Healthy fat in chips and sausages ?

A new method for digestion, extraction and analysis of fat in food samples

by

Aurelia Bertini[1], Marcel Pacheco-Moreno[1], Ulf Sengutta[2], Uwe Oppermann[3], Jürgen Schram[1]

[1] Niederrhein University of Applied Sciences, Krefeld, Germany
[2] CEM, Kamp-Lintfort, Germany
[3] Shimadzu, Duisburg, Germany

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Why is fat important?

- essential fatty acids
  - necessary for life
  - not synthesizable in organisms
  - physiological important: *unsaturated fatty acids*

- EU: **New:** Declaration of food
  - EU Nr. 1169 / 2011
  - detailed nutritional information (saturated and unsaturated fatty acids)
  - more Information for consumers in detail

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Fatty Acids - Analytics

- determination of total fat content
- analysis of saturated and unsaturated fatty acids in the total fat content
  - Identification and quantification
- method of analysis: GC-FID

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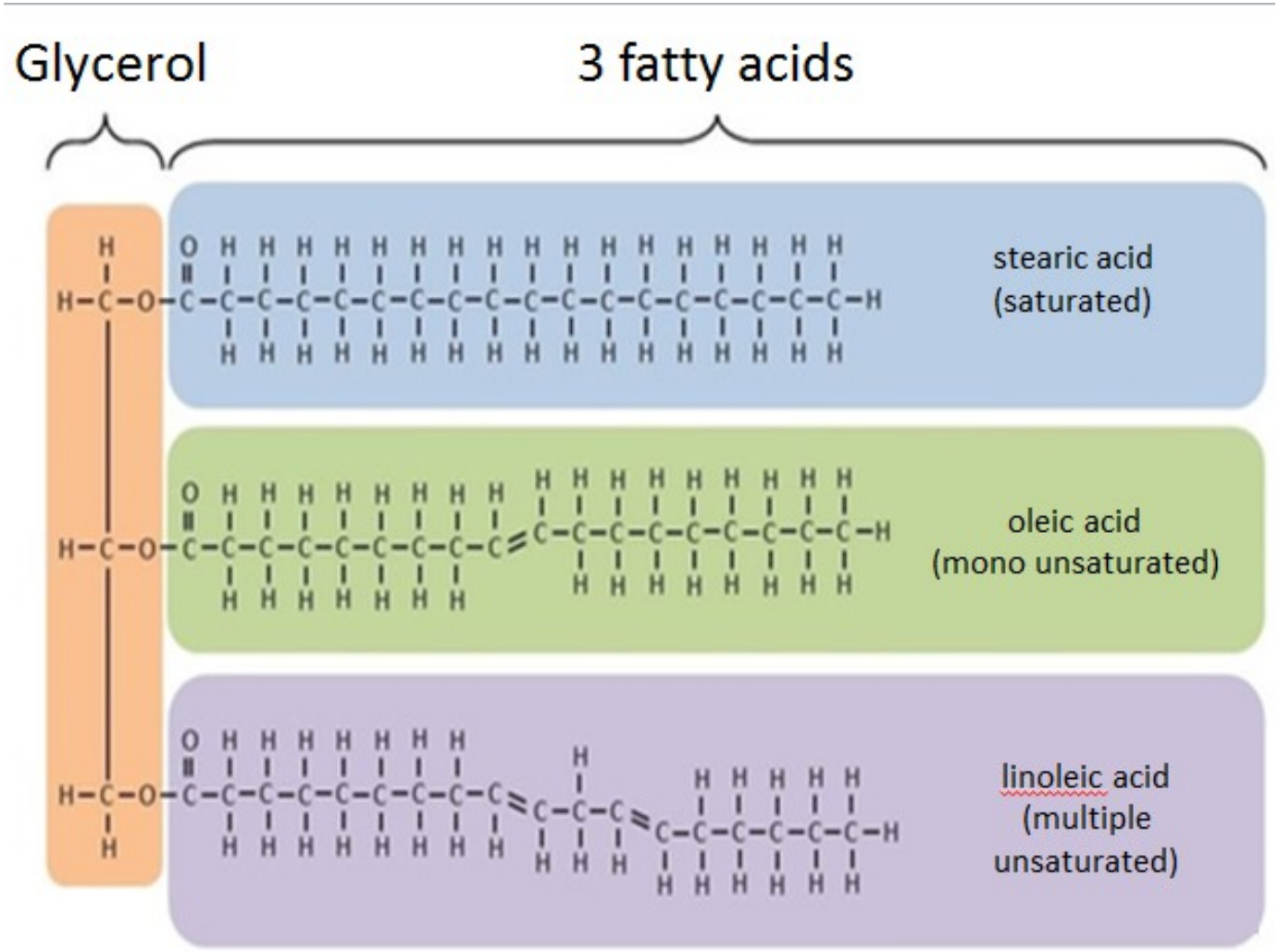
What are Fats / Fatty Acids?



literature: http://i1.wp.com/blog.foodlinx.de/wp-content/uploads/2015/06/triglycerid_schema.jpg (21.12.2015)
http://media.tumblr.com/1d0f9d3b8e2cec8753410f789bfaeca4/tumblr_inline_mn0l1r1JZF1qz4rgp.jpg (11.01.16)

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Fatty Acids in Food

name	main occurrence
saturated fatty acids	
palmitic acid, stearic acid	butter, cream, cheese, sausages, beef, coconut, palm oil
mono unsaturated fatty acids	
oleic acid	olive oil, rape oil, hazelnut, avocado
multiple unsaturated acids	
omega 6 fatty acids	
linoleic acid	thistle oil, sunflower oil, wheat germ oil, corn germ oil, sesame oil, soybean oil, chia seeds
arachidonic acid	lard, pork liver, egg yolk, tuna, liverwurst, pork meat
omega 3 fatty acids	
α-linoleic acid	linseed oil, hemp oil, walnut oil, rape oil, chia seeds

literature: https://vebu.de/fitness-gesundheit/naehrstoffe/omega-3-fettsaeuren-in-pflanzlichen-lebensmittel/ (15.07.2016

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Fatty Acids and Health

fatty acid	opesity	dia-betes	fat metabolis disorder	High blood pressur e	Disease of the coronary arteries	stroke	cancer
total fat	↑↑	○○	↑↑↑	~	○○	○	○○
saturated	n.s.	○○	↑↑↑	○○○	↑	○○	↑
mono unsaturated	~	○○	↓↓↓	~	○	○○	↓
multiple unsaturated	~	↓	↓↓↓	~	↓↓↓	↓↓	↓
Trans	n.s.	~	↑↑↑	n.s.	↑↑↑	○	~

evidence	increasing risk	risk reducing	no correlation
convincingly	↑↑↑	↓↓↓	○○○
probably	↑↑	↓↓	○○
possible	↑	↓	○

literature: [1]

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Why is derivatization necessary?

- fatty acids are preferably determined by GC
- fatty acids are not analyzable with GC without derivatization
  - volatility is too low
- fatty acids → fatty acid methyl ester **(FAME)**

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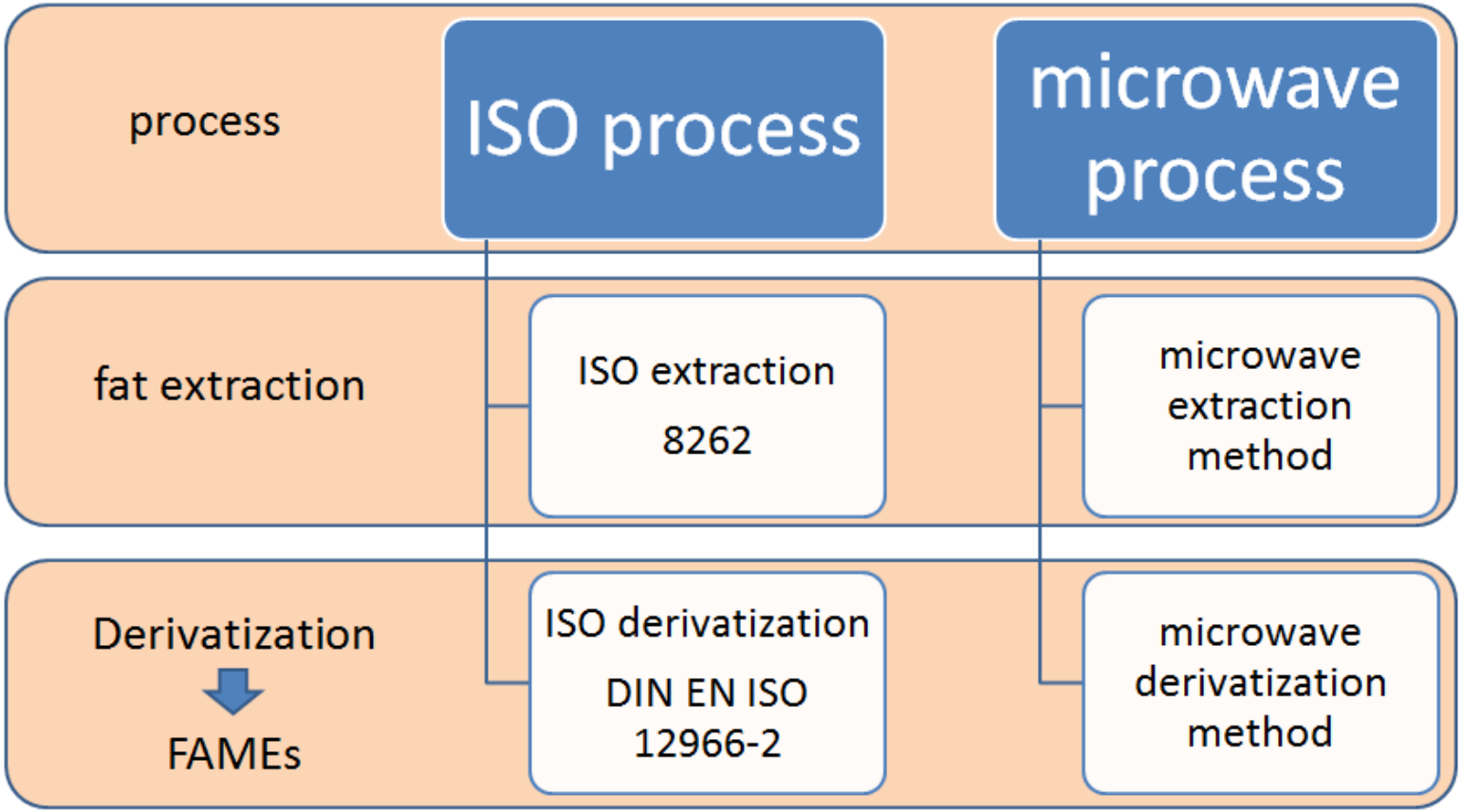
Task / Aim

- Comparison of ISO process with a new developed microwave process



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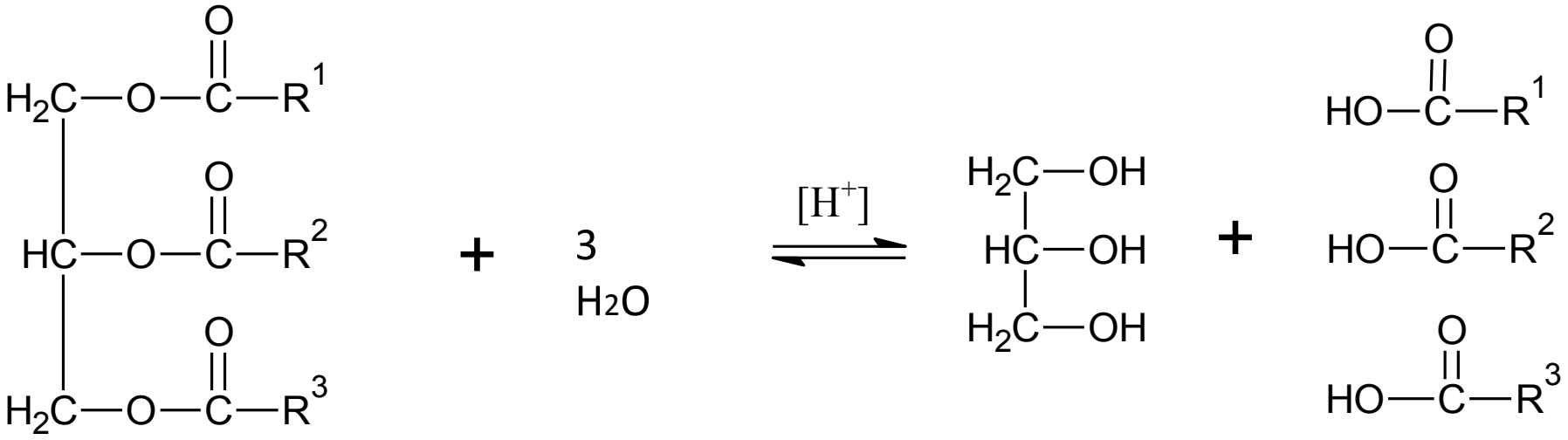
Digestion

- proteins are denaturated and release the bound fat
- tricglycerides are hydrolysed in the presence of hydrochloric acid



Triglyceride **Water** **Glycerine** **Fatty Acids**

Digestion According to Weibull-Berntrop [ISO 8262-1-3]

Digestion

- weighed sample: 5-20g
- open digestion vessel
- 100 ml HCl (4 mol/L)
- 30-60 min digestion at 100°C

