UNIVERSITY OF HOHENHEIM
Food Science and Biotechnology
Plant Foodstuff Technology and Analysis

Characterization of carotenoids and fatty acids in Costa Rican *Acrocomia aculeata* palm fruits

Steingass CB, Schex R, Lieb VM, Jiménez VM,
Esquivel P, Schweiggert RM, Carle R

FSC Regional Seminar 2017 in Latin America
San José, December 5, 2017

Vitamin A deficiency (VAD)

Vitamin A deficiency (VAD)

- Global dietary problem
- WHO (2009): 190 million affected pre-school children

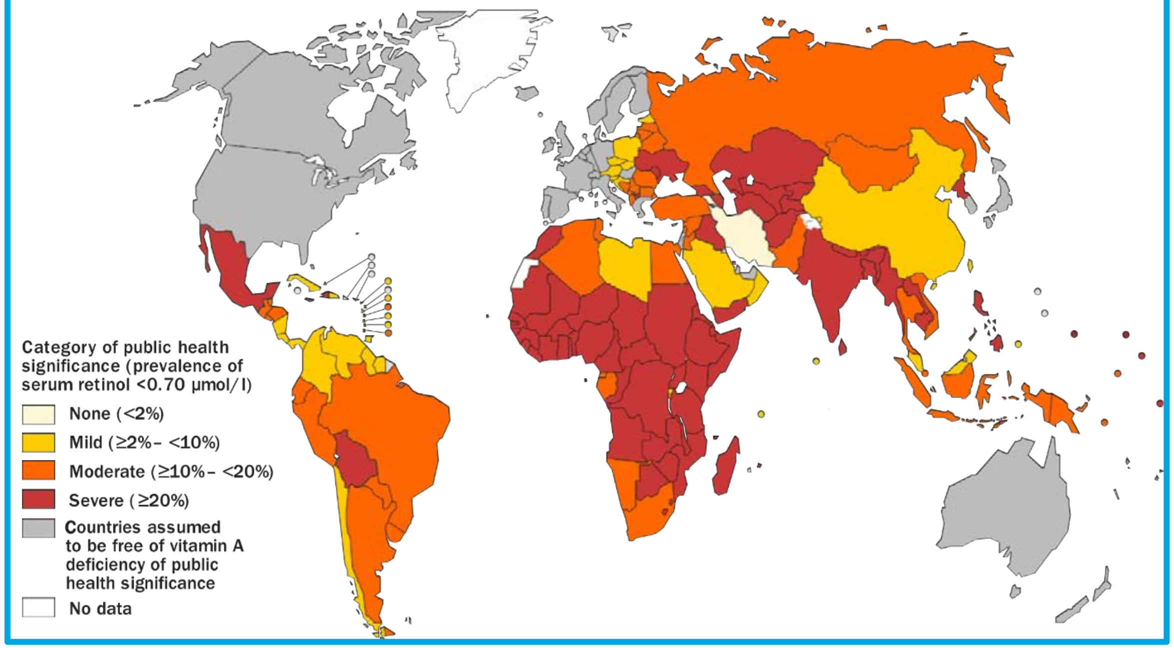
Functions of vitamin A

- Vision (rhodopsin)
- Growth and development
- Immune defense
- Reproduction

Consequences vitamin A deficit

- Increased morbidity and mortality (infections)
- Xerophthalmia, night blindness

→ Plant sources of vitamin A



Vitamin A deficiency 1995-2005: Moderate to severe health problem in **122 countries** (WHO, 2009)

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Palm oil: current situation

- World production 2016: 62–65 Mio. tonnes
- South East Asia: African oil palm (*Elaeis guineensis* Jacq.)

Drawbacks palm oil (examples)

- Deforestation (South East Asia)
- Extensive monocultures (*E. guineensis*)
- Susceptible for phytopathogens (pesticides!)

Advantages palm oil

- Broad range of applications (food, feed, cosmetics)
- High fat yields (50–70% dry matter)
- High melting point (~36 °C)
- Lipid fractions: **kernel fat, mesocarp oil, carotenoids (E160a)**
- Red palm oil: "functional food": squalene, **vitamin E, carotenoids**

⇨ **Diversification of monocultures**

⇨ **Alternative fat sources**

Acrocomia sp. (Arecaceae)

Macáuba/Coyol (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.)

- Origin: Central and South America
- Tropical and **temperate zones:** silvopastoral cultivation
- Harvest yield: ~40 t/ha (*Elaeis* sp. ~35 t/ha)

⇨ **Alternative fat sources**

HPLC-DAD-APCI/ESI-MS^n analysis

Method development: *Acrocomia aculeata*

Isolation: carotenes, xanthophylls & tocochromanols

1. Freeze-dried mesocarp

⇩ + $CaCO_3$

2. Extraction: acetone (3×)

⇩

3. Saponification: 2 mL 20% KOH (in MeOH)
 + 2 mL diethylether
 + 0.2 mL 20% ascorbic acid solution

⇩ Stirring 3h, room Temp.

4. Washing and LLE: diethylether

⇩

5. Evaporation to dryness

⇩

6. Dissolution in MeOH/MTBE 1/1 *v*/*v*

HPLC analysis

	System 2: *Acrocomia*
Column (C30)	250×4.6 mm i.d., 3 µm
Eluent A	80:18:2 MeOH/*t*BME/H_2O
Eluent B	8:90:2 MeOH/*t*BME/H_2O
Column Temp.	23 °C
Flow rate	0.6 mL/min
Injection volume	10 µL
Total run time	45 min

Recoveries

violaxanthin 90%
lutein 98%
β-carotene 95%
α-tocopherol 91%

HPLC-DAD-APCI/ESI-MS^n analysis

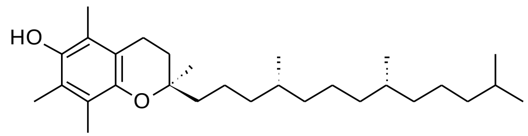
Identification criteria

- ✓ Mass spectrum (at least: molecular ion)
- ✓ Chromatographic behaviour (t_R)
- ✓ UV/vis absorption spectrum

Mass spectrometry

Background: TAGs etc. → **saponification**

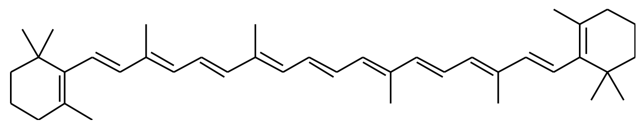
Tocochromanols: ESI(-)



