Almond Flavor, Sensory and Shelf-Life Preservation

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Chemical Characterization of Oxidative Changes in Roast Almonds

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Dec 8, 2016
Almonds & Rancidity

- Rancidity is the unpalatable odor and flavor of deteriorating edible fats and oils in foods
- Occurs via two chemical reaction pathways in almonds:
  - **Oxidation**
    - Oxidation of triglycerides
  - **Hydrolysis**
    - Addition of *water* across triglycerides and release of fatty acids (FFAs)
    - oxidation
A Closer Look at the Fats in Almonds

• By weight, almonds are roughly 50% fat
• These fats are composed of triglycerides
• A triglyceride is a molecule that is composed of 3 fatty acids attached to glycerol

Glycerol is a simple sugar alcohol
• Three hydroxyl groups that can react with fatty acids
• Backbone of all triglycerides
What is a Fatty Acid?

• A fatty acid is a long chain of carbon and hydrogen with an carboxylic acid group at the end of the chain

\[
\begin{align*}
\text{Acid} & \quad \text{Long hydrocarbon chain – hydrophobic and fat like}
\end{align*}
\]
A Closer Look at Fatty Acids

- Fatty acids can be saturated (no double bonds) or unsaturated (double bonds)
- Double bonds give fluidity (kinks) to fats and are the site of oxidation

![Saturated fat](image)

![Monounsaturated fat (MUFA)](image)

![Polyunsaturated fat (PUFA)](image)
The Fatty Acids in Almonds

- Oleic 60-75%; linoleic 19-30%; steric 1-3%; palmitic 0.5-8%
Rancidity in Almonds

• Rancidity occurs primarily via the oxidation of oleic [18:1] and linoleic [18:2] acids
  – Initiated by exposure to heat (e.g. pasteurization, blanching, roasting, etc.), moisture and/or oxygen (e.g. during storage)

• When fatty acids are oxidized they form lipid hydroperoxides

• These break down into volatiles that can be detected by the nose
Measuring Rancidity

• Although rancidity is a real problem confronting processors, there is no completely objective chemical method for determining rancidity
  – Industry relies on several analytical methods for routine estimates of oxidation however, there is no uniform or standard method for detecting oxidative changes

• Challenges:
  – Lipid oxidation is a dynamic process and levels of chemical markers of lipid oxidation change throughout the lipid oxidation process
  – Little is known regarding correlations between chemical measures of rancidity and consumer acceptance/perception of almonds
Chemical Measures

* moving analytical targets *

I. Oxidative Rancidity:

- Peroxide Value (PV)
  - Lipid peroxides are the first product of oxidation and are used as an indicator of the early oxidative changes
  - Measures initial stages of rancidity
  - Almonds PV < 5 meq/Kg is considered the benchmark

- However:
  - PV levels decrease as oxidation progresses and lipid peroxides break down
  - Low levels can be present when there is extensive lipid oxidation
Chemical Measures

moving analytical targets

• Conjugated Dienes (CDs)
  – Measures initial stages of rancidity
    • When linoleic acid is oxidized, it can rearrange to form a CD
    • CDs absorb UV light at 232–234 nm which can be measured

• However:
  – CD levels can decompose as oxidation progresses
  – No industry values for CDs for almonds
II. Hydrolytic Rancidity

• Free Fatty Acids (FFA):
  – Hydrolytic or enzymatic release of FFAs
    • More susceptible to oxidation
  – Industry standard is < 1.5% FFAs
• Found to correlate with sensory evaluation in butter
  – No studies in almonds
Chemical Measures

moving analytical targets

III. Head Space Volatiles

- Measures the later stages of oxidation
- Lipid peroxides breakdown to form volatile compounds
- Generated during roasting for flavor

• Hexanal
  - Most commonly measured
  - No industry standard for hexanal in almonds
  - A wide range of volatile compounds exist in raw, roasted, and stored almonds
Linking Rancidity with Consumer Linking

Study Design

• **Objective 1:**
  • Monitor common markers of lipid oxidation and volatile aroma profiles in light and dark roasted almonds undergoing accelerated shelf-life conditions that promote rancidity development over 12 months

• **Objective 2:**
  • Determine the consumer hedonic response (degree of liking) of light and dark roast almonds to determine how consumer liking correlates with the chemical measures during rancidity development during accelerated shelf-life storage
Samples

- De-hulled, raw Nonpareil almonds obtained from Blue Diamond Growers (Sacramento, CA)
- Almonds were dry roasted at 115 ± 6°C for 60 min or 152 ± 6°C for 15 min to achieve a ‘light’ or ‘dark’ degree of roast respectively
- Almonds were stored at 39 ±1°C and RH of 15 ±1%
- Almonds were randomized and stored in open bags to maximize oxidation during storage
Analytical Measurements

• Primary Oxidation Products
  – Peroxide Value
  – Conjugated Dienes
• Hydrolytic Rancidity
  – Free Fatty Acids
• Vitamin E
  – Tocopherols (α, β + γ and δ)
• Head Space Volatiles
• Sensory Measures
  – Consumer Hedonic Analysis
Volatile by SPME-HS GC/MS

- Sample Preparation
  - A 50 g sample of almond was ground/particle sized at 841 microns
  - 5 g of this sample was placed in a 20-mL SPME vial
  - Headspace was equilibrated for 4 hours at room temperature

- Sample Extraction
  - CTC Combi/PAL autosampler (Zwingen, Switzerland)
  - Samples were agitated at 500 rpm and pre-equilibrated at 40°C for 45 min, after which they were extracted with a 1 cm 30/50um StableFlex DVB/CAR/PDMS fiber exposed for 45 mins at 250 RPM
Consumer Hedonic Analysis

• Untrained consumers (99) between the ages of 14 and 80 and who consumed almonds at least once a month, were recruited

• Consumers were served samples of 6-7 almonds at room temperature, with randomly generated 3-digit numbers as sample identifiers

• Consumers were asked to indicate their liking of samples by marking a 9-pt hedonic scale with accompanying phrases as anchors
Hedonic Ratings

• Significant differences in liking related to the storage time
  – Consumers had a significant difference in liking between samples aged 0, 2, 4, and 6 months, while there was no significant difference found between samples aged 6, 8, and 10 months

• No significant difference was found in liking related to roast level

<table>
<thead>
<tr>
<th>Analysis</th>
<th>0 months</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>8 months</th>
<th>10 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Roast</td>
<td>7.4 ± 1.4 a</td>
<td>6.6 ± 1.5 b</td>
<td>5.8 ± 1.55 c</td>
<td>4.9 ± 1.9 d</td>
<td>4.9 ± 2.0 d</td>
<td>4.7 ± 2.1 d</td>
</tr>
<tr>
<td>Dark Roast</td>
<td>7.2 ± 1.7 a</td>
<td>6.8 ± 1.4 b</td>
<td>5.8 ± 1.7 c</td>
<td>4.7 ± 2.0 d</td>
<td>4.5 ± 2.0 d</td>
<td>4.2 ± 2.0 d</td>
</tr>
</tbody>
</table>

*A rating of 5 indicates that the consumer is indifferent to the product and below this begins to dislike the product*
## Correlation with Measures of Lipid Oxidation

### Light Roast Almonds

<table>
<thead>
<tr>
<th>Analysis</th>
<th>0 months</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>8 months</th>
<th>10 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxide Value (mEq/g oil)</td>
<td>0.57 ± 0.04</td>
<td>1.13 ± 0.14</td>
<td>1.38 ± 0.20</td>
<td>2.84 ± 0.02</td>
<td>2.14 ± 0.18</td>
<td>2.70 ± 0.08</td>
<td>1.26 ± 0.08</td>
</tr>
<tr>
<td>Free Fatty Acid Value (% oleic)</td>
<td>0.21 ± 0.00</td>
<td>0.25 ± 0.00</td>
<td>0.28 ± 0.00</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>Conjugated Dienes (%)</td>
<td>0.213 ± 0.000</td>
<td>0.222 ± 0.00</td>
<td>0.255 ± 0.000</td>
<td>0.242 ± 0.003</td>
<td>0.261 ± 0.008</td>
<td>0.267 ± 0.002</td>
<td>0.302 ± 0.001</td>
</tr>
<tr>
<td>Alpha Tocopherol Conc. (mg/kg oil)</td>
<td>435 ± 4</td>
<td>424 ± 16</td>
<td>406 ± 1</td>
<td>387 ± 3</td>
<td>362 ± 4</td>
<td>363 ± 32</td>
<td>334 ± 11</td>
</tr>
<tr>
<td>Beta + Gamma tocopherol Conc. (mg/kg)</td>
<td>34.3 ± 0.5</td>
<td>34.0 ± 0.2</td>
<td>34.1 ± 0.3</td>
<td>32.8 ± 0.5</td>
<td>31.9 ± 0.4</td>
<td>33.5 ± 1.0</td>
<td>31.7 ± 0.9</td>
</tr>
<tr>
<td>Mean Consumer Hedonic Score</td>
<td>7.4 ± 1.4 a</td>
<td>6.6 ± 1.5 b</td>
<td>5.8 ± 1.55 c</td>
<td>4.9 ± 1.9 d</td>
<td>4.9 ± 2.0 d</td>
<td>4.7 ± 2.1 d</td>
<td></td>
</tr>
</tbody>
</table>
## Correlation with Measures of Lipid Oxidation