Soxhlet-Extraction

- 300 ml Petroleum Ether (40-60°C)
- extraction time 4 hours

Microwave-Assisted Digestion and Extraction

Digestion and Extraction

- weighed sample: **0,5-1,0 g**
- **11 ml** HCl (4 mol/L)
- **5 ml Cyclohexane**
- **closed** digestion vessel
- **15 min** digestion at 115°C
- 3 further extractions
- extraction time **1 min**

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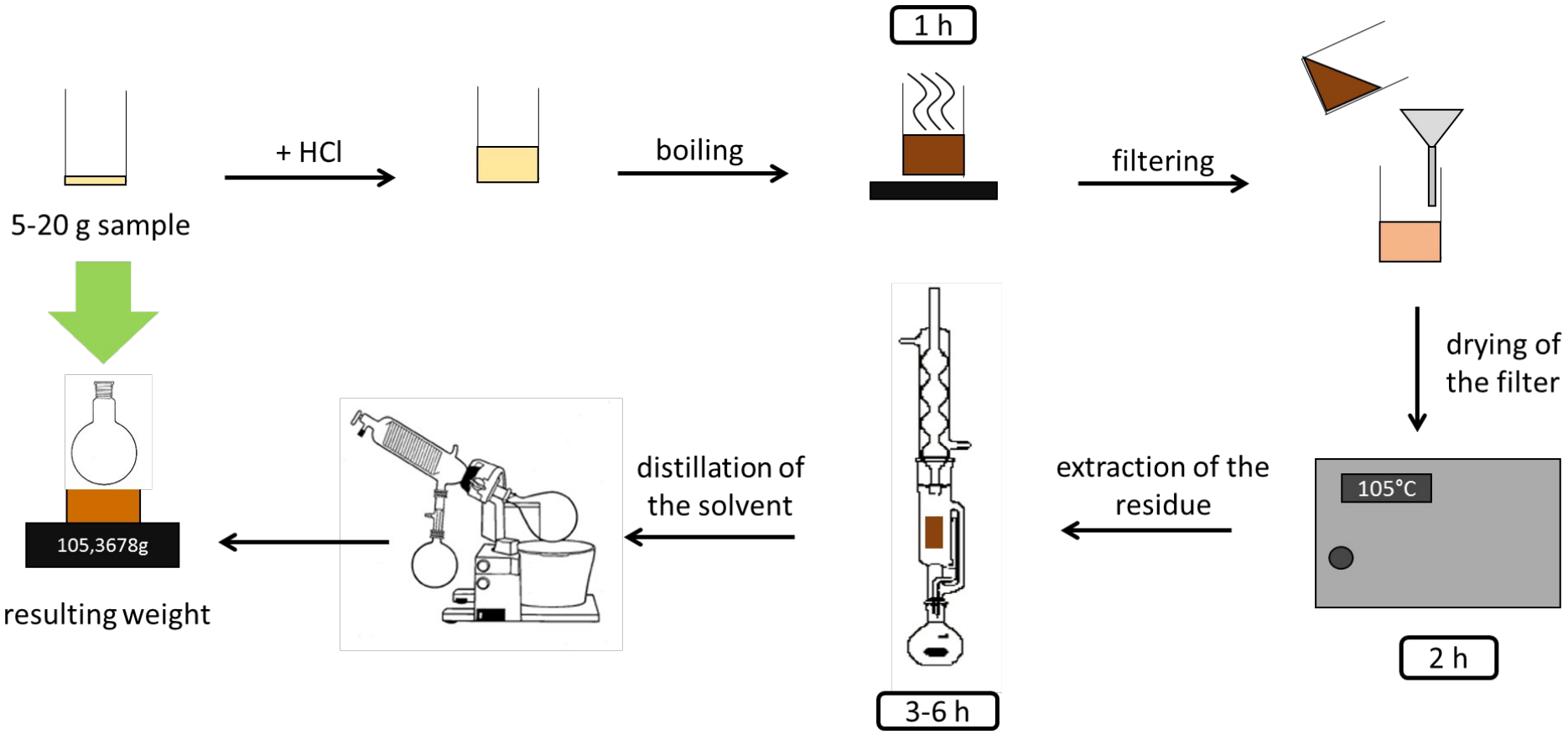
Total Fat Content according to *Weibull-Berntrop* [ISO 8262-1-3]



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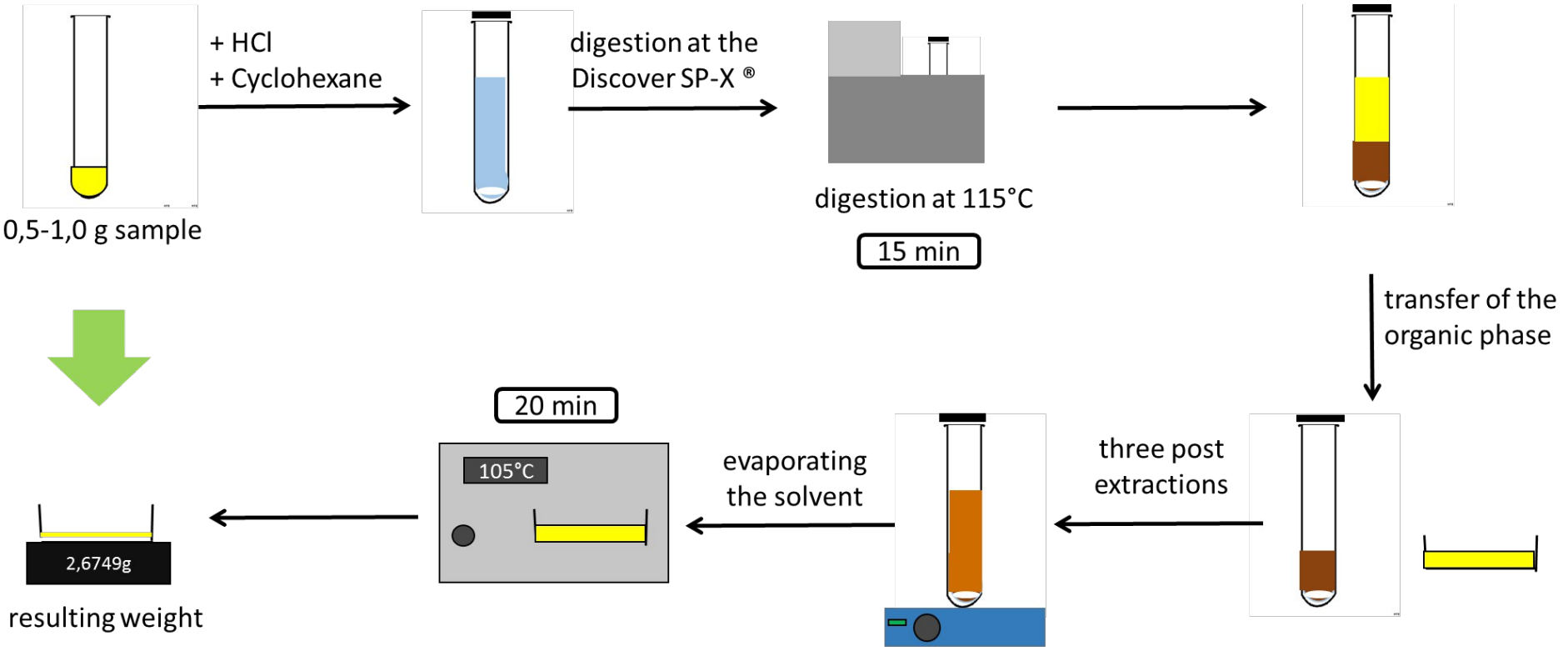
Microwave-Assisted Digestion and Extraction



0,5-1,0 g sample

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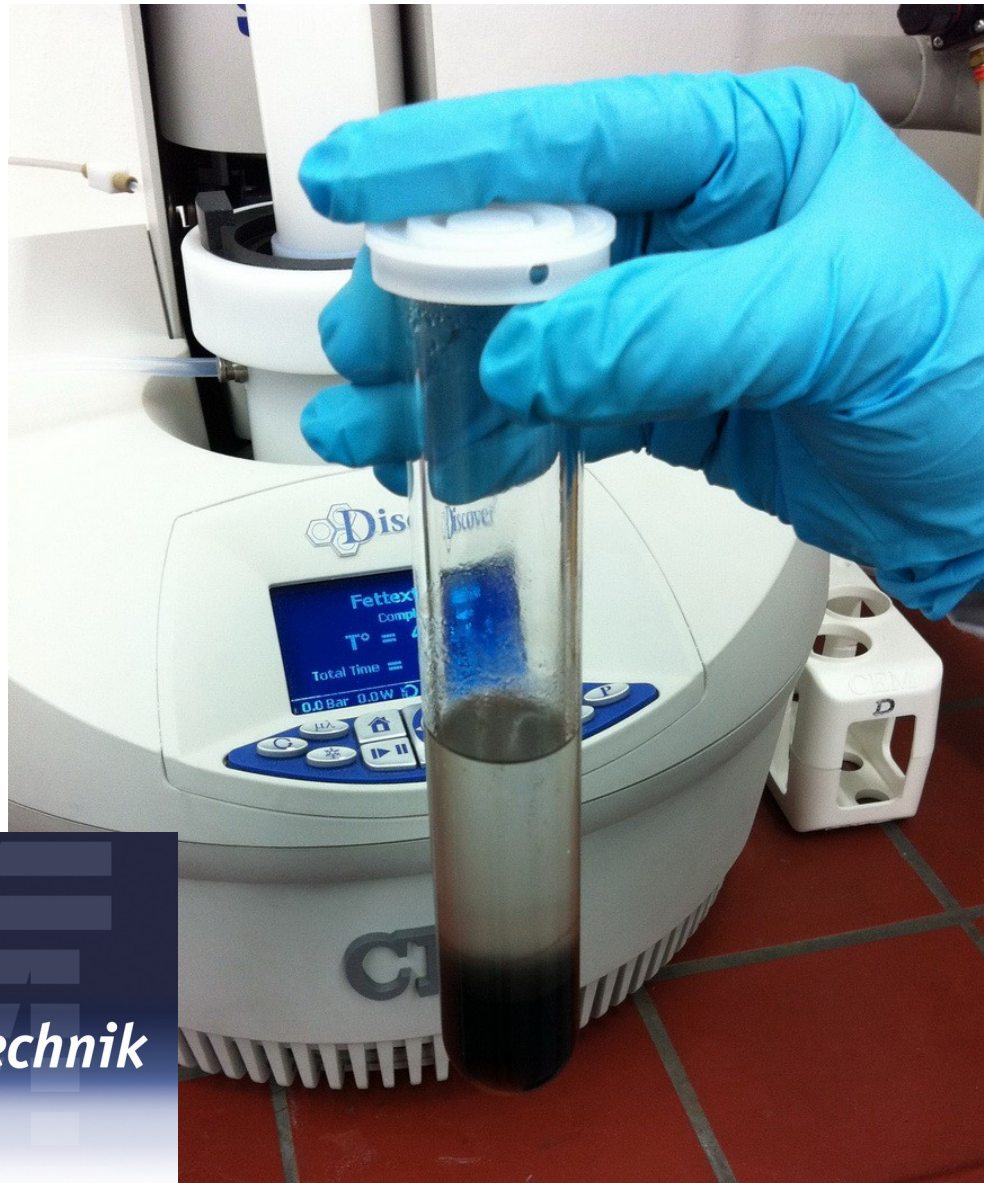
Fat Analysis realised with:

Discover SP-D ®



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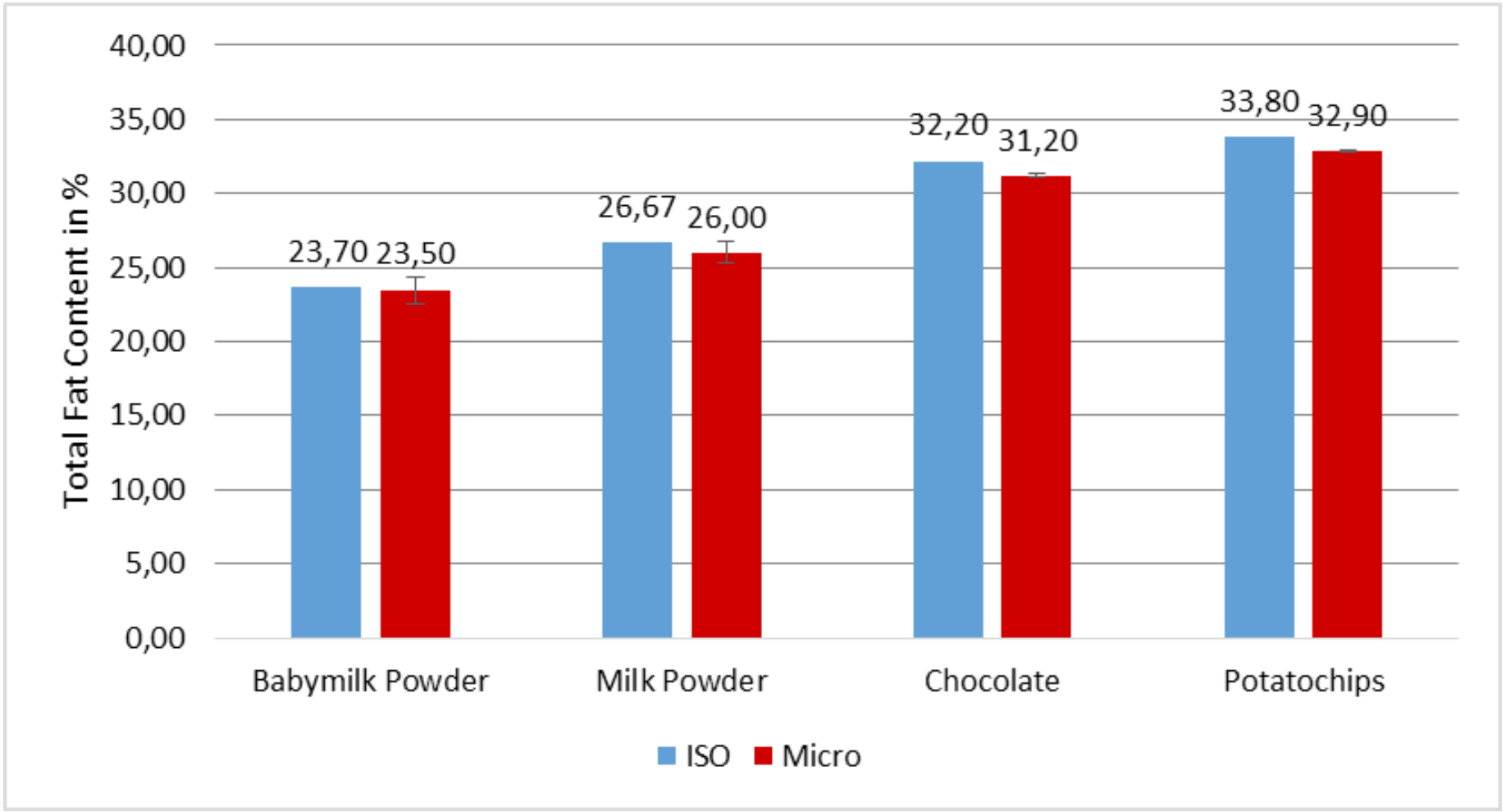
Total fat content via Microwave-Assisted Digestion

