TRACKING LIPID OXIDATION IN STORED SHELLED AND INSHELL RAW ALMONDS BY HS-SPME-GC

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Abstract

HS-SPME-GC-FID and -MS were used to track the extent of lipid oxidation in shelled and inshell raw almonds, stored under different temperature/relative humidity (RH) conditions, over 24 months. In total, 5 aldehydes, 13 alcohols, 4 alkanes, and 10 miscellaneous organic volatiles were identified and quantified. The extraction temperature, extraction time, and desorption period of major almond volatiles were optimized. Quantitation was carried out in two ways: calibration curves of hexanol-d_{13} and hexanal-d_{12}, relating peak area to concentration, were developed; and the standard addition method was performed using these isotopologic-deuterated standards and an ultrapycnometer to determine the density of ground almond powder (GAP). With both methods of quantitation yielding <12% differences in calculated µg/mL concentrations of volatiles, the employment of deuterated isotopologues of analytes-of-interest was validated. Relative quantitation of these volatiles indicated that storage parameters (*i.e.*, temperature and % RH) were influential in their development, especially for volatiles that were known secondary lipid oxidation products of linoleic acid. To illustrate, hexanal levels within the first 8 mo of storage at 25 °C achieved levels of 5.5 and 7.2 µg/g GAP when stored under low- and high-humidity conditions, respectively. Hence, the comparison of volatile contents between inshell and shelled almonds throughout storage highlights a potential for the utilization of inshell storage as a means of promoting flavor stability, although this efficacy may be dependent on shell characteristics of the almond cultivar in question.

Tentative identifications of aroma-active cmpds in raw almonds after 24-mo

Numbering	Analyte	Odor Description
Aldehydes		
7	pentanal	fermented, nutty, fruity, berry
15	hexanal	green, grassy, leafy, sweaty
18	benzaldehyde	sweet, bitter, almond, cherry
24	octanal	aldehydic, citrus, green, fatty
29	nonanal	aldehydic, rose, peely, waxy
Alcohols		
4	1-propanol, 2-methyl-	ethereal, winey
6	1-butanol	oily, sweet, balsamic, whiskey
8	3-methylbut-3-en-1-ol	sweet, fruity
9	3-methyl-1-butanol	fusel, whiskey, fruity, banana
10	1-butanol, 2-methyl-, (S)-	ethereal, fresh
11	1-pentanol	fusel, oily, sweet, balsamic
12	prenol	fruity, green, lavender, yeasty
13	2,3-butanediol, {R-(R*,R*)}-	
14	2,3-butanediol	creamy
16	1-hexanol	herbal, fruity, alcoholic, sweet
26	benzyl alcohol	floral, rose, phenolic, balsamic
30	phenylethyl alcohol	floral, rose, dried rose
32	1-nonanol	fresh, floral, rose, orange, oily
Alkanes		
2	pentane, 3-methyl-	gasoline-like
3	*n*-hexane	odorless when pure, gasoline
5	cyclopentane, methyl-	gasoline-like
27	cyclooctane	camphoraceous
Misc. cmpds		
21	hexanoic acid	sour, fatty, sweaty, cheesy
1	acetone	ethereal, apple, pear, solvent
17	oxime-, methoxy-phenyl-_	
19	benzene, 1-ethyl-2-methyl-	
20	isopropylbenzene	gasoline-like
22, 23, 25	benzene, 1,2,3-trimethyl- isomers	aromatic
28	benzene, 1-ethyl,2,4-dimethyl-	plastic
31	benzene, 1,2,3,4-tetramethyl-	

Materials & Methods

Raw shelled and inshell almonds (varieties: 'Nonpareil' & 'Butte') were stored in woven PP bags at varying temperature and % RH conditions in SP Scientific environmental chambers for up to 24 mo.

Ground Almond Powder (GAP) for GC Analyses

- At 8-mo intervals, almonds were randomly sampled, shelled using a nut cracker (for inshell samples), immersed in liquid N_2, ground, and then sieved using an 18-mesh Tyler standard screen to give a ground almond powder (GAP).
- GAP samples (2.5 g) were transferred into 20-mL amber glass HS vials, sealed with an Al HS cap and PTFE/silicone liner, and stored at -80°C until GC analysis.

