Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V.

Flavour Stability
Flavour Stability

• The retaining of the original character of the beer, from filling until consumption
• Beer is often stored for several weeks (even month) before it is sold
• Important ➔ flavour and taste of the beer constant over long period
• Changes ➔ divided in two groups:
  – Change of palatefullnes and bitternes, loss of the original harmony
  – Change of the aroma ➔ lightstruck and stale flavour
• Oxidation ➔ high oxygen content in wort and beer
• Thermal influences ➔ long boiling times, pasteurisation, high storage temperatures
• Influence of light (wave length of 350-500nm) ➔ lightstruck flavour
Journal-No. / Date of Arrival: #

Sample:

<table>
<thead>
<tr>
<th>Storage time and</th>
<th>weeks</th>
<th>°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>storage temperature:</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
</tr>
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</table>

**GENERAL IMPRESSION**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**ODOUR**

<table>
<thead>
<tr>
<th>Quality</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**FLAVOUR**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Quality</th>
<th>Palate fullness</th>
<th>Bitterness</th>
<th>Intensity</th>
<th>Quality</th>
<th>Fizziness</th>
<th>Intensity</th>
<th>Fruitiness</th>
<th>Intensity</th>
<th>Harmony of the beer (palate fullness/bitterness)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**OFF-FLAVOURS**

- Oxidized, paper, cardboard
- Oxidized, rancid
- Oxidized, madeira/sherry
- Scratchy, harsh, clinging bitterness
- Adstringent
- DMS, cabbage, celery
- Diacetyl
- Sulfury (-SH)
- Sulfitic (SO₂)
- Others....................
- Others....................
- Others....................

not present 1 2 3 4
Flavour Changes during Storage

![Graph showing changes in intensity over time with annotations for bitter taste, ribes, sweet aroma, sweet taste, toffee-like aroma and flavor, and cardboard flavor.]

Dalgliesh1977
Possible Pathways on the Formation of Stale Flavour Aldehydes

- Oxidation of higher alcohols
- Oxidative decomposition of bitter substances
- Maillard reaction and Strecker degradation
- Aldol condensation
- Secondary autoxidation of aldehydes
- Oxidation of unsaturated fatty acids
  - Photooxidation
  - Radical induced autoxidation
  - Enzymatic oxidation (lipoxygenase)
Carbonyl Compounds

- Aldehydes and Ketones
- Characteristic → complete change of flavour, due to minor changes
- Very low flavour threshold in ppb-range

Example:
Moving a double bound one step along the carbon chain changes the flavour from cardboard-like to cucumber-like

Tech. Quart, No. 3, 135 – 141, 1974
# Flavour Thresholds of chosen Aldehydes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold [ppm]</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>25</td>
<td>Green leaves, fruity</td>
</tr>
<tr>
<td>Furfural</td>
<td>150</td>
<td>Paper, husk</td>
</tr>
<tr>
<td>n-Hexanal</td>
<td>0.35</td>
<td>Bitter, vinous</td>
</tr>
<tr>
<td>2E-Hexenal</td>
<td>0.6</td>
<td>Bitter, astringent, green leaves</td>
</tr>
<tr>
<td>3Z-Hexenal</td>
<td>0.02</td>
<td>Green leaves, freshly cut grass</td>
</tr>
<tr>
<td>5-Methylfurfural</td>
<td>20</td>
<td>Almonds, burnt/phenolic</td>
</tr>
<tr>
<td>n-Heptanal</td>
<td>0.075</td>
<td>Vinous, bitter, very unpleasant</td>
</tr>
<tr>
<td>n-Octanal</td>
<td>0.04</td>
<td>Orange peel, bitter, vinous</td>
</tr>
<tr>
<td>2E-Octenal</td>
<td>0.0002</td>
<td>Bitter, stale</td>
</tr>
<tr>
<td>2E-Nonenal</td>
<td>0.00011</td>
<td>Papery (cardboard), oxidized, stale</td>
</tr>
<tr>
<td>2E,4E-Nonadienal</td>
<td>0.0005</td>
<td>Oily, rancid</td>
</tr>
<tr>
<td>T-2-c-6-Nonadienal</td>
<td>0.00005</td>
<td>cucumber</td>
</tr>
<tr>
<td>2E-Decenal</td>
<td>0.001</td>
<td>Bitter, rancid, stale</td>
</tr>
<tr>
<td>2E,4E-Decadienal</td>
<td>0.0003</td>
<td>Oily, deep-fried</td>
</tr>
</tbody>
</table>

