KANTONALES LABOR
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Analysis of trans fatty acids: how and what to analyze

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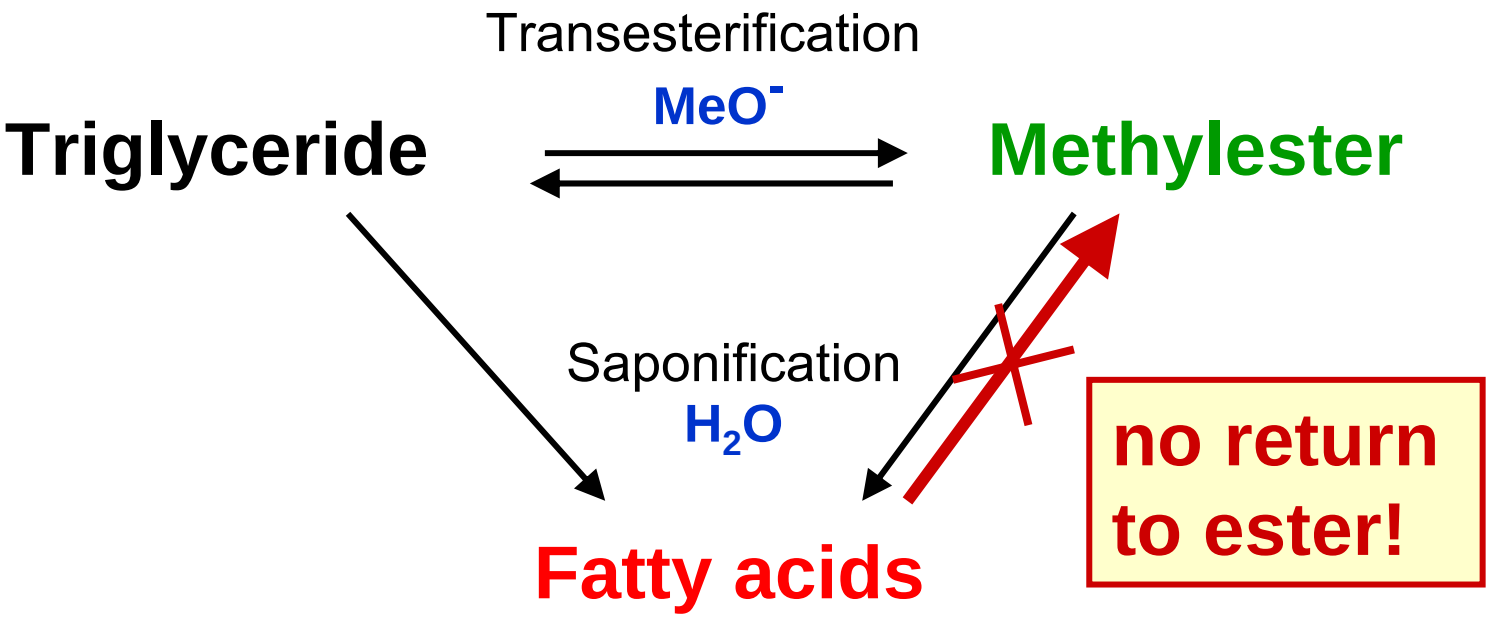
Formation of fatty acid methyl esters (FAME)

- Alkali (alcoholates)
  - Ester to ester, free acids are not esterified
  - Fast: 1 min/RT, mild conditions
  - Tolerates water if stopped before saponification
- BF_3/alcohol
  - Hydrolysis followed by esterification
  - Direct transesterification (~ 2 h at 80 °C)
  - Includes free fatty acids
  - Tolerates >30 % water