(Samples Higher than 20% Fat)



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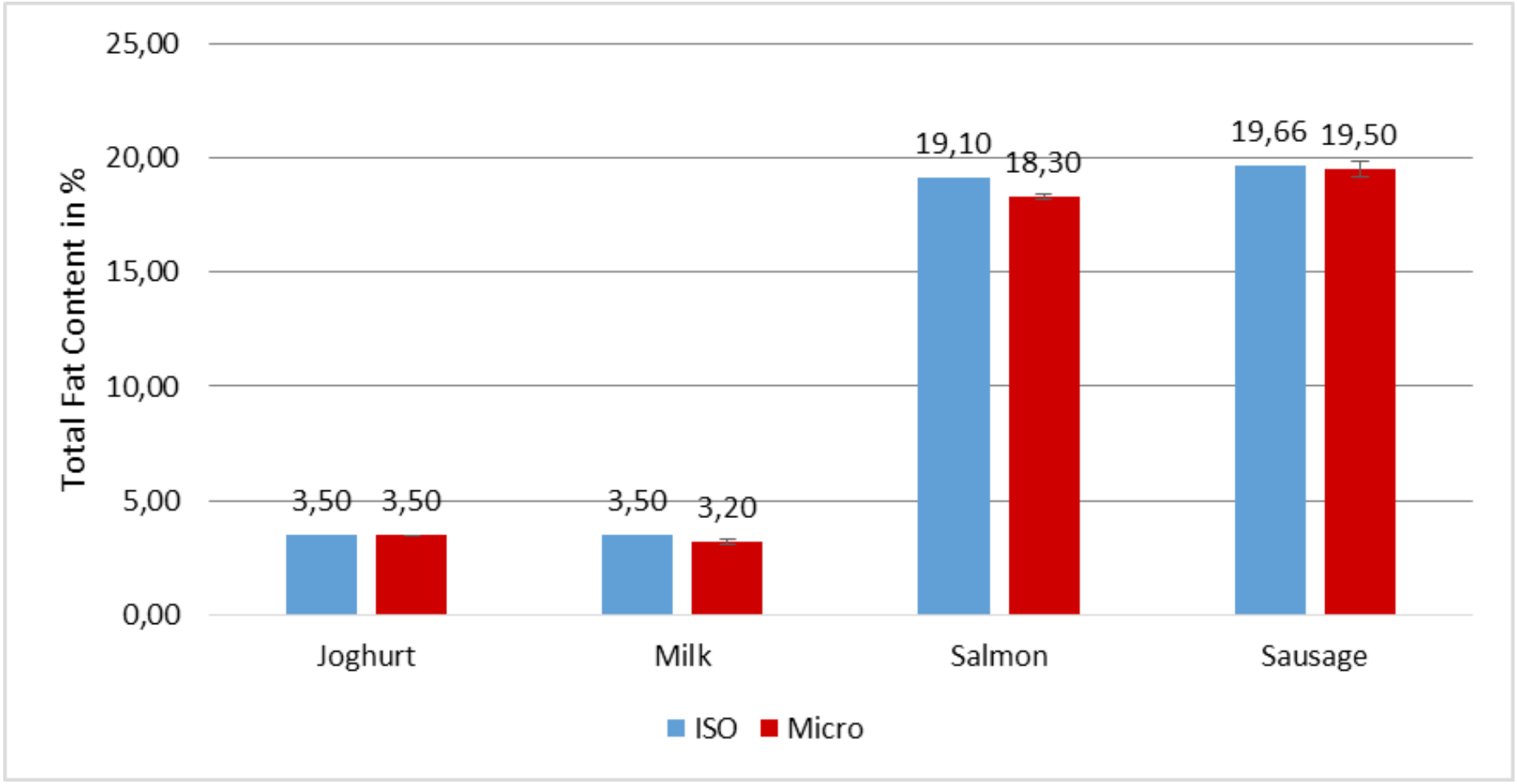
Total Fat Content via Microwave-Assisted Digestion

(Samples Less than 20% Fat)



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Recovery of Fat in all analysed food samples

Food Sample	Total Fat Content [%]	Recovery [%]
Joghurt	3,50	100,1 ± 0,1
Milk	3,50	91,8 ± 2,4
Salmon	19,10	90,7 ± 0,6
Sausage	19,66	99,9 ± 0,5
Babymilk Powder	23,70	97,3 ± 4,2
Milk Powder	26,67	98,2 ± 2,7
Chocolate	32,20	97,1 ± 0,5
Potatochips	33,80	97,5 ± 3,5

http://freepngimages.com/wp-content/uploads/2015/12/Glass-of-milk-transparent-background.png (15.07.16)

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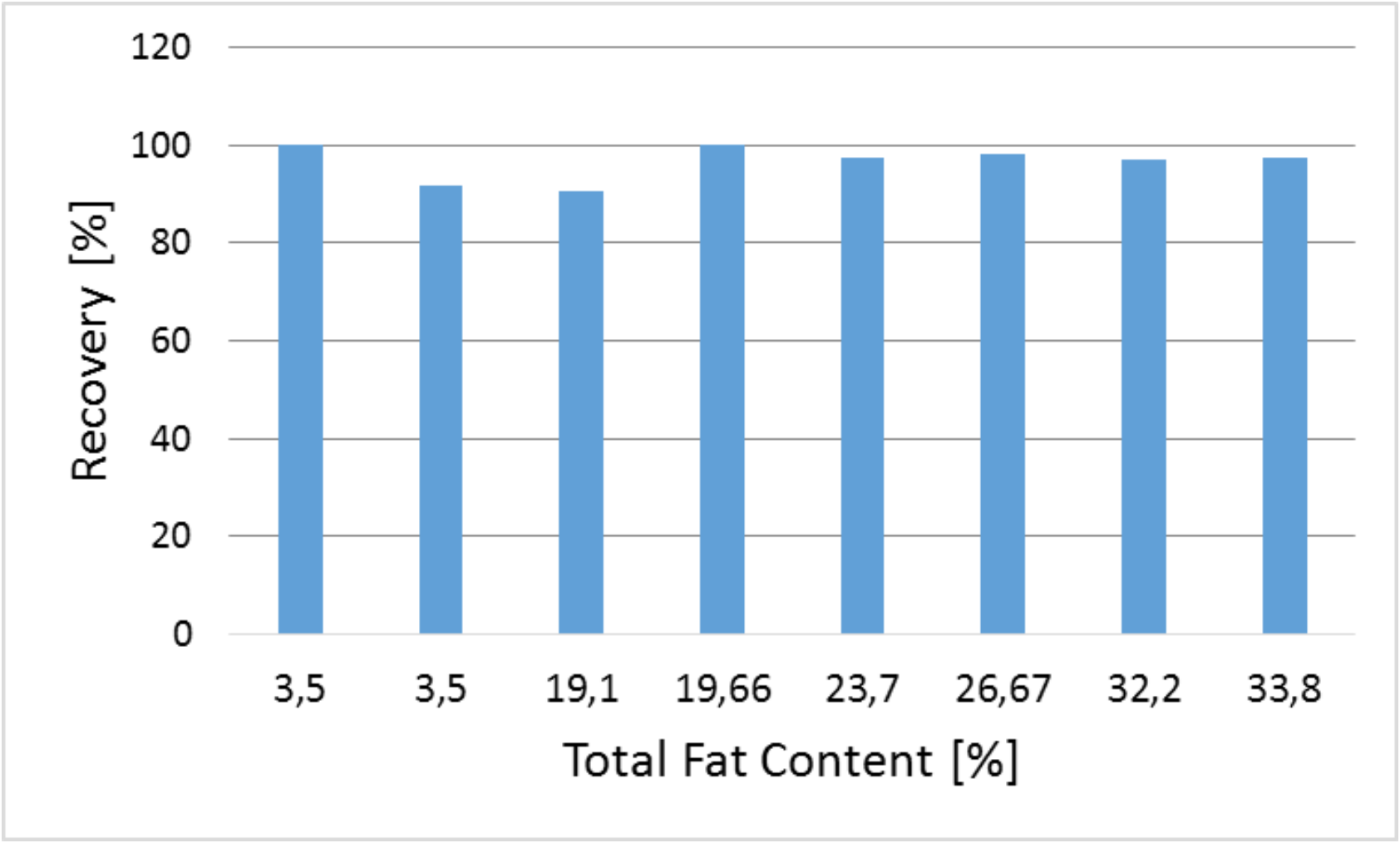
Correlation of Recovery and Total Fat Content of a Food Sample



Interim results – Total fat extraction

- rate of recovery of the microwave process:

  > 90 weight %

- no correlation between the total amount of fat of a sample and the rate of recovery

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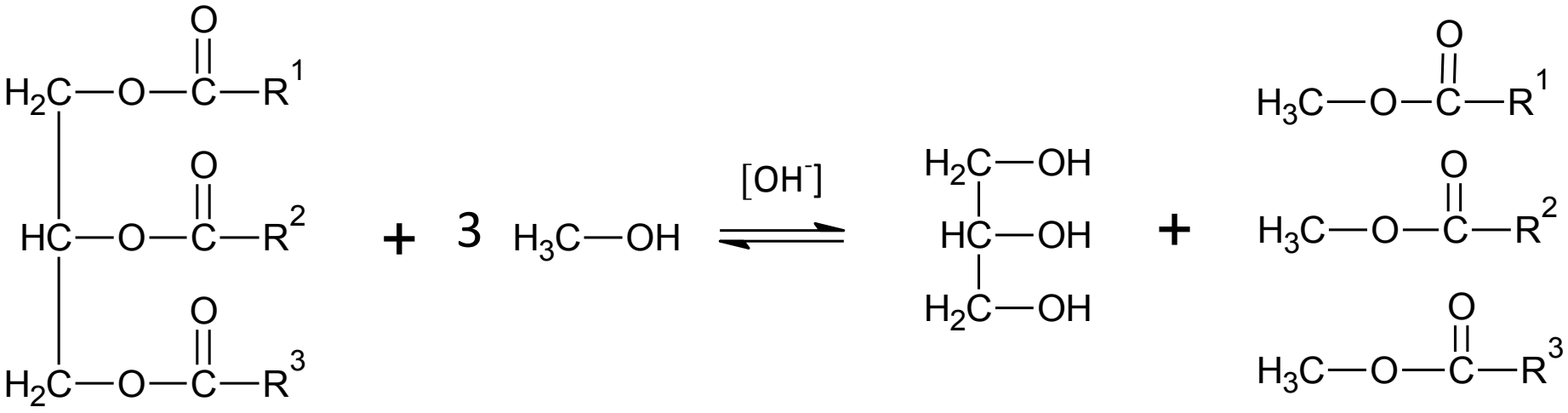
Alkaline derivatization

- transmethylation of bound fatty acids



Triglyceride **Methanol** **Glycerine** **Fatty Acid Methyl Ester**

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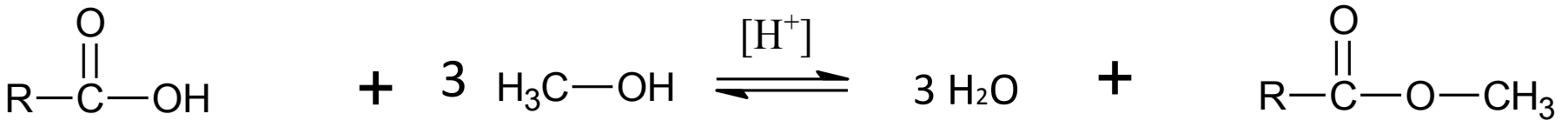
Acidic derivatization

