < 0.05$. ND, not detected; SEM, standard error of the mean.

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Funding

The Almond Board of California provided financial support for this study. We also acknowledge the support of the John Kinsella Endowed Chair in food, nutrition, and health.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Thomas Gradziel (Plant Science, UC Davis) and Franz Niederholzer (UC Farm Advisor Colusa/Sutter/Yuba Counties) for many thoughtful conversations on almond flavor and breeding and for providing almond samples. Additionally, we thank Dr. Susan Ebeler and Anna Hjelmeland from the UC Davis Food Safety and Measurement Facility.

ABBREVIATIONS USED

CD, concealed damage; NCD, no concealed damage; HS-SPME-GC/MS, headspace solid phase microextraction gas chromatography/mass spectrometry

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■ NOTE ADDED AFTER ASAP PUBLICATION

This article published September 8, 2015 with a mistake in the spelling of an author name. Guangwei Huang's name was amended, and the article reposted September 14, 2015.