### Dark Roast Almonds

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Storage Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 months</td>
</tr>
<tr>
<td>Peroxide Value (mEq/g oil)</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td>Free Fatty Acid Value (% oleic)</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Conjugated Dienes (%)</td>
<td>0.216 ± 0.00</td>
</tr>
<tr>
<td>Alpha Tocopherol Conc. (mg/kg oil)</td>
<td>444 ± 1 a</td>
</tr>
<tr>
<td>Beta + Gamma tocopherol Conc. (mg/kg)</td>
<td>35.0 ± 0.1 a</td>
</tr>
<tr>
<td>Mean Consumer Hedonic Score</td>
<td>7.2 ± 1.7 a</td>
</tr>
</tbody>
</table>
Peroxide Values

- PV measures early oxidation, and almonds are considered to not have undergone significant lipid oxidation if values are < 5 mEq/kg
- Doesn’t correlate with consumer liking in light roast almonds
Free Fatty Acids
(% Oleic)

- FFAs indicate hydrolytic rancidity in almonds and the “recommended” maximum value is < 1.5 % oleic
- Never exceeded, however roasting would inactivate lipase enzymes
Conjugated Dienes

- Significant increases were observed at 2 months for the DR samples and at 3 months for LR samples
- Correlate with consumer liking however sensitivity is very low
All isomers were found also to correlate significantly with consumer liking at $p < 0.01$, though alpha tocopherol had the highest $r^2$ value at 0.814, whereas beta + gamma and delta tocopherol had a correlation $r^2$ values of 0.721 and 0.625 respectively.
Volatile Compounds

- A total of 99 volatile compounds were identified in roasted almonds over the 12 months of storage.
- Compounds include: 8 acids, 17 alcohols, 19 aldehydes, 5 alkanes, 4 esters, 11 ketones, 13 pyrazines, 3 terpenes and 20 other compounds.
- Authentic standards were available to confirm identities of 63 compounds.
- Tentative identities of the remaining 33 compounds were made by comparing MS spectra with the NIST Library and calculated Kovat’s retention indices (KI) with literature values of standards chromatographed under comparable conditions.
Trends in Total Volatiles by Class

Alcohols 4 or Less

Alcohols 4 or More

Total Acids

Total Aldehydes More than 4

Total Alkanes

Total Esters

Total Heterocycles

Total Ketones 6 or Less

Total Ketones 6 or More

Total Lactones

Total Oxiranes

Total Pyrazines

Total Sulfur

Total Terpenes

Storage Time (months)
Changes from 0 to 2, 4 months of select compounds with significant correlation (p < 0.01) to consumer liking

<table>
<thead>
<tr>
<th>Name</th>
<th>Correlation Value</th>
<th>Concentration in DR</th>
<th>Concentration in LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>methoxymethyl oxirane</td>
<td>0.939</td>
<td>3.7 ± 0.0</td>
<td>2.27 ± 0.03</td>
</tr>
<tr>
<td>ethylpyrazine</td>
<td>0.921</td>
<td>5.56 ± 0.08</td>
<td>3.45 ± 0.09</td>
</tr>
<tr>
<td>2,3-dimethylpyrazine</td>
<td>0.918</td>
<td>3.67 ± 0.04</td>
<td>2.36 ± 0.11</td>
</tr>
<tr>
<td>2-ethyl-6-methylpyrazine</td>
<td>0.908</td>
<td>5.41 ± 0.10</td>
<td>3.59 ± 0.10</td>
</tr>
<tr>
<td>2,5-dimethylpyrazine</td>
<td>0.886</td>
<td>80.1 ± 1.4</td>
<td>49.2 ± 1.29</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>0.886</td>
<td>92.9 ± 0.8</td>
<td>64.4 ± 0.89</td>
</tr>
<tr>
<td>2-methylbutanal</td>
<td>0.884</td>
<td>225 ± 3</td>
<td>146 ± 2.37</td>
</tr>
<tr>
<td>2,6-dimethylpyrazine</td>
<td>0.870</td>
<td>13.7 ± 0.31</td>
<td>8.45 ± 0.23</td>
</tr>
<tr>
<td>2-ethyl-5-methylpyrazine</td>
<td>0.861</td>
<td>2.11 ± 0.06</td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td>methylpyrazine</td>
<td>0.855</td>
<td>44.6 ± 1.78</td>
<td>21.8 ± 0.48</td>
</tr>
<tr>
<td>pyrazine</td>
<td>0.842</td>
<td>2.49 ± 0.14</td>
<td>1.19 ± 0.04</td>
</tr>
</tbody>
</table>
Change from 0 to 2, 4 months of select compounds with significant correlation (p < 0.01) to consumer liking

<table>
<thead>
<tr>
<th>Name</th>
<th>Correlation Value</th>
<th>Concentration in DR</th>
<th>Concentration in LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Months 2 Months 4 months</td>
<td>0 Months 2 Months 4 months</td>
</tr>
<tr>
<td>hexanoic acid</td>
<td>-0.900</td>
<td>1.05 ± 0.06 14.7 ± 3.00 59.8 ± 2.11</td>
<td>1.96 ± 0.32 12.3 ± 0.15 56.3 ± 1.22</td>
</tr>
<tr>
<td>pentanal</td>
<td>-0.902</td>
<td>3.92 ± 0.04 62.8 ± 0.59 110 ± 1.26</td>
<td>5.5 ± 0.06 41.3 ± 0.84 111 ± 0.79</td>
</tr>
<tr>
<td>hexanal</td>
<td>-0.902</td>
<td>58 ± 0.34 716 ± 16.30 1360 ± 15.9</td>
<td>77.9 ± 1.8 492 ± 9.25 1390 ± 8.25</td>
</tr>
<tr>
<td>2-pentylfuran</td>
<td>-0.909</td>
<td>4.86 ± 0.14 13.9 ± 0.23 28.8 ± 0.58</td>
<td>6.48 ± 0.33 16.3 ± 0.42 32.4 ± 0.35</td>
</tr>
<tr>
<td>2-hexenal</td>
<td>-0.922</td>
<td>0.393 ± 0.01 2.93 ± 0.17 5.13 ± 0.27</td>
<td>1.23 ± 0.01 2.67 ± 0.02 5.28 ± 0.03</td>
</tr>
<tr>
<td>2-heptenal</td>
<td>-0.928</td>
<td>0.799 ± 0.03 6.51 ± 0.23 17.6 ± 0.32</td>
<td>1.1 ± 0.057 5.12 ± 0.07 19.2 ± 0.34</td>
</tr>
<tr>
<td>1-butanol</td>
<td>-0.931</td>
<td>0.542 ± 0.03 1.48 ± 0.03 2.78 ± 0.07</td>
<td>0.623 ± 0.01 1.05 ± 0.02 2.62 ± 0.02</td>
</tr>
<tr>
<td>3-octen-2-one</td>
<td>-0.934</td>
<td>0.465 ± 0.01 5.58 ± 0.15 17.2 ± 0.34</td>
<td>0.755 ± 0.05 5.6 ± 0.09 14 ± 0.35</td>
</tr>
<tr>
<td>styrene</td>
<td>-0.943</td>
<td>1.53 ± 0.02 3.85 ± 0.05 5.02 ± 0.17</td>
<td>1.79 ± 0.05 2.47 ± 0.05 5.28 ± 0.02</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>-0.946</td>
<td>3.79 ± 0.06 16.8 ± 0.22 36.9 ± 0.81</td>
<td>6.31 ± 0.14 11.7 ± 0.13 34.6 ± 0.27</td>
</tr>
</tbody>
</table>
Compounds with significant correlation with consumer liking (p < 0.05) and an absolute regression slope of >2 μg/kg change in concentration per unit liking