Monatsschr. F. Brauwiss., No. 10, 396 – 401, 1985
Changes in Aldehyde Values during Storage [ppb]

<table>
<thead>
<tr>
<th>Compound</th>
<th>0 °C</th>
<th>30 °C</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 Weeks</td>
<td>4 Weeks</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>2-Methylpropanal</td>
<td>6.1</td>
<td>20.0</td>
<td>30.6</td>
</tr>
<tr>
<td>3-Methylbutanal</td>
<td>1.8</td>
<td>3.1</td>
<td>4.2</td>
</tr>
<tr>
<td>3-Methylbutanal</td>
<td>12.2</td>
<td>17.2</td>
<td>20.7</td>
</tr>
<tr>
<td>Pentanal</td>
<td>0.3</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Hexanal</td>
<td>1.0</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Furfural</td>
<td>28.8</td>
<td>202.8</td>
<td>362.0</td>
</tr>
<tr>
<td>Methionial</td>
<td>2.8</td>
<td>3.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>6.6</td>
<td>9.9</td>
<td>10.1</td>
</tr>
<tr>
<td>2E-Nonenal</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Vesely et al. (2003)
Oxidation of Higher Alcohols by Melanoidins

Higher alcohols can be oxidized by oxygen. This reaction can be accelerated by the presence of Melanoidins. In addition to that this oxidation can be accelerated by higher temperatures and a low pH value.

Brew. Digest., No. 4, 48 – 57, 1972
# Oxidation of Higher Alcohols

<table>
<thead>
<tr>
<th>Higher Alcohols</th>
<th>Aldehydes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propyl</td>
<td>Propylaldehyde</td>
</tr>
<tr>
<td>Iso-butyl</td>
<td>Iso-butylaldehyde</td>
</tr>
<tr>
<td>n-butyl</td>
<td>n-butylaldehyde</td>
</tr>
<tr>
<td>Amyl</td>
<td>Amylaldehyde</td>
</tr>
<tr>
<td>Iso-amyl</td>
<td>Iso-amylaldehyde</td>
</tr>
</tbody>
</table>

Brauwelt *114, Nr. 10*, 159-161, 1974
FORMATION OF ALDEHYDES
Strecker Degradation

\[
\begin{align*}
\text{α-Diketone} & \quad \text{Amino acid} & \quad -\text{C}=\text{O} + \text{R} \quad \text{H}_2\text{N} \quad \text{C} \quad \text{COOH} & \quad \text{+ H}_2\text{O} \quad -\text{H}_2\text{O} \quad \text{+ H}_2\text{O} & \quad \text{−C}=\text{N} \quad \text{C} \quad \text{COOH} & \quad \text{+ H}_2\text{O} & \quad \text{−C}=\text{O} \quad \text{−C}=\text{O} & \quad \text{H}_2\text{O} \quad \text{−C}=\text{O} \\
\text{Amino ketone} & \quad \text{Aldehyde} & \quad \text{H}_2\text{N} \quad \text{C} \quad \text{COOH} & \quad \text{+ R-CHO} + \text{CO}_2 & \quad \text{C} \quad \text{NH}_2 & \quad \text{H}_2\text{O} & \quad \text{C} \quad \text{NH}_2 & \quad \text{H}_2\text{O} \\
\text{Melanoidins} & \quad \text{Condensation}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Corresponding aldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>Leucine</td>
<td>3-Methylbutanal</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2-Methylbutanal</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2-Phenylethanal</td>
</tr>
<tr>
<td>Valine</td>
<td>2-Methylpropanal</td>
</tr>
<tr>
<td>Methionine</td>
<td>Methional</td>
</tr>
<tr>
<td>Glycine</td>
<td>Formaldehyde</td>
</tr>
</tbody>
</table>
Non-oxidative Aging of Beer
(Strecker Aldehydes)

+ TBI as indicator for heat stress is giving a measure for amount of maillard products
  - Directly proportional to amount of HMF (Hydroxy-Methyl-Furfural)
  - Sugar and amino acids reacting
+ Amount of FAN has been found to have an impact on intensity of this „aging pathway“. Excessive amounts of FAN are as well not beneficial to taste, foam, bitterness.
+ Just consider the right amount of FAN to make the yeast happy. Todays understanding of yeast metabolism: about 100 – 140 ppm FAN is taken up. This can be easily calculated when knowing the wort and final beer FAN.
+ Raw material mix, mashing, yeast food (as additive)…. Has an influence on the FAN amounts.
### Important Strecker Aldehydes