- methylation of free fatty acids



Fatty Acid **Methanol** **Water** **Fatty Acid Methyl Ester**

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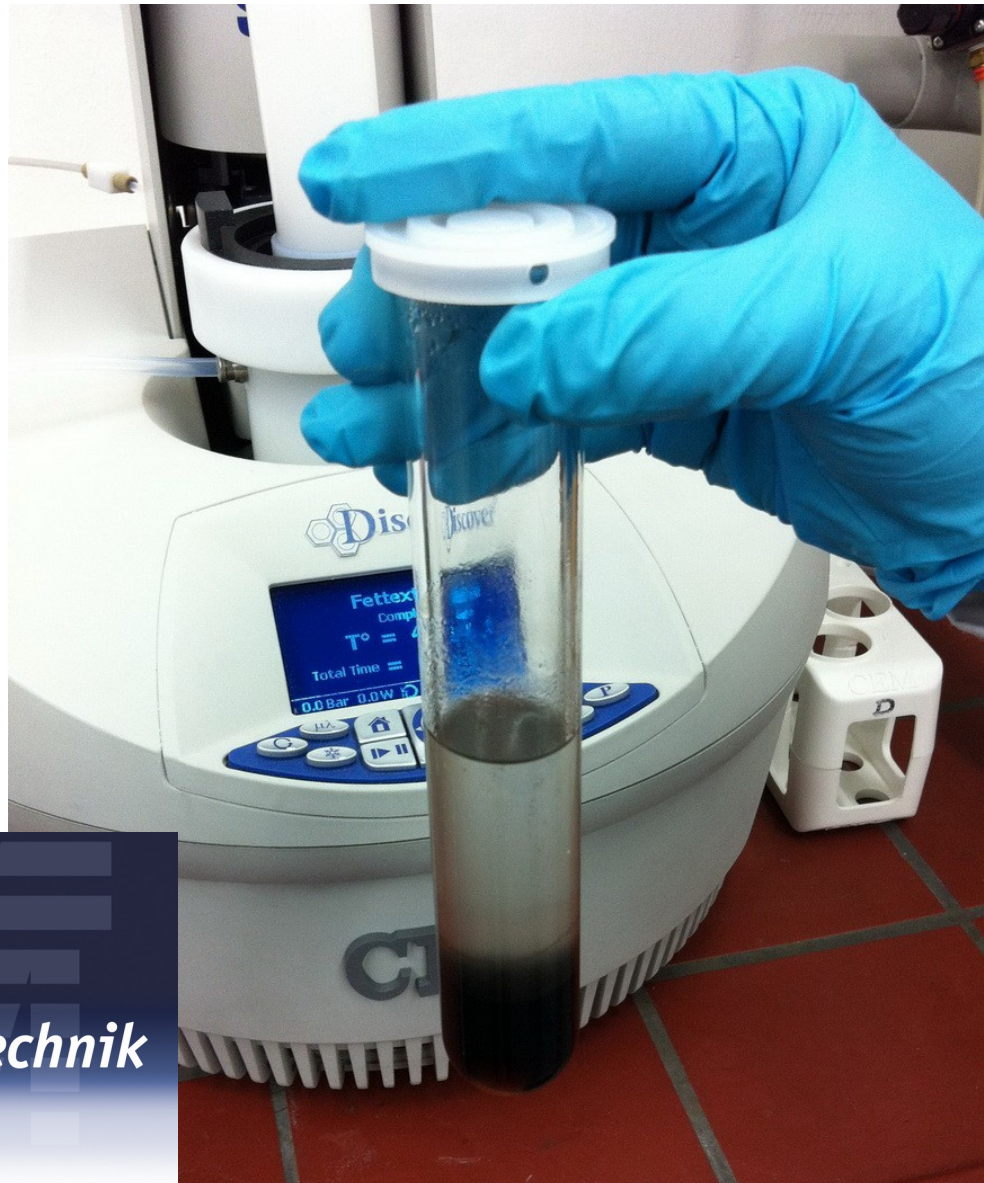
Fat Analysis realised with:

Discover SP-D ®



Derivatization According to ISO 12966-2:2011

Alkaline Derivatizaion

- weighed sample: up to 50g
- MeOH + NaOH (0,2 mol/L)
- boiled 5-20 min unter reflux
- open vessel

Acidic Derivatization

- MeOH + H_2SO_4 (1,0 mol/L)
- boiled 5 min under reflux
- open vessel

Extraction of FAMEs

- 4 ml of a saturated NaCl solution
- 5 ml n-hexane

Microwave-Assisted Derivatization

Alkaline Derivatization

- weighed sample: **0,10-0,15 g**
- MeOH + KOH (0,4 mol/L)
- boiled 10 min at 90°C
- **closed** vessel

Acidic Derivatization

- MeOH + HCl (1,0 mol/L)
- boiled 6 min at 120°C
- **closed** vessel

Extraction of FAMEs

- 10 ml of a saturated NaCl solution
- 5 ml n-hexane

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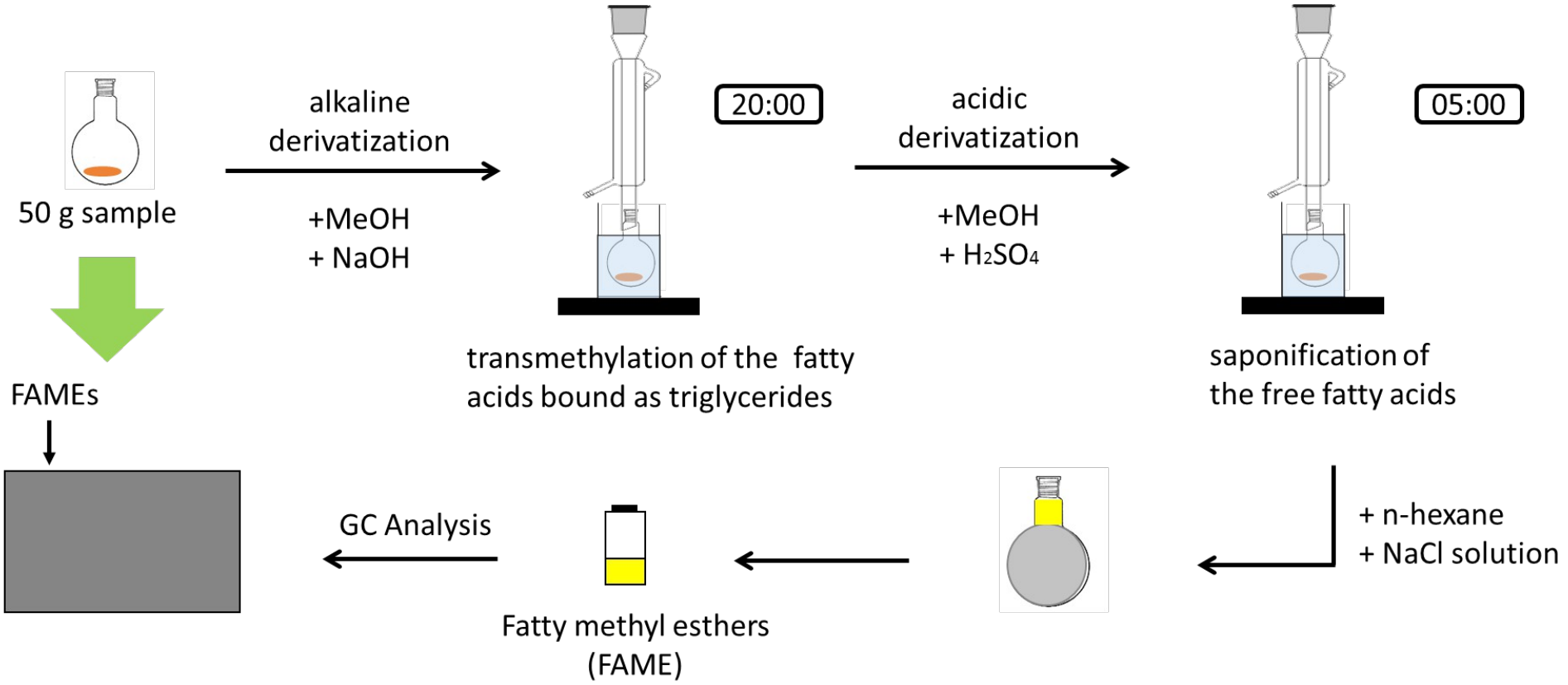
Conventional Derivatization

[ISO 12966-2:2011]



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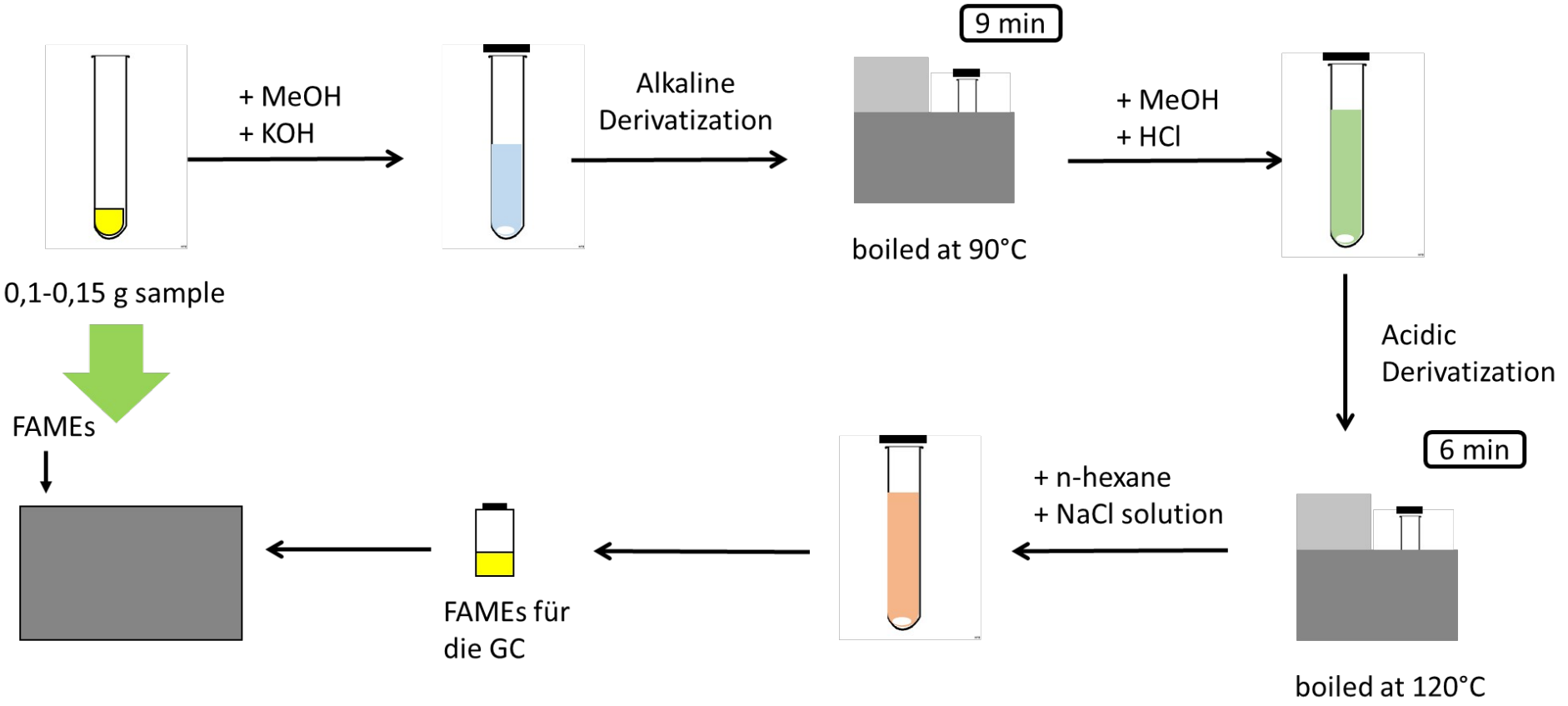
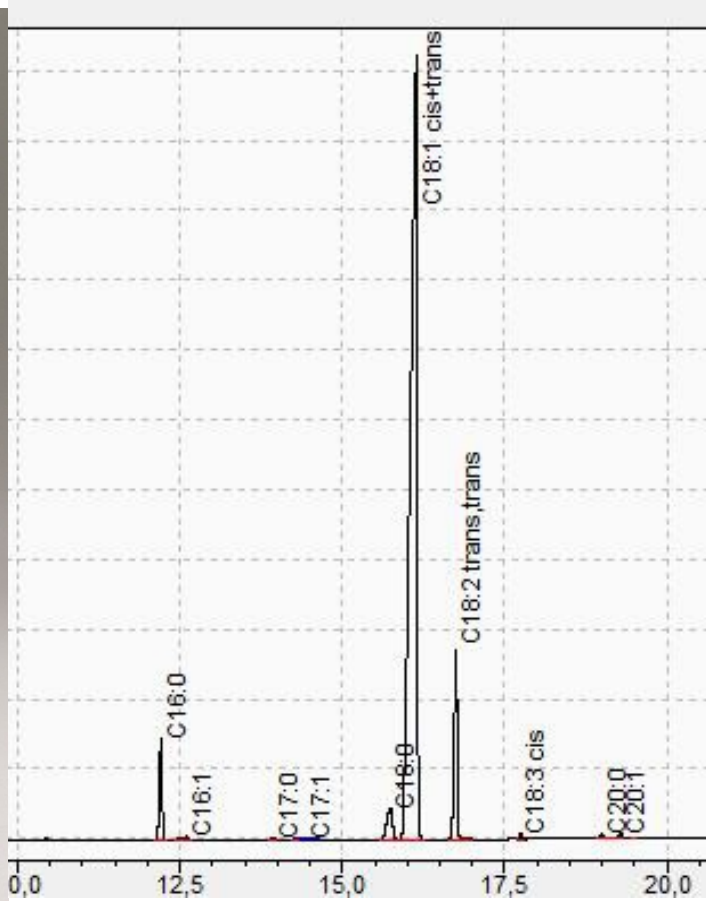
Microwave-Assisted Derivatization