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Linear R² value</th>
<th>Slope of Conc. (μg/kg) vs. liking</th>
<th>Aroma Quality</th>
<th>Aroma Threshold (μg/kg)</th>
<th>DR 4 month</th>
<th>DR 6 month</th>
<th>LR 4 month</th>
<th>LR 6 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>heptanal</td>
<td>0.952</td>
<td>-82.55</td>
<td>fatty, oily, powerful, rancid, citrus, sharp</td>
<td>50¹,0</td>
<td>85.4 ± 2.0</td>
<td>187 ± 3</td>
<td>87.1 ± 0.9</td>
<td>137 ± 2</td>
</tr>
<tr>
<td>octanal</td>
<td>0.885</td>
<td>-93.24</td>
<td>citrus-like, soapy, penetrating</td>
<td>55¹,0</td>
<td>69.5 ± 1.5</td>
<td>158 ± 5</td>
<td>69 ± 1</td>
<td>127 ± 3</td>
</tr>
<tr>
<td>hexanal</td>
<td>0.814</td>
<td>-736.91</td>
<td>fatty, green, grassy</td>
<td>75¹,0</td>
<td>1360 ± 15.9</td>
<td>2380 ± 34.6</td>
<td>1390 ± 8.25</td>
<td>1480 ± 6.5</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>0.753</td>
<td>-11.27</td>
<td>mushroom, earthy, green, oily, fungal</td>
<td>1⁴,W</td>
<td>7.29 ± 0.237</td>
<td>14.9 ± 0.365</td>
<td>5.75 ± 0.158</td>
<td>10 ± 0.207</td>
</tr>
<tr>
<td>benzaldehyde</td>
<td>0.586</td>
<td>-2.53</td>
<td>artificial almond, sweet, cherry</td>
<td>350⁴,W</td>
<td>8.79 ± 0.334</td>
<td>10.6 ± 0.568</td>
<td>9.1 ± 0.0877</td>
<td>8.71 ± 0.195</td>
</tr>
<tr>
<td>furfural</td>
<td>0.631</td>
<td>4.63</td>
<td>sweet, woody, almond, bread</td>
<td>3,000⁴,W</td>
<td>5.2 ± 0.219</td>
<td>3.95 ± 0.12</td>
<td>4.55 ± 0.0394</td>
<td>3.78 ± 0.0424</td>
</tr>
<tr>
<td>2-methylbutanal</td>
<td>0.781</td>
<td>83.19</td>
<td>fruity, dry, slightly green, chocolate, nut</td>
<td>10⁵,0</td>
<td>43.7 ± 0.691</td>
<td>23.1 ± 1.8</td>
<td>47.9 ± 1.17</td>
<td>18.8 ± 0.159</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>0.785</td>
<td>35.66</td>
<td>musty, chocolate, nutty, malty</td>
<td>5.4⁵,0</td>
<td>19.6 ± 1.09</td>
<td>11.2 ± 0.675</td>
<td>20.3 ± 0.63</td>
<td>8.28 ± 0.141</td>
</tr>
</tbody>
</table>
Conclusions

• All chemical measures of rancidity behaved significantly different between LR and DR almonds

• PV does not relate to consumer liking and 5 mEq/kg is not a good measure of rancidity development in roasted almonds

• FFA remain below recommended 1.5% oleic indicating this is not a good measure of rancidity development in roasted almonds

• Consumer liking was not different based on roast level
  – Consumers changed liking significantly by 2 months, by 4-5 months consumers neither liked nor disliked the almonds
  – Consumers disliked almonds on average by 6 months for both LR and DR

• At 2 months, significantly decreases were observed in compounds related to roasted flavor (pyrazines) indicating that flavor fade occurs rapidly

• Headspace volatiles correlate better with consumer liking than PV, CD, and FFA, and at 6 months significant increases in aldehydes were observed with the strongest correlation with heptanal, followed by octanal and hexenal
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  • Lillian Franklin, M.S.
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• John Kinsella Endowment / Almond Board of California
Sensory Evolution of Rancidity in Roasted Almonds

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Covance Food Solutions, Livermore CA

Dr Alyson Mitchell & Lily Franklin
UC Davis, Davis CA
Content

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• Methodology
• Results
• Key Findings
About Covance

As of October 2015, we are part of the Covance portfolio of leading food solutions.

Heritage in food safety, process validation and commercialization. Providing services across the product development continuum: product & process development, food safety & quality, consumer & sensory

Foundation in product & process development and commercialization in the food, beverage & nutritionals industries
## Product Design and Integrity

Solutions for the life cycle of your product

<table>
<thead>
<tr>
<th>Concept</th>
<th>Development</th>
<th>Launch</th>
<th>Quality Programs</th>
<th>Brand Protection</th>
</tr>
</thead>
</table>

### Product Design
- Idea Generation
- Culinary Services
- Product and Process Development
- Consumer Research
- Sensory Evaluation
- Regulatory Considerations
- Commercialization and Scale-Up

### Product Integrity
- Nutritional Chemistry Solutions
- Microbiology and Food Safety
- Adulterant and Non-Targeted Analyses
- Contaminants/Pesticides/Residues
- Risk Assessments
- Challenge Studies and Process Validations
- Shelf-Life and Stability Testing
Sensory Evaluation

Our Approach:

• Tap into our pool of 40 highly trained panelists with an average of 5+ years of experience.
  • These are not consumers and they do not provide their liking or opinions.
  • Skilled at describing sensory characteristics and intensity ratings of a wide variety of products.
  • Screened for olfactory & gustatory acuity and ability to describe flavor nuances.
  • Extensively screened and provided with 3+ months of training before qualification.

• Overseen by experienced panel leaders
  • Advanced degrees (Master’s or Ph.D. in Sensory Science)
Sensory Evolution of Rancidity in Roasted Almonds
Roasting Changes Volatile Profile of Almonds

**Maillard Reaction**
(non-enzymatic browning)

- Ketones
- Aldehydes
- Pyrazines
- Alcohols
- Thiols
- Sulfides
- Furans
- Pyrroles
Processing & Environment Changes Volatile Profile of Almonds

Lipid Oxidation
- Peroxides
- Conjugated Dienes
- Aldehydes (ie. hexanal)
- Ketones
- Alcohols (1-octen-3-ol)

RANCIDITY
Background & Objectives

Background:

• As demand for almonds continues to grow, products may be shipped longer distances and stored over extended periods of time.

• It is important to monitor the flavor of almonds over time, to ensure the quality and value of exports and products are preserved.

Objectives:

• To identify changes in sensory profile throughout storage.

• To identify volatile compounds related to key sensory attributes, and assess which of these are most related to consumer liking.
Product Descriptions

• Raw Non-pareil almonds with skin (2014 harvest).
• Dry roasted – 239 ± 6°F for 60 min => LIGHT Roasted (LR)
  – 305 ± 6°F for 15 min => DARK Roasted (DR)
• Samples were stored at 15% ± 1% relative humidity and 102 ± 1°F for intervals of 1 to 12 months.
  – 12 accelerated storage times + Control (13 samples total) at two roast levels.
  • All samples were analyzed using HS-SPME-GC-MS.
  – 6 samples (Control + 5 storage times) were assessed by trained panelists & consumers.

<table>
<thead>
<tr>
<th>Storage time (months)</th>
<th>0 (Control)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptive Analysis &amp; Consumer Testing</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS-SPME-GC-MS</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
Methodology – Descriptive Analysis

• Descriptive testing was conducted by Covance using trained panelists.
• 10 panelists, 3 replications.
• Panelists participated in one 2-hour orientation session to discuss the samples and review the references.
• The light roast samples were assessed before the dark roast samples.
• The control sample in each roast level was used as a reference to rate the degree of difference and was anchored to the scoresheet.
• Samples were identified by 3-digit codes and were served in a randomized and balanced order within each roast level.
• Panelists rated attribute intensities on 15-point line scales.
Methodology – Sensory Attributes

- **Overall Degree of Difference** (vs. Control)
- **Aroma and Flavor**
  - Total Aroma/Flavor Intensity
  - Clean Nutty
  - Clean Roasted
  - Total Oxidized
    - Cardboard *
    - Painty / Solvent *
    - Soapy *
    - Bitter *
- **Texture – Initial**
  - Hardness
- **Mouthfeel**
  - Pungent / Irritation / Burning
  - Astringent

* Flavor only

---

**FLAVOR:**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Flavor Intensity</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Clean Nutty</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Clean Roasted</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Total Oxidized</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Cardboard</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Painty / Solvent</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Soapy</strong></td>
<td>0-15</td>
</tr>
<tr>
<td><strong>Bitter</strong></td>
<td>0-15</td>
</tr>
</tbody>
</table>

R1 = Light Roast Control (LCON)
Methodology – Consumer Test

- Central Location Testing was performed at UC Davis.
- 99 consumers, 1 replication.
- Consumers consumed almonds at least once a month, were not pregnant and aged 14-80 years.
- Consumers were instructed to taste at minimum of 3 almonds before making an assessment.
- Consumers evaluated 12 samples (6 samples for each roast level).
- Serving order of all 12 samples was randomized to control for position biases.
- All products were served unbranded.