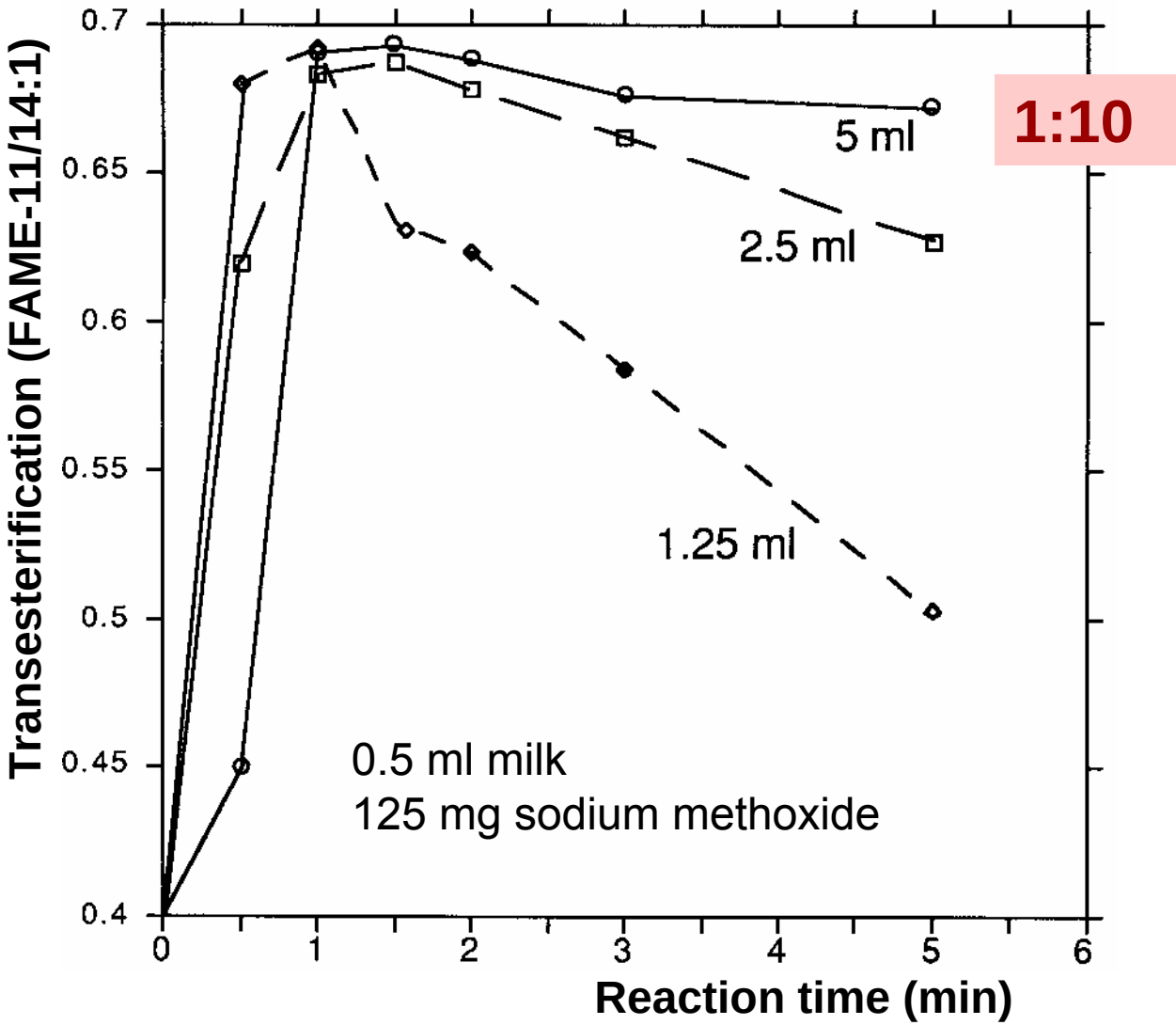
Importance of free fatty acid

Transesterification against saponification

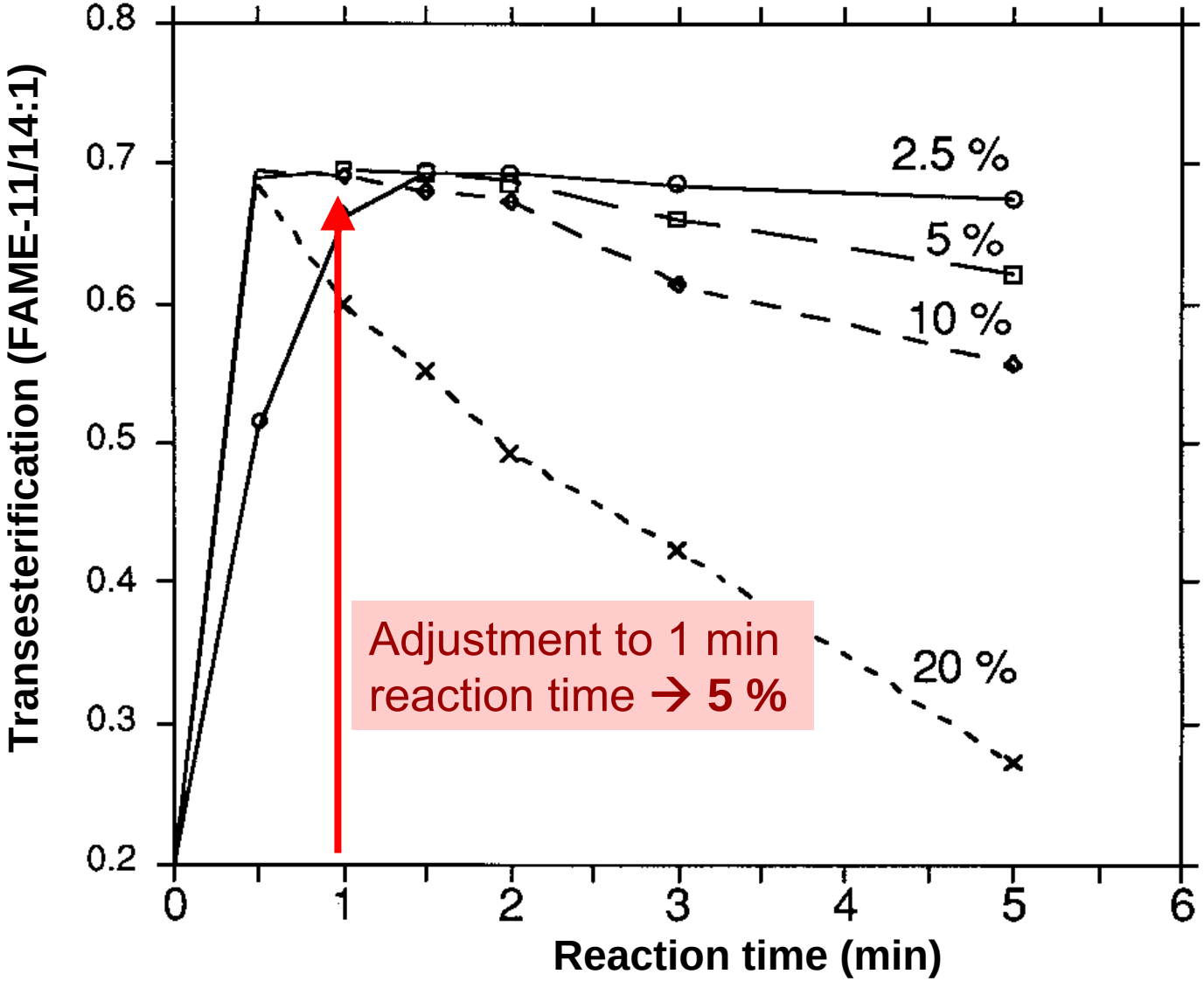


- Transesterification faster than saponification
- Elaboration of reaction kinetics → optimized conditions
- Stopping the reaction before relevant onset of saponification

Transesterification: water/methanol ratio



Dependence on the methoxide concentration



Method

1. <500 mg homogenized sample
2. pretreatment to access fat
3. 5 ml dioxane (mediator water-fat-reagent)
4. 5 ml 5 % methoxide/methanol
5. 1 min (40 s – 3 min)
6. 15 ml heptane
7. 10 ml aqueous disodium hydrogen citrate

Determination of fat content and fatty acid composition through 1 min transesterification in the food sample; principals. B. Suter, K. Grob, and B. Pacciarelli. Z. Lebensm. Unters. Forsch. 204 (1997) 252-258.

Determination of fat content and fatty acid composition through 1 min transesterification in the food sample; II. Solubilization of the fat, results. B. Suter, K. Grob, B. Pacciarelli, and A. Novoselac. Mitt. Gebiete Lebensm. Hyg. 88 (1997) 259-276.

Simultaneous determination of milk fat (butyric acid) and total fat by 1-min transesterification directly in the food. B. Suter, K. Grob and B. Pacciarelli. Mitteilungen aus Lebensmitteluntersuchung und Hygiene 90 (1999) 149-166

Determination of total fat and fatty acid composition through 1-min transesterification directly in the foods: collaborative studies. K. Grob, B. Suter, U. Buxtorf, and A. Dieffenbacher. Mitt. Lebensm. Hyg 91 (2000) 224-233.

Pretreatment to solubilize the fat

None	Slurry with water	Refluxing with DMF
edible oil/fat	milk powder	meat/meat products
milk	infant foods	cheese
curd, yoghurt		soups/ready meals
chocolate		bakery products
ice cream		cocoa powder, nuts
		cereals

Refluxing in DMF (bp. 153 °C)
opens up cells containing fat

GC-Analysis

- GC-FID
  - Assumption of equal response > C10 acid (convention)
  - Difficult calibration of response (instability of standards)
- On-column injection
- Optimum separation by double bond
  - Stationary phase of high polarity
  - Avoided squeezing out effect (no Carbowax <20,000 D)
  - Masking the polarity of acid group
  - Minimum elution temperature

→ Methyl esters

→ Cyano polysiloxanes: trans before cis
  - preferable for separation of unsaturated FA

→ Carbowax: trans after cis
  - preferable for butyric acid and iso/anteiso acids

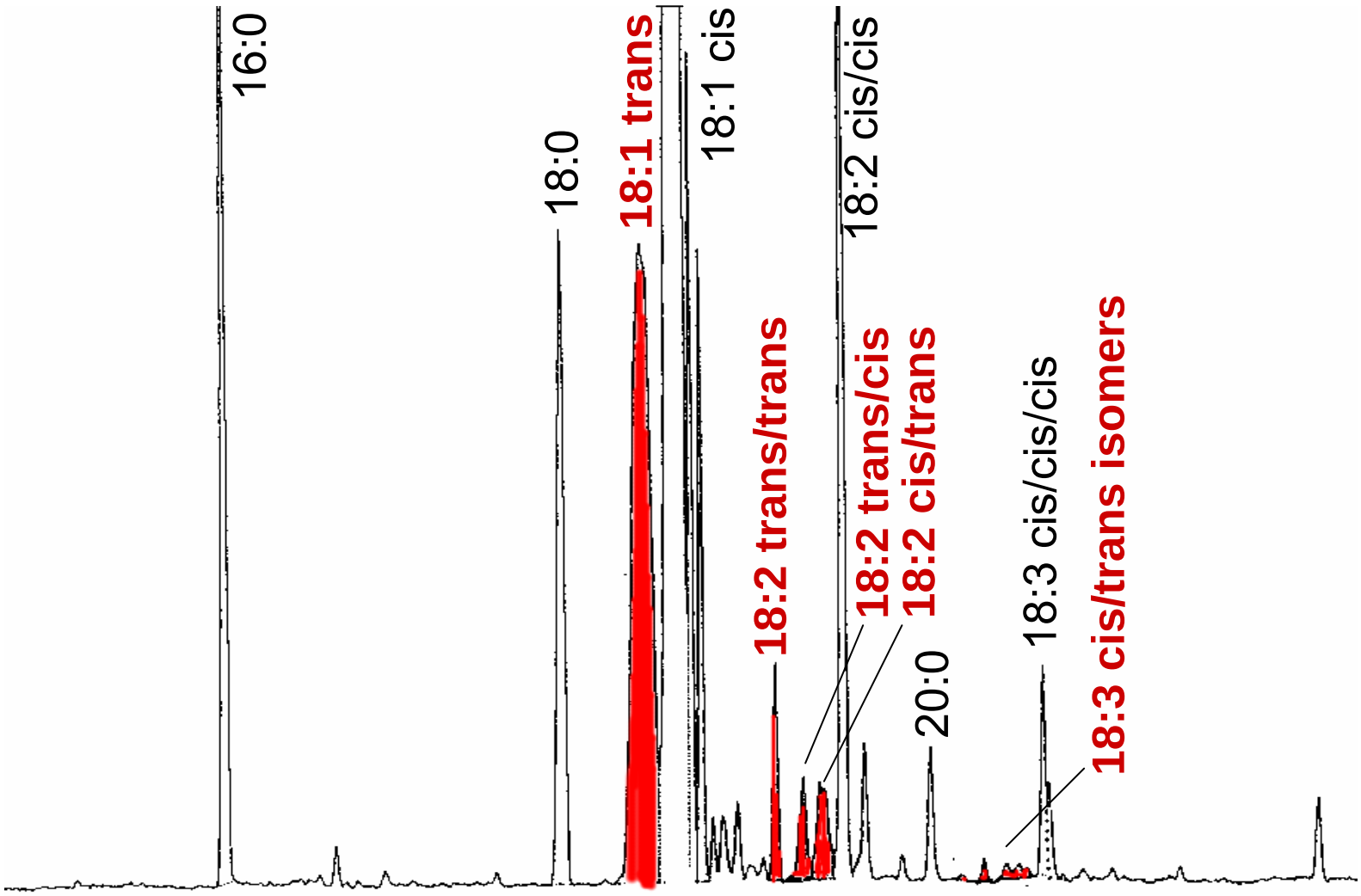
→ 100 m x 0.25 mm i.d. column: capacity (overloading)