Fat analysis realised with:

GC 2010 plus A1

SHIMADZU
Excellence in Science



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GC method based on ISO 12966-4

Parameter	Settings
Injector	SPL (Split)
Injection Volume	1 µL
Injector Temperature	250 °C
Split-Ration	1:100
Column Type	FAME WAX
Column Length	30 m
Inner Diameter	0,25 mm
Film Thinkness	0,25 µm
Detector	FID
Detector Temperature	250 °C
Mobile Phase	Helium
Carrier Gas Gpeed	35 cm/second

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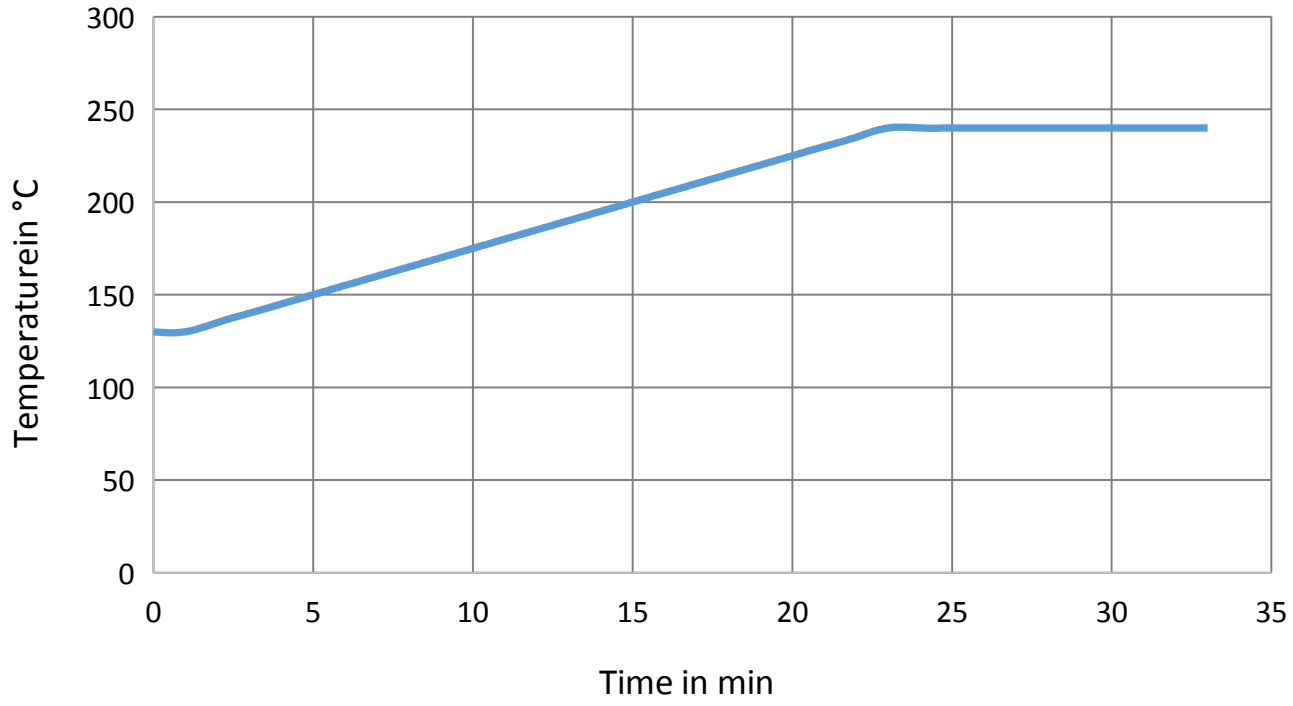
GC-Analysis: Temperature Programm



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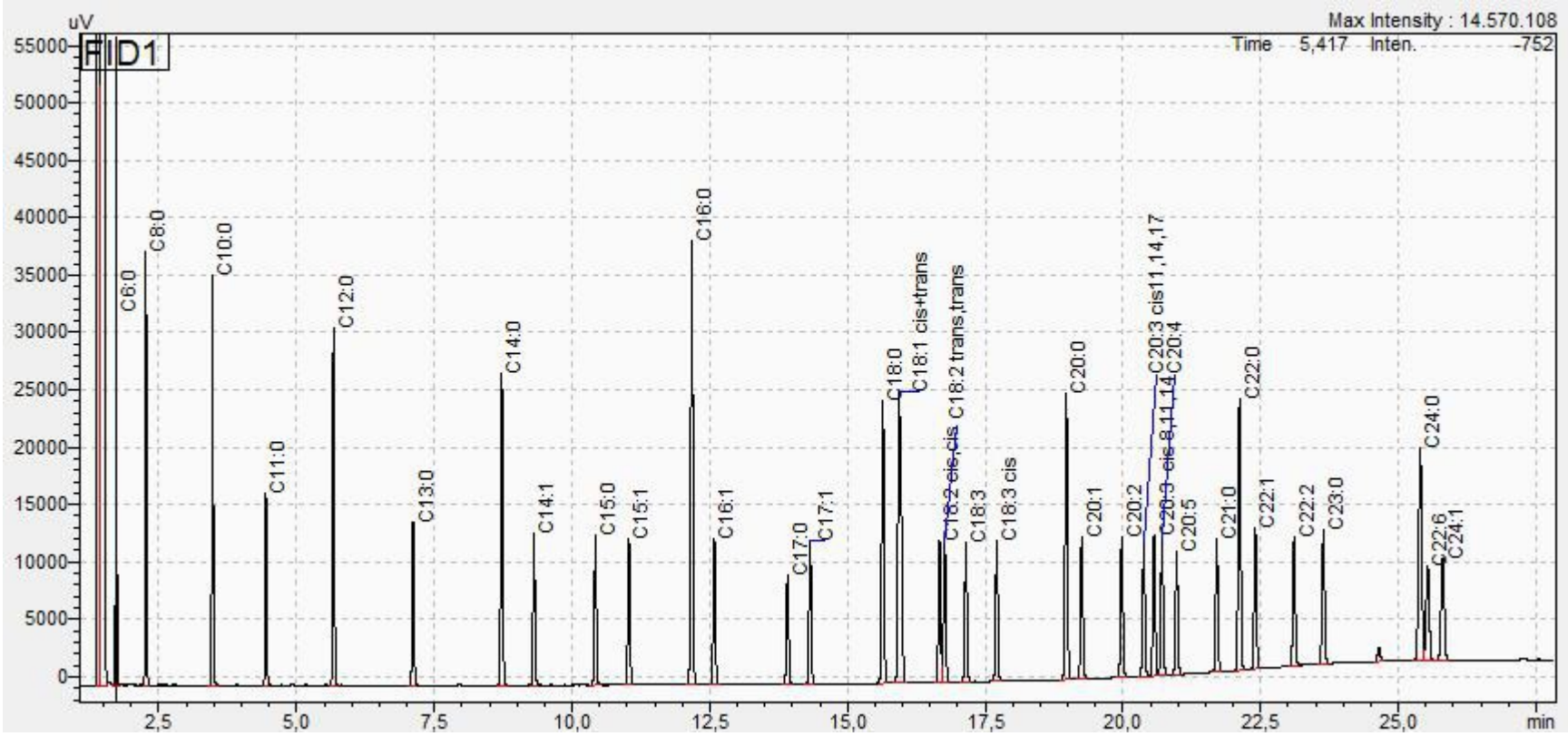
Chromatogram of 37 component FAME Mix

by Supelco



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FAME Standard (Part 1)

ID#	Name	Ret. Time	Conc.	Unit	Area	Height
1	C6:0	1,727	419,1	µg/mL	38495	24238
2	C8:0	2,268	420,7	µg/mL	43787	28045
3	C10:0	3,482	413,7	µg/mL	47021	26696
4	C11:0	4,454	209,0	µg/mL	23961	12610
5	C12:0	5,684	426,5	µg/mL	50317	23717
6	C13:0	7,133	208,9	µg/mL	25230	10935
7	C14:0	8,744	418,5	µg/mL	52190	21190
8	C14:1	9,331	209,8	µg/mL	25504	10137
9	C15:0	10,446	208,3	µg/mL	26382	10062
10	C15:1	11,055	211,8	µg/mL	26170	9823
11	C16:0	12,198	638,6	µg/mL	81506	29877
12	C16:1	12,606	205,0	µg/mL	26826	9742
13	C17:0	13,937	166,7	µg/mL	20804	7380
14	C17:1	14,347	208,3	µg/mL	27282	9811
15	C18:0	15,667	418,2	µg/mL	55843	19288

Unsaturated fatty acid methylester | **Saturated fatty acid methylester**

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FAME Standard (Part 2)

ID#	Name	Ret. Time	Conc.	Unit	Area	Height
16	C18:1 cis+trans	15,963	630,3	µg/mL	83560	20031
17	C18:2 cis,cis	16,697	210,4	µg/mL	27234	9543
18	C18:2 trans,trans	16,79	203,8	µg/mL	26893	9384
19	C18:3	17,177	208,7	µg/mL	27043	9413
20	C18:3 cis	17,735	206,0	µg/mL	27458	9487
21	C20:0	18,997	421,2	µg/mL	57036	19177
22	C20:1	19,277	206,2	µg/mL	28081	9415
23	C20:2	20,003	209,0	µg/mL	27696	9374
24	C20:3 cis11,14,17	20,404	213,2	µg/mL	27280	9198
25	C20:3 cis 8,11,14	20,588	191,8	µg/mL	29012	9697
26	C20:4	20,736	215,6	µg/mL	28259	9357
27	C20:5	21	199,5	µg/mL	24815	8248
28	C21:0	210,7	210,7	µg/mL	26446	8812
29	C22:0	22,138	421,5	µg/mL	57781	19129
30	C22:1	22,426	210,1	µg/mL	28797	9509
31	C22:2	23,131	193,4	µg/mL	26128	8386
32	C23:0	23,662	210,9	µg/mL	29063	8839
33	C24:0	25,427	438,8	µg/mL	59198	14947
34	C22:6	25,565	203,5	µg/mL	26263	6625
35	C24:1	25,833	209,9	µg/mL	29277	7015

Unsaturated fatty acid methylester

Saturated fatty acid methylester

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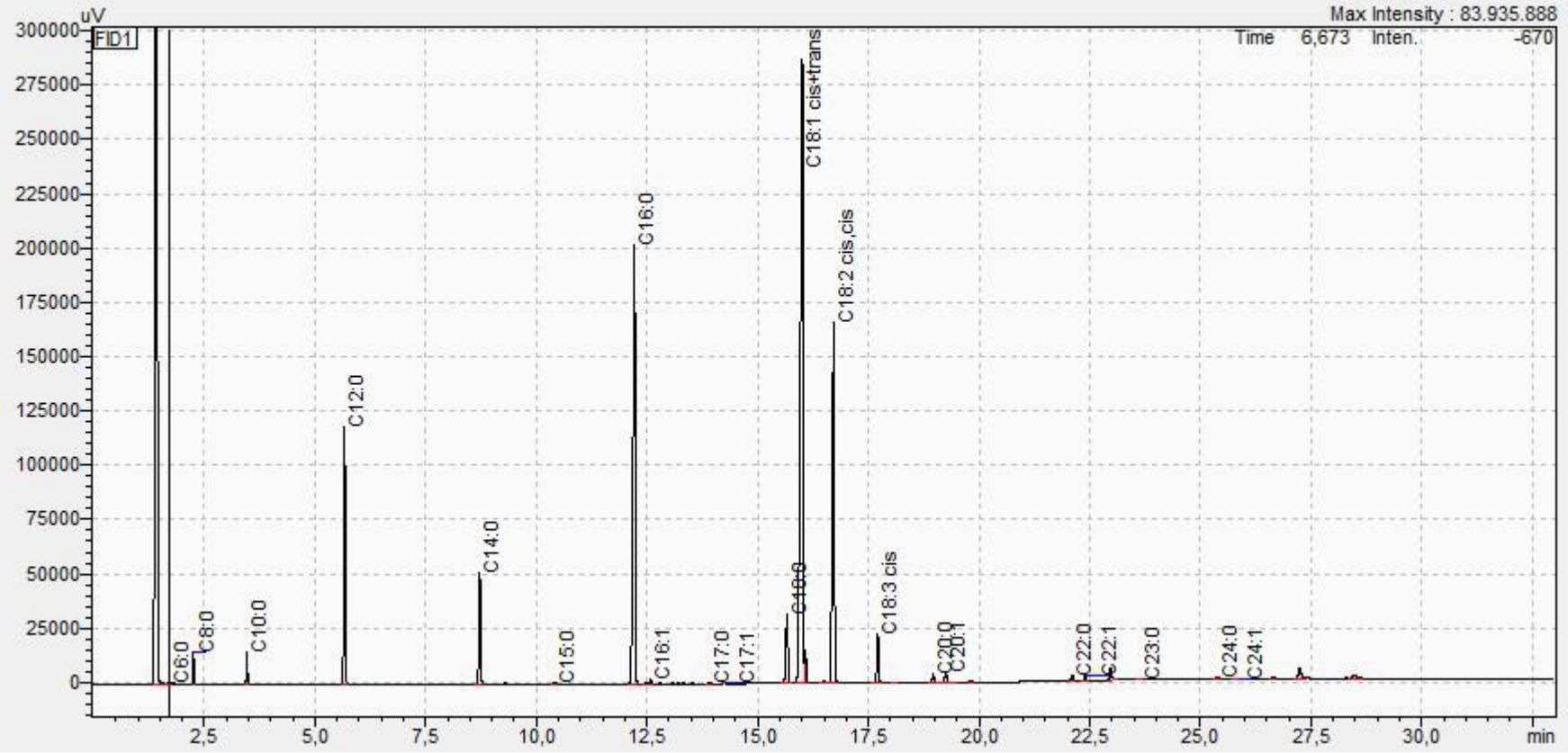
Chromatogram of a Babymilk Powder sample (Microwave)



