Biggest Mistakes in Lipid Analysis Laurence Eyres AAOCS September 2017

Agenda

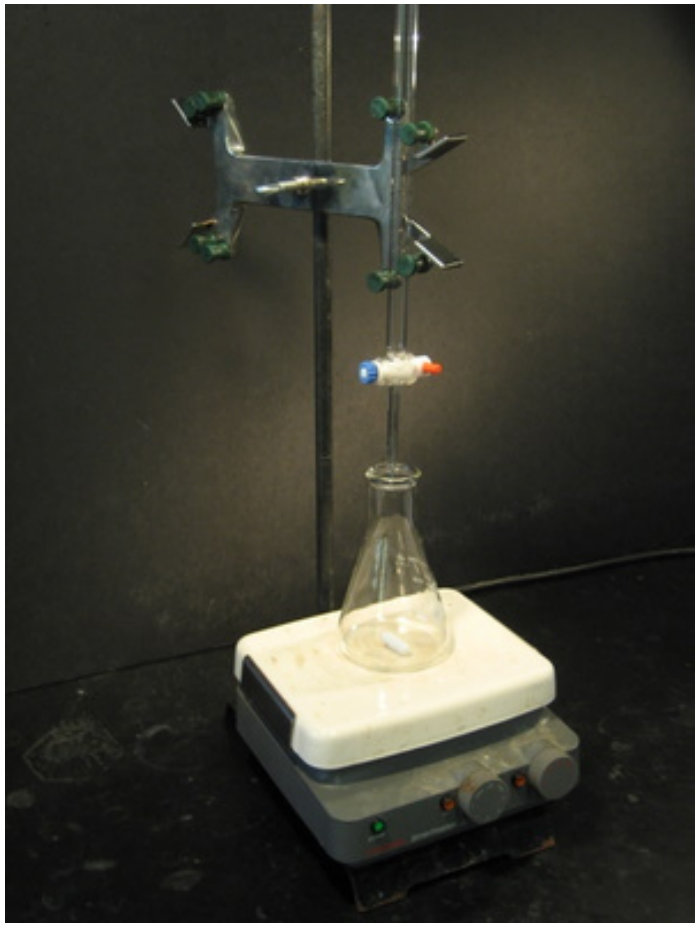
Summary of where lipid analyses go wrong-my experience, Abels Margarine, Massey uni. Auckland Uni, Bluebird Foods, NZ Dairy Foods

Worked examples

Conclusions

Recommendations

FFA titration (supposedly simple)



Grade A burettes

Reading the meniscus

Standardizing reagents

Analar

Colour change-end points

Peroxide value – Oxidation

A very old wet chemistry technique. Prone to errors

Sample size

Molarity of sodium thiosulphate solution/m/500 preparation

Stability of reagent

Reaction time

Oxygen in solvents

Burette calibration

Solvent

Starch indicator

Peroxide value

The classic iodometric method, is not without weaknesses, one

of them being the use of acetic acid in most of its numerous modifications. Acetic acid is easily oxidised and is a source of peroxides

As stated by Stansby (8), some lots of this acid, even

though meeting ACS specifications, give high and irregular

blanks, while some may react with iodine. This, and the high

“oxygen error” (liberation of iodine from potassium iodide by

atmospheric oxygen)

Anisidine Value - oxidation

Quality of p-Anisidine absolutely paramount-recystallisation almost always necessary

Discard when turns an off colour away from water white

Solvent purity a must

Interference from aldehydic flavouring agents

Frying Fat Analyses-50 years of endeavour

Using the following to evaluate the state of the used fat :

Peroxide value

Colour

FFA alone

Non eluted off a silica column

TLC

Testo for polar material

Smoke points

Many suppliers quote spurious inaccurate smoke points.

Within a broad range smoke point is proportional to FFA

The **smoke point** of an oil or fat is the temperature at which, under specific and defined conditions, an oil begins to produce a continuous bluish smoke that becomes clearly visible

Has to be measured under standard conditions AOCS standard method

American Oil Chemists' Society (2011). "AOCS Official Method Cc 9a-48, Smoke, Flash and Fire Points Cleveland Open Cup Method". *Official methods and recommended practices of the AOCS -* (6th ed.). Champaign, Ill. : American Oil Chemists' Society.