- Auto-MS^n

→ ESI(-): $[M-H]^-$

Carotenoids: APCI alternating polarity



- Auto-MS mode: MS1 spectra

→ APCI(-): $[M]^{\bullet -}$

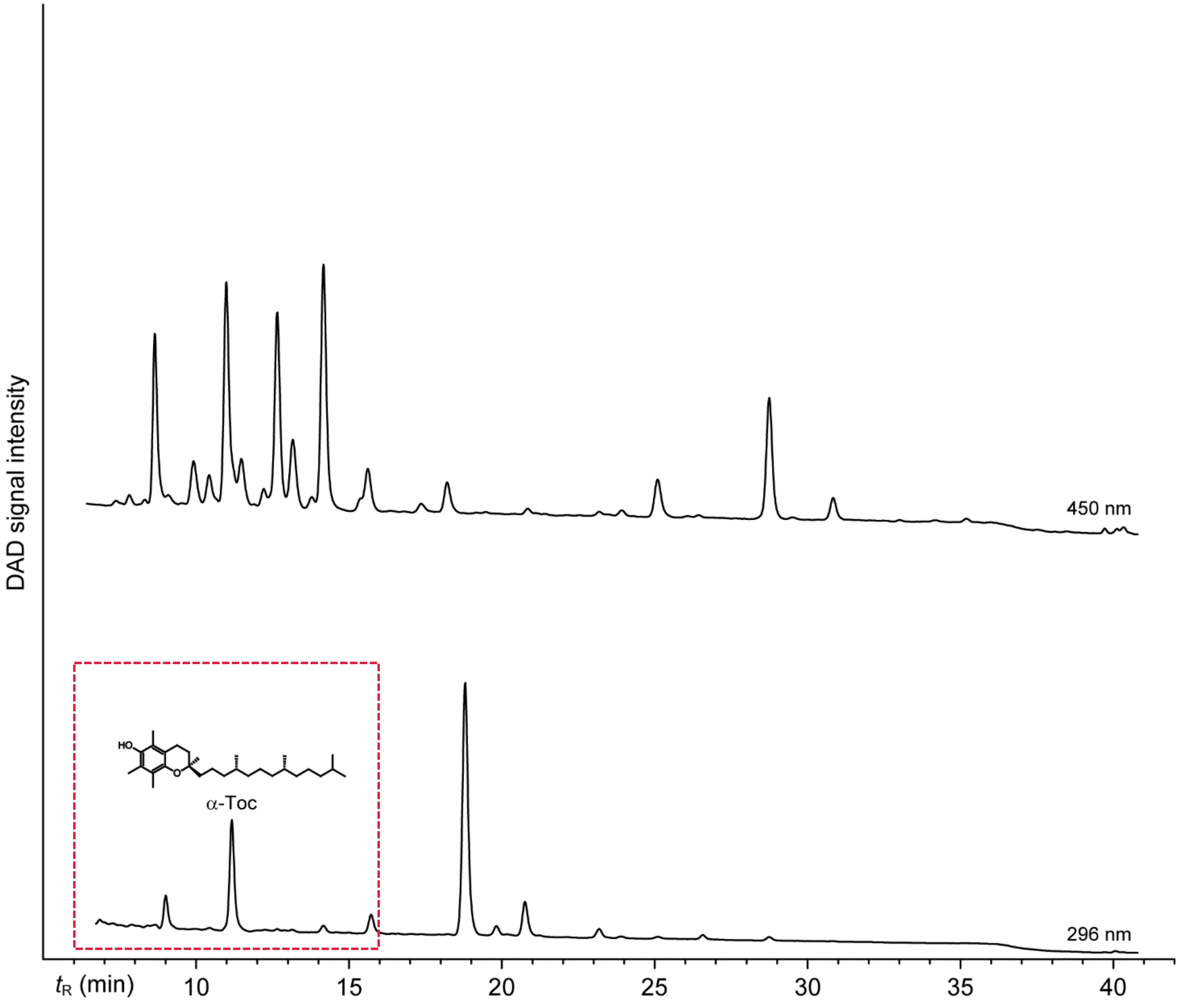
→ APCI(+): $[M+H]^+$

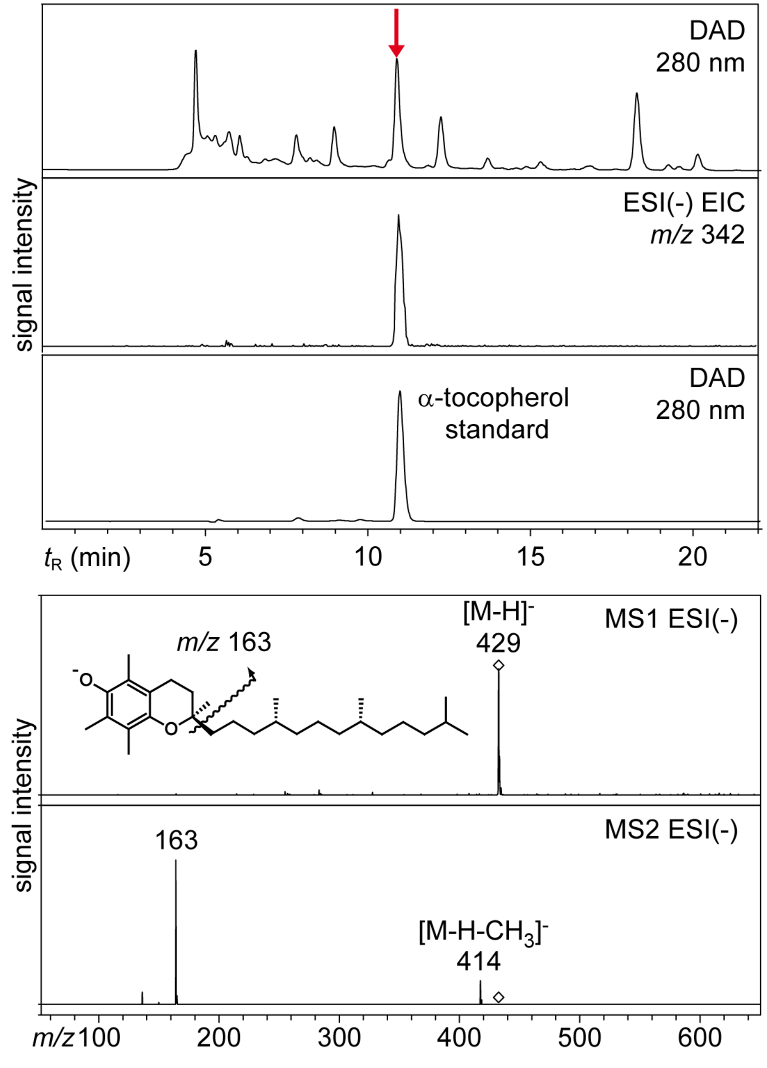
- MS/MS experiments

→ APCI(+): indicative mass fragments

HPLC-DAD-ESI-MS^n analysis

Tocochromanols: *Acrocomia aculeata*

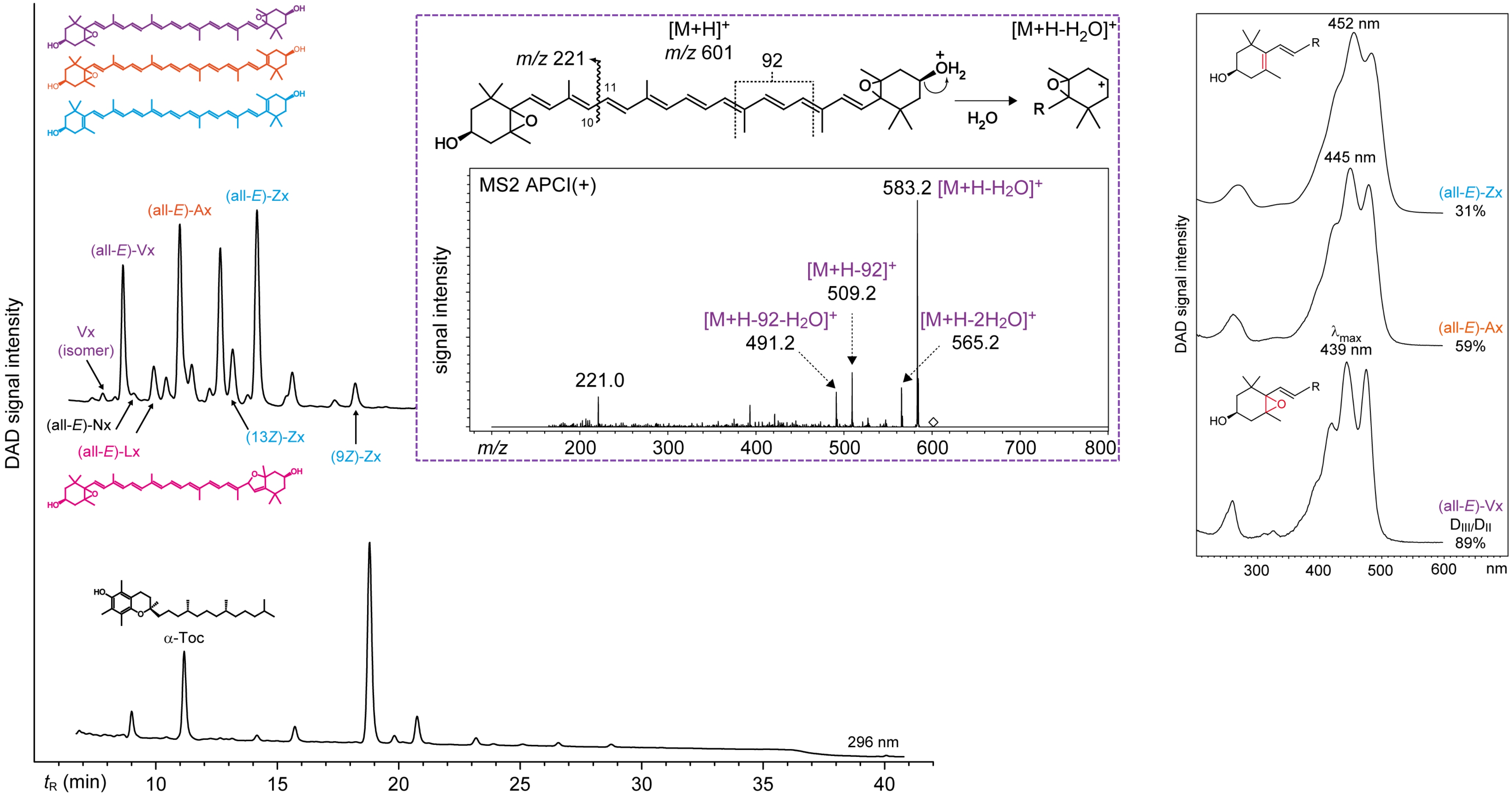




Schex *et al.*, *Food Res. Int.* DOI 10.1016/j.foodres.2017.11.041