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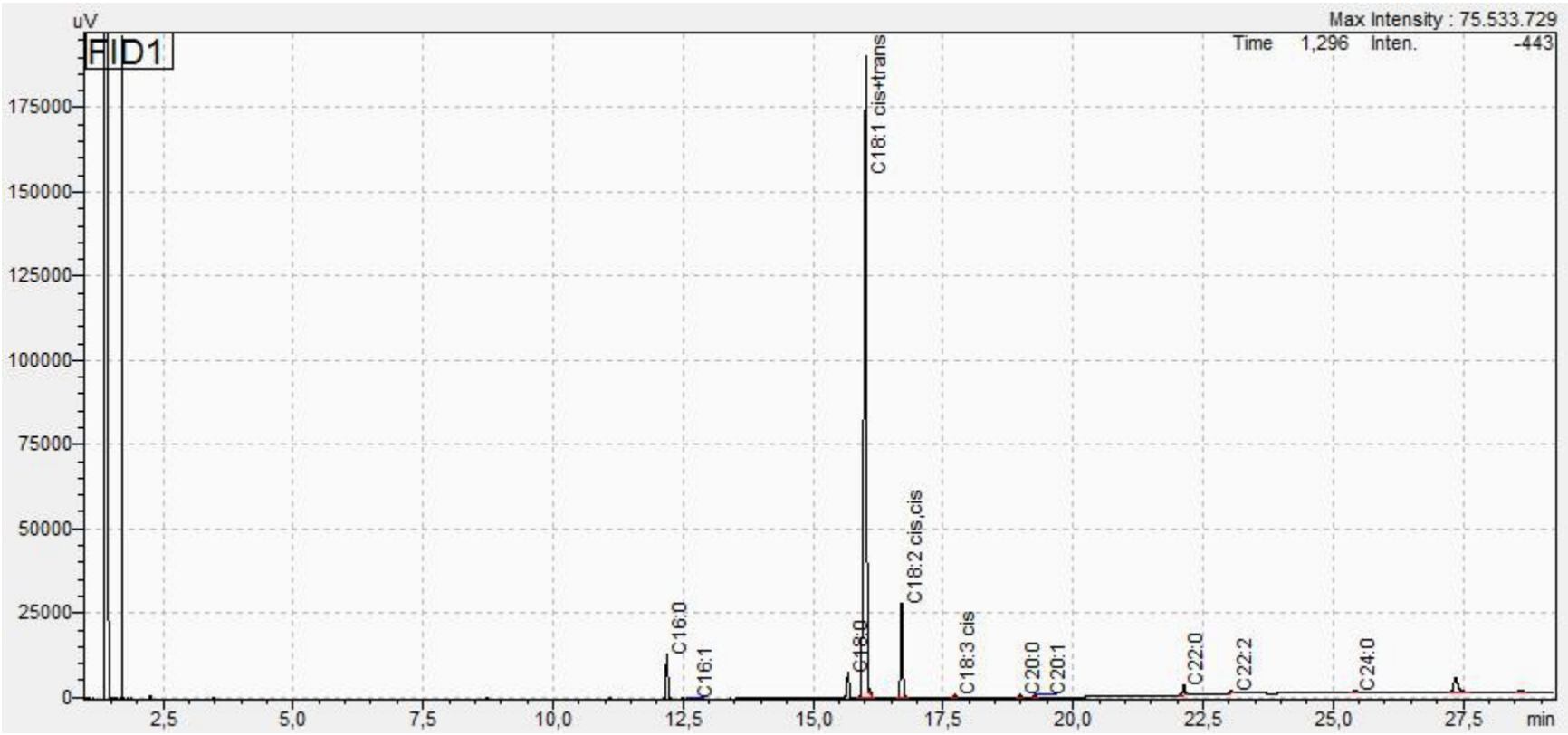
Chromatogram of a Potatochips Sample (Microwave)



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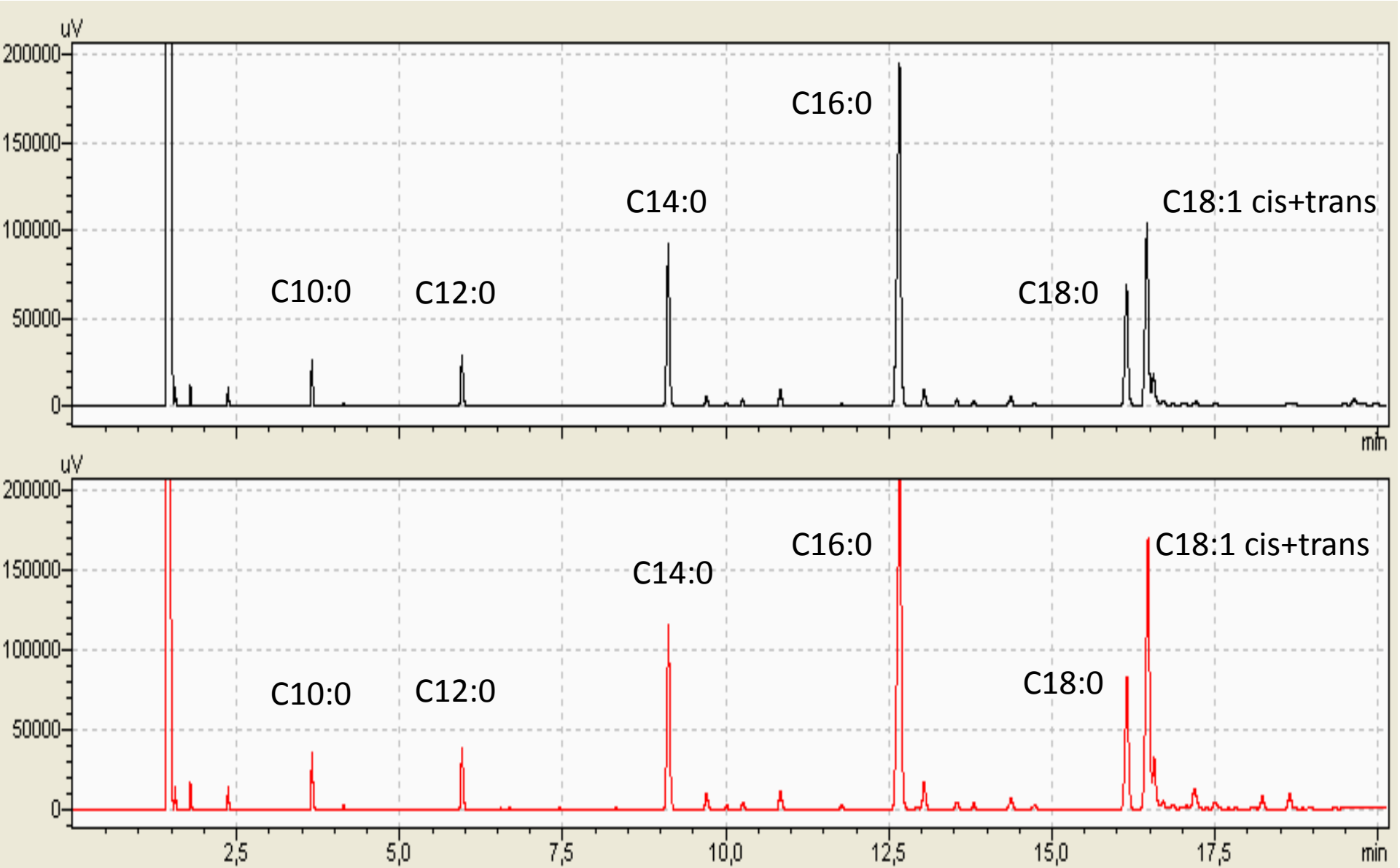
ISO vs. Microwave: Milk Powder



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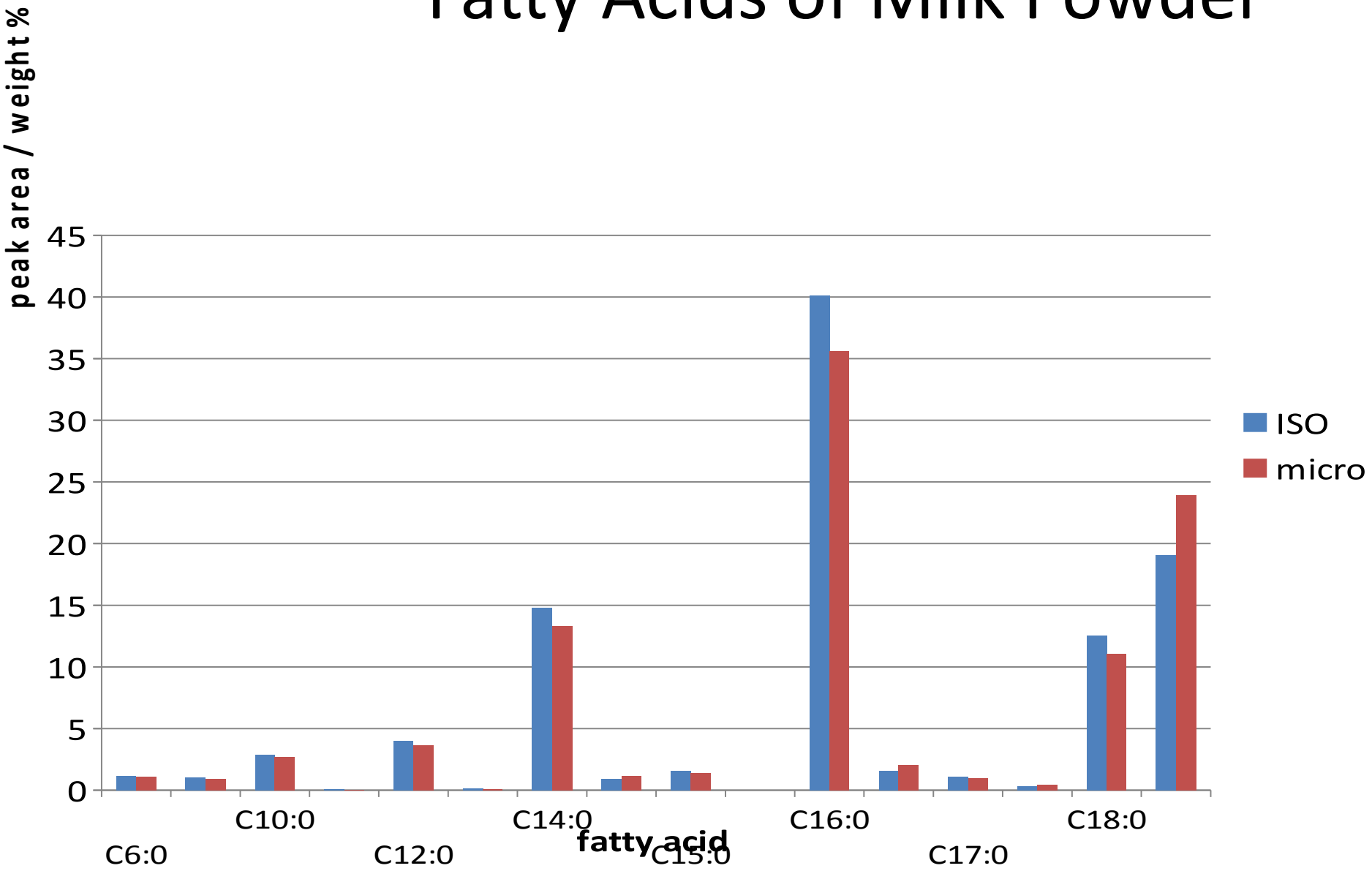
Sum of Peak Area (Weight %) Fatty Acids of Milk Powder