Melting point

Melting-Point Determination of Fat Products

Melting point is generally imprecise and depends on method

Barnicoat falling ball method is the most reproducible and useful technique

Simple apparatus

Correlates well with Mettler automated

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Analytical results from nil analyses

The infuriating claims for coconut oil based on containing MCT

Triglyceride analysis of true MCT oil.C8 and C10 fatty acids

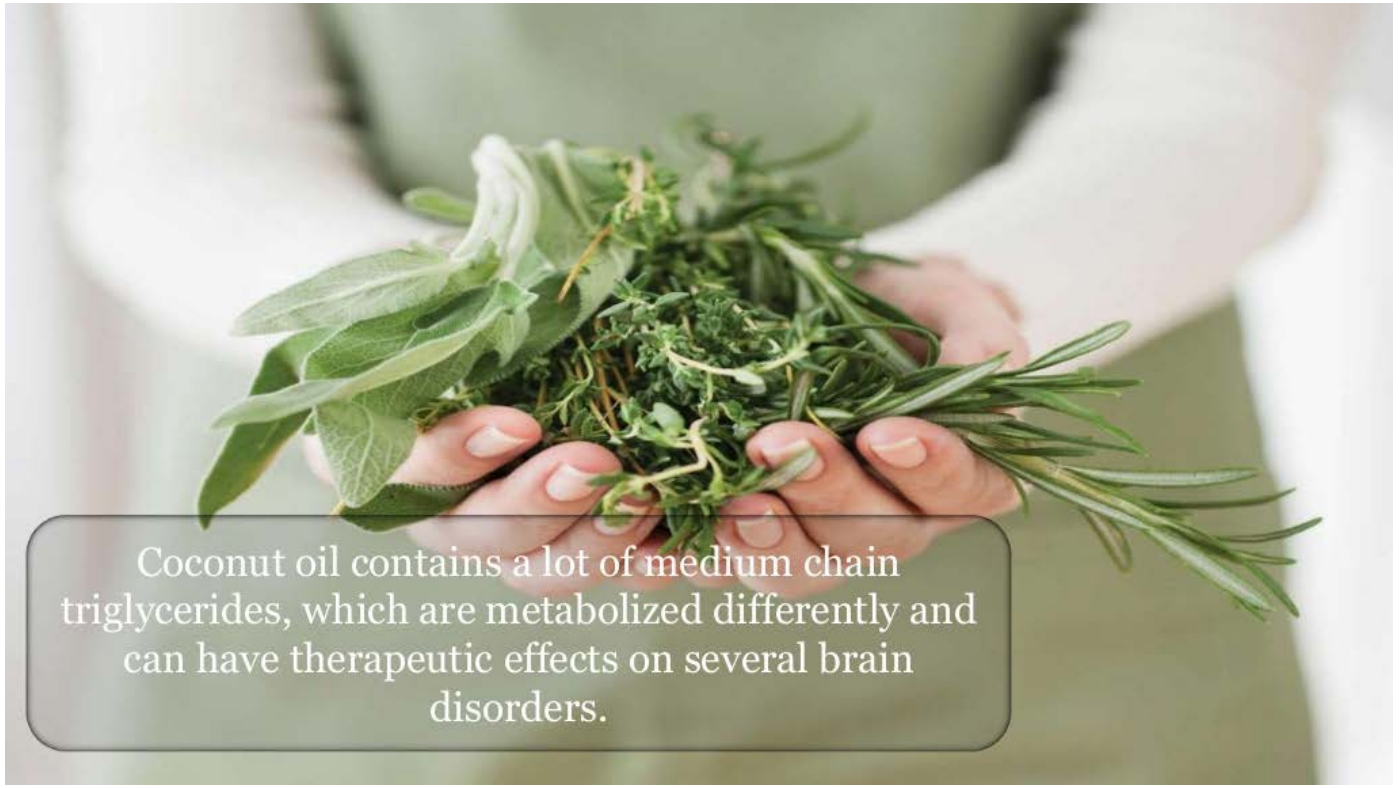
Major peaks C24-C30

Coconut oil has hardly a TAG in this range

This does not stop the spurious claims of marketers

Spurious claims for coconut oil

Oh No it does not!!!!



Difference between CNO and MCT

PHYSICAL DIFFERENCE MCT AND CNO AT AMBIENT

Total fat content in fat powders(infant formulae)

Extraction problems typical of difficult- to- extract matrices

Spray drying objective is to get free fat as low as possible

It is bound to the matrix like the proverbial you know what

Inefficient extraction only gets at the loosely bound fat which leads to errors in quantification, composition and oxidative state.

