The Chemistry of Almond Quality

Understanding Rancidity Development

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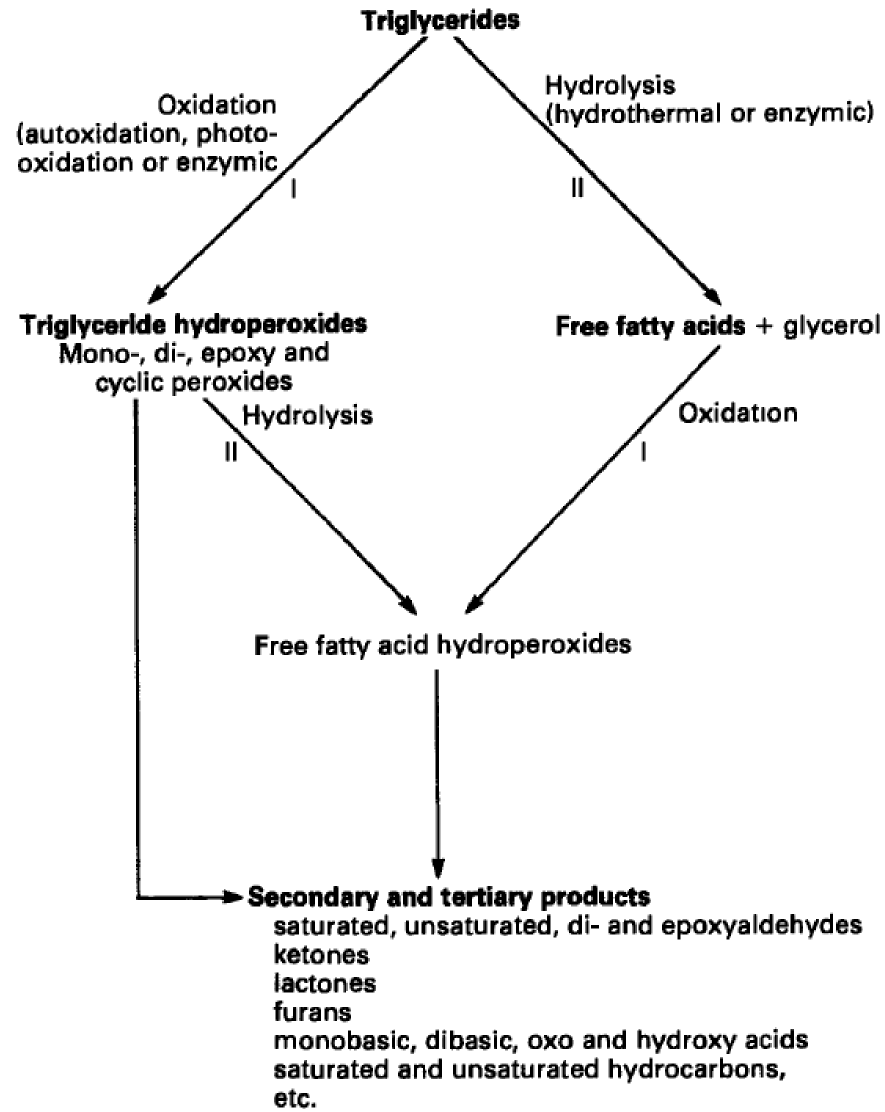
Rancidity

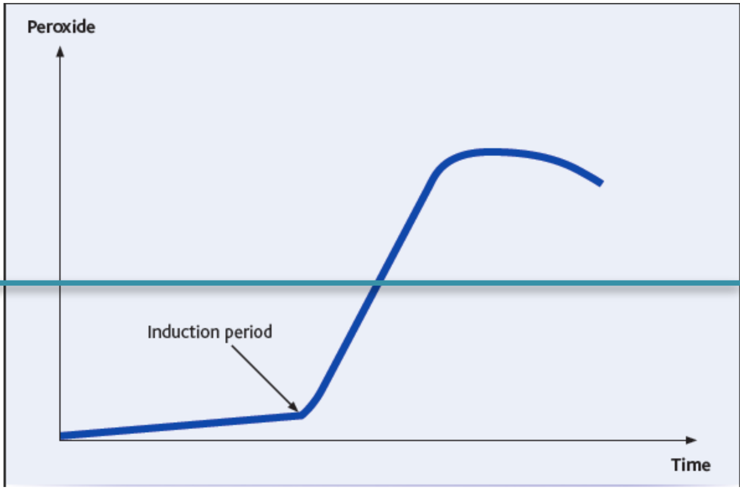
- Rancidity is the unpalatable odor and flavor of deteriorating edible fats and oils in foods
- Rancidity occurs via two chemical reactions:
- Autoxidation
  - oxygen attack of trigylcerides
- Hydrolysis
  - Addition of *water* across triglycerides and release of FFAs



Scheme 1. Over-all reaction scheme for (I) oxidative and (II) hydrolytic rancidity

Robards et al., Analyst, 1988 v 113

Oxidative Rancidity:
Three Phase Process



1. Initiation Phase
   - Molecular oxygen combines with unsaturated fatty acids to produce hydroperoxides and free radicals
   - Requires an oxidative initiator (e.g. heat, light, metals, enzymes, etc.,)
2. Propagation Phase (autoxidation phase)
   - The reactive products of the initiation phase react with additional lipid molecules to form other reactive chemical species
3. Termination Phase
   - Relatively unreactive compounds are formed including hydrocarbons, aldehydes, and ketones (volatile odors)