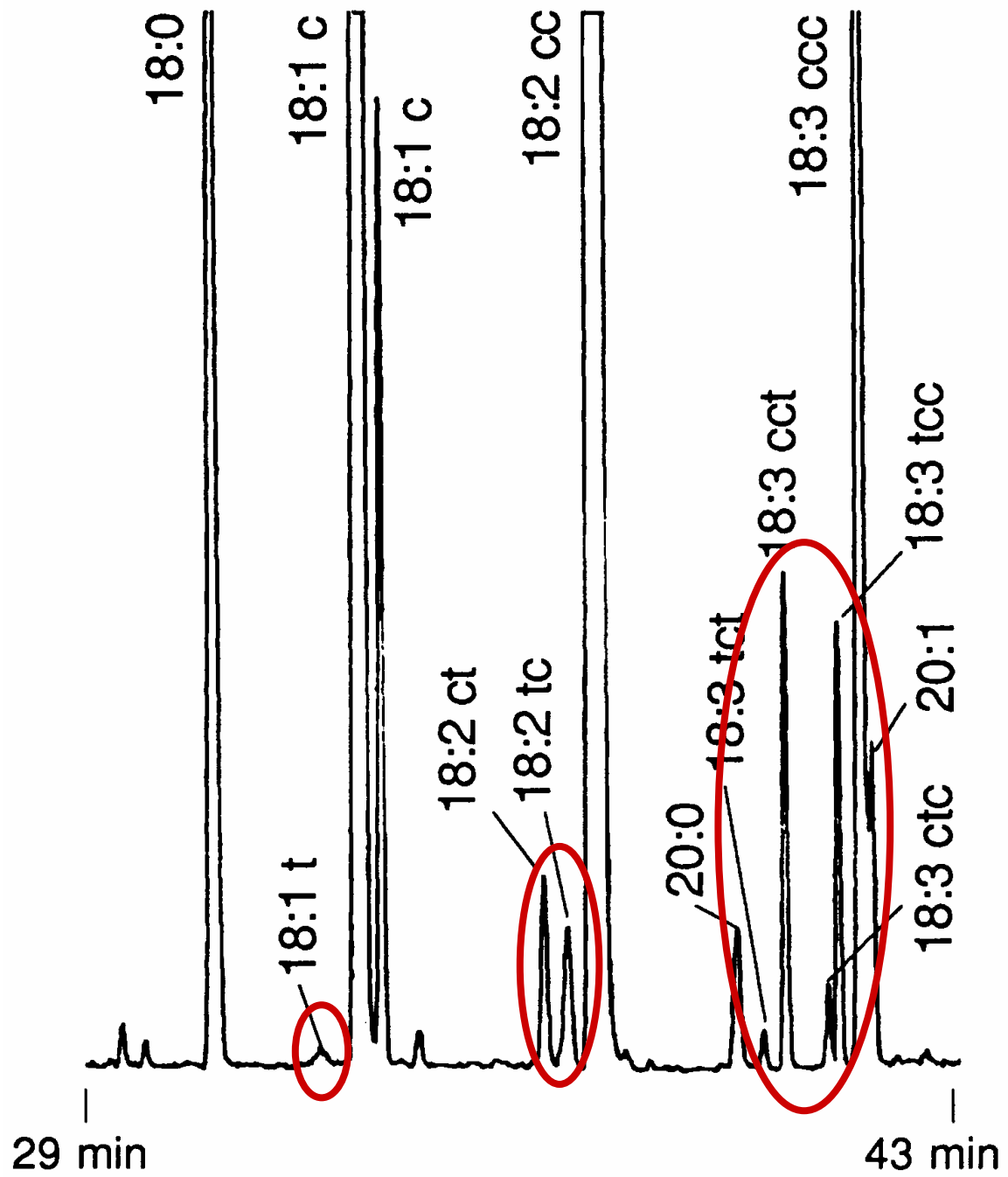
Two subjects of interest

- Partial hydrogenation → trans monoenes
  - margarines
  - bakery products
  - soup preparations
- Deodoration at high temperature → trans di- and trienes
  - all edible oils
  - margarines

...and the cis-isomers?

Partially hydrogenated fat → monoenes



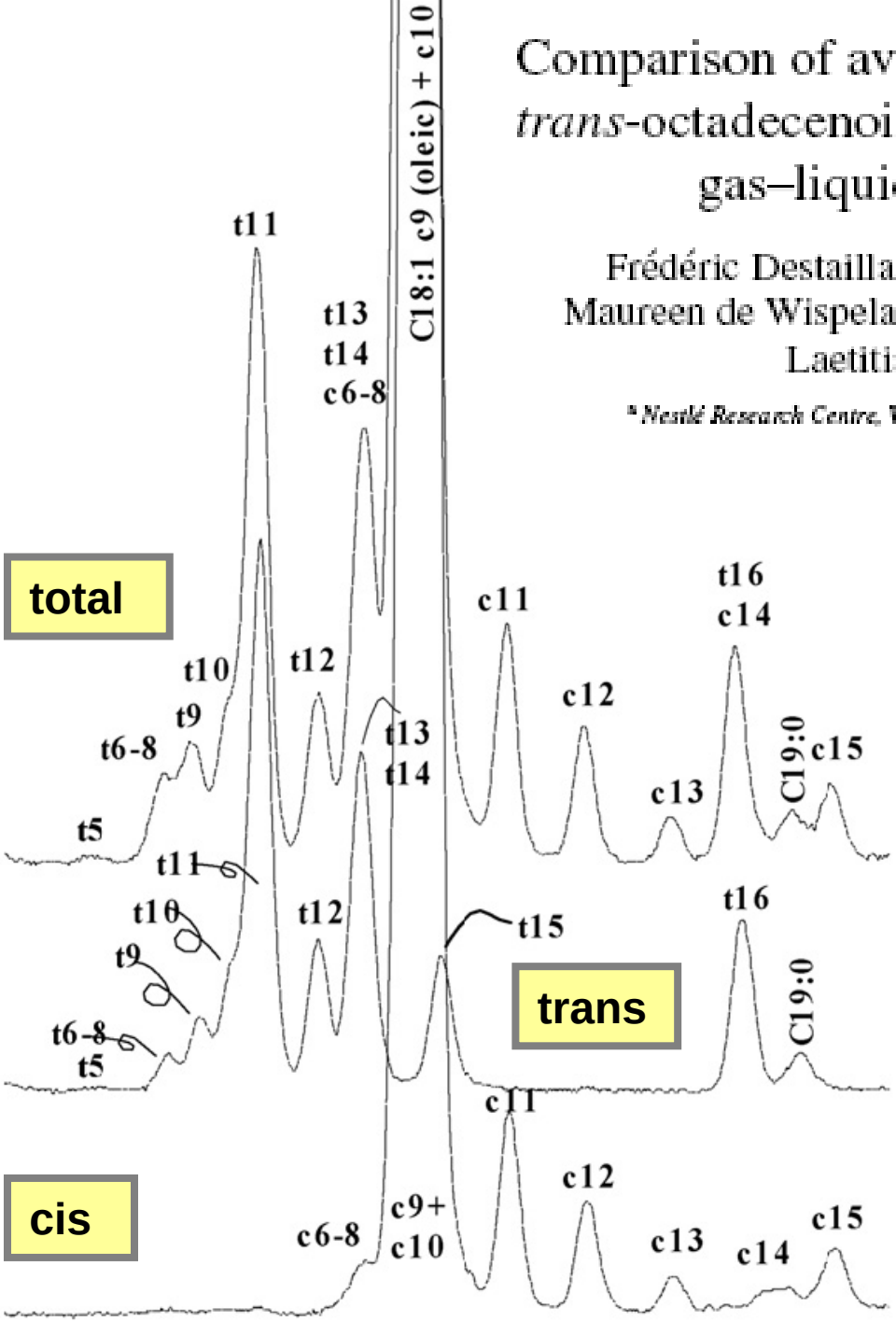


High deodoration temperatures → di and trienes

Comparison of available analytical methods to measure *trans*-octadecenoic acid isomeric profile and content by gas–liquid chromatography in milk fat

Frédéric Destaillats [a,*], Pierre-Alain Golay [a], Florent Joffre [b], Maureen de Wispelaere [a], Bernadette Hug [a], Francesca Giuffrida [a], Laetitia Fauconnot [a], Fabiola Dionisi [a]

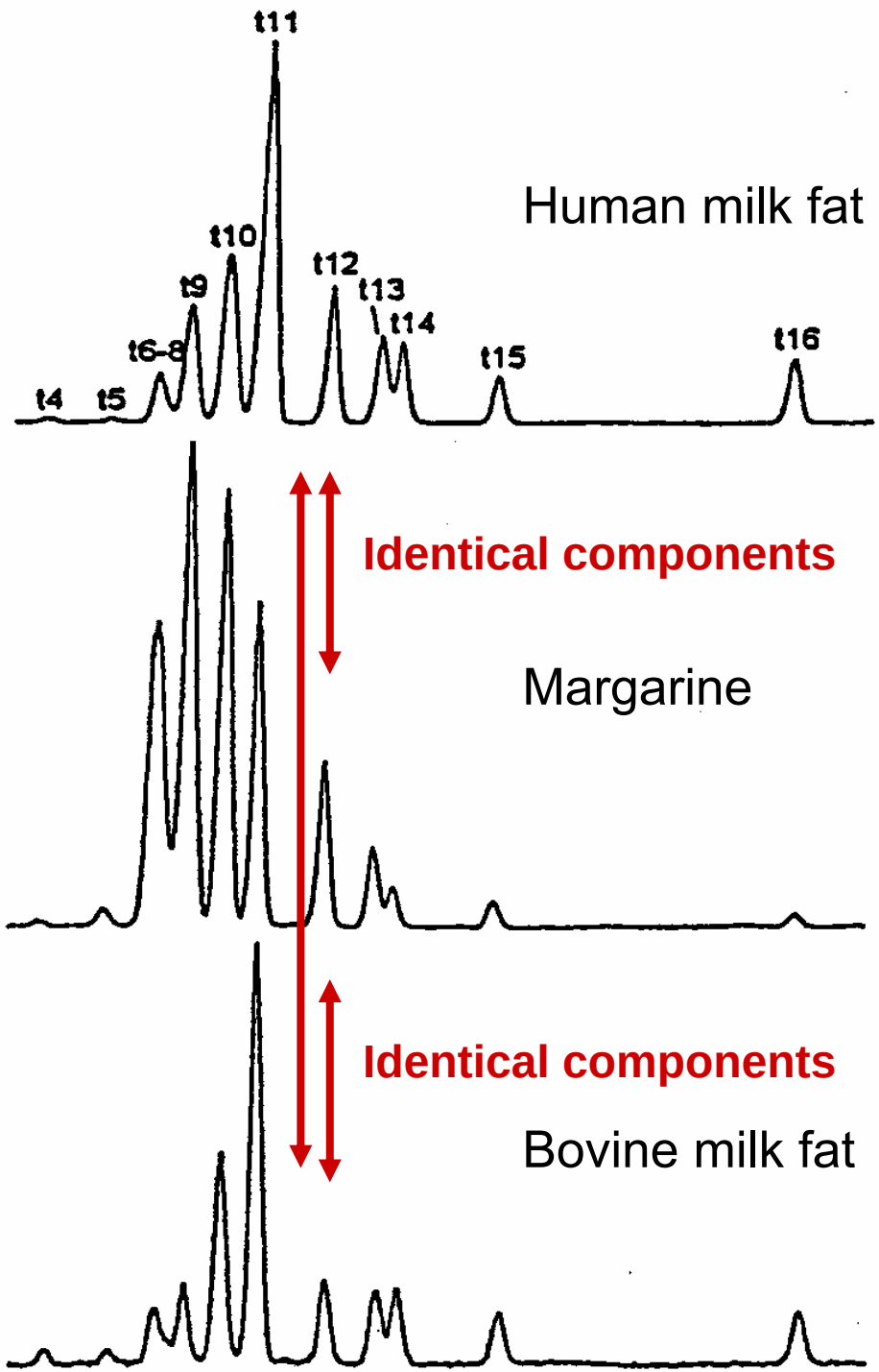
[a] *Nestlé Research Centre, Vers-chez-les-Blanc, P.O.Box 44, CH-1000 Lausanne 26, Switzerland*
[b] *Omega 21, Marsannay le Bois, France*



Prefractionation by Ag-TLC is not really necessary

100 m x 0.25 mm i.d. cyanopropyl polysiloxane

Preseparation for monoenes?