Volatile extraction and HS-SPME Conditions

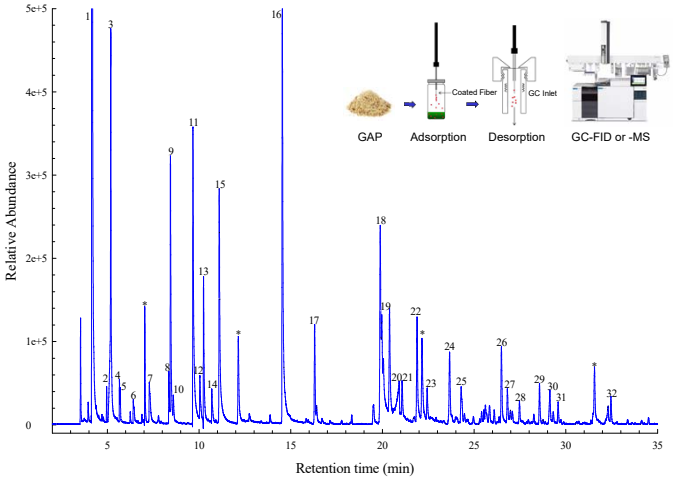
- To GAP samples, 1.7 mL of a saturated NaCl solution, 80 µL of hexanol-d_{13}, and 90 µL of hexanal-d_{12} (internal standards both at 40 µg/mL) were added; contents were vortexed for 60 s.
- CTC Combi PAL autosampler set at 55°C for 40 min using a 50/30 µm DVB/CAR/PDMS SPME fiber was employed to adsorb volatiles.

GC-FID and –MS Conditions

- Agilent 7890A GC with FID at 275°C; Combi PAL autosampler; J&W DB-5 column (60 m x 0.25 mm; 0.25 µm); inlet at 250°C; split ratio 5:1; 8 min desorption from SPME fiber; 1 mL/min He flowrate; oven from 38°C to 175°C at 2.5°C/min, then to 250°C at 20°C/min.
- Same setup with MS detector using NIST mass spectral library.

Quantitation of Volatiles from Calibration Curves of the Iss

- Standard addition method with hexanol-d_{13} and hexanal-d_{12} was used to quantitate endogenous levels of hexanol and hexanal in the GAP samples.
- A Quantachrome ultrapycnometer 1000 was used to determine the averaged density (g/cm^3) of prepared GAP samples by using argon gas to employ Archimedes' principle of fluid displacement.



Results & Discussion

- From the Tables & Figure: 5 aldehydes, 13 alcohols, 4 alkanes, and 10 miscellaneous organic HS volatiles were identified for shelled and in-shell almonds, and quantified at 8, 16, and 24 mo of storage.
- Greatest change in HS levels was for hexanal: its concentrations at 8, 16, and 24 mo (unless the sample failed sensorially) are given.
- Calibration curves for hexanol-d_{13} and hexanal-d_{12} in devolatilized GAP samples had R^2 values of 0.992 and 0.982, respectively.
- Using an ultrapycnometer and the 'standard addition method' with the deuterated standards, HS volatile concentrations were different by <12% relative to values calculated from the calibration curves.
- As temp/%RH conditions became more abusive (e.g., 25°C, 70% RH *vs* 15°C, 70% RH), hexanal levels were found to be greater.
- The shell of 'Nonpareil' almonds (INP) afforded significantly (p<0.05) more protection against secondary lipid oxidation volatile development compared to shelled (SNP) counterpart samples.

	8 mo		16 mo		24 mo
Condition	INP	SNP	INP	SNP	INP
	Average relative concentration of hexanal				
10°C, 65%RH					
hexanal	0.99 ± 0.061	1.68 ± 0.103	1.01 ± 0.182	2.43 ± 0.147	1.84 ± 0.146
15°C, 55%RH					
hexanal	1.01 ± 0.048	2.83 ± 0.107	1.88 ± 0.017	3.29 ± 0.027	1.98 ± 0.099
15°C, 70%RH					
hexanal	1.19 ± 0.103	3.50 ± 0.238	2.02 ± 0.116	3.58 ± 0.055	Failed*
25°C, 55%RH					
hexanal	1.57 ± 0.106	5.52 ± 0.246	2.44 ± 0.072	10.5 ± 0.11	Failed*
25°C, 70%RH					
hexanal	1.61 ± 0.082	7.17 ± 0.221	Failed*	Failed*	Failed*

Failed means that in-shell (I) or shelled (S) Nonpareil (NP) almond samples were unacceptable by a consumer panel.

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