HPLC-DAD-APCI-MS^n analysis

Xanthophylls: *Acrocomia aculeata*



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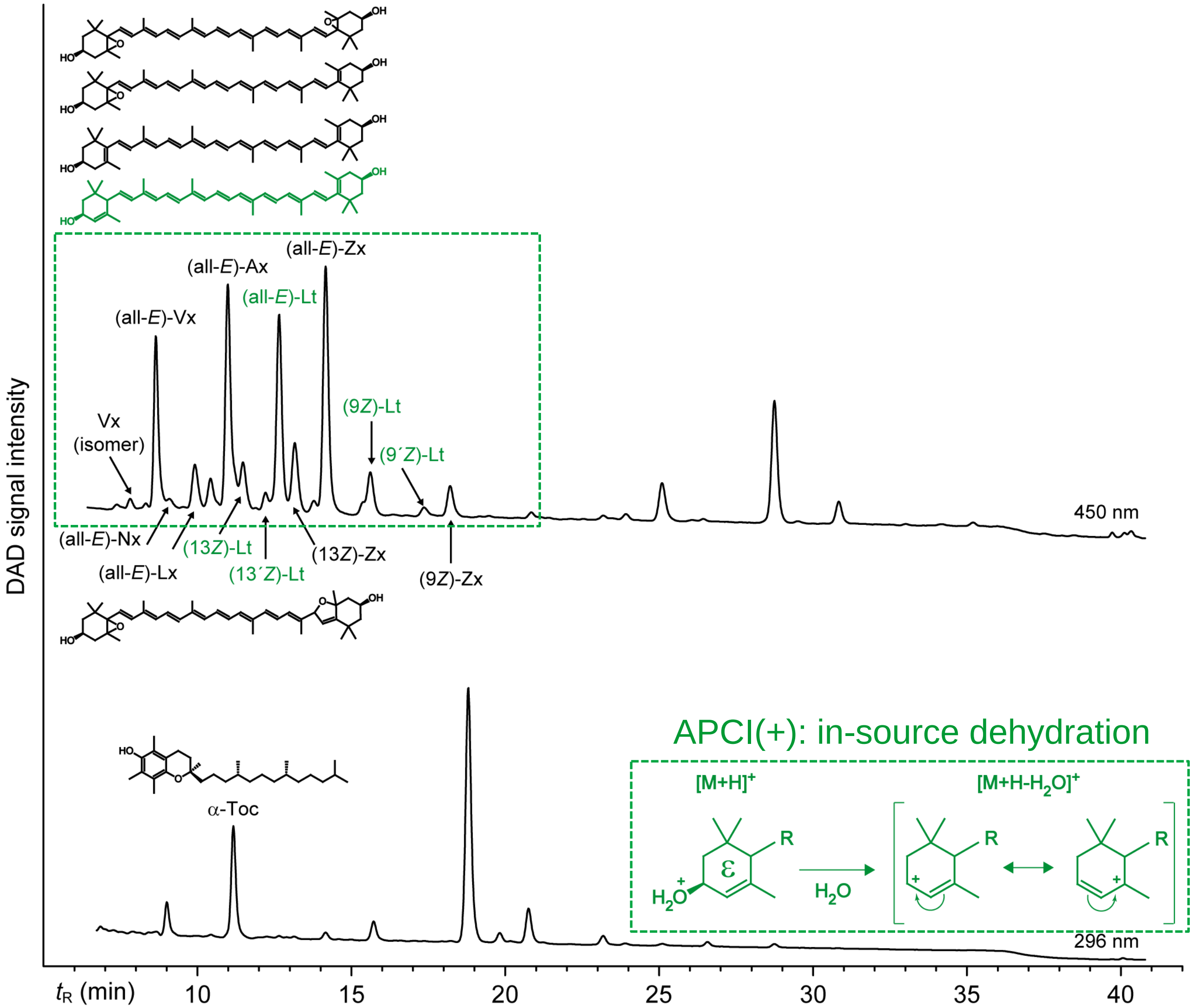
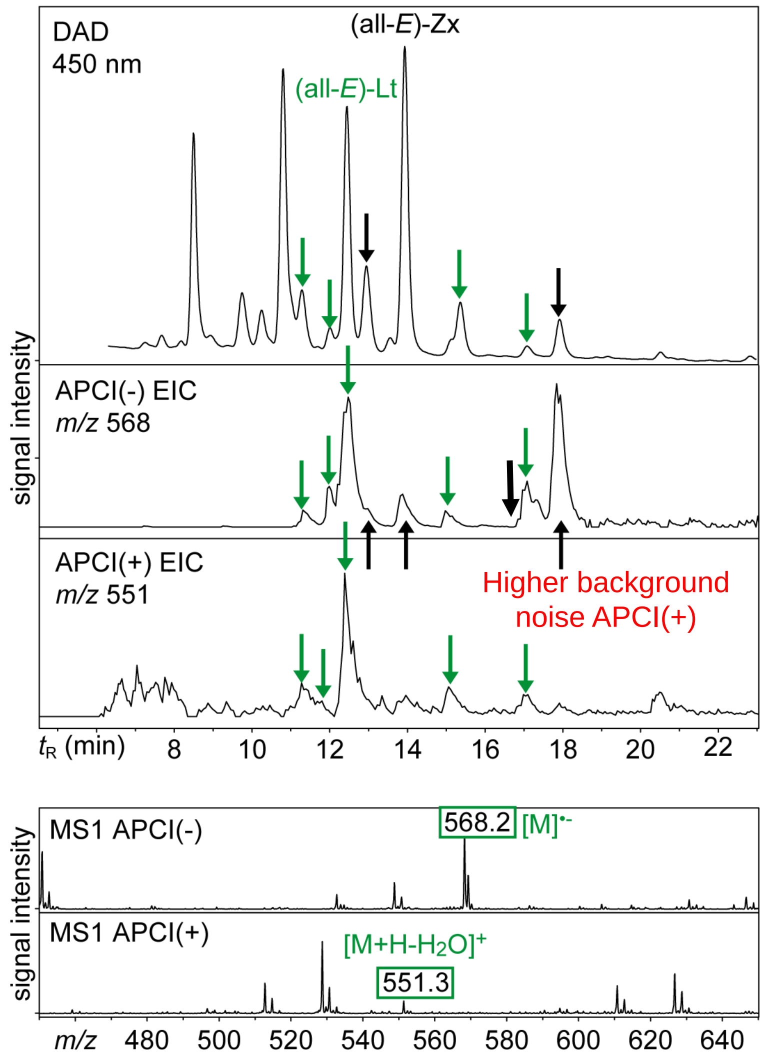
HPLC-DAD-APCI-MS^n analysis

Xanthophylls: *Acrocomia aculeata*

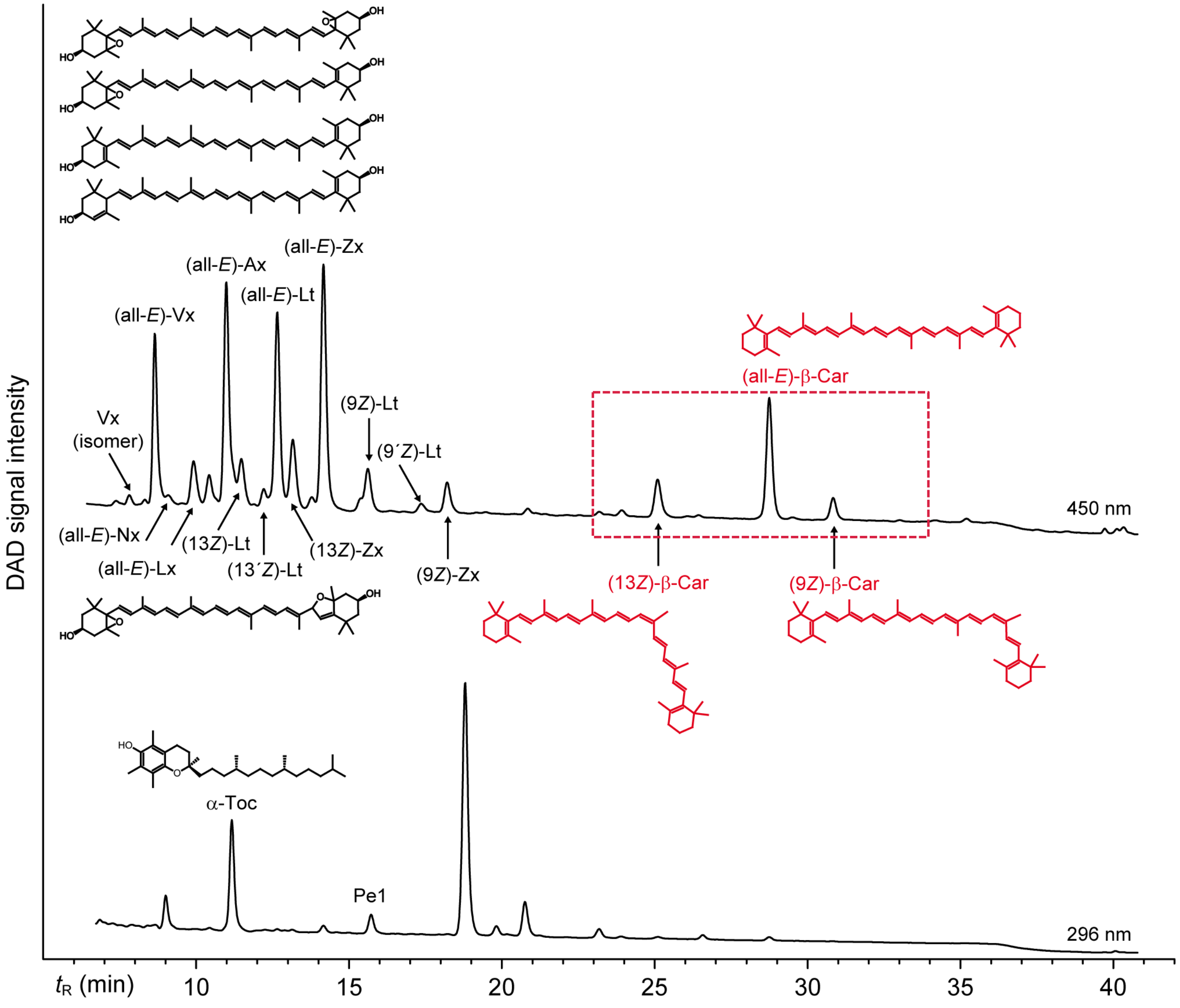




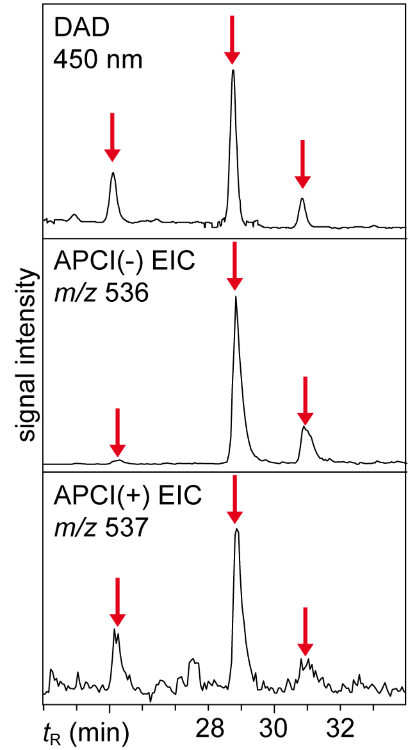
Schex *et al.*, *Food Res. Int.* DOI 10.1016/j.foodres.2017.11.041