How much do you like this sample overall?

- Dislike Extremely
- Dislike Very Much
- Dislike Moderately
- Dislike Slightly
- Neither Like Nor Dislike
- Like Slightly
- Like Moderately
- Like Very Much
- Like Extremely
Methodology – HS-SPME-GC-MS

- Headspace Solid-Phase Microextraction Gas Chromatography/Mass-Spectrometry (HS-SPME-GC/MS)
  - Measured at UC Davis, 3 replicates
  - 98 volatile compounds, most confirmed with authentic standards
    - 8 acids
    - 17 alcohols
    - 19 aldehydes
    - 5 alkanes
    - 2 esters
    - 11 ketones
    - 13 pyrazines
    - 3 terpenes
    - 20 other compounds

http://www.elcalabs.com/GCMS.html
Descriptive Analysis Results
Clean Nutty & Clean Roasted Flavors Decreased Over Time

**Clean Nutty flavor**

- Light and dark roast samples decreased in **Clean Nutty flavor** over time.

**Clean Roasted flavor**

- Dark roast samples higher in **Roasted flavor** than light roast samples.
- Light and dark roast samples decreased in **Roasted flavor** over time.
Oxidized Flavor & Bitter Taste Increased Over Time

**Oxidized flavor**

- Light and dark roast samples increased in Oxidized flavor over time.

**Bitter taste**

- Dark roast samples higher in Bitter taste than light roast samples.
Consumer Test Results
All Consumers Preferred Fresher Almonds

**POSITIVE CORRELATIONS**
- Clean Nutty aroma and flavor
- Clean Roasted aroma and flavor

**NEGATIVE CORRELATIONS**
- Oxidized aroma and flavor
- Cardboard flavor
- Painty/Solvent flavor
- Soapy flavor
- Bitter taste
- Pungent/Irritation/Burning mouthfeel

There were no substantial differences in liking between the light and dark roast samples.
Three Clusters of Consumers with Different Liking Patterns

Cluster 1 (55%)
- Liking decreased substantially up to 6 months for both roast levels

Cluster 2 (24%)
- Less discriminating in liking – less offended by oxidative notes in the aged almonds

Cluster 3 (21%)
- Light roast almonds liked more than dark roast samples
- Liking decreased substantially up to 4 months for light roast and 8 months for dark roast almonds

No difference in liking between the roast levels
Relating Descriptive & Consumer Data

Individual Consumer liking scores (n=99)

Fresh samples → Aged samples

D1 (88%)

D2 (10%)

Light Roast

Dark Roast

Ingredients:
- Clean Roasted flavor
- Clean Roasted aroma
- Total Aroma Intensity
- Astringent
- Bitter
- Total Oxidized flavor
- Cardboard flavor
- Painty Solvent flavor

Light Roast:
- L2
- L4
- L6
- L8
- L10

Dark Roast:
- D2
- D4
- D6
- D8
- D10

Dark Roast:
- DCON

Light Roast:
- LCON
Analytical Results
2-Methylbutanal decreased over time.

2-Methylbutanal, 3-Methylbutanal & 2,5-Dimethylpyrazine:
- Highly positively correlated with Clean Nutty and Clean Roasted flavors, as well as liking of Total Respondents, and Clusters 1 and 3.
Analytical Measures – Lipid Oxidation Breakdown

**Peroxide Value (PV)**
- Dark roast samples were substantially higher in PV than light roast almonds.
- PV increased over time in dark roast samples.
- **Peroxide Value:**
  - <5.0 is a recommended industry rejection standard.
  - None of the light roast samples were greater than 5.0, despite being perceived as rancid at the later time points.
  - PV peaked at 9 months for dark roasted samples, while rancidity continued to increase.

**Conjugated Dienes (CD)**
- CD increased over time in dark roast samples.
- **Conjugated Dienes:**
  - No common industry standard currently exists for CD.
  - There was very little change in light roast samples, despite being perceived as rancid at the later time points.
Analytical Measures – Lipid Oxidation Breakdown (Con’t)

Free Fatty Acids (FFA):
- <1.5% Oleic is a recommended industry rejection standard.
- No samples were greater than 1.5% Oleic, despite being perceived as rancid at the later time points.

Hexanal:
- Commonly used as an indicator of oxidative rancidity in industry (currently no standard).
- Highly positively correlated with Total Oxidized flavor.
- Negatively correlated with liking for Total Respondents, and Clusters 1 and 3.
Analytical Measures – Lipid Oxidation Breakdown (Con’t)

Octanal
- Increased over time, slightly more in dark roast almonds
- Highly positively correlated with Total Oxidized flavor
- Negatively correlated with liking for Total Respondents, and Clusters 1 and 3
- Not previously been reported as an oxidative indicator in nuts

Nonanal
- Highly positively correlated with Total Oxidized flavor
- Negatively correlated with liking for Total Respondents, and Clusters 1 and 3
- Not previously been reported as an oxidative indicator in nuts
Key Findings – Analytical Measures

• Current **industry indicators of oxidation did not do a great job** at predicting sensory rancidity and consumer liking.
  – For light roast samples, Peroxide values were less than 5.0 mEq and Conjugated dienes had very little change, despite being perceived as rancid at the later time points.
  – All almond samples had Free Fatty Acid levels less than 1.5% Oleic, despite being perceived as rancid at the later time points.

• **Rancidity** attributes were related to the volatile compounds **hexanal**, **heptanal**, **octanal** and **nonanal**.
  – These compounds were **negatively** correlated to consumer liking.
  – Heptanal, octanal and nonanal have not previously been reported as an oxidative indicator in nuts.

• **Clean Nutty** and **Clean Roasted** attributes were related to **2-methylbutanal**, **3-methylbutanal** and **2,5-dimethylpyrazine**.
  – These compounds were **positively** correlated to consumer liking.
Key Findings – Descriptive & Consumer Testing

- **Storage time** had a **strong influence** on consumer liking, as well as almost all descriptive attributes and headspace compounds in both light and dark roasted almonds.

- In contrast, **degree of roasting did not affect** consumer liking and only influenced approximately half the descriptive attributes and ~75% of the headspace compounds significantly.

- Oxidative flavor changes tended to **peak after 6 months at 102°F for light roasted samples**, and **8 months at 102°F for dark roasted samples** and remained stable for up to 12 months of storage.

- Three clusters of consumers had different liking patterns, however they **all preferred the fresher almond samples**, regardless of roast level.
  - Total Respondents and Clusters 1 and 3 liking were **negatively correlated with lipid oxidation by-products**, however, **Cluster 2 consumers were less offended by oxidative notes**.
  - Cluster 3 consumers preferred **light roast almonds more than dark roast samples**, which may be due to a **negative correlation with bitter taste**.
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CHEMICAL DRIVERS FOR NUT QUALITY DETERIORATION

Ronald B. Pegg
Department of Food Science & Technology
December 8, 2016
Susceptible to ...

- Microbial spoilage ... mold
- Oxidation
- Textural degradation
Solutions...

- Microbial spoilage ... mold  ➔  Reduce moisture content to 4-6%
- Oxidation  ➔  Nitrogen flush, vacuum package
- Textural degradation  ➔  Protect against too high/too low RH

What is needed to achieve this? $
Factors that Affect Shelf Life

- Almonds are relatively low-moisture, high-oil-containing nuts with a long shelf life when properly handled.
- Almond quality and shelf life can be influenced by three general factors: the product characteristics, the environment during distribution and storage, and the package.
- These factors interact in many ways to influence almond quality and to impact shelf life.
- Shelf life guidance for almonds must specify the product and the storage conditions.