Rancidity in Almonds

- Rancidity in almonds occurs primarily via the autoxidation of unsaturated fats (oleic, linoleic)
- Initiated by exposure to heat (pasteurization, blanching, roasting), or oxygen exposure (e.g. during storage)
- Autoxidation processes produce various breakdown products that can cause off-flavors and rancidity development in almonds and include:
  - Volatile compounds (off odors/aromas)
  - Non-volatile compounds (degradation products)

Measuring Rancidity

- Although rancidity is one of the most pressing problems confronting processors, there is no completely objective chemical method for determining rancidity
- Industry relies on several analytical methods for routine estimates of oxidation in almonds however, there is no uniform or standard method for detecting oxidative changes
- The biggest challenge:
  - Lipid oxidation is a dynamic process and levels of chemical markers of lipid oxidation change throughout the lipid oxidation process
  - Each method measures something different

Chemical Measures

moving analytical targets

I. Oxidative Rancidity:

- Peroxide Value (PV)
  - Measures initial stages of rancidity
    - Peroxides are the first product of autoxidation and are used as an indicator of the initial stages of oxidative changes
  - Almonds PV < 5 meq/Kg is considered the benchmark
    - However:
      - PV levels can decrease as oxidation progresses (decompose)

Chemical Measures
moving analytical target

- Thiobarbituric Acid (TBA):
  - Measures the later stages of oxidation
  - Low TBA may indicate that aldehydes may have not yet formed or that volatile aldehydes may have been lost during processing and storage

- Almonds Volatiles
  - Hexanal or is usually monitored
    - Later stages of oxidation
    - Data is needed on a variety of samples of the same product (cultivar) to establish a correlation between hexanal concentration and product quality

Chemical Measures

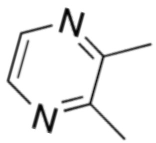
moving analytical targets

II. Hydrolytic Rancidity:

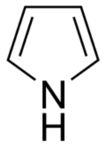
- Free Fatty Acids (FFA):
  - Easiest method for monitoring hydrolytic rancidity
  - Reported as % by mass of free fatty acids expressed as oleic acid
- For almonds <1.5% FFAs
  - Found to correlate with sensory evaluation in butter

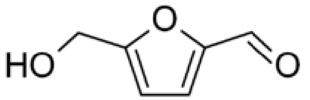
Measuring Volatiles in Roasted Almonds

- Evaluate the volatile composition in raw and roasted almonds and changes that occur during storage
- Goal: is to identify potential markers of rancidity development



- Limited research available on the composition of volatiles in roasted almonds
  - Takei et al., 1974
    - Solvent extraction
  - L. Vazquez-Araujo, et al., 2008, 2009
    - Simultaneous steam distillation extraction





- Limited information on volatiles changes during storage

Experimental Design

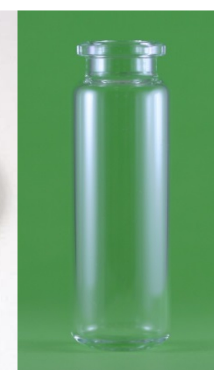
- Measuring the impact of roasting and storage on volatiles
  - Cultivar: *Prunus dulcis* cv. Butte/Padre
  - Dry roasting temperature: 138°C
  - Roasting Time: 28 min, 33 min and 38 mins

- Storage Conditions
  - Temperature: 35°C
  - Humidity: Ambient and 65% RH
  - Time: Evaluated at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24 weeks
- Analysis:
  - Headspace solid-phase micro-extraction (HS-SPME)
    - *No solvents, heat or artifact generation*
  - GC/MS

