

17TH MAY TEAGASC, CORK

THE LATEST IN INFANT FORMULA ANALYSIS

QUANTIFICATION OF ALDEHYDES ASSOCIATED WITH OXIDATIVE RANCIDITY IN DAIRY POWDERS AND INFANT MILK FORMULA BY HS-SPME GC-FID

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Overview



- Background information on lipid oxidation
 - Types
 - Reaction
 - Secondary oxidation products
 - Controlling oxidation
- Measurement of lipid oxidation
 - Quantification of Aldehydes
 - HS SPME GCFID
 - Future Work

Lipid Oxidation in dairy products



- Lipid oxidation is a common flavour defect in dairy products but a major defect in fat filled powders, often generally described as “oxidised flavour”.
- However the defect is characterised by a very broad range of sensory descriptors; *“cardboardy”, “grassy”, “painty”, “fatty”, “fishy” “beany” “tallowy” and “metallic”*.

Lipid Oxidation in dairy products

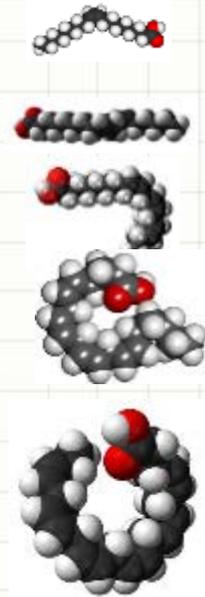


- Lipid oxidation also leads to a decrease in essential polyunsaturated fatty acids and vitamins, thus also impacts on nutritional quality.
- 3-Types of Lipid oxidation
 - Light induced (sunlight or sunshine flavour)
 - Wet cardboard odour - Presence of light or a photosensitizer such as riboflavin (pro-oxidant).
 - Metal induced (metallic or cardboard flavour)
 - Naturally occurring metals in the milk participate in the initiation of auto-oxidation reactions.
 - Spontaneous (Main type in dairy products)
 - Balance of anti-oxidative and oxidative factors

Lipid Oxidation in dairy products



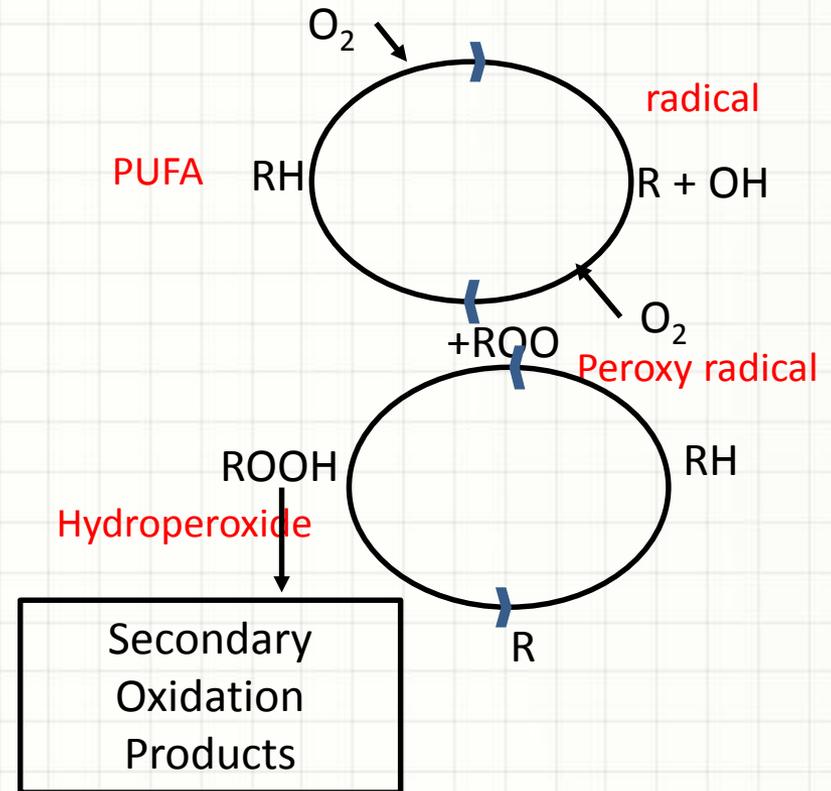
- The important lipids involved in oxidation are the unsaturated fatty acid moieties
 - Oleic acid
 - Linoleic acid
 - Linolenic acid
 - Arachidonic acid (AA)
 - Docosahexaenoic acid (DHA)
- The rate of oxidation increases with degree of unsaturation.