**Major Factors Influencing Almond Quality and Shelf Life (ABC 2014)**

<table>
<thead>
<tr>
<th>Product characteristics</th>
<th>Environment</th>
<th>Package</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition; Water activity; Form.</td>
<td>Temperature; Humidity; Oxygen; Processing conditions; Insects, pests, microorganisms.</td>
<td>Physical protection; Moisture barrier; Gas barrier.</td>
</tr>
</tbody>
</table>
ABC Recommendations - Storage Conditions and Handling Practices

• Storage for all almond forms in cool and dry conditions (<50°F/<10°C and <65% relative humidity) is recommended.
• The optimal goal of the recommended storage conditions is to maintain <6% MC, which helps preserve shelf life.
• A cool temperature of <50°F/<10°C is optimal, but a higher temperature that does not stimulate insect activity may work as well to control moisture migration (and also minimize lipid oxidation).
• Almonds are a shelf-stable nut that can have more than two years of shelf life when stored at the recommended conditions.

High Quality → Moisture <6%, aw 0.25-0.35, Free fatty acids <1.5%, PV <5 meq/kg
• Rancidity is a general term for the unacceptable off-flavors and off-odors that eventually develop as fats breakdown through various reactions.

• Before, what we call, true rancid notes develop, we often sense cardboardy, painty or stale notes associated with the product … or sometimes desirable flavor notes are lost or are masked by developing secondary products of lipox.

• This is oxidative rancidity.

• Hydrolytic rancidity occurs when fatty acids are released from fat triglycerides (by naturally occurring enzymes) and are now considered free.

• Free fatty acids are more susceptible to oxidation than triglycerides. (The presence of moisture enhances this type of rancidity).
Expeller-pressed Oil

- Oil is recovered with a Carver press
Lipid Oxidation

- Lipid oxidation (lipox) is a complex series of undesirable reactions that cause the breakdown of fats and oils.

- In almonds, the oxidation reactions lead to a loss of quality as the nuts develop “rancid” flavors and odors.

- During lipox, $O_2$ reacts spontaneously with the fatty acids in fats to form primary breakdown products (e.g., peroxides, conjugated dienes) and, as oxidation progresses, secondary products (e.g., volatile aldehydes, ketones) are formed that give rise to off-flavors and off-odors.

- Oxidation can be measured by testing for the presence or accumulation of one or more of these primary and secondary products.
Initiation

\[
\text{LH} \rightarrow \text{L}^* + \text{H}^*
\]

Propagation

\[
\text{L}^* + \text{O}_2 \rightarrow \text{LO}_2^*
\]

\[
\text{LO}_2^* + \text{LH} \rightarrow \text{LO}_2\text{H} + \text{L}^*
\]

Termination

\[
\text{L}^* + \text{L}^* \rightarrow \text{L} - \text{L}
\]

\[
\text{L}^* + \text{LO}_2^* \rightarrow \text{LOOL}
\]

Hydroperoxide - unstable

\[
\text{LOOH}
\]

aldehydes

ketones

alcohols

hydrocarbons
• High storage temperatures, increased moisture, light, and some metal ions (e.g., iron) may promote lipid oxidation in almonds and reduce shelf life.

• Processing also makes almonds more susceptible to oxidation — blanching and cutting increase the surface area exposed to $O_2$, and roasting changes the almond microstructure which allows more oil within the cells to be exposed to $O_2$.

• Water activity ($a_w$) level affects lipid oxidation rates — lipid oxidation is typically lowest when almond $a_w$ is ~0.25 to 0.35 (~3-4% moisture content), and increases above or below that $a_w$ range.
Moisture >8% or relative humidity >75%:
Stimulate biological activities and texture changes
Accelerate lipid oxidation, enzymatic activities, non-enzymatic browning

Moisture 6 - 8% or relative humidity 65-75%:
High temperatures (>20°C) may promote lipid oxidation, enzymatic activities and non-enzymatic

Moisture 3 - 6% or RH 20 – 65%: Optimal conditions for minimum reactions

Different Varieties and Sizes of Almonds Absorb Water from Environment Similarly

Moisture <3% or relative humidity < 20%: Concerns for lipid oxidation increase
Peroxide value (PV)

• Peroxides (LOOH) can be measured by techniques based on their capability to liberate iodine ($I_2$) from potassium iodide (KI). Their content is usually expressed in terms of milliequivalents of active $O_2$ per kilogram of fat.

• Although the PV is applicable for following LOOH formation at the early stages of oxidation, it is, nevertheless, highly empirical. The accuracy is questionable, the results vary with details of the procedure used, and the test is extremely sensitive to temperature changes.

• During the course of oxidation, peroxide values reach a peak and then decline.

\[
\text{LOOH} + 2H^+ + 2\text{KI} \rightarrow I_2 + \text{LOH} + H_2O + 2K^+
\]

\[
I_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2\text{NaI} \text{ (starch indicator)}
\]
As oxidation progresses, PV will peak and then decline as the peroxides quickly break down, so a low PV is not always a sign of freshness or good quality.
Conjugated Dienes (CDs)

- CDs are carbon compounds (with 2 double bonds separated by a single bond) produced when PUFAs break down during the initial stages of lipid oxidation.

- They give rise to an absorption peak at ~233 nm in the UV region.

- In almonds they arise predominantly from rearrangement of the double bonds of linoleic acid.

- An increase in UV absorption reflects theoretically the formation of primary oxidation products in fats.

- Good correlations between CDs and PVs have been found in edible oils ... BUT these tend to be oils rich in PUFAs.
• In almonds, CDs are generated as a result of oxidation of linoleic acid (~10-18%)

• PVs develop from all mono- and polyunsaturated fatty acids undergoing oxidation.
Volatile Reaching our Nose

- Hexanal is an important secondary product of lipox of linoleic acid.
- It is a volatile aldehyde with a “fatty, green, grassy” note.
- It forms as almonds oxidize during storage; it can be measured and used as an indicator of oxidative rancidity.
- Other volatile compounds exist in raw, roasted, and stored almonds.
- Emerging research is showing that selected volatiles other than hexanal might be sensitive markers of early oxidation in almonds.
Headspace — Solid Phase Microextraction — Gas Chromatography — Mass Spectrometry Analysis
12 mo - Roasted Almond Sample in a PP Bag @35 °C/65% RH
Method for Texture & Sound Analysis

• Texture analysis can be performed using a Texture Technologies TA-XT2i texture analyzer.

• The fracturability of whole almonds can be evaluated using the texture analyzer with a compression disk.

• The audio can be recorded during texture analysis and analyzed to provide a more complete fracturability profile.
Description of textural factors extracted from the force/displacement curves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Textural association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of force peaks (FP)</td>
<td>Crispness, Brittleness</td>
</tr>
<tr>
<td>Average gradient (AG)</td>
<td>Chewiness</td>
</tr>
<tr>
<td>Average drop-off (AD)</td>
<td>Crispness, Brittleness</td>
</tr>
</tbody>
</table>
### Study Design

The effects of environmental storage conditions on roasted and raw almond quality characteristics were investigated with an incomplete factorial design \((n = 25)\) over 16-mo and 24-mo, respectively.