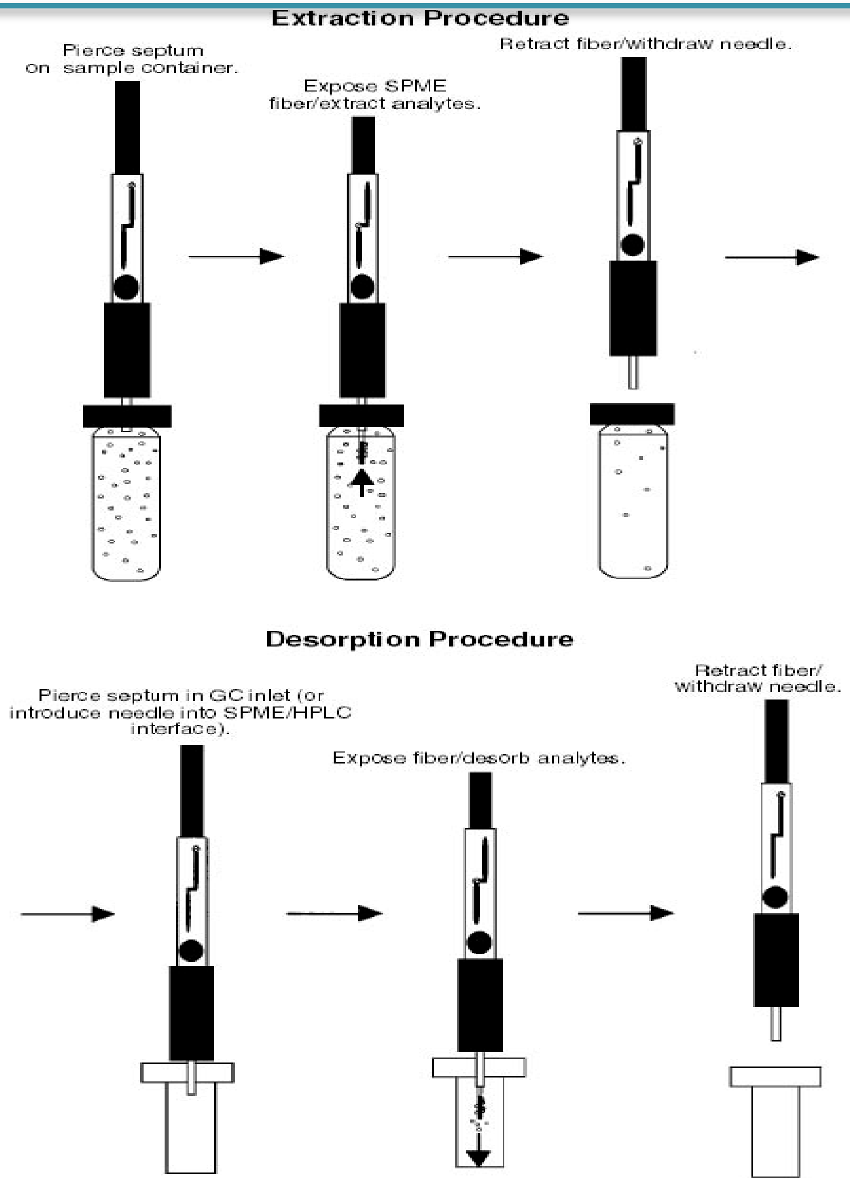
Sample Preparation

- Preparation: Cold room at 4°C
  - 50 g almonds were homogenized
  - Sifted through a 16 meshes screen for particle size control
  - 5 g was transferred to a 20 mL headspace vial
  - 100 ml internal standard (500 ppb) added and the vial was sealed
  - All samples were prepared in triplicate



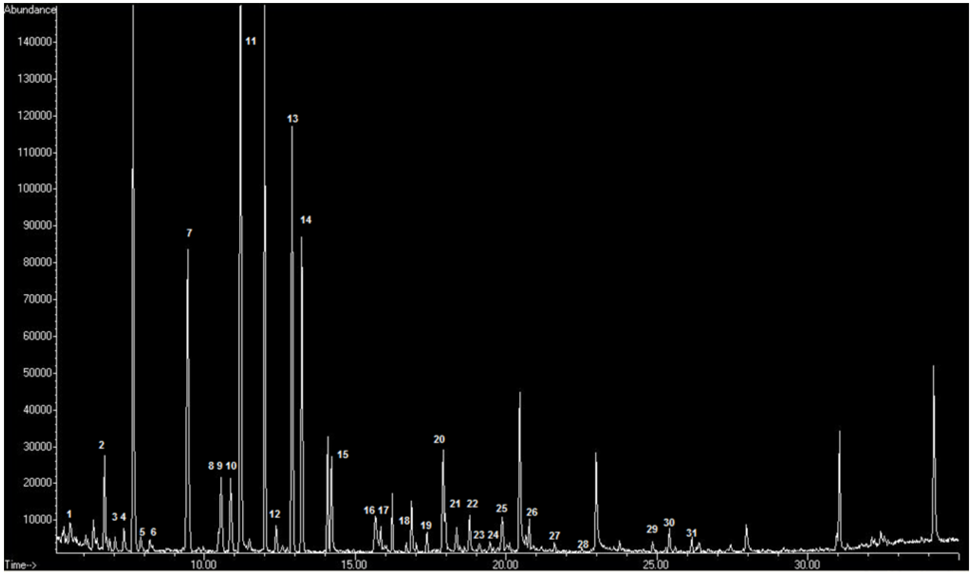
Headspace solid-phase microextraction (HS-SPME)



- Sample was placed in vial and equilibrated for 30 minutes
- Exposed to a 1 cm 50/30 μm coated fiber for 30 min at room temp
- Desorption (230°C): 10 min in injection port

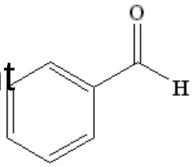
GC Chromatogram of Almond Volatiles

- Peak Identification:
- GC/MS
- Comparison of mass spectra and t_R with standards (38) or by comparing MS and Kovats Index with NIST MS database with 80 % cut-off (no standard)

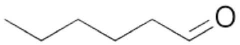


Volatiles Identified in Raw Almonds

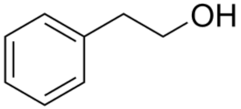
- 41 Identified Compounds: 13 carbonyls, 1 pyrazine, 20 alcohols, and 7 additional volatiles
- Benzaldehyde, the breakdown product of amygladin, was the predominant volatile in raw almonds (2,934.6 ± 272.5 ng/g)
  - Almond-like aroma



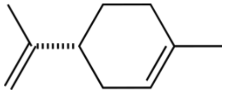
- Hexanal (422.6 ± 97.9 ng/g); found in other nuts as well
  - Fruity/green (cut grass)



- 2-phenylethanol (6.2 ± 0.6 ng/g); deamination of amino acids in plants
  - Floral