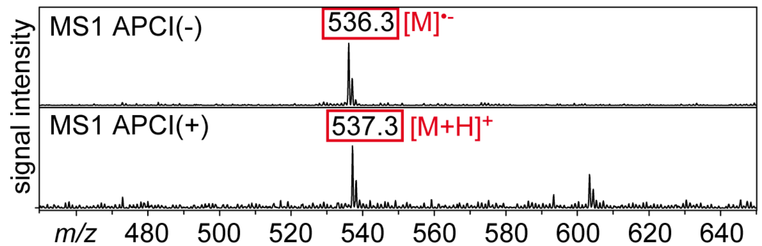
HPLC-DAD-APCI-MS^n analysis

Carotenes: *Acrocomia aculeata*



Higher background noise APCI(+)





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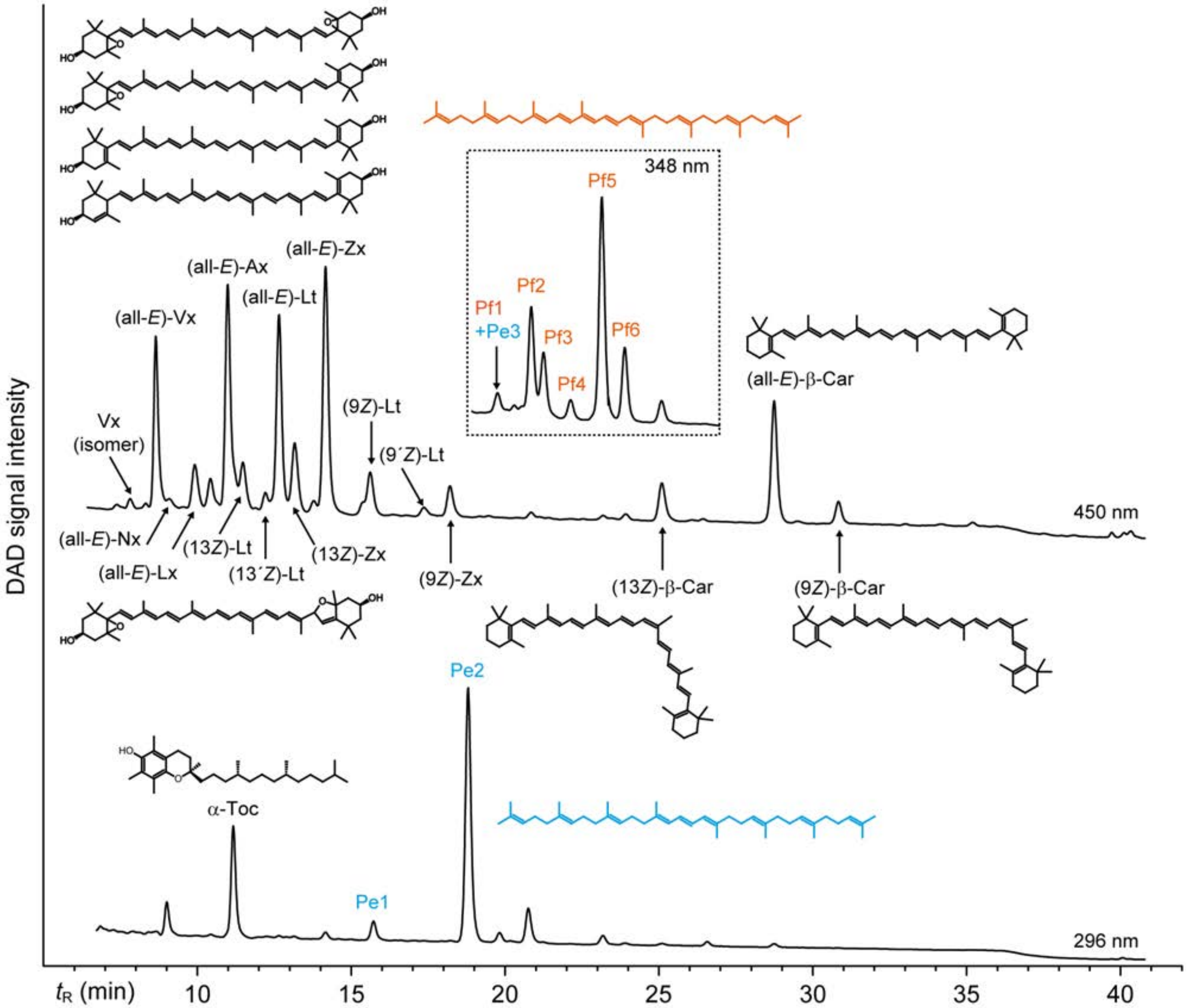
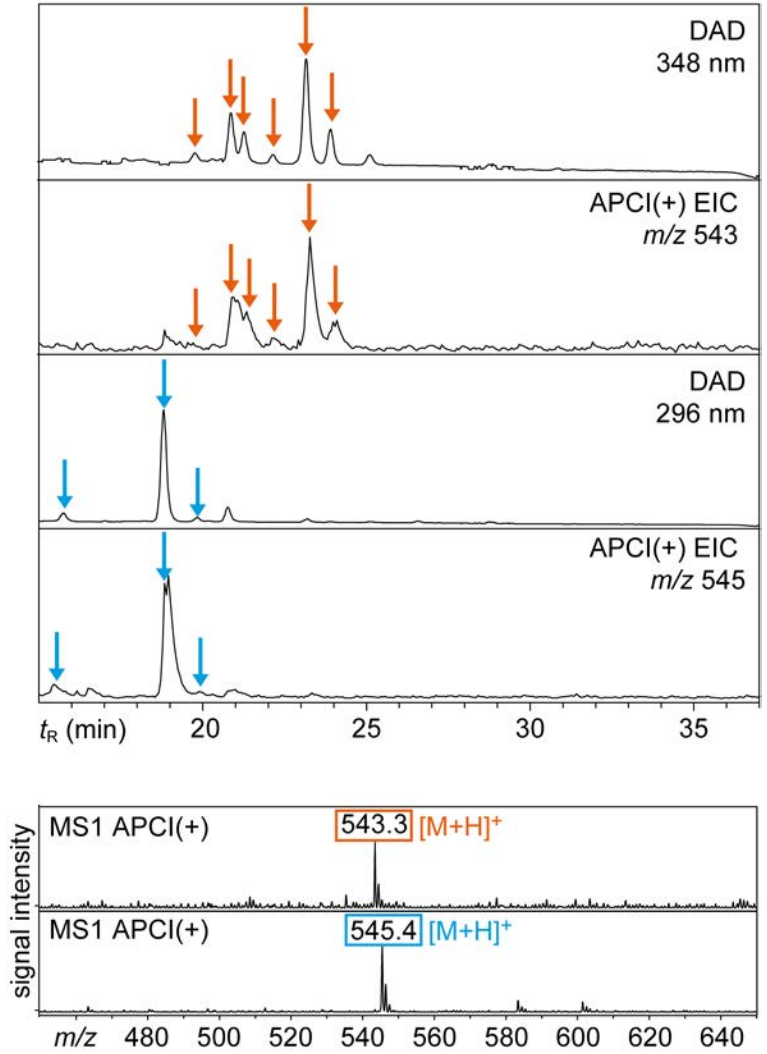
HPLC-DAD-APCI-MS^n analysis

Carotenes: *Acrocomia aculeata*





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HPLC-DAD-APCI/ESI-MS^n analysis