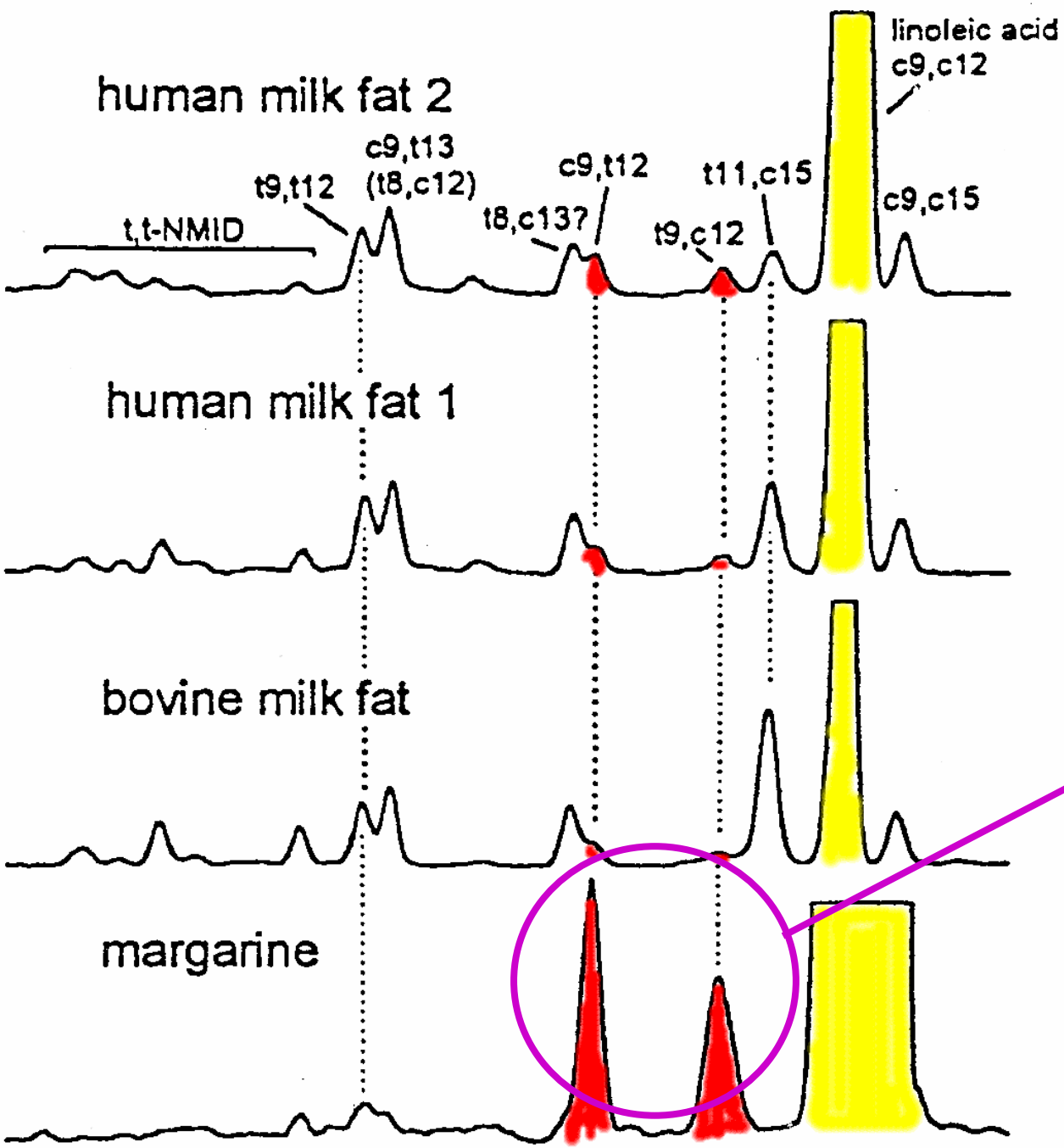


18:1 trans

Preseparated on Ag-TLC

Is milk fat dangerous?

Precht, Molketin, 1999
Nahrung 43, 233-244



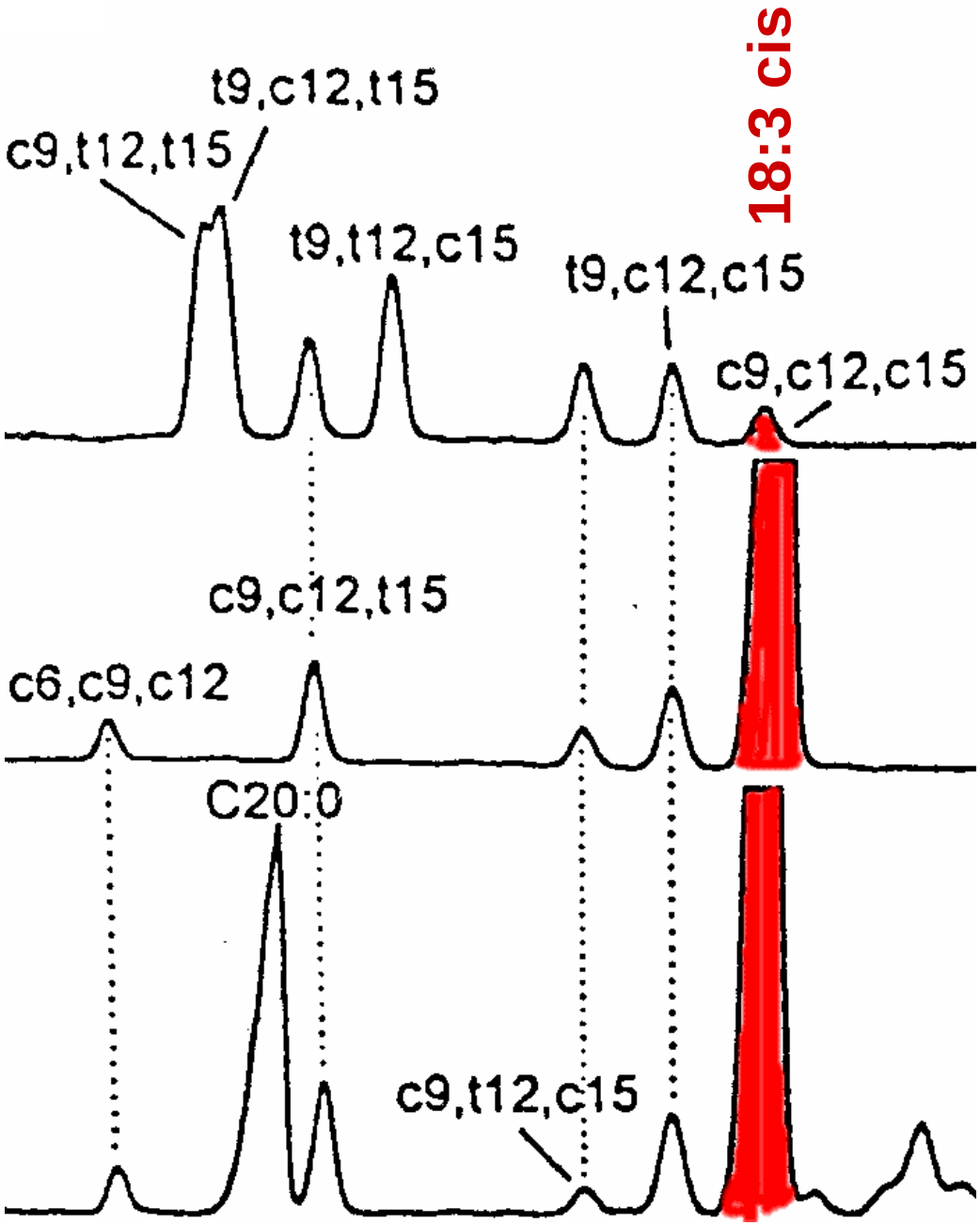
18:2

Preseparated on Ag-TLC

± inexistant in nature

Precht, Molketin, 1999

18:3



Standards

Fractionated human milk fat

Human milk fat

Precht, Molketin, 1999

What do we really know?