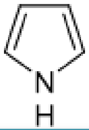
- α-Pinene (15.0 ± 0.1 ng/g) and limonene (16.6 ± 0.5 ng/g); terpenes
  - Pine/citrus

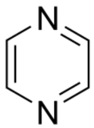


- 1,2-propane diol (propylene glycol)
  - Fumigation (breakdown product of propylene oxide)

Xiao et al., J. Food Chemistry 151 (2014) 31-39

Volatiles in Roasted Almonds





- An additional (13-17) volatiles were formed during roasting
  - Most related to flavor: branch-chain aldehydes, alcohols, pyrazines, heterocyclic and sulfur containing compounds
    - Maillard reaction products / some lipid oxidation products

- An additional 17 new compounds, absent in raw and freshly roasted almonds, but detectable after 10-16 weeks of storage were identified
  - ketones, aldehydes, alcohols, oxiranes and short-chain acids

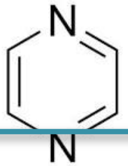
Changes in Volatile Aldehydes and Keytones

possible compounds	roasting time raw	28 min	33 min	38 min	increase[b] (%)
aldehydes and ketones					
butanal	19.6 ± 2.7	27.6 ± 1.5*	29.3 ± 0.6*	40.8 ± 2.1***	67
2-methylbutanal [chocolate/nutty]	14.3 ± 0.3	1468.6 ± 25.7**	5000.3 ± 241.1***	6573.7 ± 275.0***	30216
3-methylbutanal [chocolate]	32.4 ± 0.5	911.4 ± 50.9*	2867.4 ± 71.1***	4268.9 ± 381.8***	8167
2,3-butanedione [sweet/butter]	8.0 ± 0.3	100.3 ± 0.8***	163.7 ± 1.3***	226.3 ± 13.7***	1940
pentanal	50.4 ± 5.7	223.0 ± 8.6***	169.0 ± 5.1***	264.1 ± 15.9***	334
hexanal	422.6 ± 97.9	983.0 ± 133.7**	689.0 ± 78.1	1140.8 ± 3.8**	122
2-heptanone	50.0 ± 4.7	72.0 ± 7.3*	71.0 ± 6.3*	123.6 ± 3.0***	78
heptanal	40.5 ± 8.9	75.2 ± 16.2*	57.1 ± 4.0	114.8 ± 3.0**	103
2-hexenal [almond/green leaf]	ND[c]	14.6 ± 2.7**	11.3 ± 2.2*	14.1 ± 2.7**	New
2-methyloxolan-3-one [rummy/nut]	ND	15.4 ± 1.3	86.3 ± 4.2***	128.1 ± 11.0***	New
3-hydroxybutan-2-one [buttery]	ND	2.2 ± 0.2**	3.0 ± 0.1***	3.8 ± 0.6***	New
octanal	25.2 ± 4.7	31.1 ± 7.3	18.5 ± 6.3	42.0 ± 3.0	21
1-hydroxypropan-2-one	1.3 ± 0.0	9.0 ± 0.9*	11.0 ± 0.0**	13.7 ± 3.0**	771
(Z)-2-heptenal	19.1 ± 0.9	65.6 ± 13.2**	36.5 ± 4.6	61.9 ± 1.6**	186
nonanal	36.6 ± 4.9	55.9 ± 13.3	34.6 ± 4.0	70.5 ± 18.9	47
(E)-2-octenal	7.3 ± 0.9	12.5 ± 2.1	8.3 ± 0.1	15.9 ± 2.0*	67
furfural [brown/caramel]	ND	103.2 ± 8.7**	366.1 ± 13.2***	460.0 ± 21.4***	New
decanal [aldehydic]	ND	6.9 ± 2.3*	5.0 ± 1.6	4.6 ± 1.0	New
benzaldehyde [almond/marzipan]	2934.6 ± 272.5	368.8 ± 41.2***	246.7 ± 53.0***	331.9 ± 65.4***	-89
(Z)-2-nonenal [green]	ND	ND	ND	5.3 ± 1.7**	New
2-phenylacetaldehyde [honey/floral]	ND	107.5 ± 20.3*	284.0 ± 22***	491.3 ± 45.4***	New

- Generated through lipid oxidation and the Maillard reaction
- Most compounds increase with roasting (exception is benzadehyde)

Changes in Volatile Pyrazines

Roasted Nutty Aromas



Compound	Roasting time				increase[b]
	raw	28 min	33 min	38 min	(%)
Pyrazines					
2-methylpyrazine	ND	4.1 ± 0.3*	21.5 ± 0.6***	26.5 ± 1.8***	New
2,5-dimethylpyrazine	11.4 ± 0.5	16.2 ± 0.6***	53.3 ± 0.3***	66.5 ± 0.4***	298
2,6-dimethylpyrazine	ND	ND	2.8 ± 0.4**	4.2 ± 0.6***	New
2-ethylpyrazine	ND	ND	2.6 ± 0.1***	3.2 ± 0.1***	New
2,3-dimethylpyrazine	ND	ND	1.0 ± 0.1***	1.4 ± 0.1***	New
2-ethyl-6-methylpyrazine	ND	ND	1.7 ± 0.1***	2.2 ± 0.0***	New
trimethylpyrazine	ND	ND	4.5 ± 0.3***	6.1 ± 0.2***	New

chocolate; meaty; nutty; green

meaty; nutty; medicinal; woody

butter; musty; woody; nutty

almond; green; meaty; coffee

chocolate; meaty; nutty; green peanut

- Six new pyrazines were identified in roasted almonds
- Generated through the Maillard reaction
- Most have low odor thresholds and increased with the degree of roast