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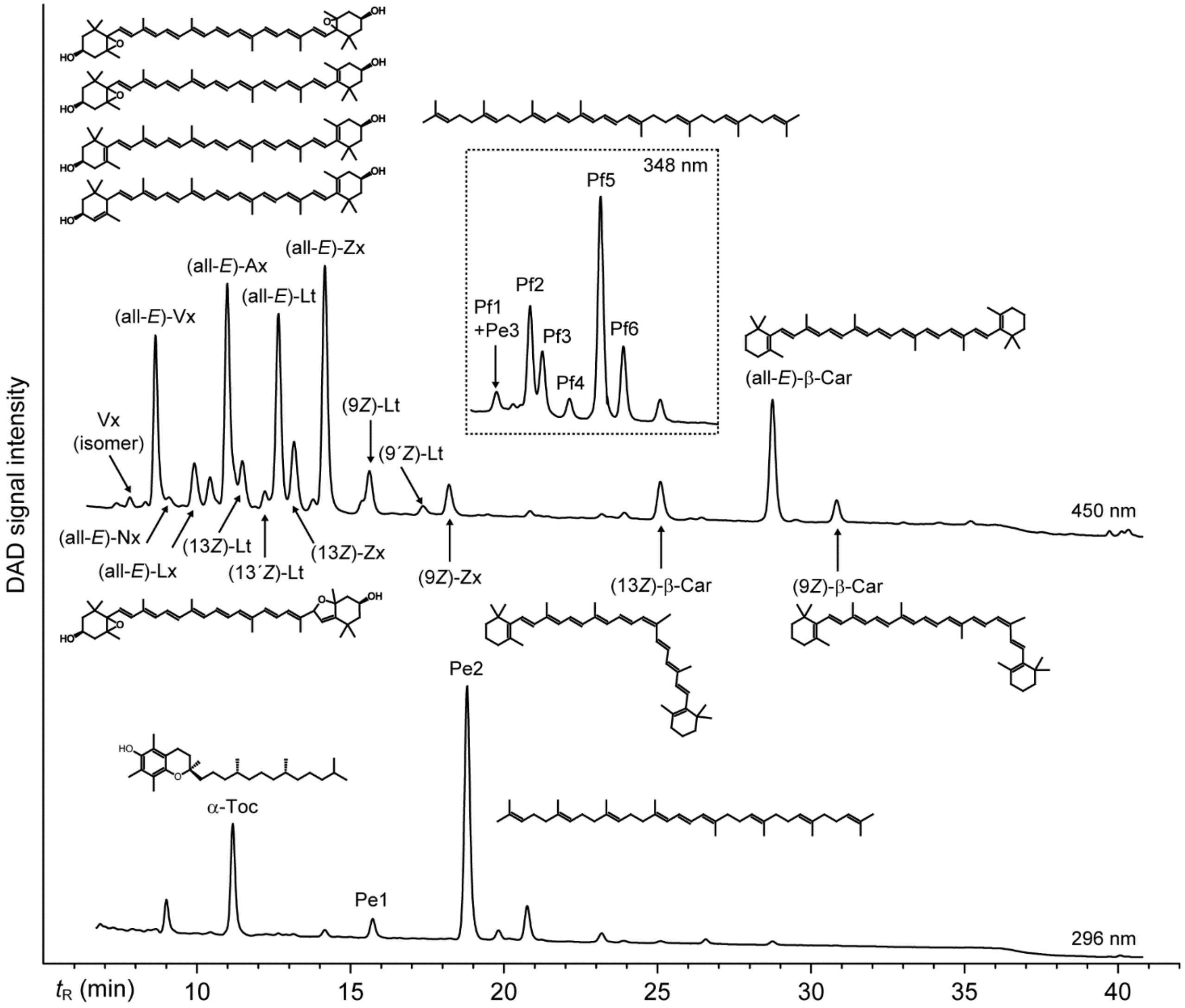
Acrocomia aculeata: α-tocopherol and 25 carotenoids



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HPLC-DAD-FLD: selective detection

DAD: carotenoids (vis absorption)

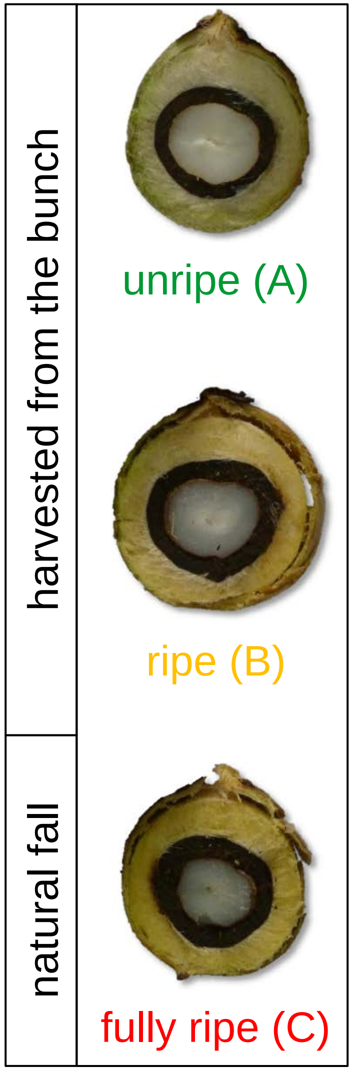
FLD: tocochromanols (fluorescence)

→ No interference

→ Simultaneous quantitation

HPLC-DAD profiling

Carotenoid profiles: *Acrocomia aculeata* pulp (µg/100 g fresh weight)



Mesocarp	Maturity A	Maturity B	Maturity C
Xanthophylls			
Violaxanthin isomer	15 ± 16a	10 ± 5a	14 ± 3a
(all-*E*)-Violaxanthin	23 ± 15b	100 ± 91ab	168 ± 46a
(all-*E*)-Neoxanthin	18 ± 11a	22 ± 10a	25 ± 6a
(all-*E*)-Luteoxanthin	1 ± 2b	5 ± 6ab	11 ± 6a
(all-*E*)-Antheraxanthin	7 ± 5b	24 ± 13b	109 ± 50a
(13*Z*)-Lutein	11 ± 3b	19 ± 9ab	26 ± 4a
(all-*E*)-Lutein	120 ± 95a	81 ± 51a	62 ± 23a
(13*Z*)-Zeaxanthin	tr.	1 ± 1b	10 ± 6a
(all-*E*)-Zeaxanthin	5 ± 2b	14 ± 5a	57 ± 29a
(9*Z*)-Lutein	1 ± 1b	tr.	12 ± 6a
Carotenes			
(13*Z*)-β-Carotene	tr.	tr.	4 ± 5
(all-*E*)-β-Carotene	7 ± 4b	12 ± 7b	41 ± 18a
(9*Z*)-β-Carotene	0 ± 1a	n.d.	2 ± 3a
Precursors			
Phytofluene 2	tr.	22 ± 18b	60 ± 6a
Phytofluene 5	n.d.	4 ± 8b	23 ± 3a
Phytoene 1	n.d.	5 ± 9a	20 ± 15a
Phytoene 2	1 ± 2c	71 ± 59b	228 ± 25a

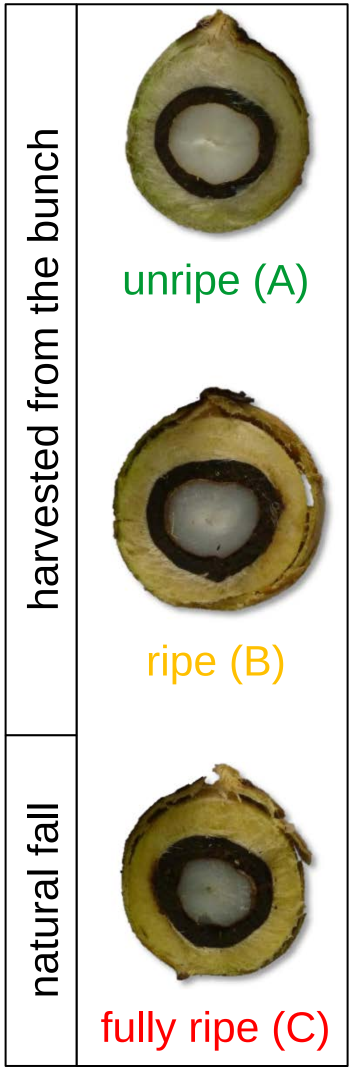
Maturation:

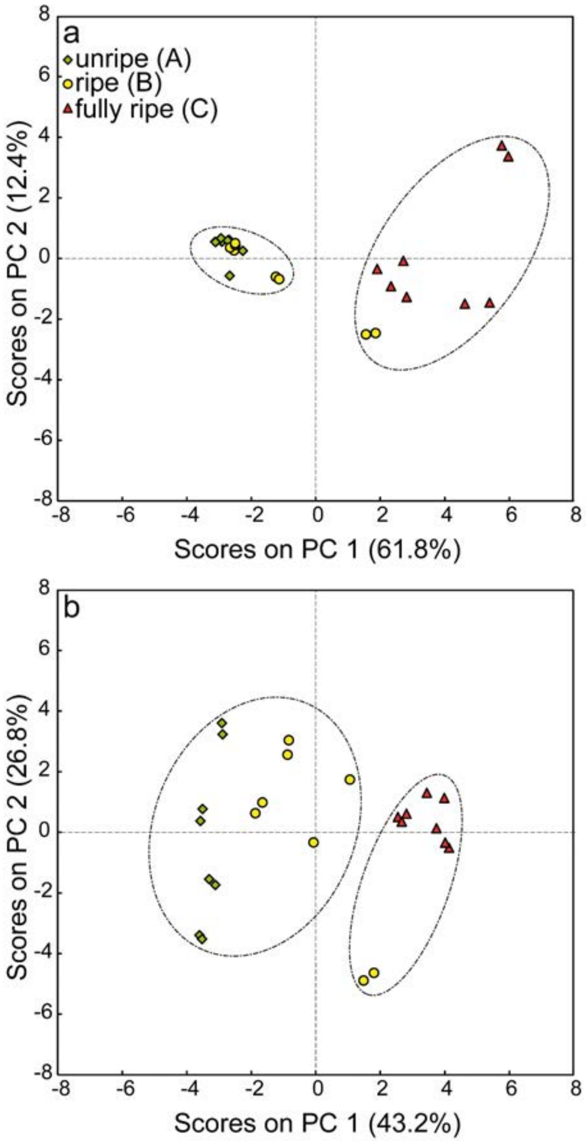
- Xanthophyll cycle pigments (Vx, Ax, Zx)
- β-Carotene
- Precursors (Pf, Pe)