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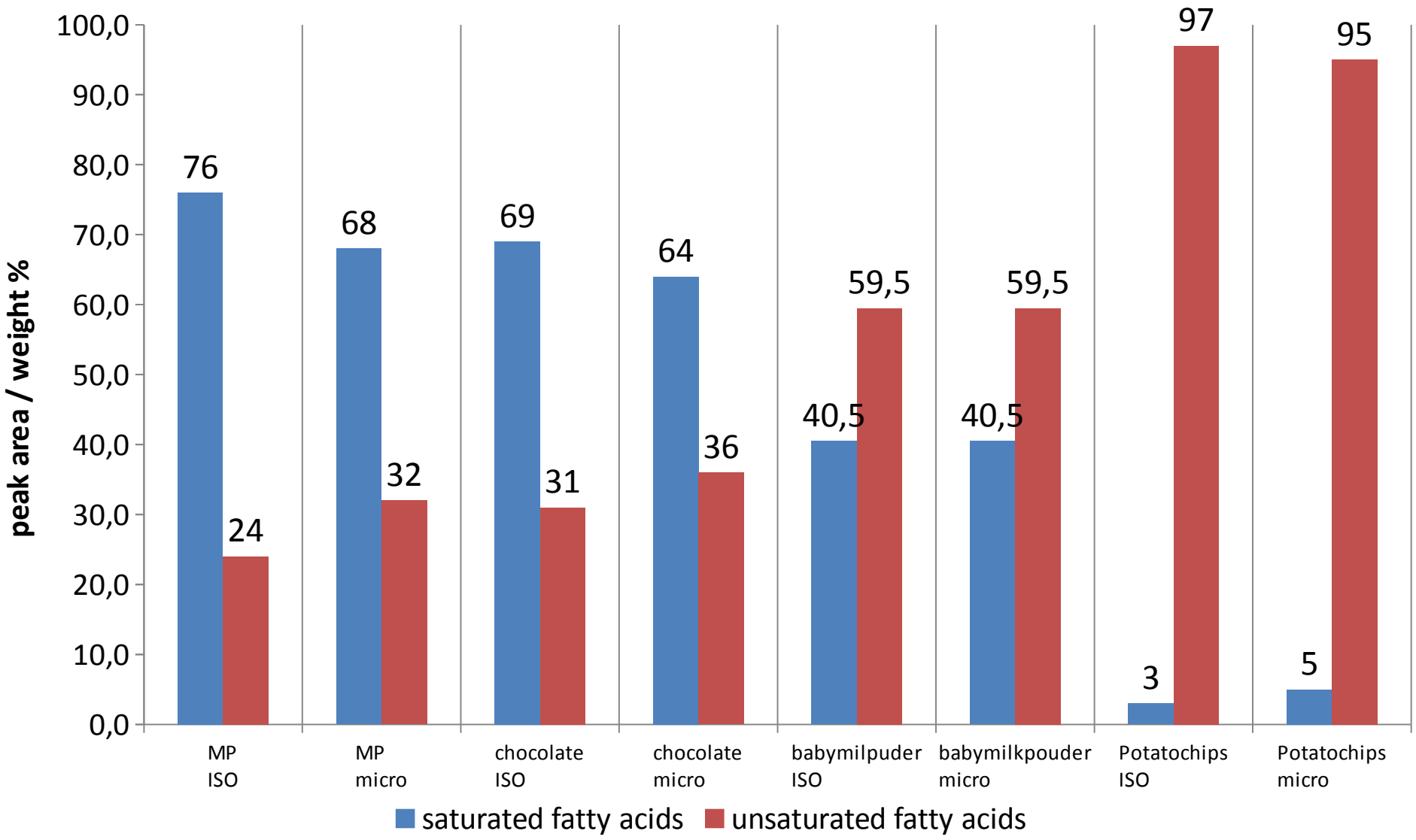
Sum of Peak Area (Weight %) ISO vs. Microwave



Conclusion- Fatty Acids ISO vs. Microwave

- babymilk powder & potatochips:
  - no signifikant difference between ISO and microwave
- milk powder & chocolate:
  - higher results in unsaturated fatty acids by using microwave process

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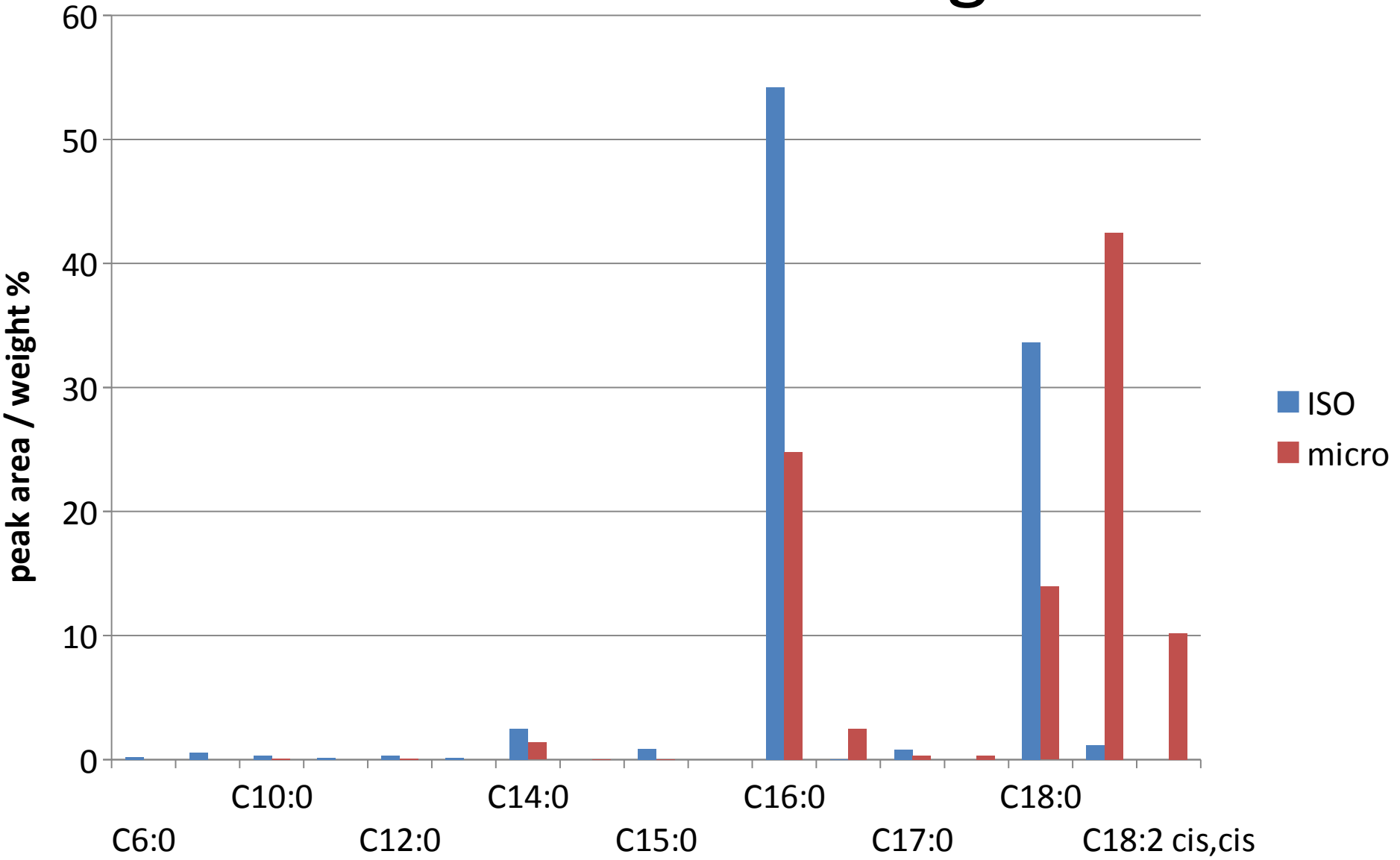
ISO vs. Microwave: Sausage



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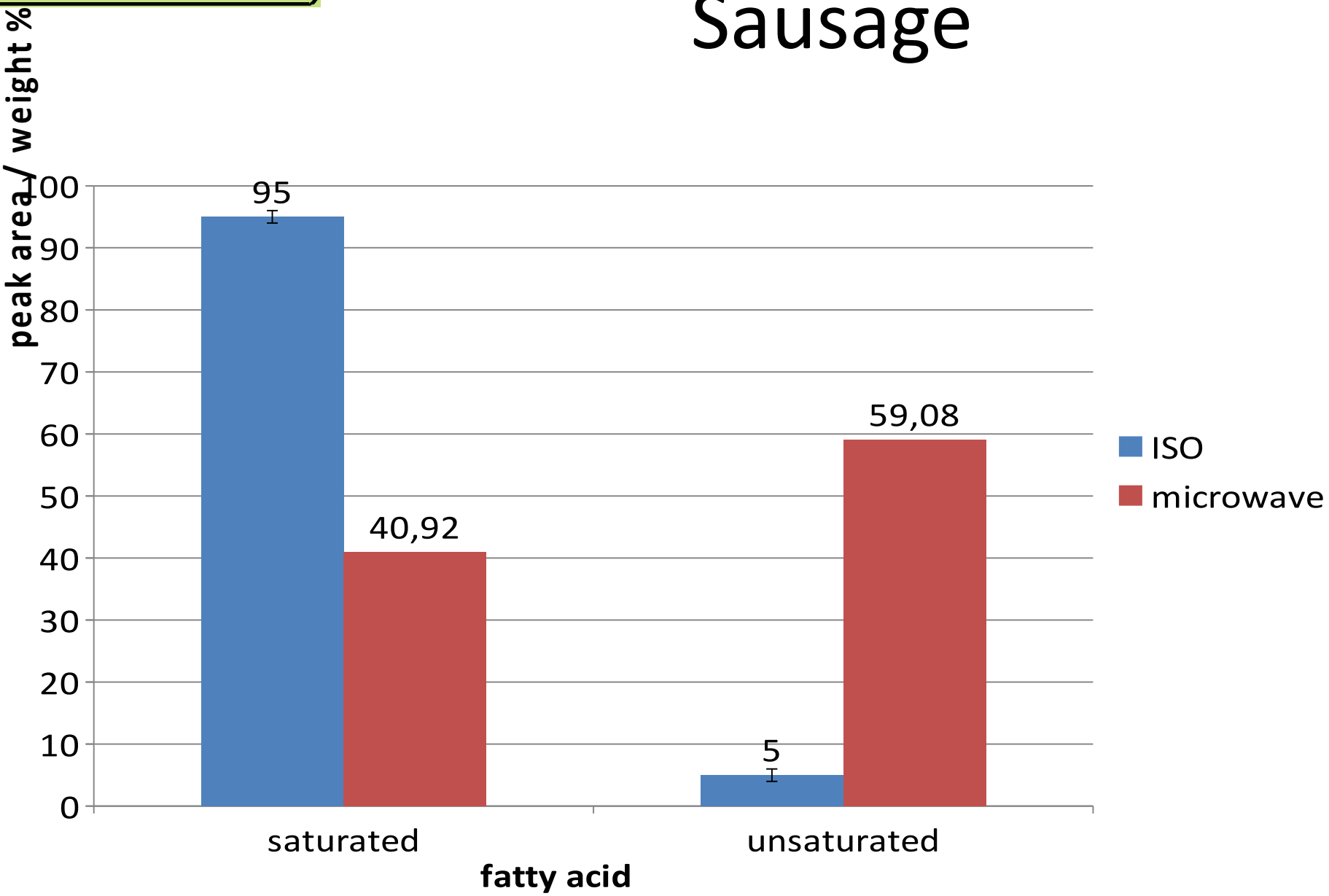
ISO vs. Microwave: Sausage



ISO vs. Microwave - Sausage

- possible explanation for the differences of the unsaturated fatty acids between the ISO and microwave process
  - open ISO is susceptible for oxidative processes
  - auto oxidation (peroxidation), protonation unsaturated fatty acids
  - Possible products: aldehydes, ketones, acids, hydrocarbons

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Comparison Methods in detail

digestion and extraction		
	ISO	**microwave**
system	open	closed
extraction time	9,4 h	1,4 h
Chemical use	600 ml H_2O 100 ml HCl (37%) 300 ml solvent	7,5 ml H_2O 3,5 ml HCl (37%) 20 ml solvent
food sample	5-20 g	0,5-1,0 g

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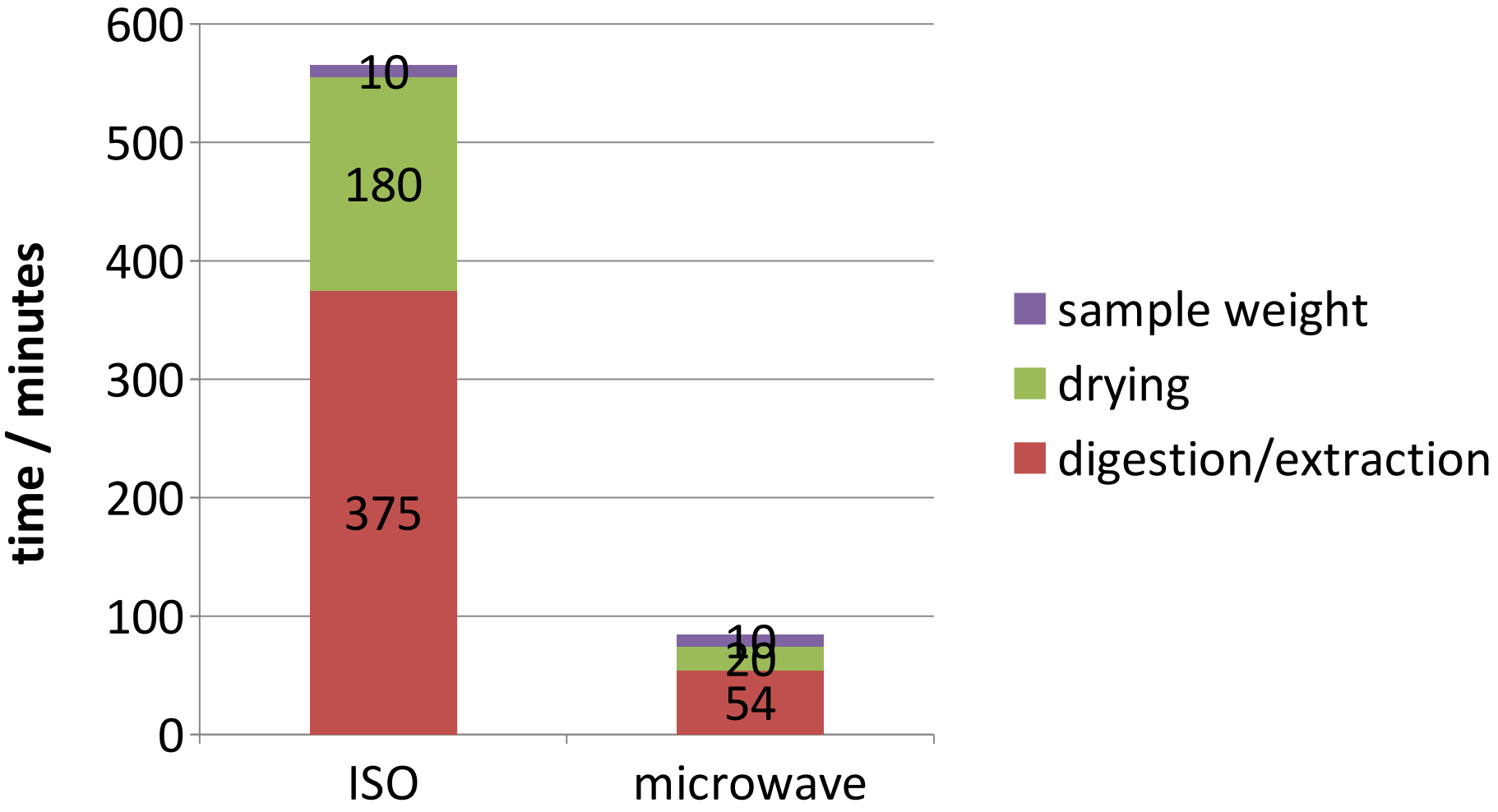
Total Fat Extraction – Time Comparison