Changes in Volatile Alcohols

Compound	Roasting time raw	28 min	33 min	38 min	increase[b] (%)
Alcohols					
2-butanol	2.9 ± 0.4	ND	ND	ND	ND
2-methyl-1-propanol	3.6 ± 0.3	1.3 ± 0.1***	1.1 ± 0.0***	1.1 ± 0.1***	-68
3-pentanol	ND	0.8 ± 0.1*	2.4 ± 0.1***	2.7 ± 0.3***	New
2-propenol	ND	2.0 ± 0.0***	2.0 ± 0.1***	2.2 ± 0.1***	New
1-butanol	8.4 ± 2.3	11.2 ± 1.1	7.2 ± 0.0	10.7 ± 0.4	15
3-methyl-butanol	86.4 ± 3.3	19.1 ± 0.3***	15.3 ± 1.0***	17.2 ± 0.6***	-80
1-pentanol	30.3 ± 4.4	45.6 ± 2.9*	37.7 ± 3.0	54.3 ± 1.3***	51
1-chloro-2-propanol	106.2 ± 5.4	161.9 ± 2.8***	111.8 ± 2.2	149.6 ± 7.6**	33
3-methyl-2-butenol	17.3 ± 0.9	ND	ND	ND	ND
1-hexanol	47.0 ± 1.1	53.1 ± 5.5	42.2 ± 4.7	70.1 ± 0.7**	17
2-chloro-1-propanol	41.9 ± 3.5	59.5 ± 0.3**	40.9 ± 0.8	53.4 ± 2.2*	22
1-methylthio-2-propanol	12.8 ± 1.3	247.2 ± 23.9**	307.0 ± 27.9**	325.0 ± 53.1***	2190
1-heptanol	3.2 ± 0.4	3.8 ± 1.0	3.0 ± 0.1	6.0 ± 0.4*	32
2-ethyl hexanol	2.4 ± 0.1	ND	ND	ND	ND
2-ethyl thioethanol	1.0 ± 0.0	20.5 ± 3.1**	24.3 ± 1.8**	29.2 ± 3.9***	2321
1-octanol	0.8 ± 0.0	1.2 ± 0.2	0.9 ± 0.0	1.6 ± 0.1**	45
1,2-propanediol	269.1 ± 2.5	789.4 ± 72.3***	510.0 ± 16.1*	647.0 ± 73.8**	141
furfuryl alcohol	0.6 ± 0.0	1.2 ± 0.1***	4.4 ± 0.3***	5.2 ± 0.4***	491
benzyl alcohol	3.9 ± 0.0	ND	ND	ND	ND
2-phenylethyl alcohol	6.2 ± 0.6	0.9 ± 0.0***	0.7 ± 0.0***	0.9 ± 0.2***	-86

- Aliphatic alcohols are generated through lipid oxidation
- Most alcohols increased with roasting time

Changes in Additional Volatiles

Compound	roasting time				increase[b]
	raw	28 min	33 min	38 min	(%)
additional volatiles					
ethyl acetate	ND	12.0 ± 1.2***	3.8 ± 0.6*	9.9 ± 0.8***	New
α -pinene	15.0 ± 0.1	16.5 ± 1.5	11.4 ± 0.2***	14.6 ± 0.9	-5
methylsulfanylmethane	ND	4.5 ± 0.7	7.8 ± 1.9*	6.1 ± 2.0*	New
limonene	16.6 ± 0.5	14.5 ± 1.4	7.1 ± 0.2***	13.0 ± 0.5*	-31
2-pentylfuran	2.4 ± 0.8	16.6 ± 1.2***	25.2 ± 0.6***	30.0 ± 0.2***	905
pyrrole	ND	0.6 ± 0.1*	2.7 ± 0.1***	2.1 ± 0.3***	New
γ -dihydrofuran-2(3H)-one	0.7 ± 0.0	1.8 ± 0.2***	2.0 ± 0.0***	2.2 ± 0.1***	179
γ- oxepan-2-one	1.2 ± 0.3	1.6 ± 0.2	1.4 ± 0.1	2.4 ± 0.3*	49
caproic acid	1.8 ± 0.6	5.7 ± 1.3*	4.0 ± 0.7	6.7 ± 0.1**	207
2-acetylpyrrole	ND	0.2 ± 0.0*	0.6 ± 0.0***	0.7 ± 0.1***	New

- Products of both lipid oxidation and Maillard reaction
- Caproic acid: oxidation of hexenal