<table>
<thead>
<tr>
<th>Precursor Aminoacid</th>
<th>Strecker-Aldehyd</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucin</td>
<td>3-Methylbutanal</td>
<td>malty</td>
</tr>
<tr>
<td>Isoleucin</td>
<td>2-Methylbutanal</td>
<td>malty</td>
</tr>
<tr>
<td>Methionin</td>
<td>Methional</td>
<td>potato, cooked</td>
</tr>
<tr>
<td>Phenylalanin</td>
<td>Phenylacetaldehyde</td>
<td>Honey-like, floral</td>
</tr>
</tbody>
</table>
## Important Carbonyles

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Carbonyl</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycin</td>
<td>Formaldehyde</td>
<td>Caramel</td>
</tr>
<tr>
<td>Alanin</td>
<td>Acetaldehyde</td>
<td>Caramel</td>
</tr>
<tr>
<td>Valin</td>
<td>Isobutanal</td>
<td>Breadcrum</td>
</tr>
<tr>
<td>Leucin</td>
<td>Isovaleral</td>
<td>Chocolate</td>
</tr>
<tr>
<td>Phenylalanin</td>
<td>Phenylacetaldehyde</td>
<td>Viola</td>
</tr>
</tbody>
</table>
Aldol Condensation of Acetaldehyde and Heptanal

Hashimoto, Kuroiwa (1975)
Degradation of Lipids

- Degradation of fatty acids ➔ aroma active components responsible for stale flavour
- High-molecular unsaturated fatty acids (linoleic acid and linolenic acid) ➔ Precursors for stale flavour
- Degradation autooxidative (chemical oxidation) or enzymatic oxidation
- Enzymatic degradation ➔ during mashing (lipoxidase activity ➔ 50-65°C) ➔ degradation to hydroxyacids ➔ long chained unsaturated aldehydes
- Aldehydes ➔ cardboard flavour
- Aldehydes:
  - T-2-nonal, decanal, 2-octenal, 2,4-nonadienal
Possible Routes for the Formation of Trans-2-nonenal

[Diagram showing the enzymatic and non-enzymatic pathways leading to the formation of trans-2-nonenal from lipids through reactions involving lipase, lipoxygenase, and autooxidation.]
Formation of the Hydroperoxy Fatty Acids 9-LOOH and 13-LOOH by Autoxidation of Linoleic Acid

Belitz and Grosch, 1999
Sen $\rightarrow$ Sen*
Sen* + $^3$O$_2$ $\rightarrow$ Sen + $^1$O$_2$ (singulet)
$^1$O$_2$ + CH$_3$-(CH$_2$)$_7$-CH=CH- (CH$_2$)$_7$-COOH

\[ \text{CH}_3-(\text{CH}_2)_6-\text{CH}=\text{CH}- (\text{CH}_2)_7-\text{COOH} \]

\[ \text{CH}_3-(\text{CH}_2)_6-\text{CH}=\text{CH}- (\text{CH}_2)_7-\text{COOH} \]

\[ \text{CH}_3-(\text{CH}_2)_5-\text{CH}=\text{CH}-\text{CH}- (\text{CH}_2)_7-\text{COOH} \]

\[ \text{CH}_3-(\text{CH}_2)_5-\text{CH}=\text{CH}-\text{CHO} \]

trans-2-nonenal

OHC-(CH$_2$)$_7$-COOH

9-oxonanoic acid

Photo Oxidation of Fatty Acids
Formation of Lightstruck Flavour

Isohumulone + 3-Methyl-2-butenyl-radical + Carbon monoxide → 3-Methyl-2-butenyl-mercaptan

R + Sulphur containing amino acids/Sulphhydryl groups (-SH)
Structures of Iso- Alpha Acid and Reduced Iso- Alpha Acids

Tetra- Iso - alpha

Hexa-iso-alpha

Iso alpha

Rho-iso-alpha
Geraniol (ppb)

Weeks

ppb

4 °C PES
RT PES
4 °C PEB
RT PEB
4°C Cans
RT I Cans
Oxygen in the Brewing Process

- Brewhouse: oxygen uptake during mashing, pumping, lautering
- Boiling: kettles should only be filled from the bottom
- Closed wort boiling systems: ➔ lower yield in bitter substances but better flavour stability
- Open boiling systems: ➔ darker beers, harsher taste, less favourable polyphenolic composition
- Minimize oxygen uptake ➔ keep the oxygen limit low during fermentation, filtration and filling
  ➔ Pressurisation of the tanks, pipes and vessels with CO₂
  ➔ Evacuation and CO₂ pressurisation during filling
Wort Clarification