Too often the extraction is undertaken in light and air for many hours resulting in oxidation

Oil extraction and analysis (2004),AOCS,D.L. Luthria

Infant formulae analysis

Difficult matrix to extract

Care with oxidation

Check common sense result

How much fat in the manufacturing formula?

Have an idea about result

Among all the tested methods, n-hexane/isopropanol 3:1 mixture gave quantitative yields in fat extraction from reconstituted milk. This method combined with the classic iodometric titration gave the best result in terms of reproducibility, detecting peroxide values down to 1.1 (n = 5, RSD = 10%) and 1.3 meq O2/kg for 4 and 10 g of milk respectively.
Influence of fat extraction methods on the peroxide value in infant formulas (PDF Download Available). Available from: https://www.researchgate.net/publication/257422918_Influence_of_fat_extraction_methods_on_the_peroxide_value_in_infant_formulas [accessed Jul 25, 2017].

Fatty acid methyl Ester preparation

TRANSESTERIFICATION VERSUS ESTERIFICATION

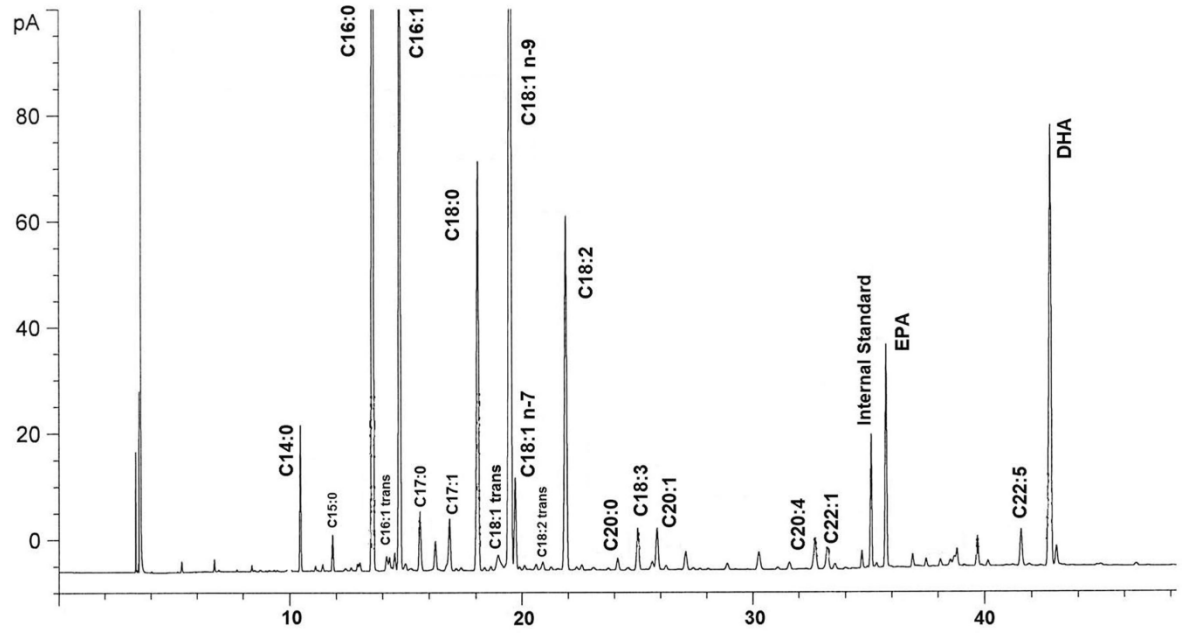
Running and quantifying FAMEs

Easy way is to normalise area% on a recording integrator

Area % is not necessarily wt% (response factors)

Wt% is not the same as mg/g of oil

Internal standard needs to be chosen wisely



Incorrectly identifying peak components

E.g. Misidentifying fatty alcohols as omega-3 FAME-a common mistake

After esterification need to separate the fatty alcohols from the FAME

When doing new oils need to check the assignment of peaks using GC/MS ,not relying on retention time.