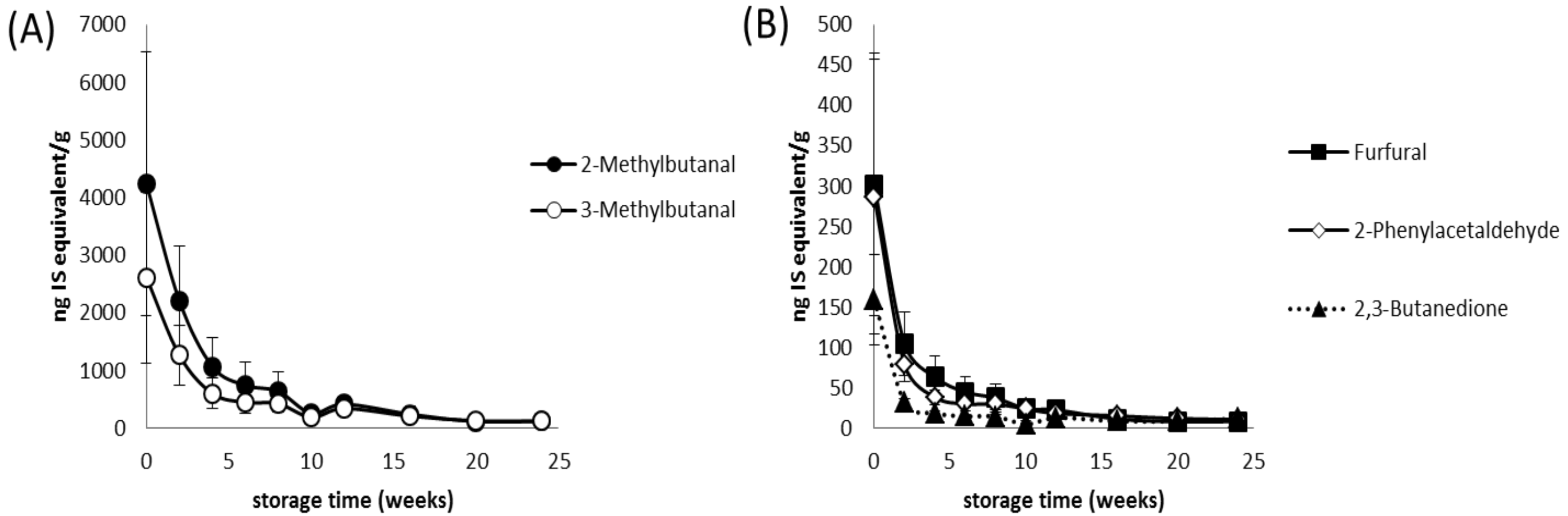
Influence of Storage on Volatiles

- Once the volatile compounds were identified by HS-GC/MS, the influence of roasting time (28 and 38 min) and storage at 35 °C and 65% relative humidity over 24 weeks was evaluated

- The goal was to evaluate early changes in volatiles during storage and to possibly identify markers of early rancidity development in almonds

Decreases in Select Volatiles During Storage

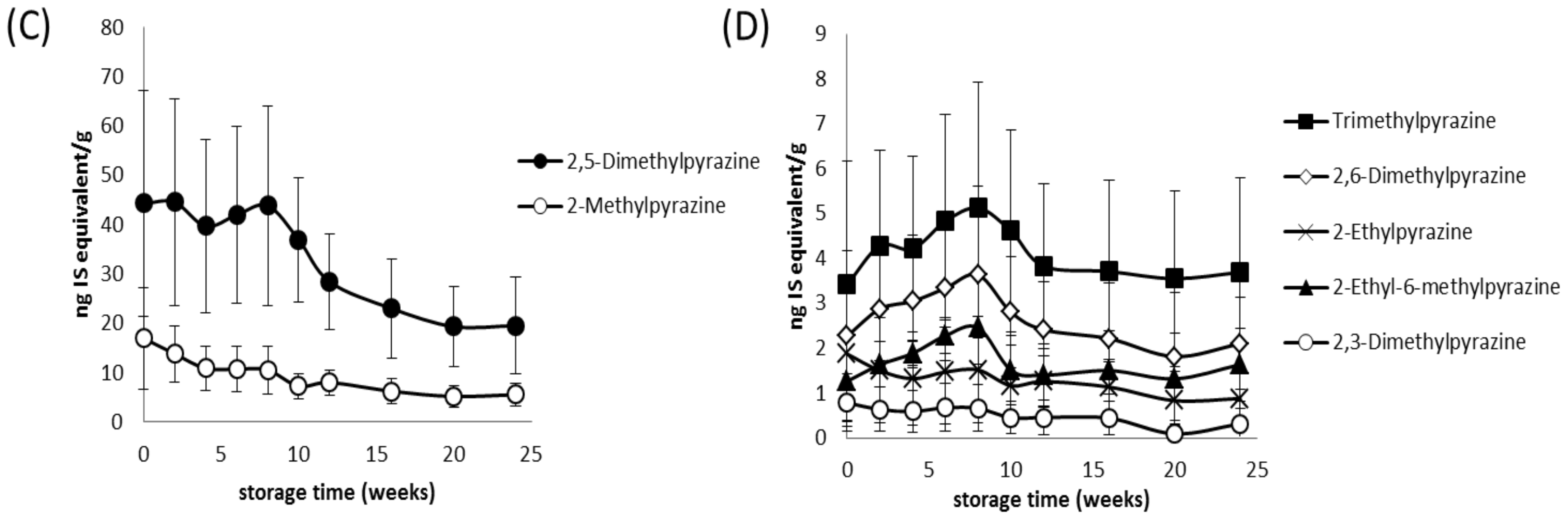
Aldehydes and Keytones



- Levels of aldehydes (e.g. butanal, pentanal, hexanal) were significantly higher than raw almonds immediately after roasting
- These are products of lipid oxidation generated in response to thermal processing
- Levels decreased significantly (75-88%) over the first 4 weeks of storage
- After 20 weeks of storage, the levels of some aldehydes increased again reflecting lipid oxidation due to storage