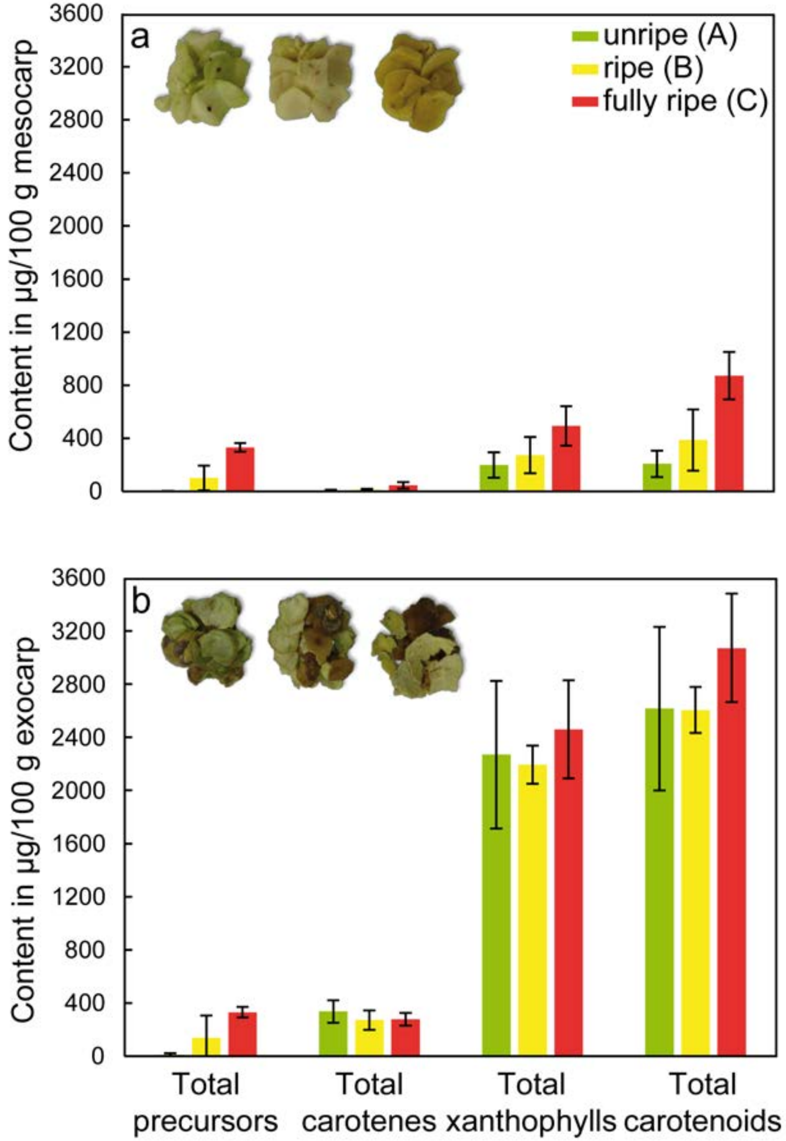
Schex *et al.*, *Food Res. Int.* DOI 10.1016/j.foodres.2017.11.041

HPLC-DAD profiling

Carotenoid profiles: *Acrocomia aculeata* maturity stages (peel, pulp)



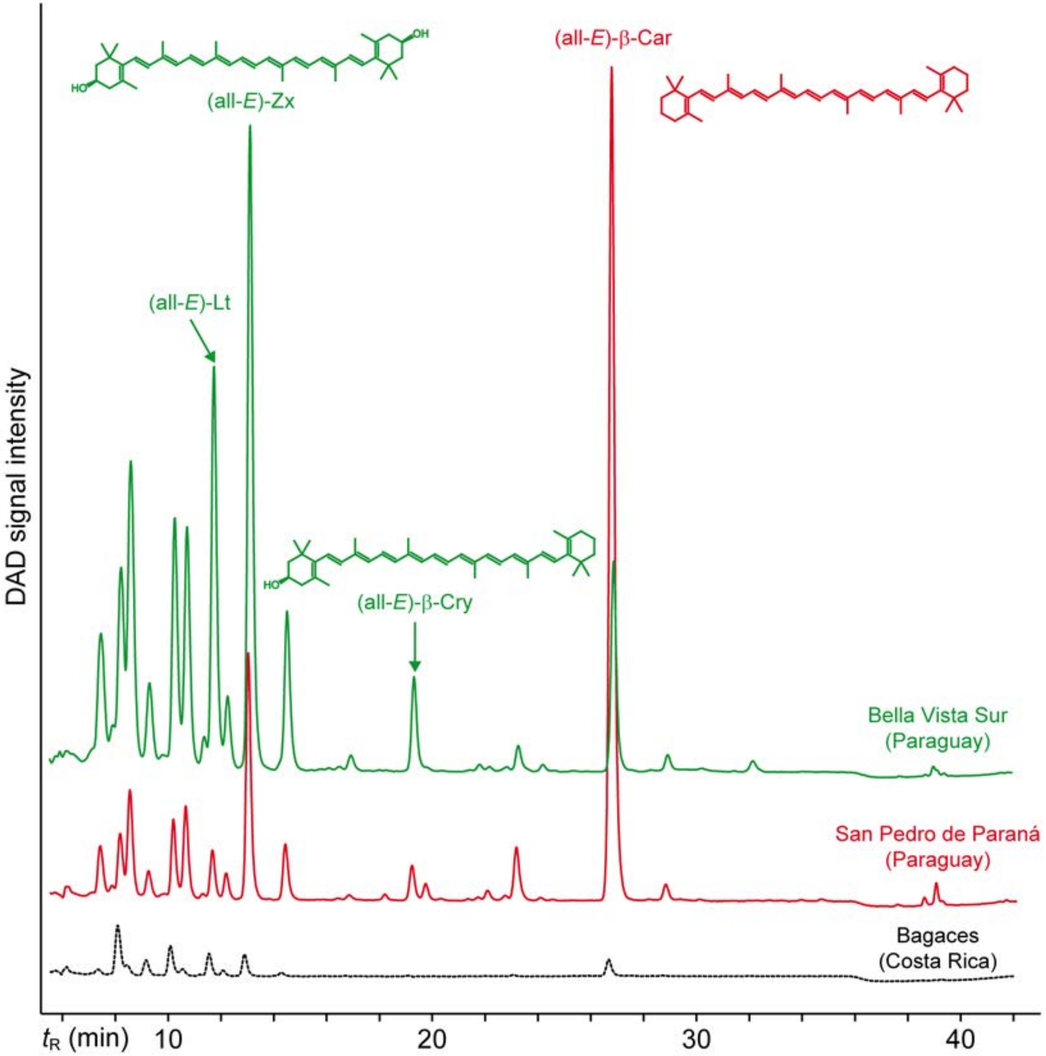




Schex *et al.*, *Food Res. Int.* DOI 10.1016/j.foodres.2017.11.041

HPLC-DAD profiling

Carotenoid profiles: *Acrocomia aculeata* from different provenances



- **Preliminary results**

✓ Different chemotypes
→ Xanthophyll type
→ β-Carotene type
→ Others?

✓ Broad natural diversity
→ Qualitative composition
→ Absolute concentrations

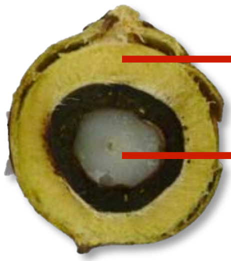
✓ Further studies needed

GC analysis

Identification criteria

- ✓ Mass spectrum
- ✓ Chromatographic behaviour (linear retention index, LRI)
- ✓ Reference standards

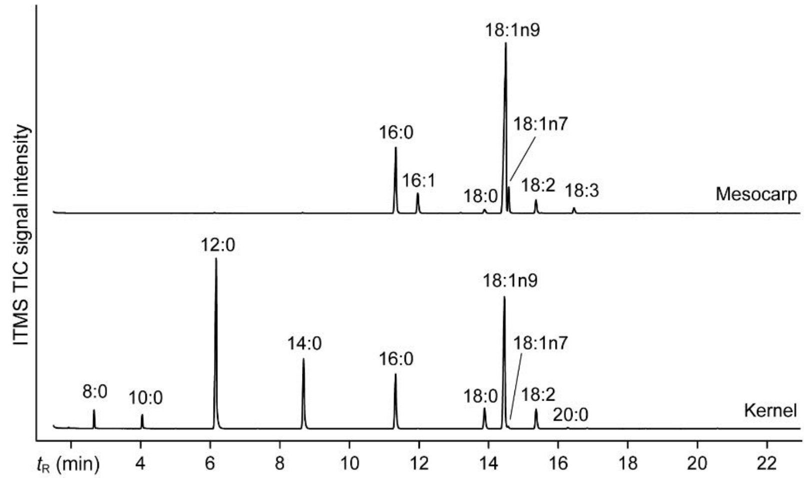
Gas chromatography



Soxhlet extraction

Total lipids (% DM, *w/w*)