Lipid Oxidation in dairy products



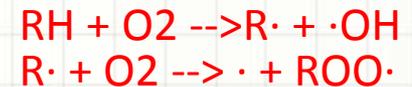
- The overall mechanism of lipid oxidation consists of three phases:
 - (1) initiation, the formation of free radicals; R
 - (2) propagation, the free-radical chain reactions; and
 - (3) termination, the formation of nonradical products.
- Hydroperoxides are flavourless and very unstable.
 - They break down to form alkoxyl radicals which are precursors of aldehydes, ketones and alcohols which are flavour/odour active.



Lipid Oxidation in dairy products



- Since the Initiation reaction is thermodynamically difficult the production of the first few radicals necessary to start the propagation reaction must occur by some catalytic means,
 - such as hydroperoxide decomposition, oxygen, light and heat exposure or metal catalysis.
 - Thus these factors drive the reaction
- Lipid oxidation is an autocatalytic reaction
 - Once started its self propagating & self accelerating!
- Factors known to influence lipid oxidation in dairy products are:
 - Animal feed, season
 - Processing operations & storage conditions
 - Water activity, moisture, packaging, light & temperature





Secondary Oxidation Products

- Aldehydes

- Especially important - very low sensory thresholds (0.002ppm in milk).

Free Fatty Acid	Potential Associated Secondary Oxidation Aldehydes
Oleic acid	Octanal, Nonanal, Decanal, 2-Decanal, 2-Undecanal
Linoleic acid	Hexanal, Pentanal, 2-Octanal, 3-Nonenal, 2,4-Decadienal
Linolenic acid	Propanal, 3-Hexenal, 2,4-Heptadienal, 3,6-Nonadienal, 2,4,7-Decatrienal
Arachidonic acid	Hexanal, Pentanal, 2-Octanal, 3-Nonenal, 2,4-Decadienal, 2,5-Undecadienal & 2,5,8-Tridecatrienal
Docosahexaenoic acid	Propanal, 2-Propenal, 4-Heptenal, 2,4-Heptadienal, 2,4,7-Decatrienal

Secondary Oxidation Products



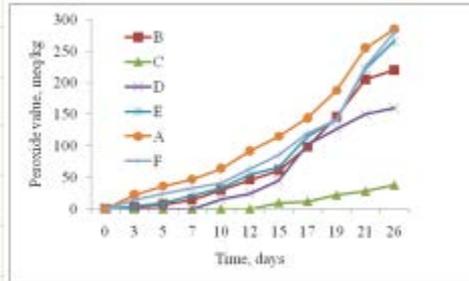
- Ketones
 - Threshold (0.4 ppm in milk)
 - “*metallic*”, “*musty*” & “*mushroom-like*”
- Alcohols
 - Threshold (varies ~similar to ketones)
 - “*grassy*”, “*green*”, “*fatty*”, “*rancid*” and “*stale*”

Controlling Oxidation!



