STKM 2622:
Advanced Chemical Analysis of Food Laboratory

Determination of Solid Fat Content (SFC) in Oils and Fats by pulsed-NMR Analyzer

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Introduction
Lipid

What is a *lipid*?

- organic compounds soluble in non-polar solvent and insoluble in water
- formed from hydrocarbon backbone
- constitutes
  - triacylglycerols (fats and oils)
  - phospholipids
  - sterols
  - lipo-protein
  - fat-soluble vitamins
Triacylglycerols that are liquid at room temperature = OILS
Triacylglycerols that are solid at room temperature = FATS
Sometimes terms LIPIDS and FATS are used interchangeably
Fats and Oils

- **Fats** are **solid** at room temperature
- **Oils** are **liquid** at room temperature
- made of 2 components = glycerol + 3 fatty acids
Effect of Lipid Composition and Processing on Functionality of Foods

Major Components | Effects/Functions | Specifics
--- | --- | ---
QUALITY ATTRIBUTES | Color, Flavor, Odor, Texture
FUNCTIONALITY | Shortenings, Margarine, Spreads, Emulsions, Structured lipids, Confectionary products
NUTRITIVE VALUE | Linoleic acid, Linolenic acid, Vitamins: A, D, E, K, Antioxidants

Composition and Processing

Affect Chemical and physical properties

Applications

Essential Nutrients
Oils and fats application

- Food (shortenings, frying, salad etc)
- Pharmaceutical
- Animal feed
- Biodiesel
- Cosmetic
- Lubricant
- Oleochemical
Food Products with Palm Oil
Cocoa Butter Alternatives

Lauric Fats

Cocoa Butter Substitute (CBS)
- Vegetable lauric Fat used to substitute the cocoa butter.
- High percentage of lauric acid C12:0 > 54%.
- It is not compatible with cocoa butter, it’s possible to add only up to 5% of cocoa butter in the fat phase.
- High contraction degree.
- Fast crystallization, growing B’ stable crystals that impart high gloss and gloss retention.
- It doesn’t need tempering.

Non Lauric Fats

Cocoa Butter Replacements (CBR)
- Vegetable non lauric fat used to replace the cocoa butter.
- High percentage of elaidic acid C18:1 trans > 50%.
- Partially compatible with cocoa butter, adding up to 30% of cocoa butter in the fat phase.
- Contraction is lower than CBS but still good for moulding applications (TCR-05).
- Fast crystallization, growing B’ stable crystals that impart high gloss and gloss retention. It’s not necessary to make tempering phase.

Cocoa Butter Equivalents (CBE)
- Vegetable non lauric fat which has a similar triglyceride composition (POS, SOS, POP) than cocoa butter.
- Fully compatible to cocoa butter, it has a 100% tolerance in whichever proportion.
- The crystallization process occurs slower than in CBR and CBS.
- Because of its polymorphic behavior, it has to be tempered to get the B’ stable crystals.
Palm stearine

Palm Oil

PKS & PKO

Pork Farms
Pork Pie

Ginsters

Palm Oil

Rapeseed oil

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Physicochemical characteristics of fat and oils

• physicochemical properties:
  – such as mouth feel
  – flavor
  – texture
  – appearance

• analytical techniques
  – Solid Fat Content
  – Melting point
  – Cloud point
  – Smoke, Flash and Fire Points
  – Rheology
Application of Pulsed-Nuclear Magnetic Resonance (p-NMR) from Bruker
Determination of Solid Fat Content by Benchtop NMR is an internationally accepted procedure and an important analysis for producers and users of fats and oils in the food industry.
Application: Food and Non Food

1. Determination of Solid Fat Content (SFC) and melt profile of fat compositions and margarines
2. Moisture content in margarine
3. Determination of polar parts in deep-frying oils
4. Oil or fat content in foods, confectionery products, and animal feeds
5. Simultaneous determination of oil and moisture in oilseeds
6. Fat and Moisture in milk powders and milk powder products
7. Moisture determinations in rice and a variety of foods
8. Total oil and/or moisture in emulsions
9. Water distribution in dispersions and gels
10. Droplet size distribution in water in oil emulsions
11. Investigation of freezing processes
12. Unilever water droplet size application (released)
13. Total Casein content in Milk
Polymer Applications

1. Density of Polyethylene
2. Changes in cross-link density
3. Finish or oil on natural fibers, yarns, and synthetic fibers
4. Xylene solubles in Polypropylene
5. R21-values of polypropylene
6. Plasticizers or elastomers in polymers
7. Rubber content in polymer blends
8. Copolymer ratios
9. Solid content of rubber latex
10. Glass in nylon
11. Monitoring of polymerization reactions
12. Viscosity measurements on polymerization reaction mixtures
13. Total fluorine analysis in polymers
14. Fluorine-finish on fibres
Pharmaceutical Applications