Saponification & methylation

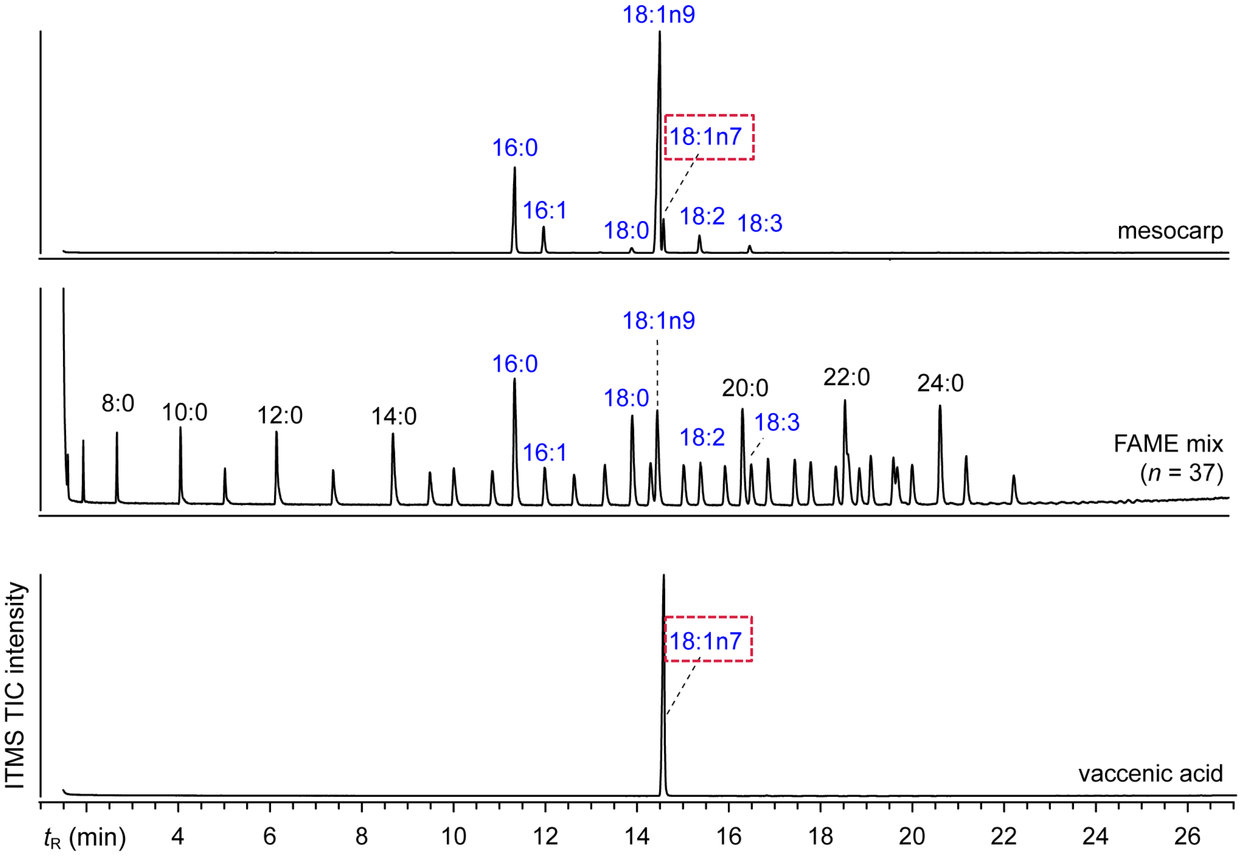


GC-ITMS/GC-FID analysis

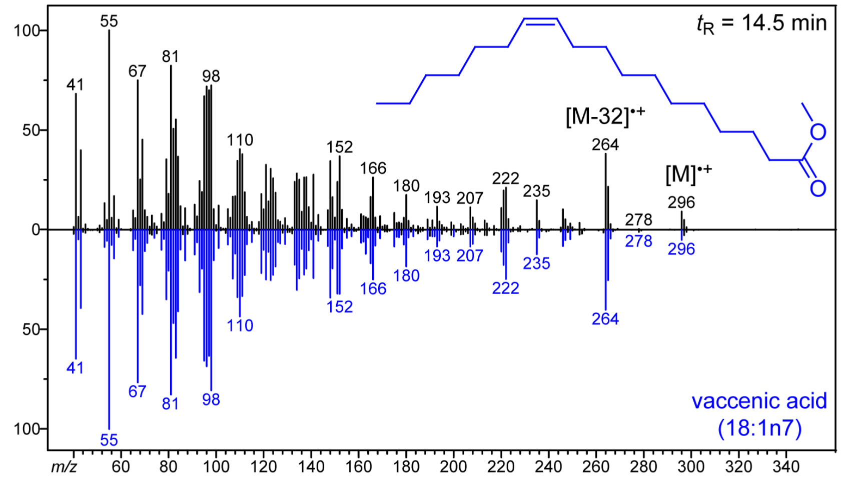
Fatty acid composition (area % of total peak area)

GC analysis

Identification: GC-ITMS



GC-ITMS TIC chromatograms: *Acrocomia* mesocarp oil, FAME mix, and vaccenic acid methyl ester (Column: SLB IL82. Total run time: 32 min)



GC-ITMS mass spectrum: FAME with t_R = 14.5 min *vs*. vaccenic acid methyl ester (in-house database)

GC-FID profiling

Fatty acid profiles: *Acrocomia aculeata* mesocarp oil

		Maturity A	Maturity B	Maturity C	Min	Max
Total lipids (% DM)		9.6 ± 5.7b	20.9 ± 3.4a	22.2 ± 5.2a	3.3	27.1
Total lipids (% FW)		2.6 ± 1.7b	8.3 ± 2.0a	10.3 ± 2.7a	0.7	13.2
Fatty acid composition (%)						
Palmitic acid	C16:0	16.9 ± 0.8a	18.1 ± 1.8a	18.7 ± 1.6a	16.3	20.1
Palmitoleic acid	C16:1n7	1.7 ± 0.6a	3.9 ± 1.7a	4.3 ± 1.5a	0.9	6.2
Stearic acid	C18:0	3.6 ± 0.6a	1.9 ± 0.4b	1.8 ± 0.5b	1.3	4.5
Oleic acid	C18:1n9	65.0 ± 5.8a	67.1 ± 4.4a	65.5 ± 3.7a	57.0	71.4
Vaccenic acid	C18:1n7	2.0 ± 0.5b	3.7 ± 0.7a	4.1 ± 0.7a	1.3	4.8
Linoleic acid	C18:2n6	5.9 ± 3.5a	3.0 ± 1.1a	3.1 ± 0.8a	1.9	10.3
α-Linolenic acid	C18:3n3	3.8 ± 2.6a	1.5 ± 0.3a	1.4 ± 0.3a	1.2	7.4
Others [a]		1.3 ± 0.6a	0.7 ± 0.1a	0.9 ± 0.3a	0.6	2.2
SFA (% of total fatty acids)		21.5 ± 1.1a	20.5 ± 1.4a	21.2 ± 1.0a	18.9	22.9
MUFA (% of total fatty acids)		68.9 ± 6.8a	75.0 ± 2.1a	74.2 ± 1.8a	59.4	77.3
PUFA (% of total fatty acids)		9.6 ± 6.0a	4.5 ± 1.3a	4.5 ± 1.1a	3.1	17.7
SFA/UFA		0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	0.2	0.3