Decreases in Select Volatiles During Storage

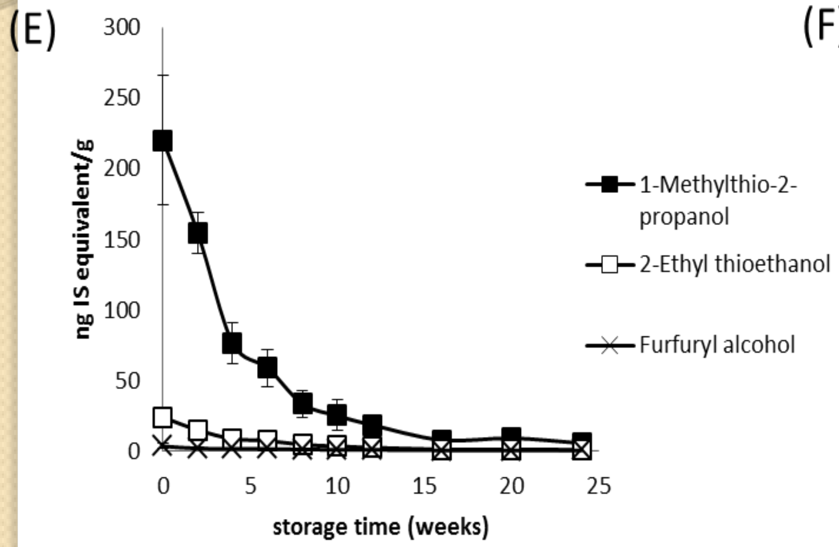
Pyrazines (roasted almond flavor)

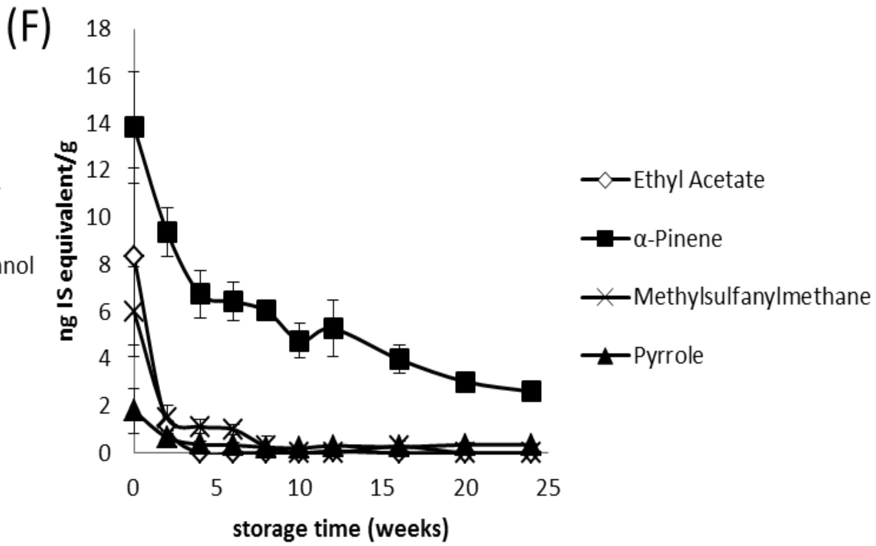


- The levels of 2,5-dimethylpyrazine and 2-methylpyrazine decreased more slowly during storage than did levels of other volatiles
- Other pyrazines did not decrease