1. Moisture in powders (free and bound)
2. Moisture in catalysts
3. Oil or liquids in solid matrices or powdered chemicals
4. Diffusion of liquids in liquids, powders, rock cores, zeolites and other hosts
5. Liquid or waxy coatings on solid particles
6. Viscosity of liquids
7. Determination of the extent of nitration of alcohols
SFC analyses

• Solid Fat Content (SFC)
• Solid Fat Index (SFI) Measurements
SFC

• The first version of an AOCS official method for SFC determination by low resolution NMR was published in 1993 (AOCS Official Method Cd 16b-93). Bruker has fully supported the NMR Committee and its work.
Official SFC Methods

• Solid Fat Content (SFC) is the generally accepted analysis of fats and oils in the food industry.

• SFC – influence to sensory and physical properties, such as spread ability, firmness, mouth feel, processing and stability.  e.g., margarine and butter.

• The traditional methods for SFC determination are slow, irreproducible and require additional chemicals
SFC by NMR

• Solid Fat Content determination by NMR is based on direct ratio measurement between the solid and liquid parts of the sample observed in the NMR.

• is defined as the percentage of the total lipid that is solid at a particular temperature, i.e. SFC = \(100\frac{M_{\text{solid}}}{M_{\text{total}}}\), where \(M_{\text{solid}}\) is the mass of the lipid that is solid and \(M_{\text{total}}\) is the total mass of the lipid in the food.
Reference Method

• Direct measurements of SFC by NMR can be performed quickly and accurately. NMR as a method of analysis has been established by the following standards:

• Presently, the AOCS official methods are:
  – AOCS Cd 16b-93 revised in 2000; Direct Method
  – AOCS Cd 16-81 revised in 2000, Indirect Method

• Official methods for SFC in Europe:
  – ISO 8292
  – IUPAC 2.150
A variety of methods of Solid Fat Content

- **density** of solid fat is higher than the density of liquid oil, so density increase when fat crystallizes and decrease when it melts - determine the solid fat content - temperature profile:
  - The density is usually measured by density bottles or dilatometry.
  - **NMR** - quicker and simpler to carry out but expensive.
  - **differential scanning calorimetry** (DSC) - measure the heat evolved or absorbed by a lipid when it crystallizes or melts. By making these measurements over a range of temperatures it is possible to determine the melting point, the total amount of lipid involved in the transition and the SFC-temperature profile.
Advantages of p-NMR

- NMR is very accurate and reproducible
- Analyses take less time to complete than SFI determinations
- NMR yields a SFC value from a single measurement when the Direct Method is employed
- SFC procedure does not overly depend on operator technique and judgement
- The procedure can be automated
- NMR can be used to obtain relative values for quality control even on finished products that contain water
- NMR is non-destructive; the same sample can be measured numerous times or used for other analytical tests.
- Approved AOCS methods
Solid Fat Index (SFI)

• Popular in US, - traditional method used for measuring the solids content of edible oils by dilatometry
• SFI is an empirical value that is derived from expansion of a fat as a chilled sample is warmed. In the process of melting, previously crystallized parts of the sample become liquefied
• Dilatometry does not directly measure the solids content of fat at any given time, rather it measures the change in volume compared to the starting point.
• SFI measurements depend on consistent operator skill and judgement for accuracy and reproducibility.
• Leaky dilatometer burettes, bubble formation and other artifacts can ruin an entire series of SFI determinations.
• SFI values for fats that contain emulsifiers are not very accurate due to some dissolution of emulsifiers into the indicator at the fat/indicator boundary
Solid Fat Index

• SFI is an empirical value that is derived from expansion of a fat as a chilled sample is warmed.
• In the process of melting, previously crystallized parts of the sample become liquefied.
• Since the fat molecules in a liquid state are less efficiently arranged in space compared to closely packed crystalline regions, liquid fat takes up more volume.
• Therefore, the degree of expansion is related to the change in the solid content.
Disadvantages of SFI

- Dilatometry does not directly measure the solids content of fat at any given time, rather it measures the change in volume compared to the starting point.
- SFI measurements depend on consistent operator skill and judgment for accuracy and reproducibility.
- Leaky dilatometer burettes, bubble formation and other artifacts can ruin an entire series of SFI determinations.
- SFI values for fats that contain emulsifiers are not very accurate due to some dissolution of emulsifiers into the indicator at the fat/indicator boundary.
- A strong impetus therefore exists to adapt an alternative method.
Interpretation
What we get from p-NMR?