Samples were analyzed at 2-mo intervals until consumer rejection or conclusion of the study, and compared to baseline values.
## Rejection timeline for raw and roasted almond samples

**Rejection timeline (mo) for all samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mo</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>&gt;&gt; 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Raw PPB)</td>
<td></td>
<td>35/65</td>
<td>35/50</td>
<td>35/65</td>
<td>25/65</td>
<td>15/65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (Raw UC)</td>
<td></td>
<td>35/65</td>
<td>35/50</td>
<td>4/90</td>
<td>25/65</td>
<td>15/65</td>
<td>25/50</td>
<td>15/50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (Roast PPB)</td>
<td>Mo</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>&gt;&gt; 24</td>
</tr>
<tr>
<td>D (Roast HBB)</td>
<td>Mo</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>&gt;&gt; 24</td>
</tr>
</tbody>
</table>

**Samples that were not rejected**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rejection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 25/50</td>
<td>22.5%</td>
</tr>
<tr>
<td>A 15/50</td>
<td>10.0%</td>
</tr>
<tr>
<td>A 4/90+</td>
<td>12.7%</td>
</tr>
<tr>
<td>C 25/50</td>
<td>18.6%</td>
</tr>
<tr>
<td>C 15/65</td>
<td>20.6%</td>
</tr>
<tr>
<td>C 15/50</td>
<td>8.9%</td>
</tr>
<tr>
<td>C 4/90</td>
<td>9.8%</td>
</tr>
<tr>
<td>D 25</td>
<td>2.9%</td>
</tr>
<tr>
<td>D 15</td>
<td>7.9%</td>
</tr>
<tr>
<td>D 4</td>
<td>5.9%</td>
</tr>
</tbody>
</table>

**Shelf-life:**

- HBB >> PPB > UC
- At 35 °C, roasted > raw
Summary

Chemical
• ↑ values → ↓ overall acceptability and ↑ rejection rate
• Univariate analysis revealed ...
  • Overall acceptability: $a_w >$ FFAs $>$ PVs
  • Rejection rate: FFAs $>$ PVs

Textural
• ↑ FPs, ADs → ↑ overall acceptability and ↓ rejection rate
• ↑ AGs → ↓ overall acceptability and ↑ rejection rate
• Univariate analysis revealed ...
  • FPs $>$ ADs
Lipid oxidation is lowest at $a_w$ of ~0.2 to 0.4

**Water Activity ($a_w$) - Stability Diagram**

source: www.aqualab.com
Overall Study Conclusions

• For both raw and roasted almonds, an interaction of chemical and textural parameters predicted shelf life.

• Both temperature and humidity are important to regulate during storage ...
  – Almonds stored at higher $T_s$ degraded more rapidly than counterparts at lower $T_s$.
  – Almonds stored at higher %RH degraded more rapidly than counterparts at lower %RH.

• Using univariate analysis, $a_w$ and MC were determined to be the better predictors of overall acceptability and rejection rate.
Acknowledgments

Almond Board of California
Guangwei Huang
Karen Lapsley
William Kerr,
University of Georgia
The Role of Packaging and Storage Conditions on the Shelf-life of Almonds

William Kerr
Professor, Department of Food Science & Technology
Director, Food Product Research and Development Laboratory
University of Georgia, Athens GA
Why Packaging?

- Portion control
- Protect product from outside world
- Marketing
- Product information
- Ingredient, nutritional labeling, traceability
- Protect from quality changes
Almonds – What Can Go Wrong

- Many chemical changes can occur during storage
- Lipid oxidation
- Loss of aromatic compounds that contribute to flavor
- Loss of nutrients
- Changes in color
- Changes in texture
A Good Place to Start - The Role of Moisture

- The rate of chemical reactions in foods depends on temperature and moisture.

Moisture Content
Weight of water in food compared to total weight
Range: 0 to 100 gH₂O/100g

Water Activity
Related to number of water molecules
Measured by vapor pressure
Range: 0 to 1
- Many chemical reactions need water to proceed
  - Vitamin C loss, enzymatic browning, non-enzymatic browning
- Lipid oxidation is tricky. Sometimes too little water can be a problem.
- Water activity seems to be a better predictor for chemical reactions
- Water activity is the basis for several food regulations e.g.
- "If the water activity of food is controlled to 0.85 or less in the finished product, it is not subject to the regulations of 21 CFR Parts 108, 113, and 114."
Food Stability Map

- Reaction Rate
- Water Activity
- Moisture Content (gH₂O/100g)

- Region I: Lipid Oxidation
- Region II: Isotherm
- Region III: NonEnzymatic Browning

- Microorganisms
- Enzyme Activity

Graph showing the relationship between moisture content, water activity, and reaction rate, with distinct regions indicating different types of food stability issues.
Moisture Isotherm – shows how moisture content varies with water activity

Almonds < 6%

Blanched almonds, 4.51%

Roasted 2.41%
Moisture Isotherm – shows how moisture content varies with water activity

- Moisture > 8%. $a_w > 0.75$
- Oxidation, chemical reactions
- Texture problems
- Yeast and molds

Stable region $a_w$ 0.20 – 0.65

Most stable to lipid oxidation $a_w$ 0.25 – 0.35
Storing at different relative humidity causes sample to lose or pick up moisture.

Original Sample \( a_w = 0.25 \)

At 65% RH, sample picks up moisture until \( a_w = 0.65 \)

At 15% RH, sample loses moisture until \( a_w = 0.15 \)
Peroxide Values and 2-Methyl Pyrazine at 12 wks - Peanuts

Perception of oxidation may increase as roasted flavors decrease

\[ a_w = \begin{array}{ccc} 
0.22 & 0.33 & 0.45 \\
Poasted Peanut & 42.9 & 37.5 & 27.1 \\
Rancid & 11.4 & 11.7 & 15 \\
Cardboard & 16.2 & 17.7 & 22 
\end{array} \]

Texture is Also Affected by Moisture

Peanuts: Baker et al 2002

Dry Biscuit: Castro-Prada et al 2008
Most chemical reactions are faster at higher temperatures.

Induction period for lipid oxidation

Onset of rancidity occurs rapidly after the induction period (PV = 10).

Q\textsubscript{10} – How much faster a reaction occurs when temperature is increased by 10\degree C

<table>
<thead>
<tr>
<th></th>
<th>$Q_{10}$ (5\degree C)</th>
<th>$Q_{10}$ (20\degree C)</th>
<th>$Q_{10}$ (40\degree C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic reactions</td>
<td>1.87</td>
<td>1.76</td>
<td>1.64</td>
</tr>
<tr>
<td>Lipid oxidation, nutrient loss</td>
<td>3.51</td>
<td>3.10</td>
<td>2.70</td>
</tr>
<tr>
<td>Nonenzymatic browning</td>
<td>6.58</td>
<td>5.47</td>
<td>4.45</td>
</tr>
</tbody>
</table>

Oxygen Also Matters

- Lipid oxidation (and some other reactions) need $O_2$ to proceed

Initiation: $RH \rightarrow R^-$

Propagation: $R^- + O_2 \rightarrow ROO^-$

$ROO^- + RH \rightarrow ROOH + R^-$

Termination: $R^- + R^- \rightarrow RR$

$R^- + ROO^- \rightarrow ROOR$

$ROO^- + ROO^- \rightarrow ROOR + O_2$
Tools at Hand

- Get the samples dry
- Control the storage humidity
- Control the temperature
- Limit oxygen
- Packaging
Store under cool and dry conditions (<10°C/50°F and <65% relative humidity)
Keep moisture at 6% or less
Avoid exposure to strong odors. Protect from insects and pests
Protect roasted products from oxygen. Nitrogen flushing and/or vacuum packaging are options.
Whole natural almonds can be stored for about 2 years if kept at (<5°C/41°F and <65% RH)
Rotate stock to optimize shelf life
<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>VOLUME</th>
<th>CONTAINER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Almonds</td>
<td>25 lbs</td>
<td>Cartons</td>
</tr>
<tr>
<td></td>
<td>50 lbs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2200 lbs</td>
<td>Fiber bulk bin</td>
</tr>
<tr>
<td>Cut Almonds</td>
<td>25 lbs</td>
<td>Cartons with plastic liner</td>
</tr>
<tr>
<td></td>
<td>1000 lbs</td>
<td>Fiber bulk bin with plastic liner</td>
</tr>
<tr>
<td></td>
<td>1500 lbs</td>
<td></td>
</tr>
<tr>
<td>Roasted Almonds</td>
<td>25 lbs</td>
<td>Cartons with vacuum-packed foil bags</td>
</tr>
<tr>
<td>In-Shell Almonds</td>
<td>50 lbs</td>
<td>Sacks</td>
</tr>
</tbody>
</table>

Source: ABC Technical Kit
The Role of Packaging in Promoting Shelf Life

- Packaging can limit the uptake of moisture and oxygen.