1. Humans do not produce trans-FA
2. Toxicology does not specify toxic isomers
   - Not even confirms that trans-monoenes are relevant
   - Composition of the fats used for tox. evaluation?
3. Trans-monoenes from cow milk are „old“
4. Trans-dienes from deodoration are ±new
5. Trans-trienes from deodoration are new

Raffination of edible oils

Pression and/or solvent extraction

Washing/degumming

Extraction with NaOH
(removal of free acids)

Bleaching (decoloration)

Deodorization 180-220 °C

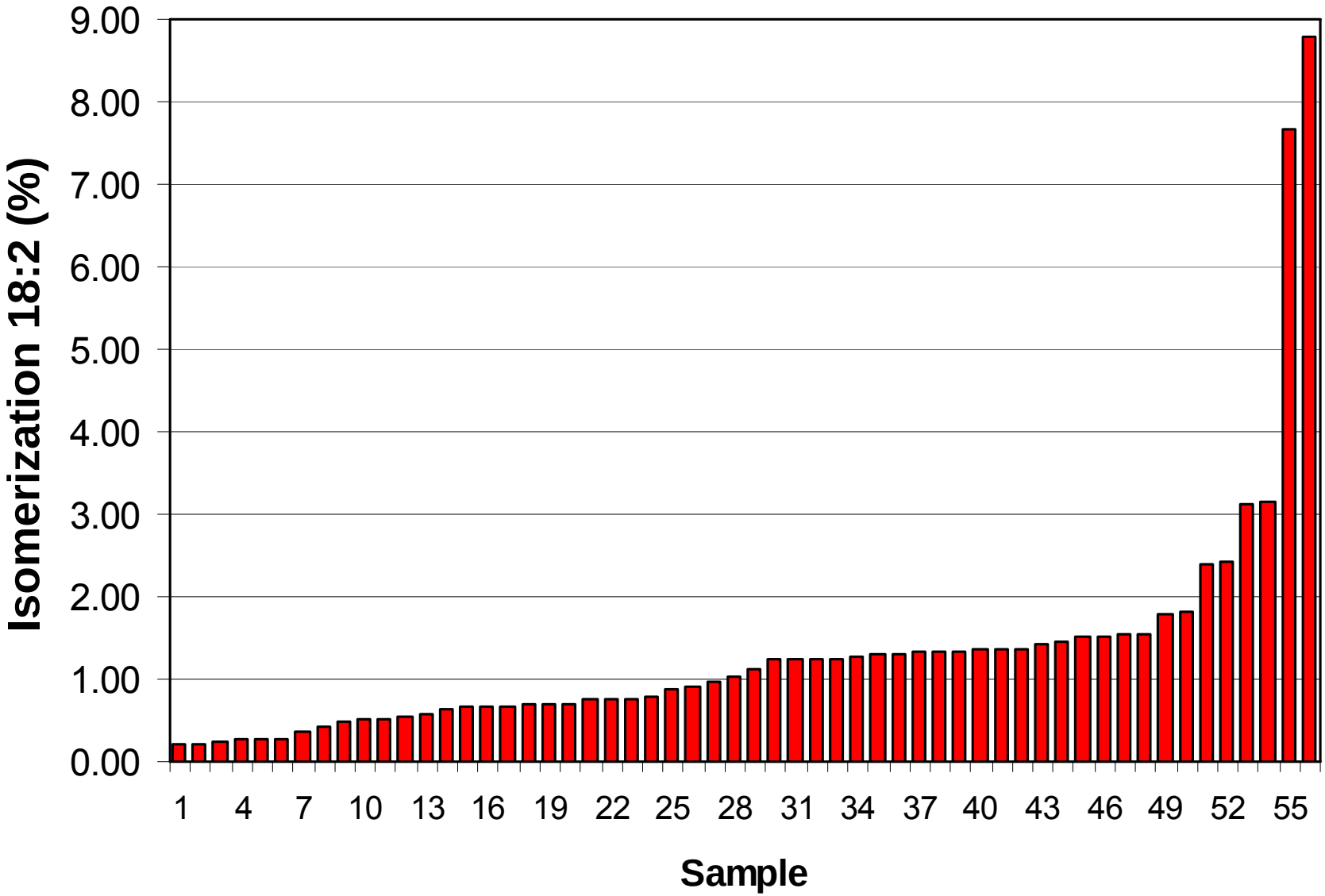
Chemical refining

Bleaching (decoloration)

Deodorization 240-260 °C
(evaporation of free acids)