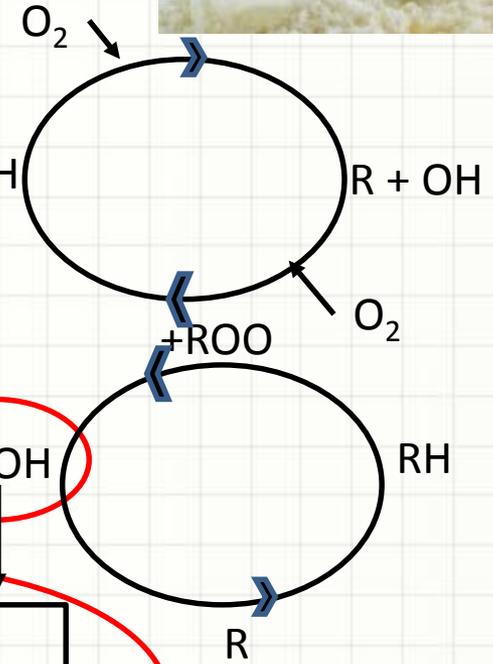
- Antioxidants function by interfering with the chain reaction.
- An antioxidant must react with free radicals more rapidly than the free radicals react with fatty acid to be effective!
- Common antioxidants
 - Butylated hydroxy anisole (BHA), Propyl Gallate, Butylated hydroxy toluene (BHT), Tertiary-butylatedhydroquinone (TBHQ) & Ascorbic acid (Vit C)
- Elimination of oxygen
 - Packaging under nitrogen, in vacuum, with an oxygen scavenger
- Elimination of the sensitive substrate
 - Replacement of polyunsaturated oils with less unsaturated oils
- Decreasing the rate of oxidation
 - Storage at low temperatures, storage in the dark, use of fats and oils that contain low levels of oxidation promoters, addition of ingredients rich in antioxidants

Measurement of Lipid Oxidation



TBARS
HS GC Methods

POV



Secondary Oxidation Products

Sensory Analysis of Off-Flavours



Descriptive Sensory Analysis

Measurement of Lipid Oxidation



- Peroxide value (POV)

- Quantifies amount of peroxides present, which are the main initial products of autoxidation.
- POV is applicable for peroxide formation at the early stages of oxidation.
- Results expressed in terms of milliequivalents of oxygen per kg fat.
- Accuracy is questionable.
- The results vary with the procedure used.
- Extremely sensitive to temperature changes which is part of the method
- Peroxides reach a peak and then decline.

Peroxide value:

Peroxide value of oil samples were determined using the method as described in AOAC,2000. And the formula is:

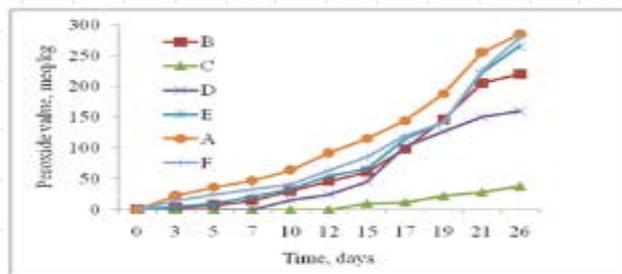
$$PV \text{ (Meq/kg)} = \frac{N \times (FS - FB) \times 1000}{\text{Wt. of sample (g)}}$$

Where,

N = normality of sod-thiosulfate, *FS* = sod-thiosulfate consumed by sample (ml), and *FB* = sod-thiosulfate consumed by blank (ml).

Unsaponifiable matter:

Similarly Unsaponifiable matter of oil samples were determined using the method as described in AOAC,2000.

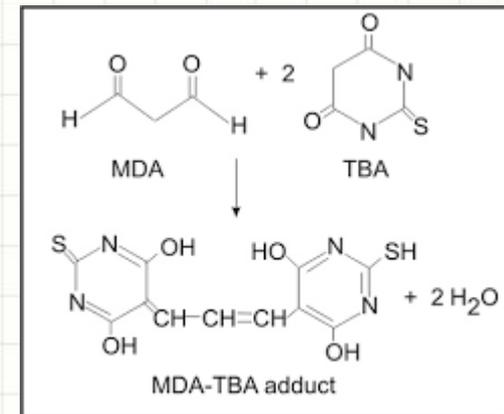


Measurement of Lipid Oxidation



- Thiobarbituric acid (TBA)

- Quantifies secondary oxidation products; aldehydes. Simple to perform.
- The principle is based on the reaction of one molecule of malonaldehyde and two molecules of TBA to form a red malonaldehyde-TBA complex, which can be quantitated spectrophotometrically (530nm).
- Non specific and insensitive for the detection of low levels of malonaldehyde.
- Sugars and other aldehydes can interfere with the malonaldehyde-TBA reaction resulting in an overestimation!