Diagram:
- High O₂ → Low O₂
- High H₂O → Low H₂O
The rate of transfer depends on key package properties

- Water vapor transmission rate (WVTR)

\[
WVTR \left( \frac{g H_2O}{m^2 \text{ day}} \right) = kA \left( \frac{P_{H2O,\text{out}} - P_{H2O,\text{in}}}{x} \right)
\]

- Oxygen transmission rate (OTR)

\[
OTR \left( \frac{cc O_2}{m^2 \text{ day atm}} \right) = KA \left( \frac{P_{O2,\text{out}} - P_{O2,\text{in}}}{x} \right)
\]
$WVTR \left( \frac{gH_2O}{m^2 \text{ day}} \right) = \frac{kA \left( P_{H_2O, out} - P_{H_2O, in} \right)}{x}$

- Area $A$
- Thickness $x$
- $P_{H_2O, out}$ Relative humidity
- Water activity
- $P_{H_2O, in}$ Relative humidity
- Water activity
Some Possibilities

- **Metal Cans**: made from tin or aluminum.
- May be coated with laquer.
- Excellent prevention of air, moisture and microorganisms
- Cost and weight can be issues
- **Glass**: Excellent prevention of air, moisture and microorganisms
- Cost, added weight, breakability are issues
- **Paper, board and foil**: relatively cheap, light packaging
- Board may be coated with polyethylene to limit interaction with contents
- Cardboard box may support plastic liner
- Foil lining can help limit H$_2$O and O$_2$
Flexible packaging

- **Polythene**: low density for film wrapping. Some resistance to moisture transfer. High density PE for greater durability.

- **Polypropylene**: can form clear bottles that can hold hot product

- **Polyamide** (nylon): good oxygen barrier. Useful for vacuum packs.

- **Polyethylene terephthalate** (PET): can form rigid bottles with heat resistance (CPET)

- **Polystyrene**: expanded polythene used for trays and insulated containers.

- **Others**: butylated polypropylene (BOPP), ethylene vinyl alcohol copolymer
PE films

Woven PE

PS trays

PP containers
Combinations of materials can be useful.

8-mil PET Prima Exo-Shell/70-ga BOPP/ink/adhesive/Metallized BOPP/adhesive/BOPP
Laminates combine performance of different materials

- Plastic layers provide strength, flexibility, printability
- Foil layers limit transfer of $\text{H}_2\text{O}$ and $\text{O}_2$
<table>
<thead>
<tr>
<th>Material</th>
<th>WVTR (g/m² 24 h)</th>
<th>OTR (cc/m² 24 h)</th>
<th>Light Barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal can</td>
<td>0</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Glass jar</td>
<td>0</td>
<td>0</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Foil</td>
<td>0</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Al laminate</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>100%</td>
</tr>
<tr>
<td>HDPE</td>
<td>145</td>
<td>53,000</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>LDPE</td>
<td>560</td>
<td>178,000</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>PP</td>
<td>260</td>
<td>81,000</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>PET</td>
<td>800</td>
<td>1500</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Cellophane</td>
<td>137,000</td>
<td>4500</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
Some Studies at UGA

- Almonds stored in different packages at temperatures between 4 and 35°C
- Relative humidity between 50 and 65%

---

Table: Packaging of Nonpareil Raw and Roasted Almonds

<table>
<thead>
<tr>
<th>Package Type</th>
<th>Raw</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardboard Box (600 ± 5 g)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Bag (300 ± 5g)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>High Barrier Bag&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bags were flushed with food-grade N₂ and sealed. Initial O₂ level was < 0.5%.
Package makes a big difference in the rate of moisture pickup

- Laminate pouch (HB) helps limit moisture pickup
- Cardboard box does not prevent moisture pickup. RH is important.
- PP provides limited protection.
Crunchiness as measured by number of fracture events

- Roasted are drier. Start with higher crunchiness.
- HB bags give most protection for roasted.
- PP bags okay if low temperature and humidity.
Peroxide values measure lipid oxidation

- Greatest lipid oxidation for raw samples in cardboard box. RH not important.
- HB bag helps protect against oxidation.
- PP bag provides some protection for roasted nuts.
- Oxidation high in raw nuts in PP bags and high temperature and humidity.
Sensory Tests

- Within 10 months, 4 samples had failed (>25% rejection)
  - Raw, 2 mo, cardboard box, 35°C/65% RH
  - Raw, 6 mo, cardboard box, 35°C/50% RH
  - Raw, 6 mo, cardboard box, 4°C
  - Raw, 6 mo, polyethylene bag, 35°C/65% RH
  - Roasted in PE bag, 35°C/65% RH (borderline)

- Samples in boxes at high RH absorb water. Flavor volatiles decrease, oxidation begins. Loses texture of crunchy almond.
Sensory Tests: Consumer Rejection

Raw almonds in PP bags

Raw almonds in Cardboard

Roasted almonds in PP bags

Roasted almonds in HB bags
Samples at 35°C/65% RH in cardboard failed (>25% rejection) within 2 months. Samples at 4°C/90%RH failed within 6 months.

Raw almonds in cardboard at 15 or 25°C and 50% RH failed after 16 months.

In PP bags, samples at 35°C/65%RH failed after 6 months.

Samples at 25°C/65%RH failed after 16 months. Those at 15°C/65%RH lasted until 24 months.
Roasted almonds: consumer rejection

- In PP bags, roasted almonds at 35°C/65%RH failed at 12 months; at 35°C/50%RH at 14 months; 25°C/65%RH at 16 months
- Samples fail for different reasons: odor, flavor, texture
- Only one sample (35°C, 65%RH) failed in HB bag after 16 months
Mexis et al. 2010. Effect of oxygen absorber, nitrogen flushing, packaging material oxygen transmission rate and storage conditions on quality retention of raw whole unpeeled almond kernels. LWT. 43: 1-11

- raw, unpeeled whole almonds
- PET/LDPE, LDPE/EVOH/LDPE bags
- some had oxygen scavenger pouches ZPT-50 (zinc pyrithione)
- measured PVs and FFAs, some volatile compounds
- $O_2$ absorber useful for preserving shelf-life regardless of temperature and exposure to light

- Whole almonds with and without PE package for 600 days. Also blanched and blanched/sliced
- Raw stored in sealed 1 lb PE bag or cartons. Blanched in PE bags.
- Not much measured changes in PVs, IVs and FFAs for natural almonds
- More dramatic changes for blanched or blanched/sliced almonds. Skins may protect FFAs from further oxidation
Conclusions

- Roasting helps lower initial moisture. Enhances crunchiness.
- Moisture, temperature and oxygen are all relevant.
- Storage of raw almonds can work if low temperature and humidity are maintained.
- Storage of raw almonds in sealed PP packages provide further protection.
- Roasted nuts in PP can last ~12 months at 35°C/65%RH. Lower temperature and humidity provide better protection.
- Roasted nuts in high barrier bags have longest shelf-life. Up to 16-24 months.

Acknowledgements: Almond Board of California
Cristian Rogel-Castillo, UC Davis
Concealed Damage caused by Moisture Exposure

Cristian Rogel-Castillo
Food Science & Technology
University of California Davis

Dec 8, 2016
Concealed Damage

- Concealed damage (CD) results in a brown to black discoloration of almond nutmeat; especially after roasting
- To date, CD cannot be detected by screening methods, and little is known about the chemistry, and relationships between moisture and CD formation, and the mechanism by which color forms
  - Lower consumer acceptance, bitter flavors and possibly lower nutritional value due to degradation of amino acids and lipids
  - May be more susceptible to lipid oxidation and rancidity development which may influence shelf-life
- Our goal was to establish a chemical understanding of CD development in almonds and how it relates to temperature and moisture exposure
Research on Concealed Damage

1. Effect of moisture and temperature in the development of CD
2. Development of a screening method for the detection of almonds with CD before roasting
3. Effect of drying and storage in almonds with CD
How Moisture and Temperature may Influence CD in Almonds

Water (Rain)
Heat (Windrows/Stockpiles)

Lipids (Oil)
Proteins
Carbohydrates

Hydrolysis

Free fatty acids
Free amino acids
Reducing sugars

Further reactions such oxidation

Volatile
Developing a Model for Evaluating CD in Raw Almonds

- Our first objective was to classify the almonds into two groups: No concealed damage (control) and those with concealed damage (CD)
- Raw almond kernels are cut in a half, numbered and placed into 2 identical racks
- One rack of is roasted [120°C/248°F for 90 min]; while, the other is not roasted
- Roasted almonds that develop color (have CD) are matched with the raw almonds
- Identify raw almonds that will develop CD
Percent CD in Raw Almonds Held at 5%, 8% and 11% Moisture at 35°C and 45°C over 18 Days
Color Development in Raw and Roasted Almonds (120°C/248°F for 90 min) Exposed to 5% (Control) and 11% Moisture (CD)
CD is a Challenge as it is a *Hidden Defect*

- Although CD is a hidden defect, the chemical changes that result in the brown pigmentation may come through the Maillard reaction.
- Moisture produces precursors for the Maillard reaction through the degradation of lipids, carbohydrates, and proteins.
- Many of these compounds break down into volatiles.