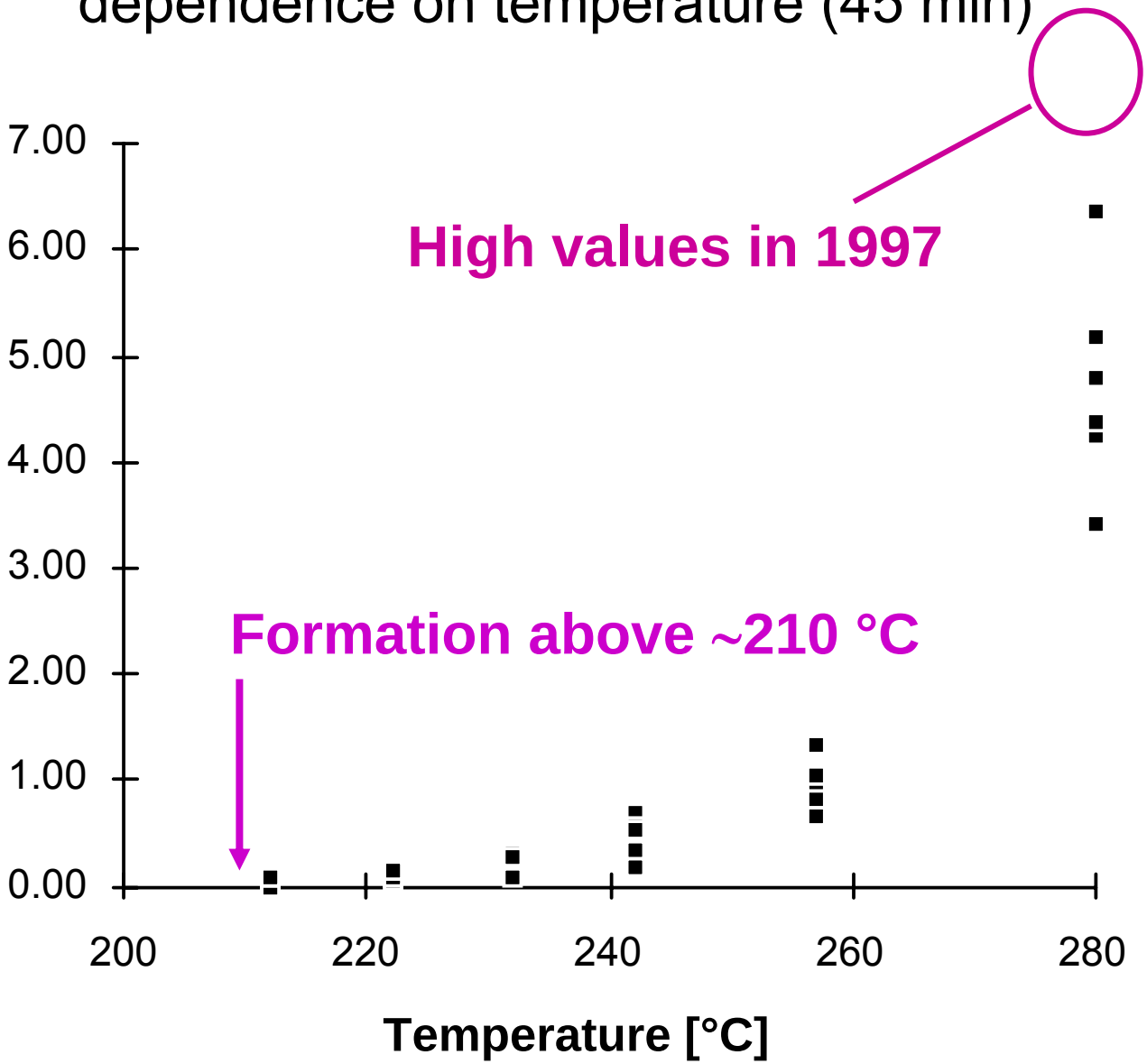
Physical refining

Edible oils from market 1997:trans 18:2

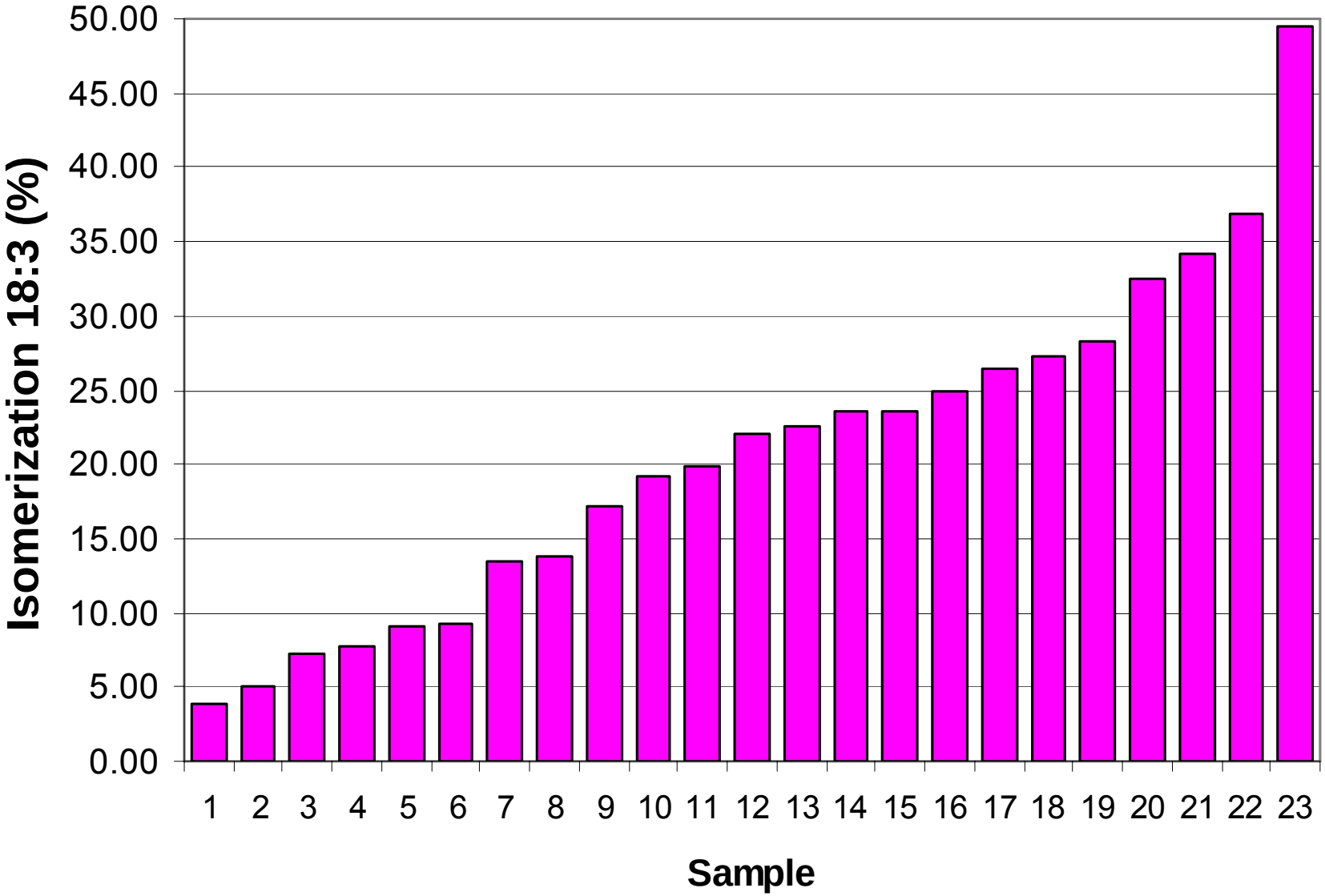


Formation of trans 18:2

dependence on temperature (45 min)

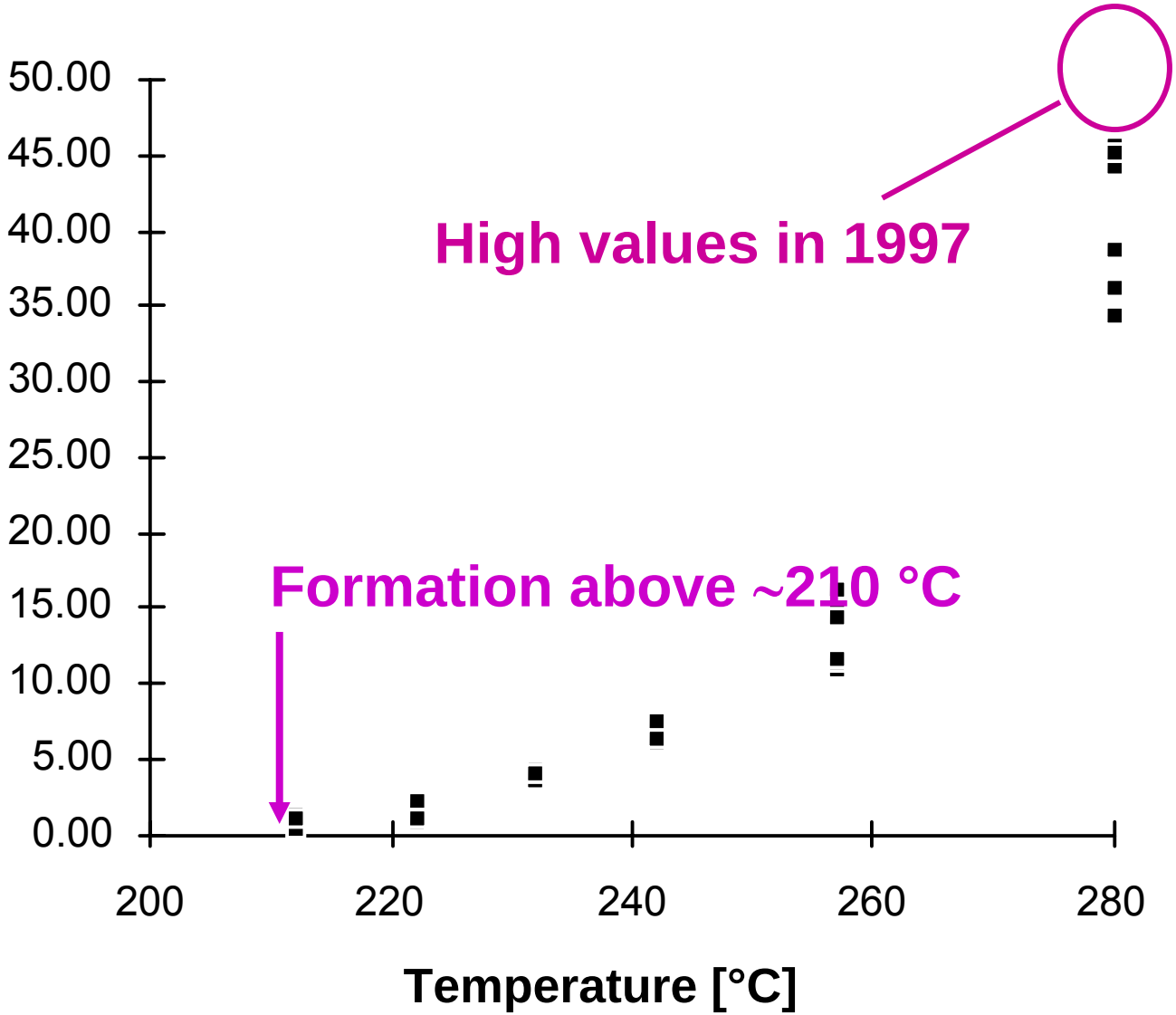


Edible oils from market 1997: trans 18:3



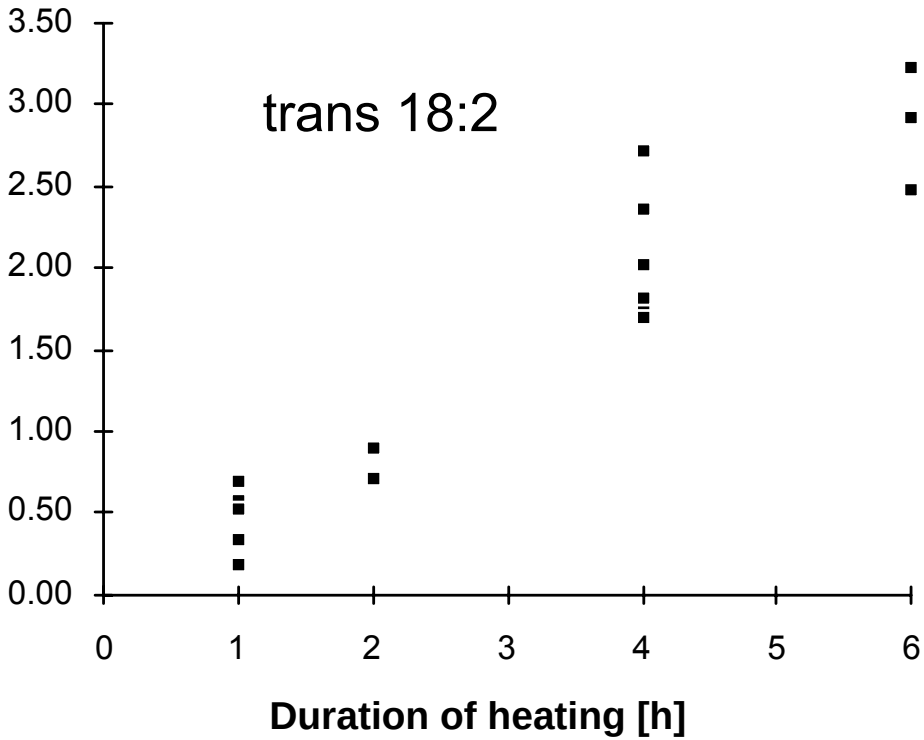
Formation of trans 18:3

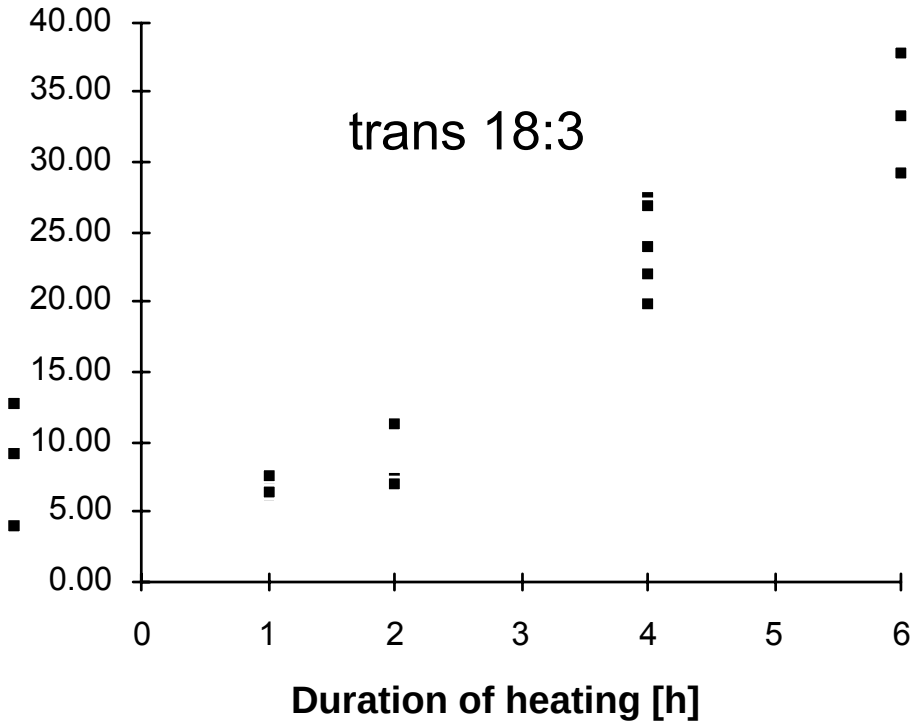
dependence on temperature (45 min)