- High correlation between the clarification of wort and flavour stability
- The brighter the wort, the less fatty acids are in the wort
- Reasons: poorer precipitation during boiling, lower trub separation in the whirlpool
- Modern lauter tuns: reduction of the solid content in wort to 20-50 ppm
- Mash filters: reduction of the solid content in wort to 50-80 ppm
- Aging of beers, made of turbid worts \(\Rightarrow\) much stronger
- Increase of acetone, t-2-butenal, iso-butanal, 2-phenylacetaldehyde, iso-valeral, hexanal, t-2-nonenal, nonanal, t-2-t4-decadienal and t-2-t-4-undecadienal
Thermal Influence

• Wort boiling, pasteurisation (flash pasteurizer, tunnel pasteur)

• Boiling: Melanoidines are formed
  – amount Melanoidines depends on the time at high temperatures
Oxidation Reactions are intensified by:

+ Higher temperatures
+ Low pH-values
+ But also co-factors like iron (Fe) and copper (Cu) from water, DE and malt accelerate oxidation reactions decisively (Fenton and Haber-Weiss-reactions)
Reactions Producing Reactive Oxygen Species (ROS) in Beer

**Reaction A: Fenton reaction**

\[
\begin{align*}
\text{Fe}^{2+} + H_2O_2 & \rightarrow \text{Fe}^{3+} + OH^- + OH^- \\
\text{Fe}^{3+} + H_2O_2 & \rightarrow \text{Fe}^{2+} + O_2^{-} + 2H^+ \\
\text{Net: } 2H_2O_2 & \rightarrow OH^- + OH^- + O_2^{-} + 2H^+
\end{align*}
\]

**Reaction B: Haber-Weiss reaction**

\[
\begin{align*}
\text{Fe}^{3+} + O_2^- & \rightarrow \text{Fe}^{2+} + O_2 \\
\text{Fe}^{2+} + H_2O_2 & \rightarrow \text{Fe}^{3+} + OH^- + OH^- \\
\text{Net: } O_2^- + H_2O_2 & \rightarrow O_2 + OH^- + OH^-
\end{align*}
\]

Kaneda et al., 1999
Influence of pH on Beer Flavour Stability

Cardboard flavour
Weeks at 30 °C

- pH 3.9
- pH 4.1
- pH 4.3
- pH 4.5
Anti-Oxidants –

They help, but do I want or need them?

+ Ascorbic acid

+ Sulfite products

+ Often in combination
ASCORBIC ACID (AH\textsubscript{2})

Dienol groups help to bind approx. 50 % of the oxygen

\[
\begin{align*}
\text{Ascorbic acid (AH}_2\text{)} & \quad + \quad O \\
\text{Dehydroascorbic acid (A)} & \quad + \quad H_2O
\end{align*}
\]

\[
\begin{align*}
\text{AH}_2 + \text{RH}_2 + \text{O}_2 & \quad \leftrightarrow \quad \text{A} + \text{R} + 2 \text{H}_2\text{O}
\end{align*}
\]

“Linked” reactions mean other reducing compounds (RH\textsubscript{2}) are simultaneously oxidized. (R = oxidized organic compounds)
Sulfite Formation by Yeast

- SO₂ formation of yeast is genetically determined and amounts up to 10 ppm
- Can be increased e.g. by reduced aeration at pitching
- In general every measure that reduces growth increases SO₂ formation
Fig. 2. *trans*-2-Nonenal-bisulfite equilibria (R = CH$_3$-(CH$_2$)$_5$-) as proposed by Barker et al (1).

Dufour, J.-P., Leus, M., Baxter, A. J., and Hayman, A. R.
FLAVOUR STABILITY
Flavour Stabilization of Bisulphite Addition to Beer

A) Effect on appearance of cardboard flavour
B) Effect on 2E-nonenal formation
C) Disappearance of sulphur dioxide during ageing
Important Influences on SO$_2$- Formation

- Wort composition (especially gravity)
- Oxygen content of pitching wort
- Yeast strain
- Physiological state of yeast

→ Influencing factors are similar to those of ester formation
FORMATION OF $\text{H}_2\text{S}$ AND FREE $\text{SO}_2$ DURING FERMENTATION AND LAGERING

Pilsen-type beer

![Graph showing the formation of $\text{H}_2\text{S}$ and free $\text{SO}_2$ during fermentation and lagering for Pilsen-type beer.](image)
Phases of SO$_2$ Formation