Example

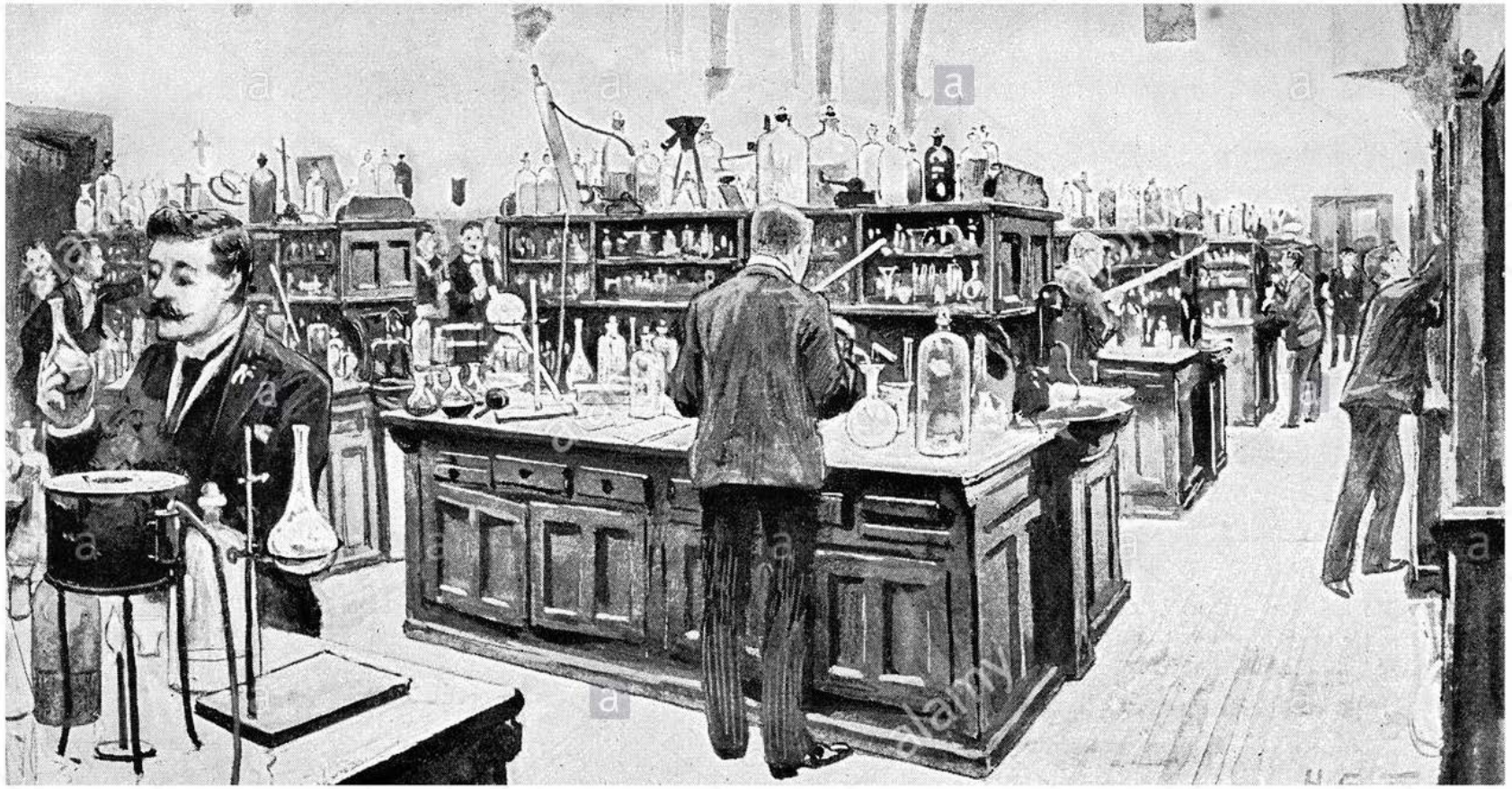
Once received the reported analysis of meadowfoam where the peaks were incorrectly assigned by a publicly registered analyst.

Sample size for GLC

One of the most unusual aspects of the dramatic changes in lipid technology in this remarkable decade from 1955 to 1965 was the reduction in the scale of operations and sample size needed to execute most analyses of fats and oils:

- 1957 *Stedman* Column 1000 g
- 1958 *Podbielniak* spinning band column 10 g
- 1959 Packed Column GLC/Katherometer 0.001 g
- 1960 Open-tubular GLC/Argon or FID 0.00001 g
- 2017 Current WCOT capillary columns