- SFC profile
Solid Fat Content is a function of temperature, temperature history, time, and composition.
SFC in chocolate products

- Vegetable fats (e.g. CB & CBA) in chocolate hardness, heat resistant, mouthfeel & flavour release
CBS Tolerance to Cocoa Butter
SFC profile for CBS

- Primarily used for molded products because of its excellent gloss and snapping.
- Ideal for making compound chocolate.
- Can be used for enrobing and panning of hard centres.

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Modification of melting profiles of CBs (SFC)
Cocoa Butter Replacer (CBR) from Fujioil, Jpn
Confectionary Fats from Blend of Hydrogenated and Interesterified Hydrogenated Palm Kernel Oil

<table>
<thead>
<tr>
<th>Fat</th>
<th>M.P.(°C)</th>
<th>SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenated palm kernel oil (PKO)</td>
<td>46.8</td>
<td>74.2</td>
</tr>
<tr>
<td>Int. hydrogenated PKO</td>
<td>35.0</td>
<td>65.0</td>
</tr>
<tr>
<td>50% hydrogenated 50% int. hydrogenated</td>
<td>41.7</td>
<td>70.0</td>
</tr>
</tbody>
</table>

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Effect of Randomization on SCI of an 80:20 Mixture of Lightly Hydrogenated Soybean Oil and Palm Stearine
Solid Content Index of Cocoa Butter before and after Interesterification
Changes in SCI of Lard by Interesterification
Principle
Solid Fat Content

• An SFC value is determined by detecting the NMR signal from both liquid and solid components in the fat sample, or by detecting the change in the liquid signal as it is displaced by solid.

• The minispec employs the "pulse" method to perform the NMR experiment. This means that a short intense burst of radio frequency (RF) energy is applied to the sample in the static magnetic field to cause excitation of the Hydrogen in the fat.
Principle of SFC Determination Using NMR

The SFC value is determined by taking two measurement points on the FID (Free Inducton Decay). FID amplitude at point S (corresponding to total solids plus liquids), and at point L (corresponding liquids only). The specific ratio can be found using equation shown on Fig. 1. This ratio is considered to be the SFC value.

\[ SFC = \frac{(f*S - L)}{f*S} \]
Application Methodology for Direct Solid Fat Content Measurements

• The fat is melted at $T = 80$ to $100^\circ C$ and held there for 15 minutes.
• The fat must be well-mixed prior to removing a representative and residue-free amount, which is used to fill a 10 mm diameter sample tubes to a height of 4 cm.
• Sample temperature is maintained at $T = 60^\circ C$ for at least 5 minutes but not more than 15 minutes.
• Transfer the sample to $T = 0^\circ C$ and maintain for $60 +/\ - 2$ minutes (must be timed accurately).
• Transfer the sample to the temperature for which a measurement is needed. Maintain this temperature for 30 to 35 minutes (must be timed accurately).
• The creation of a melting curve requires the sample to be measured at many different temperatures (e.g. $10^\circ C / 15^\circ C / 20^\circ C / 25^\circ C / 30^\circ C / 35^\circ C$ etc).
• Insert the sample tube into the minispec for a 6 second analysis. The direct SFC method consists of the single measurement at the desired temperature. The direct parallel method consists of the consecutive measurement of solid fat content at all desired temperatures.
Options for the SFC Measurement

• Two pulsed NMR methods exist for measuring the SFC of edible oils: the direct and the indirect methods.
  – The direct method measures the signal from both the solid and liquid components
  – The indirect method measures only the liquid signal and compares it to the signal from a fully melted sample.
• The direct method is very fast, reproducible and sample preparation is minimal. Melted oil is simply poured into an NMR tube to a height of approximately 4 to 5 cm and the sample is tempered in the tube. Only one NMR measurement is needed to obtain a SFC value by the direct method.
• The indirect method is also very reproducible and accurate and it is not as fast. More care must be taken when preparing samples. Four measurements must be performed at two temperatures in order to calculate the percentage of solids.
Apparatus

- Pulsed NMR Spectrometer (Bruker Minispec mq20)
- Circulating refrigerated water bath
- Reference sample for calibration (0%, 31.44%, 75.4%)
- Aluminum heating block
Sample preparation

• Sample must be melted (microwave or oven)
• Transferred to sample tube up to 3 cm but not more than 4 cm high
• Measured the ref sample
• Transfer the sample into 0°C for 90 min
• Then transfer all the samples simultaneously into circulating water bath
• After 30 min measure the SFC at specific temperature
• Normally: 0, 5, 10, 15, 20, 25, 30, 37, 40°C
Melting
Preparation in sample tube
Incubation with different temperature
Calibration
SFC Measurement
Thank you