![Phases of SO$_2$ Formation graph](image)

- **SO$_2$ (total)**
- **Yeast cell count**
- **Extract**

Time of primary fermentation

---

EBC Proc. 23, 1991
SO$_2$ - Content depending on Aeration Strategy

- All brews
- 3rd brew without
- 4th brew without
- Only 1st brew aerated

Source: C. Tenge
Aeration Strategy

Fermentation time in days

Source: C. Tenge
Influence of Packaging on Flavor Stability
Case Study from Brewery Consulting

+ **Pick-up during filling**
+ Random spot checks: 0.15 mg/L to > 0.4 mg/L (TPO)
+ Target should be: < 0.1 mg/L

+ TPO: Dissolved + headspace oxygen
Influences on Oxygen Pick-Up during Filling

- Filling pressure (high $\rightarrow$ high $O_2$)
- Filling tube
- Pre-evacuation (1x, 2x, 3x)
- $CO_2$ rinsing
- $CO_2$ in ring vessel
- Air in bottle neck
- Jetter (!)
- Time while not closed

$O_2$ uptake through filler: 0.01...0.1 mg/l (goal: 0.05 mg/l)
Oxygen Reduction during Filling

e.g. 0.5 l bottle

Contents: 520 ml air at 1 bars

Pre-pressurising using CO₂

Application of vacuum 90 % removal of air

52 ml air at 1 bars

17 ml air at 3 bars
Air in bottle neck: (500 ml bottle $\rightarrow$ 20 ml $\rightarrow$ 4.2 ml $\text{O}_2$ $\rightarrow$ 5.6 mg $\text{O}_2$)
What can I do to improve my Stability?????

Simple Approaches with little Investment Costs…..
Raw Materials

- Barley
  - Low LOX, No LOX varieties (!?)
- Malt
  - Protein and FAN content
- Yeast
  - $\text{SO}_2$ – production
- Water
  - De-aeration (< 5 ppb possible)
Check List - Brewhouse

• Reducing heat stress (Strecker aldehydes; TBI)
  – Malt
  – Wort boiling
  – Whirlpool
  – Wort cooler performance

• Reduction of reaction partners
  – Lower FAN contents of malt and less FAN release during mashing

• Oxygen uptake (?!)
  – Milling
  – Mashing-in
  – Mashing (turbulent surface)
  – Lautering
Oxygen Check List - Cold block

• Tank farm
  – Pre-pressurising and emptying with inert gas (N₂, CO₂)
  – Immediate pitching (after aeration)
  – Transfer into maturation tank without oxygen uptake
  – Check if connections to pump are tight (snorkeling of air)
  – Function of transfer pump´s mechanical shaft seal
  – Flow rate into tank too high → fountain formation

• Pipelines
  – „Snorkeling“ of air by untight connections
  – Avoidance of air cushions by filling hoses and pipes with (de-aerated) water backwards
  – Centrifuges
Oxygen Check List - Cold block

• Filter
  – De-aerated water, line de-aeration
  – Proper bleeding when sterilizing and before pre-coating
  – Inert gas and slow running stirrers in DE-dosing-vessel
  – Iron and cupper contents in DE
  – Pre- / post-runs to maturation, scrubbing with CO₂ or blending

• BBTs
  – Low oxygen in BBTs: CO₂-pressurizing from below
  – No oxygen approach: BBT filled with water and emptied with CO₂
  – Cleaning with acid under CO₂-athmosphere keeps tank oxygen-free
Filtration plant

Sources of oxygen pick-up
Oxygen Check List - Packaging

Packaging

– Correct operation of jetter (check air in head space)
– Efficient pre-evacuation
– Machine construction
  • single chamber filler, multi chamber
  • Filling tube
  • Filling pressure
– Good removal of oxygen from filler ring channel
– Removal of coarse bubbles with CO₂ flow (bubble breaker) before can seamer
– Reduce stops of filler (open bottles)
The Bottom Line

+ Avoid oxygen uptake as good as possible!
  – At any stage except for wort aeration
  – Maybe precursors for oxidation can be minimized
  – Optimize yeast and its production of natural antioxidants (SO₂)
  – The investment into a DO-meter is expensive but inevitable when optimizing the process
+ Avoid heat stress and minimize the precursors for non-oxidative staling (FAN)
+ Keep beer cold and try to minimize agitation of the product during transport

BUT: Beer is no beef jerky or dry pretzel! Its flavour will always change over time! You can only slow the process down by the above measures!
Thank you for your Attention!

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