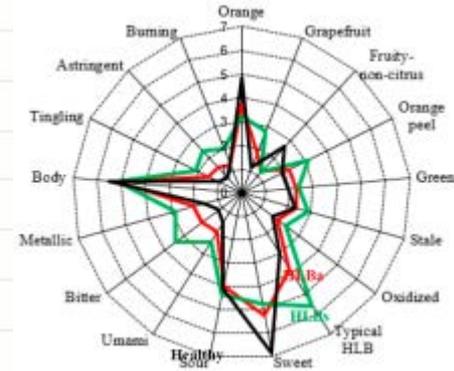


Measurement of Lipid Oxidation



- Descriptive Sensory Analysis

- This approach uses a well trained panel very familiar with the product utilizing a set of attributes/descriptors/lexicons that best describe the product incorporating the all potential off-flavours.
- The panels effectively act like a well-calibrated instrument and rate the product against the selected attributes on a scaling system.
- Can be expensive, but can operate very effectively using in-house personal.
- Potential to be coupled to other methods such as TBA or Gas chromatography techniques.



Measurement of Lipid Oxidation

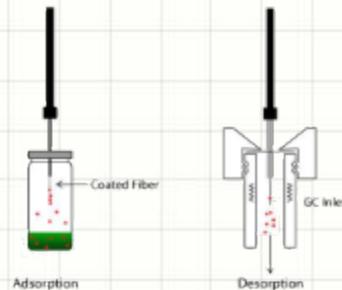
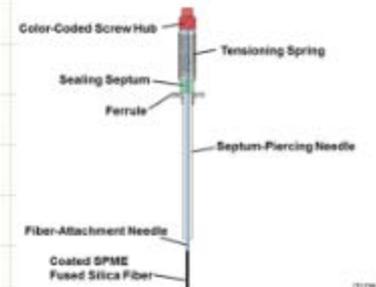


- Gas chromatography

- Best approach because of the volatile nature of the secondary products of lipid oxidation

- Sample Extraction/Concentration

- Headspace techniques are favored as sample preparation is minimal/easy, no artifacts are created, sample not destroyed & automatic.
- Most widely used method is a Static Headspace Technique – Solid Phase MicroExtraction (SPME)
- A wide range of fibres are available to target selected analytes of interest
- Vial choice and injection septa are important



Measurement of Lipid Oxidation

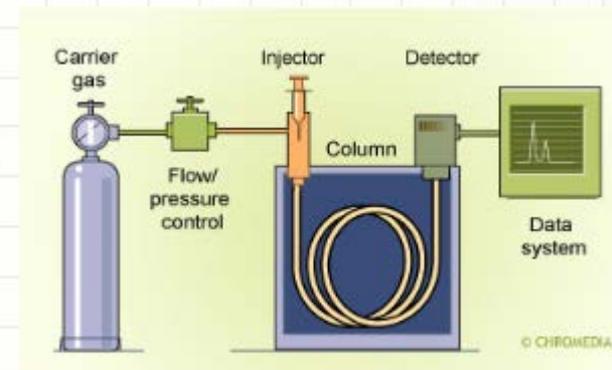


- Gas chromatography

- Fibre is thermally desorbed into a GC inlet at high temperatures and transferred to a column.
- The difference in affinity of the compounds of interest with the column phase results in separation at a fixed gas flow or pressure in a defined temperature program.
- Compounds eluting from the column enter a detector



- Flame ionization detector (FID)
- Mass spectrometer (MS)



HS-SPME GCFID - Aldehydes



- Quantification of Aldehydes
 - Hexanal, Pentanal & Propanal

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- Mianer, P.G., KOOS, Y.H., KILCawley, K.N., AUTY, M.A.E & Fenelon, M.A (2015). Levels of pentanal and hexanal in spray dried nanoemulsions