Formation of trans 18:2 and 18:3

dependence on time





Baby food

- Addition of oils increasing 18:2 and 18:3
  - To adjust, e.g., bowine milk to human milk
  - ~ 15 % 18:2, ~ 1.5 % 18:3
  - Essential fatty acids

18:01		18:2			18:3		
tr	c	c/tr	cc	% tr	tr	ccc	% tr
12	28.9	0.4	22.4	1.75	≤0.03	0.43	< 8
≤1	23.5	<0.06	37.2	<0.2	<0.03	≤1.7	?
1.2	20.9	≤0.05	13.9	<0.4	≤0.05	0.79	≤6
0.3	34.6	0.42	14.8	**2.76**	0.39	1.42	21.5
0.15	31.7	0.64	14	**4.37**	0.36	0.78	**31.6**
1.7	33.7	0.6	12.2	**4.69**	0.1	1.54	6.1
21.4	24.6	≤0.3	17.7	≤1.5	≤0.2	≤0.7	?
0.38	16.1	0.21	36.4	0.57	0.31	4.6	6.3
0.08	19	0.2	14.1	**1.40**	0.23	1.32	14.8
11.8	28.7	≤0.4	23.3	≤1.5	0.08	0.47	14.5
2.1	20.9	0.3	1.5	16.7	≤0.06	0.78	<7
1.9	32.4	0.28	12.8	2.1	<0.06	0.65	<8.5
1.1	34.4	0.42	9.7	**4.2**	0.07	1.34	5.0
1.8	33.7	0.37	9.5	**3.7**	0.24	1.15	17.3
1.5	37.1	0.56	11.9	**4.5**	0.32	1.19	**21.2**
1.9	36	0.56	11.2	**4.8**	0.1	1.44	6.5
21.3	25.7	0.3	16.5	1.8	<0.05	0.45	<20

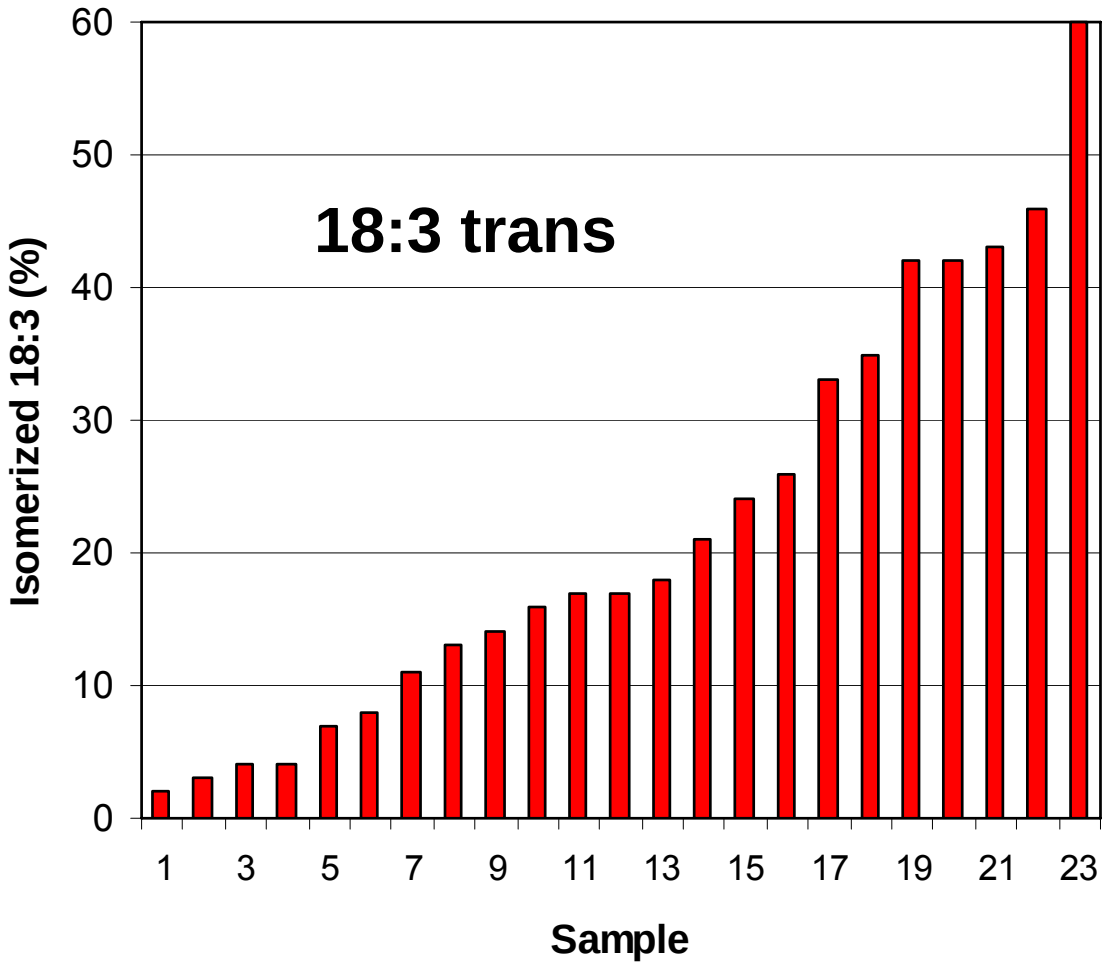
Trans 18:2 and 18:3 in baby food

1997/1998

Proportion of trans 18:2 and 18:3 in baby food

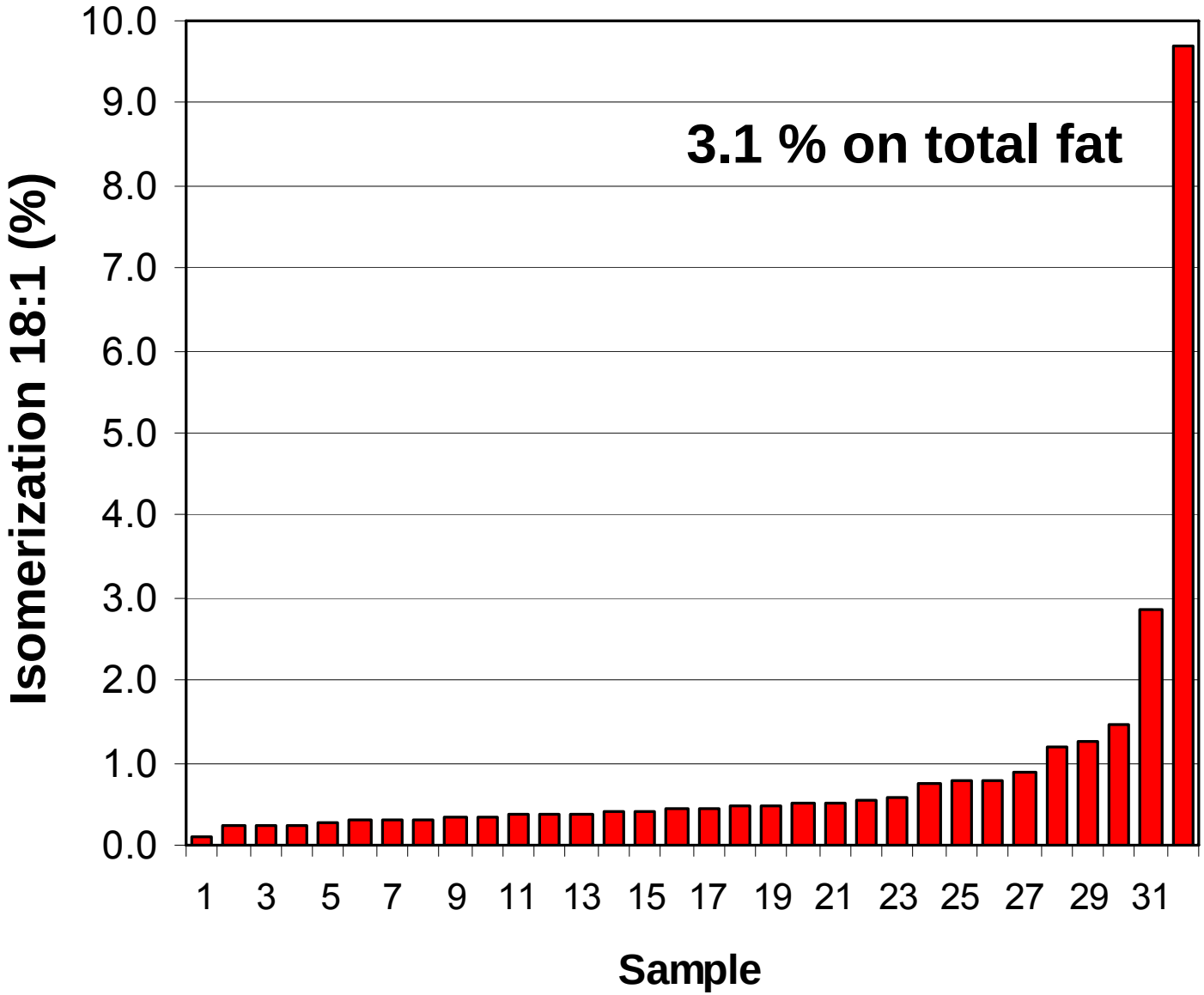
Samples from Germany 1998

	% in trans	
	18:2	18:3
1	2.2	26.3
2	1.4	46.0
3	1.6	24.4
4	2.1	42.0
5	2.6	43.1
6	2.9	60.4
7	2.4	33.3
8	1.7	35.2
9	2.7	17.6
10	1.0	41.9
11	1.6	12.3
12	3.3	11.4
13	1.8	14.5
14	1.9	15.7
15	3.2	18.2
16	2.2	16.7
17	2.5	7.8
18	2.2	7.2
19	3.1	21.3
20	2.8	0.0
21	0.5	2.1