<table>
<thead>
<tr>
<th>Raw</th>
<th>Roast</th>
<th>Raw</th>
<th>Roast</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Raw Almond" /></td>
<td><img src="image2.png" alt="Roasted Almond" /></td>
<td><img src="image3.png" alt="Raw Almond" /></td>
<td><img src="image4.png" alt="Roasted Almond" /></td>
</tr>
</tbody>
</table>

(a) raw and roasted almonds exposed to 5% moisture (control)  
(b) raw and roasted almonds exposed to 11% moisture.
Different Pathways for the Production of Volatile Compounds

Identify Changes in Volatile Compounds in Almonds with CD

CD ➔ Increase in volatile related to:

1) Lipid oxidation
   - Acetic Acid
   - Hexanal
   - Nonanal
   - Alcohols

2) Amino acid metabolism
   - 3 – methyl – 1 – butanol

Our results suggest that post-harvest moisture exposure resulting in an internal kernel moisture > 8% is a key factor in the development of CD in raw almonds, and that CD is accelerated by temperature.

Rogel-Castillo et al. Journal of Agricultural and Food Chemistry 2015 63 (37), 8234-8240
Research on Concealed Damage

1. Effect of moisture and temperature in the development of CD
2. Development of a screening method for the detection of almonds with CD before roasting
3. Effect of drying and storage in almonds with CD
Developed NIR Model for Identifying Raw Almond Kernels with CD before Roasting

• To date, there are no rapid, robust methods for detecting CD in raw almonds
• Previous studies focused on the use of UV-Vis, pH and color measurements (LCh) to predict almonds with CD before roasting without positive results
• Our studies suggest that lipids, protein and carbohydrates degrade in almonds with CD

How NIR Works

- Near Infrared (NIR) Spectroscopy was evaluated as a technique as it is a non-destructive method that can be used to measure lipids, carbohydrates and proteins in raw almonds.
- This technique may be suitable for in-line NIR sorting of raw almonds.
NIR Spectroscopy as a Tool to Classify Almonds with CD before Roasting

Raw almonds (Before roasting) → NIR Spectra Acquisition (1125 – 2153 nm) → Data pre – processing:
1) SNV
2) Second derivative

Prediction and Classification of almonds with Concealed Damage (Before roasting) → Chemometrics:
Partial Least Squares Discriminant Analysis (PLS-DA) → NIR Spectra (After data pre-processing)
A New NIR Spectroscopy Model

- Differences between “normal” almonds and “moisture damage” were in the region corresponding to lipid content as well as protein and carbohydrates.

**NIR model:**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (nm)</td>
<td>700 – 1400</td>
<td>700 – 1400*</td>
<td>700 –1400*</td>
<td>1125–2153*</td>
</tr>
<tr>
<td>% Error rate</td>
<td>12.4 – 27.5</td>
<td>5.8</td>
<td>8.8</td>
<td>8.2 – 9.2</td>
</tr>
<tr>
<td>% False positive</td>
<td>0.7 – 5.4</td>
<td>2.91 – 3.41</td>
<td>0.58 – 1.74</td>
<td>12.4 – 16.1</td>
</tr>
<tr>
<td>% False negative</td>
<td>11.1 – 23.8</td>
<td>14.81 – 62.96</td>
<td>31.48 – 53.70</td>
<td>10.6 – 17.2</td>
</tr>
</tbody>
</table>

* Selected wavelengths

Research in Concealed Damage

1. Effect of moisture and temperature in the development of CD

2. Development of a screening method for the detection of almonds with CD before roasting

3. Effect of drying and storage on almonds with CD
Moisture and Concealed Damage

• We demonstrated that post-harvest internal kernel moisture ≥ 8% is a critical factor in the development of CD in raw almonds and that CD development is accelerated with increasing temperature.

• In typical commercial practices, almonds are left in the field to dry in windrows to a kernel moisture content of < 6%. Almonds are then stored in stockpiles until they can be processed.

• However, during a rainy season, almonds may be exposed to extra moisture while in windrows and/or stockpiles.

• Under these conditions, almonds are typically dried at the hulling/shelling facility back to ~6% moisture.
  – However, almonds may experience prolonged moisture exposure due to drying limitations of processors.
Moisture and Concealed Damage

• Pearson (1998) was the first to recognize that drying wet almonds prior to roasting had the potential to reduce visible signs (pigments) associated with CD
  – Heat promotes the loss of water and facilitates the Maillard reaction leading to the formation of pigments

• However, the chemical changes related to lipid oxidation that occur in response to moisture exposure in almonds, don’t go away with drying
  – We recognized that the lipid oxidation by-products formed during moisture exposure may promote early rancidity development and decreased shelf-life in moisture-exposed almonds

• Oxidation of oleic and linoleic acids will increase production of volatile compounds related to off-odors and rancidity including: aldehydes, ketones, acids, alcohols, hydrocarbons, lactones, and esters
  – Evaluate the influence of drying moisture-exposed almonds on volatile compounds and during accelerated storage
Effect of Drying on the Visual Color of Almonds with CD

Percentage Concealed Damage

% CD (Based on L value)

Drying Temperature (°C)

CD  Dry @45C  Dry @55C  Dry @65C  Dry @75C  Dry @85C  Dry @95C
Effect of Drying Temperatures on Volatiles in Moisture Exposed Almonds

Hexanal

Nonanal

Drying temperature (°C)

Relative concentration (µg/Kg)
Effect of Accelerated Storage on Moisture Exposed and Dried Almonds

* Accelerated storage at (45°C/80% RH), 1 week = 1 month
Effect of Drying Temperatures on Volatiles in Moisture Exposed Almonds

**Acetic Acid**

- Relative concentration (µg/Kg)
- Drying temperature (°C)
- CD, Dry @ 45C, Dry @ 55C, Dry @ 65C, Dry @ 75C, Dry @ 85C, Dry @ 95C

**Benzaldehyde**

- Relative concentration (µg/Kg)
- Drying temperature (°C)
- CD, Dry @ 45C, Dry @ 55C, Dry @ 65C, Dry @ 75C, Dry @ 85C, Dry @ 95C
Effect of Accelerated Storage on Moisture Exposed and Dried Almonds

* Accelerated storage at (45°C/80% RH), 1 week = 1 month
Maillard Reaction

Reducing groups
* Reducing sugars
* Lipid oxidation by-products (Aldehydes, ketones)

Free amino acids

Early Maillard reaction / Amadori rearrangement

Advanced Maillard reaction

Melanoidins formation (brown pigments)

Amino Acid Structure

\[
\text{Aldehyde} \quad \text{Ketone}
\]

\[
\begin{align*}
\text{Amino Acid} & : \quad \text{Carbonyl group} \\
\text{Amino Group} & : \quad \text{Carbonyl Group} \\
\text{Side Chain} & : \quad \text{Amadori Product}
\end{align*}
\]
Correlation between Reducing Sugar and Amadori Compounds in Almonds Dried between 45°C and 95°C (▲: CD)

![Graph showing the correlation between reducing sugar and Amadori compounds. The graph includes data points and a trend line with a correlation coefficient of r = -0.404.](image)

1. **X-axis:** Reducing sugar (g/100 g almond db)
2. **Y-axis:** Amadori compounds (AOD/g protein db)
3. **Trend Line:** 
   - The trend line is indicated by a dashed line.
   - The equation of the trend line is: 
     \[ y = mx + b \]
   - The correlation coefficient is: \( r = -0.404 \)
Correlation between Total Aldehydes and Amadori Compounds in Almonds Dried between 45°C and 95°C ($\Delta$: CD)

$r = 0.838$
Conclusions

• Results demonstrated that drying between 45°C - 55°C reduces the visible sign of CD (i.e. pigmentation) and decrease lipid oxidation

• Increased drying temperatures above 75°C promote lipid oxidation and the Maillard reaction (i.e. pigmentation)

• Furthermore, our results suggest that lipid oxidation by-products may participate in the browning reaction instead of reducing sugars

• In addition, it is known that some of the by-products of lipid oxidation are related with off-flavors (e.g. aldehydes, ketones, and organic acids) suggesting that almond with CD after drying may present defects in quality.

• Hence, further analysis (specially, sensory analysis) should be done to test the effect of drying in the quality of almonds with CD after drying in consumer acceptance (next research in our lab).

• Moreover, alternative drying protocols and technologies should be tested in the future to find the best drying condition (temperature and time) to control CD minimizing the effect in quality.
Thank You For Your Attention
Questions?