HS-SPME GCFID - Aldehydes

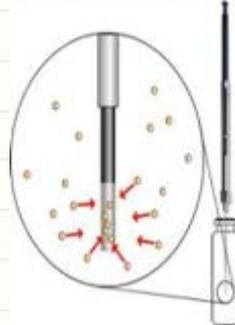


- Sample Preparation
 - Can vary slightly depending upon sample.
 - Infant Milk Formula
 - 1.25g Sample into a Amber 20ml HS Vial (silicone/PTFE septum)
 - Add 0.25 mL Internal Standard (ethyl butyrate 10 ppm) and make up to 5 g with distilled water.
 - Sample analysed in triplicate.
- Standard Preparation
 - External standards (propanal, pentanal & hexanal).
 - Standard Curve 1, 5, 10, 15 & 20 ppm.
 - Standard addition approach (standards to sample)
 - Blank sample subtracted from standard samples.
 - Standards ran in duplicate.
 - Internal Standard
 - Ethyl butyrate used to correct for any deviations

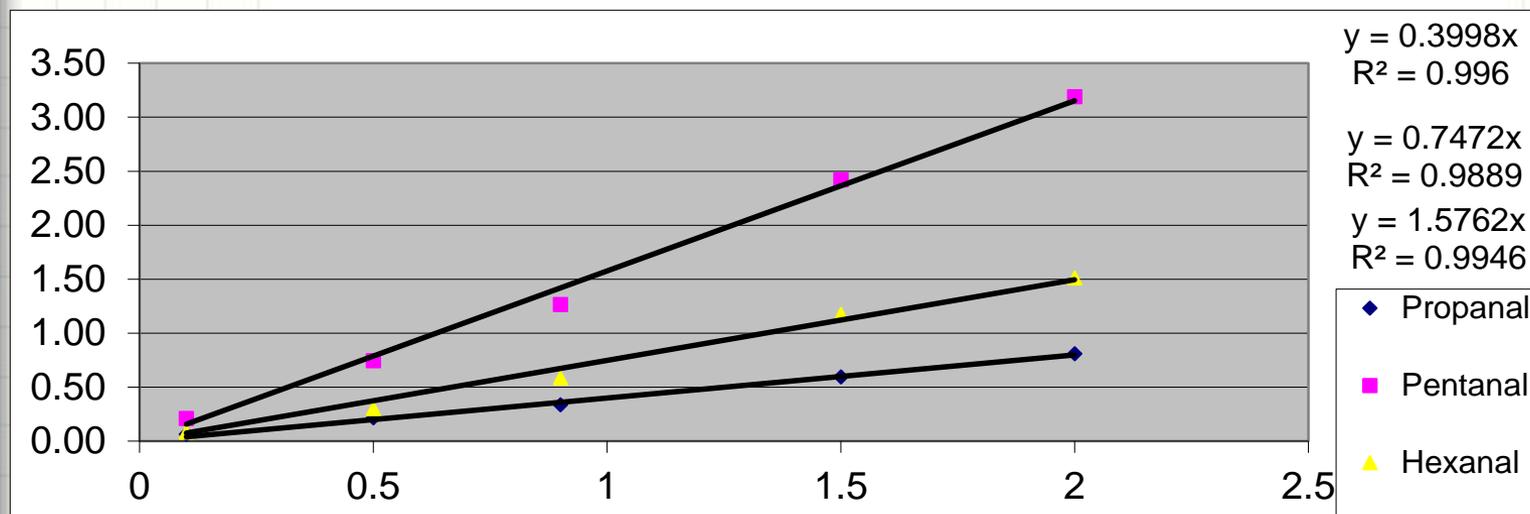
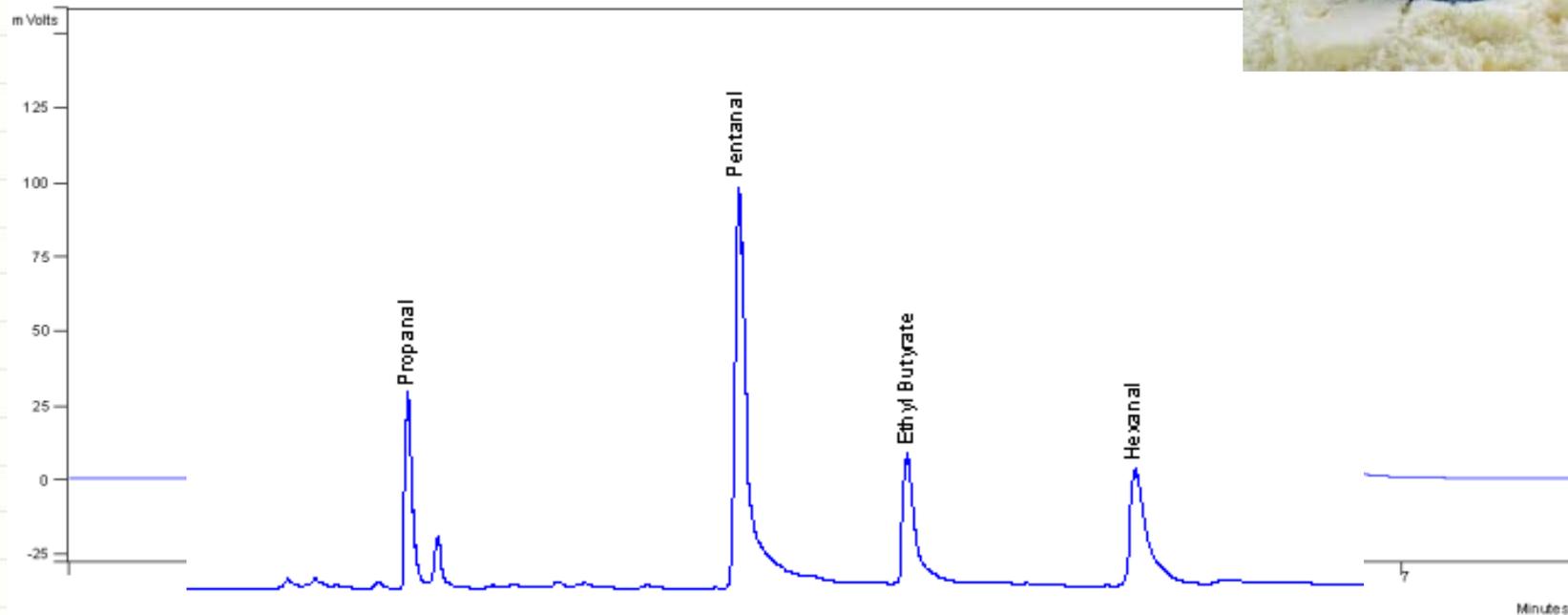
HS-SPME GCFID - Aldehydes



- SPME Conditions
 - Fibre conditioning (3 mins @ 300°C)
 - HS Saturation 20mins @ 40°C with agitation
 - > 40 °C you may get decomposition of hydroxyperoxides
 - Equilibration - 15mins @ 40°C
 - Desorption - 2mins @ 250°C
 - Multimode injector with merlin microseal
- GC
 - Injection
 - Split injection 1:10
 - Column
 - Agilent polar DB-WAX etr (30 m x 0.25 mm x 0.50 um)
 - Carrier gas
 - Helium 1 mL / min
 - Temp Program
 - 75°C for 8 min, to 240°C at 30°C per min , hold at 240 °C for 12 min with a total run time of 25.5 min
 - FID
 - 300°C



HS-SPME GCFID - Aldehydes



HS-SPME GCFID - Aldehydes



- Results
 - Expressed in ug per Kg or ppb
- Future
 - Investigate direct Immersion solid phase microextraction
 - MS detection
 - Expand quantification of other secondary oxidation products.
 - Aldehydes and ketones
 - Link with descriptive sensory analysis
 - Determine at what concentration are certain secondary oxidation products perceived
 - Sample dependent - Matrix effect
 - Link with other traditional methods
 - TBARS etc...

QUESTIONS?