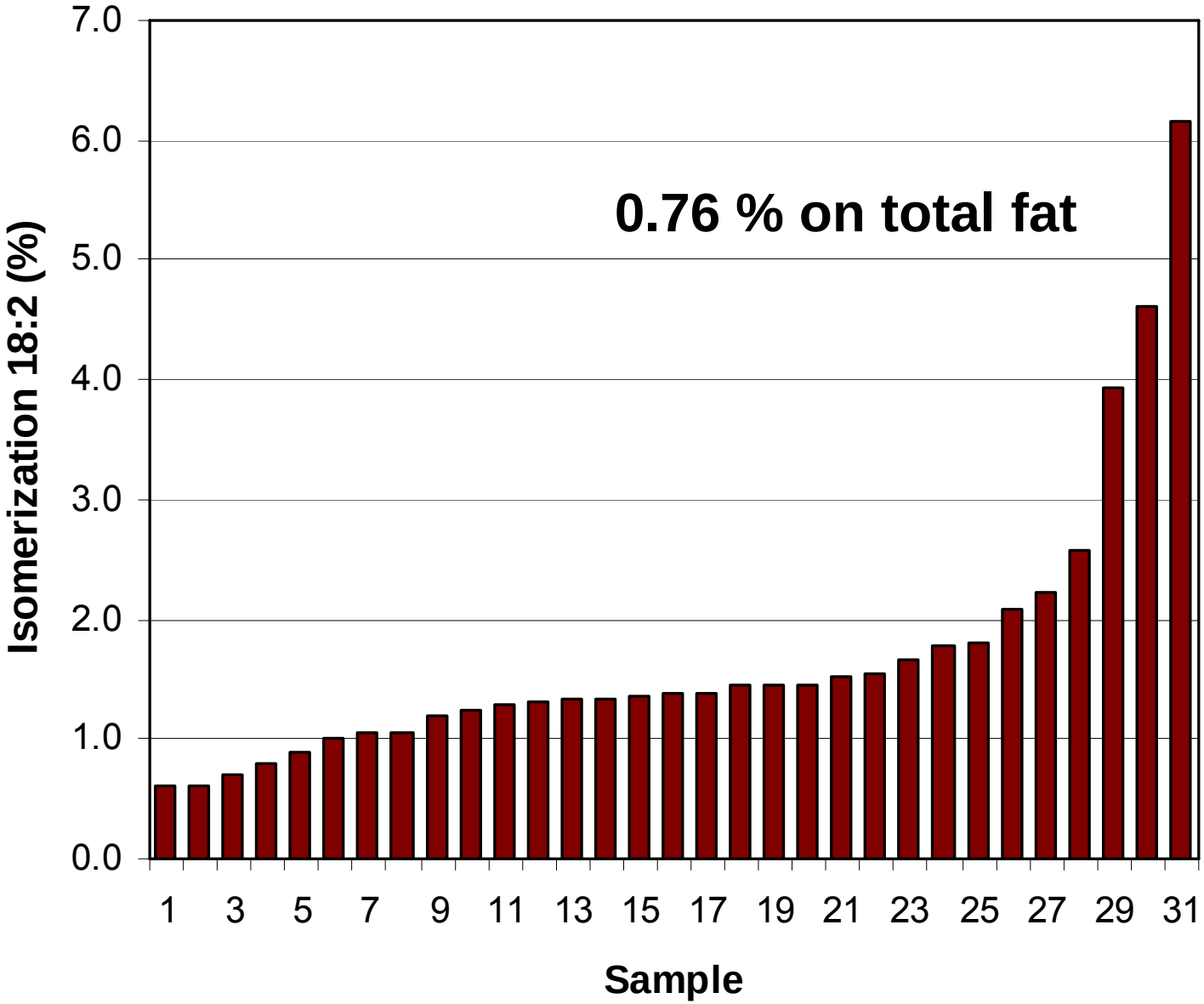
Baby food CH 2007

KLZH, April 07



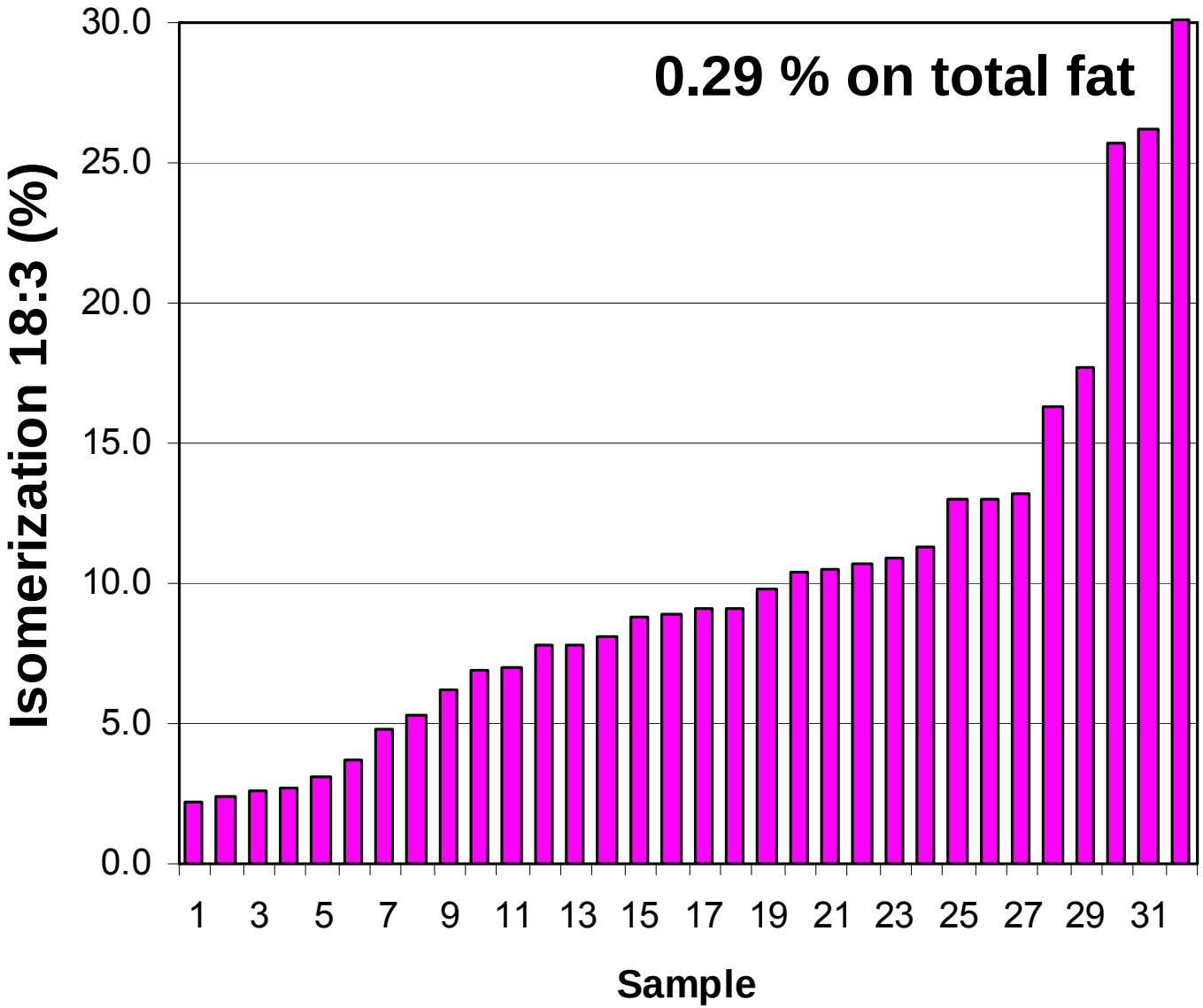
Baby food CH 2007

KLZH, April 07



Baby food CH 2007

KLZH, April 07



Concentration or proportion?

- Is the relevant quantity
  - the absolute concentration of isomerized fatty acid or
  - the proportion (degree) of isomerization?
- Metabolism uses essential fatty acids for synthesis and the risk of taking an isomerized acid depends on the proportion of isomerization

Hindering legislation

- ~1995, EU limit for the sum of the trans fatty acids in baby food of 4 %/fat
  - to prevent use of hydrogenated fats
  - bovine milk fat: up to 4 % natural trans fatty acids
- Typical contents of 18:3: 1-1.5 %, → even total isomerization does not exceed legal limit

→Limit should refer to trans 18:1 only!

→Limit for degree of isomerization

Trans-polyunsaturated FA (PUFA)

- The most relevant trans-FA are the trans-PUFA
- With physical refining a cheaper process was introduced without paying the price to ensure safety
- Deodoration at 200 °C yields no detectable isomerization
- “Gently deodorized” oils are treated <120 °C
  - But are not completely neutral in taste

- Restriction to GMP