Where controversial Fish oil analyses originate



Other analyses that have posed issues

Alpha monoglyceride levels

Phosphorus by ashing-losing material in smoke

Unsaponifiable matter-removing soap

Insoluble impurities

Iodine value-reagent age

Accuracy and Precision

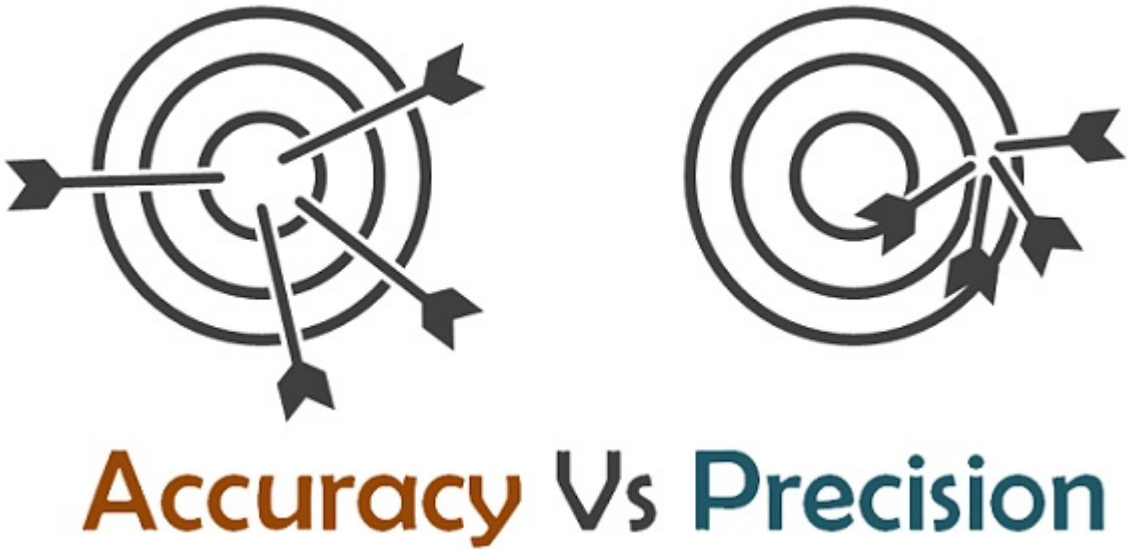
"**Accuracy normally refers to the difference (error or bias) between the mean**, %, of the set of results and the value x, which is accepted as the true or correct value for the quantity measured".

and

" **Precision relates to the reproducibility of measurements within a set**".

I must admit that the automation of analytical apparatus such as gas chromatography or atomic absorption spectrometers can produce very good precision. However how this relates to accuracy is another matter.

RG Ackman,Fette Seifien (1980)



Conclusions

Choose the appropriate standard method, AOCS or other-keep up with improvements

Study the chemistry-read up on the method

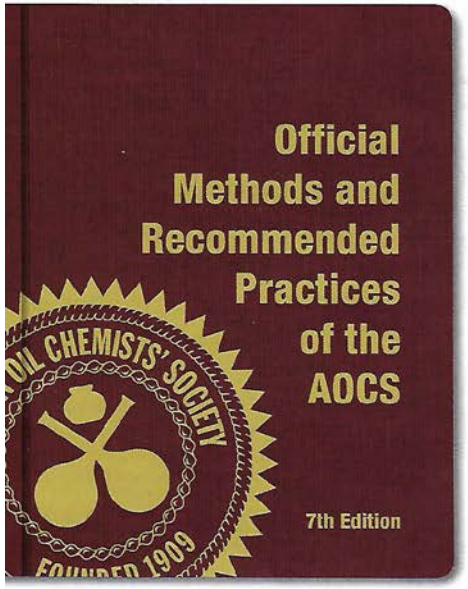
Practice with samples of approx. known analytical result.

Use common sense when inspecting the analyses.

Do Round Robin tests – GOED/AOCS have a proficiency program

Obtain exact standard materials

Use trained analysts and pay them reasonably



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New Methods

Five new methods accepted in 2016

- Ac 6-16 (Official Method) Extraction and Indirect Enzyme-Linked-Lectin-Assay (ELLA) Analysis of Soybean Agglutinin in Soybean Grain
- Cd 12c-16 (Standard Procedure) Accelerated Oxidation Test for the Determination of Oxidation Stability
- Cd 30-15 (Official Method) Analysis of 2- and 3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Oil-Based Emulsions
- Ce 12-16 (Official Method) Sterols and Stanols in Foods and Dietary Supplements Containing Added Phytosterols
- Ce 13-16 (Recommended Practice) Determination of Cyclopropenoic and Nutritional Fatty Acids in Cottonseed and Cottonseed Oil by Gas Chromatography

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