Decreases in Select Volatiles During Storage

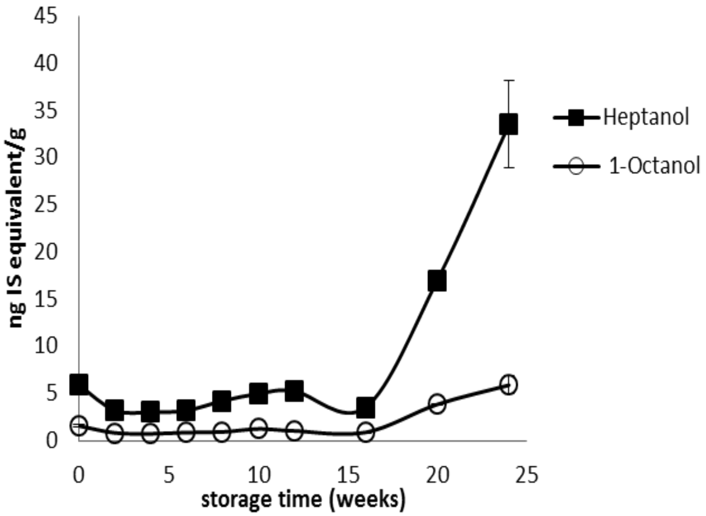
Alcohols and Others

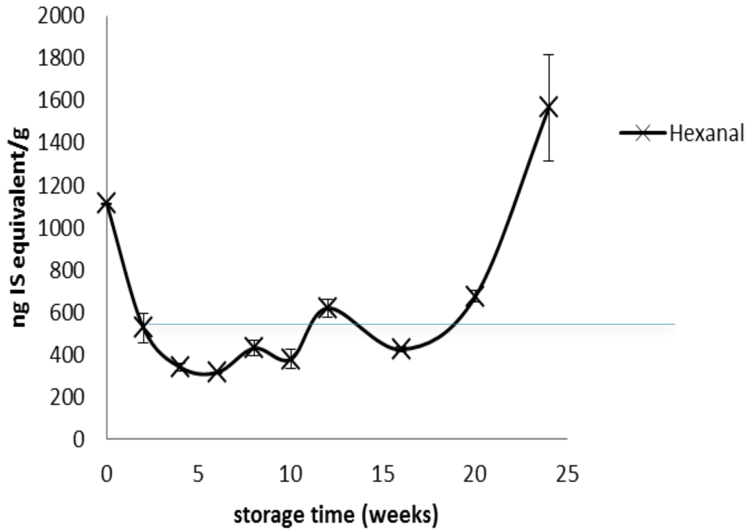




- Decreases in compounds (4 weeks) relating to roasted almond flavor (1-methylthio-2-propanol) and fresh aroma (a-pinene), ethyl acetate (sweet)

Increases in Select Volatiles During Storage: Early Markers of Rancidity





- Levels of hexenal decreased and then increased again until ~18 weeks (regardless of roasting temperature) and did not increase above baseline until after 20 weeks
  - Hexenal is a product of the oxidation of linoleic acid
- Linear increases in heptanol and 1-octanol at 16 weeks
- Heptanol has a greater response

Additional Markers of Early Changes

- Compounds that are initially absent in the roasted almonds but detectable after 16 weeks of storage
- Levels of 2-octanone, 3-octen-2-one, and acetic acid showed large increases as early as 16 weeks

volatile compounds	light roast (28 min at 138C)			dark roast (38 min at 138 C)		
	16 wk	20 wk	24 wk	16 wk	20 wk	24 wk
2-octanone	13.4 ± 1.4	52.3 ± 2.9	33.3 ± 0.1	6.4 ± 0.2	15.4 ± 0.9	34.2 ± 4.0
2-nonanone	9.8 ± 1.4	47.5 ± 0.9	31.2 ± 0.2	4.8 ± 0.3	16.4 ± 0.4	28.6 ± 4.3
3-octen-2-one	18.1 ± 1.8	41.1 ± 2.0	45.4 ± 0.9	9.1 ± 0.4	19.7 ± 0.6	33.6 ± 4.5
2-decanone	2.8 ± 0.2	13.4 ± 0.2	10.3 ± 0.4	ND[a]	7.2 ± 1.4	7.6 ± 1.3
(E)-2-decenal	2.9 ± 0.1	7.1 ± 1.1	10.9 ± 0.9	2.4 ± 0.8	5.6 ± 0.4	6.6 ± 1.4
2,4-nonadienal	10.5 ± 0.9	14.2 ± 1.2	15.4 ± 1.1	7.5 ± 0.1	10.9 ± 0.5	10.4 ± 1.6
2-undecenal	ND	2.1 ± 0.7	3.4 ± 0.3	ND	1.5 ± 0.1	1.8 ± 0.3
1-octen-3-ol	2.2 ± 0.2	7.4 ± 0.4	6.9 ± 0.1	1.4 ± 0.0	4.1 ± 0.0	8.9 ± 1.3
nonanol	0.8 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	0.5 ± 0.1	1.1 ± 0.3	1.0 ± 0.1
pentyl oxirane	9.9 ± 0.8	55.8 ± 11.1	27.8 ± 0.9	5.0 ± 0.8	9.5 ± 0.4	97.5 ± 9.3
hexyl oxirane	31.0 ± 3.1	1.7 ± 0.7	42.1 ± 0.2	9.7 ± 1.5	12.2 ± 0.6	2.9 ± 0.3
acetic acid	35.8 ± 2.7	60.8 ± 3.0	45.2 ± 2.3	43.5 ± 5.4	57.9 ± 1.5	60.2 ± 16.1
vinyl hexanoate	1.5 ± 0.2	6.8 ± 0.1	4.9 ±0.2	1.2 ± 0.0	4.1 ± 0.4	8.1 ± 1.3
pentanoic acid	0.9 ± 0.1	6.1 ± 0.2	2.5 ± 0.2	0.5 ± 0.1	3.3 ± 0.0	5.9 ± 1.9
heptanoic acid	0.6 ± 0.0	5.4 ± 2.3	4.6 ± 0.4	0.5 ± 0.1	4.1 ±0.7	2.7 ± 0.6
octanoic acid	0.4 ± 0.1	5.2 ± 2.3	5.1 ± 0.7	0.2 ± 0.0	3.6 ±0.7	2.0 ± 0.3
nonanoic acid	ND	1.3 ± 0.8	2.5 ± 0.2	ND	0.6 ± 0.2	0.4 ± 0.0

[a]ND stands for not detected.

Conclusions

- Storage (35°C):
  - Significant decreases in aroma volatiles were observed by 4 weeks of storage
    - independent of the roasting time and storage temperature
  - Oxidation occurs at 16 weeks of storage
  - Oxidation products began dominating the profile by 20 weeks
- Potential markers of early oxidative changes:
  - Compounds absent from raw and freshly roasted almonds but detectable after a short period of storage (~16 weeks) certain oxiranes, carbonyls and short chain acids
  - 1-Heptanol (and 1-octanol) as these compound compounds demonstrate robust linear changes in concentration at 16 weeks versus 20 weeks for hexenal

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