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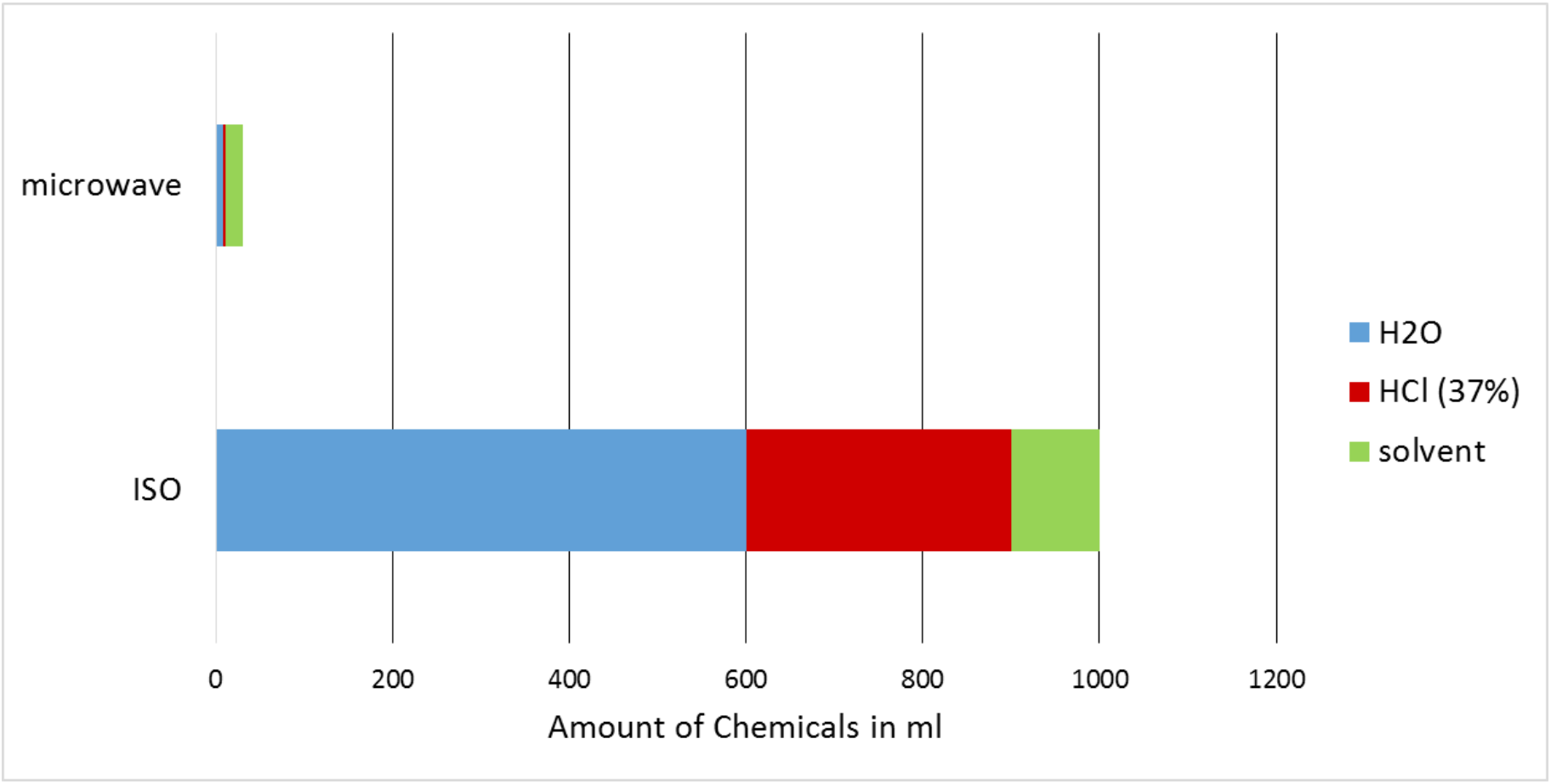
Use of chemicals in ISO and Microwave-Assisted Digestion and Extraktion



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Comparison Methods in detail

derivatization		
	ISO	**microwave**
system	open	closed
extraction time	61 min	55 min
Chemical use	6 ml MeOH 0,12 ml H_2SO_4 (96%) 0,16 g NaOH 5 ml n-hexane	24 ml MeOH 0,75 ml HCl (37%) 1,0 ml KOH (21%) 5 ml n-hexane
sample	0,2 g fat	0,10-0,15 g fat

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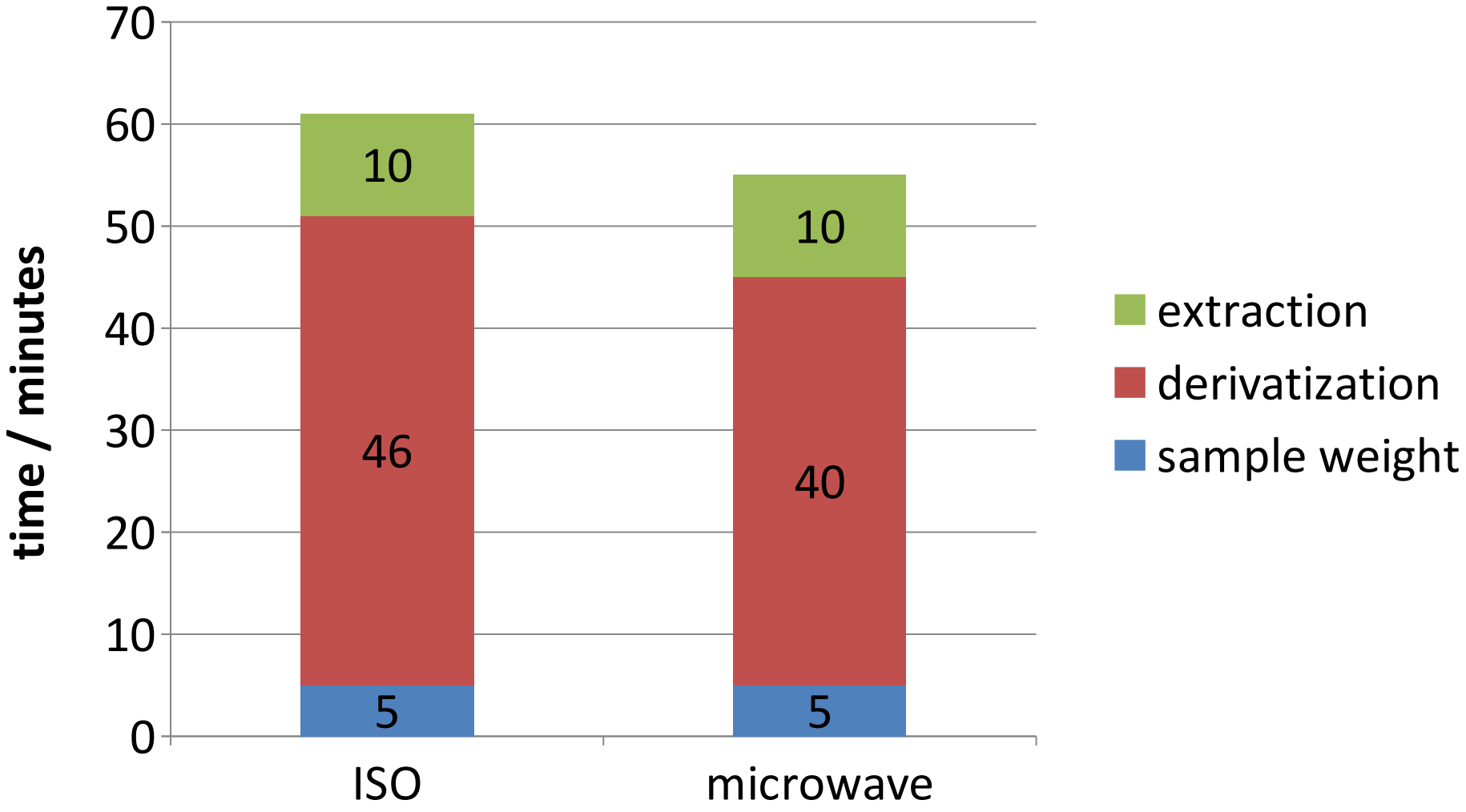
Derivatisation - Time Comparison



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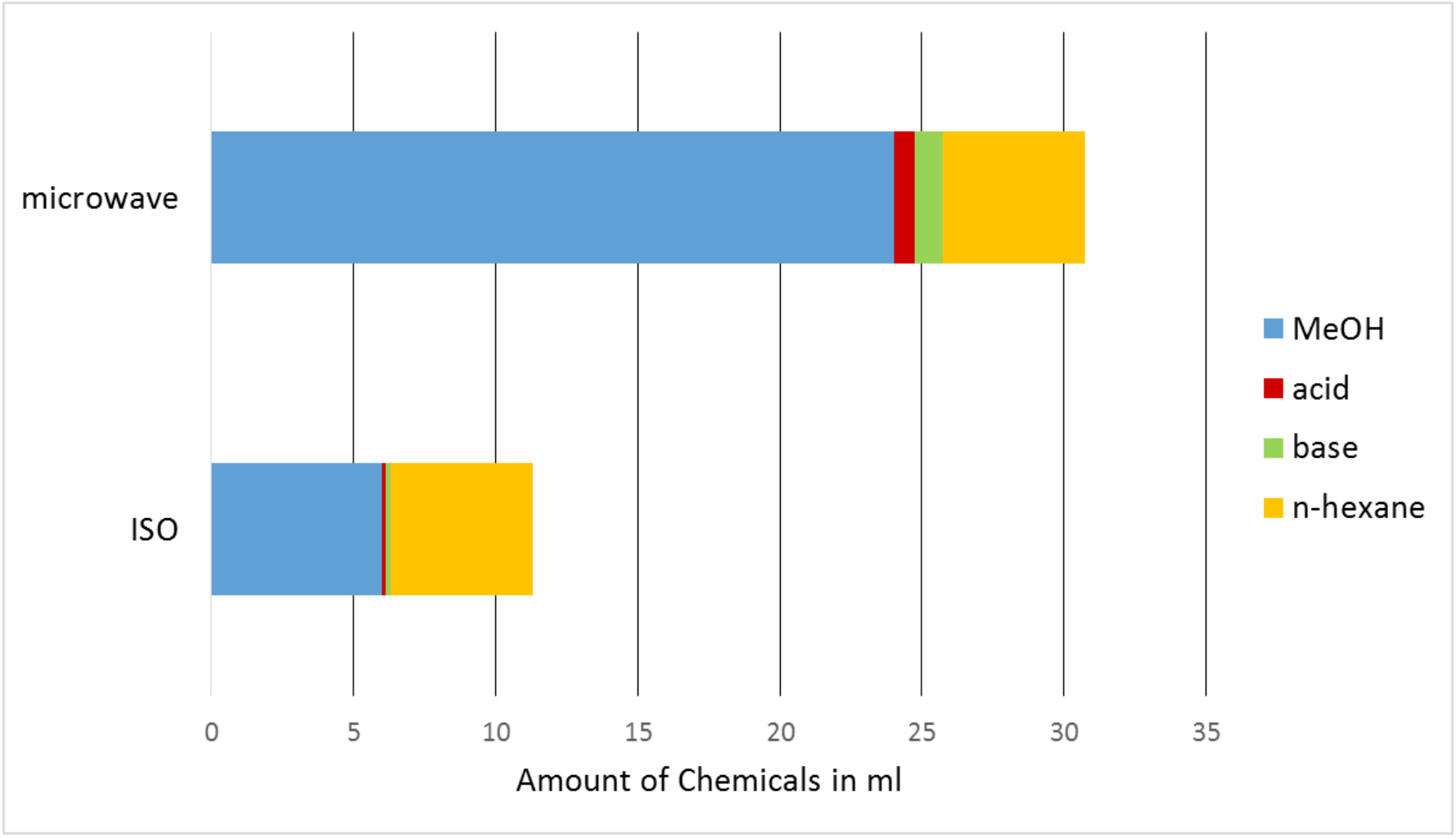
Use of Chemicals in ISO and Microwave-Assisted Derivatization



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Advantages NEW Microwave process

- Lower time expenses → higher sample throughput within a day
- Lower chemical use (in total)
- Closed system prevents losses volatile FAMEs (<C12)
- Reduce negative impacts on sensitive unsaturated fatty acids (oxidation processes)
- Inhomogeneous samples can cause significant deviations → sample homogeneity important

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Thank You for Your attention !