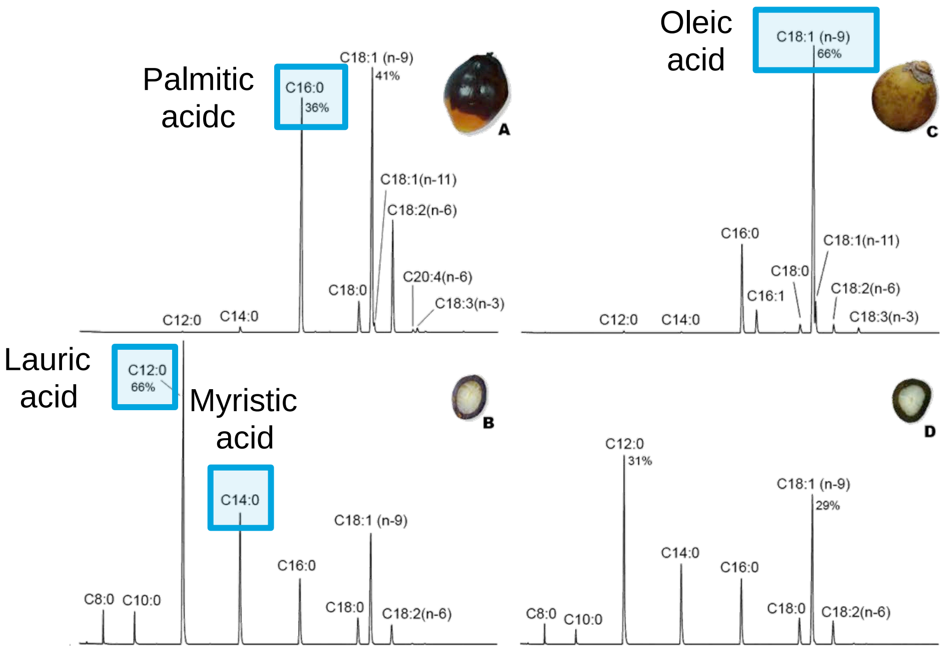
GC-FID profiling

Fatty acid profiles: *Acrocomia aculeata* kernel fat

		Maturity A	Maturity B	Maturity C	Min	Max
Total lipids (% DM)		55.5 ± 1.9a	53.4 ± 2.2a	53.5 ± 2.6a	50.3	57.4
Total lipids (% FW)		44.6 ± 0.7a	43.3 ± 2.3a	44.1 ± 2.6a	40.5	47.1
Fatty acid composition (%)						
Caprylic acid	C8:0	5.2 ± 0.5a	5.0 ± 0.3a	4.9 ± 0.4a	4.4	5.6
Capric acid	C10:0	3.3 ± 0.2a	3.2 ± 0.1a	3.1 ± 0.2a	2.9	3.5
Lauric acid	C12:0	43.3 ± 1.1a	42.5 ± 0.7ab	41.5 ± 0.6b	40.8	44.5
Myristic acid	C14:0	13.4 ± 0.3a	13.5 ± 0.4a	13.4 ± 0.5a	12.9	14.1
Palmitic acid	C16:0	8.8 ± 0.5a	9.0 ± 0.2a	9.2 ± 0.4a	8.2	9.7
Stearic acid	C18:0	3.2 ± 0.2a	3.2 ± 0.4a	3.1 ± 0.3a	2.7	3.6
Oleic acid	C18:1n9	18.6 ± 1.1a	19.5 ± 1.7a	20.5 ± 0.8a	17.5	21.8
Linoleic acid	C18:2n6	3.0 ± 0.2a	3.0 ± 0.2a	3.2 ± 0.1a	2.7	3.3
Others [a]		1.2 ± 0.0a	1.1 ± 0.1a	1.1 ± 0.1a	1.1	1.2
SFA (% of total fatty acids)		78.0 ± 1.2a	77.0 ± 1.8a	75.9 ± 0.8a	74.6	78.9
MUFA (% of total fatty acids)		19.0 ± 1.2a	19.9 ± 1.7a	21.0 ± 0.8a	17.9	22.2
PUFA (% of total fatty acids)		3.0 ± 0.2a	3.1 ± 0.2a	3.2 ± 0.1a	2.8	3.3

GC-FID profiling

Fatty acid profiles: *Acrocomia aculeata vs. Elaeis* sp.



GC-ITMS TIC chromatograms of FAMES from *E. guineensis* (left) and *A. aculeata* (right) mesocarp oil and kernel fat, respectively.

Mesocarp	*A. aculeata*[1]	*E. guineensis*[2]	*E. oleifera*[2]	*Eo × Eg* hybrids[2]
SFA [%]	15.7–31.0	54.2–54.6	22.3–31.5	42.4–49.1
MUFA [%]	55.1–77.0	34.4–35.4	47.5–66.1	39.8–44.5
PUFA [%]	4.2–14.0	10.4–11.0	5.2–22.5	11.1–13.1

Kernel	*A. aculeata*[1]	*E. guineensis*[2]	*E. oleifera*[2]	*Eo × Eg* hybrids[2]
SFA [%]	66.8–76.7	84.9–86.0	68.5–79.1	75.9–78.2
MUFA [%]	20.2–30.0	11.7–13.0	16.0–26.0	17.5–19.3
PUFA [%]	2.9–4.2	2.2–2.3	4.1–5.0	4.4–4.7

[1]Samples from Costa Rica (GIE) and Paraguay: unpublished results
[2]Samples from Costa Rica (ASD): Lieb *et al.*, *J. Agric. Food Chem.* 2017 65(18) 3617

⇨ ***Acrocomia*: a promising substitute for *Elaeis* palms**

Forthcomings

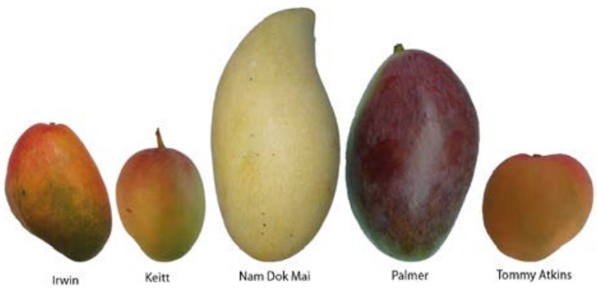
Alternative lipid sources

- **Macáuba/Coyol (*Acrocomia aculeata*)**

✓ Triglyceride and melting profiles
✓ Technology: fractionation (olein/stearin)

- **Mango (*Mangifera indica* L.) kernel fat**

✓ Fatty acid and triglyceride profiles
✓ Technological properties

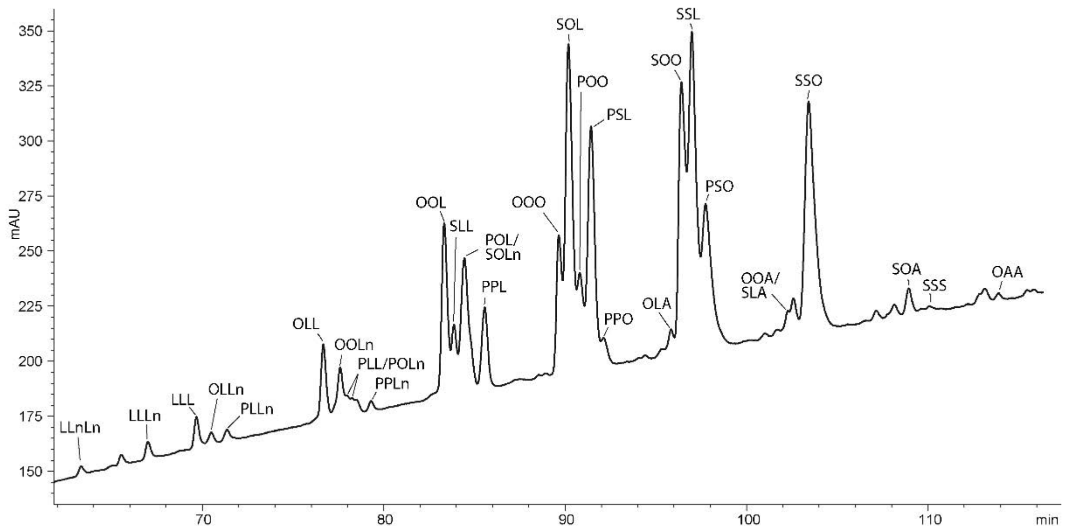


HPLC-DAD-ESI-MS^n

Elutents: Acetonitrile (1), 2-Propanol (2)

Columns: C_{18} (two 250 × 4.6 mm, 5 µm particle size)

Run time: 120 min (32 TAGs assigned)



Summary & conclusion

- **Optimized HPLC-DAD-FLD method**

✓ Simultaneous extraction and analysis: **>25 lipophilic antioxidants**
✓ C30: resolution tocochromanols/geometrical carotenoid isomers within 45 min
→ *A. aculeata*: α-tocopherol/complex carotenoid profile

- **Optimized GC method**

✓ SLB IL82: chromatographic resolution **>38 FAMEs** (C4 to C24) within 32 min
→ High-throughput screening of fatty acids

- **Conclusion: *Acrocomia aculeata***

✓ Interesting nutritional source of **provitamin A and vitamin E**
✓ Carotenoid fractions: **valuable by-products** (E 160a)
✓ Mesocarp/kernel **fatty acids** resemble those of *Elaeis* sp.
→ *Acrocomia aculeata*: **promising, sustainable oil crop**

- **Future studies**

✓ Lipid profiling: Macáuba/Coyol from different provenances
→ **Natural diversity** of *Acrocomia*

Thank you for your attention!

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UNIVERSIDAD DE COSTA RICA

ADALBERT RAPS STIFTUNG

Research project
(VML and CBS)

Green Integrated Energies

Alexander von Humboldt Stiftung/Foundation

Research group
linkage program

Hanns Seidel Stiftung

Travel grant
(RS)

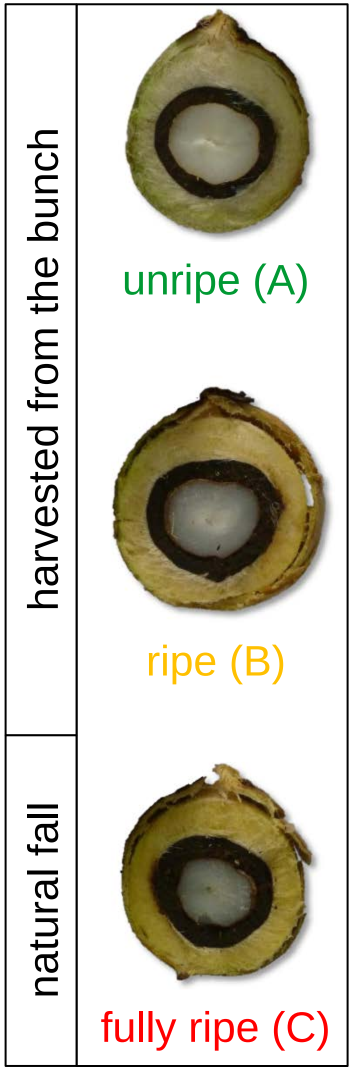
DAAD

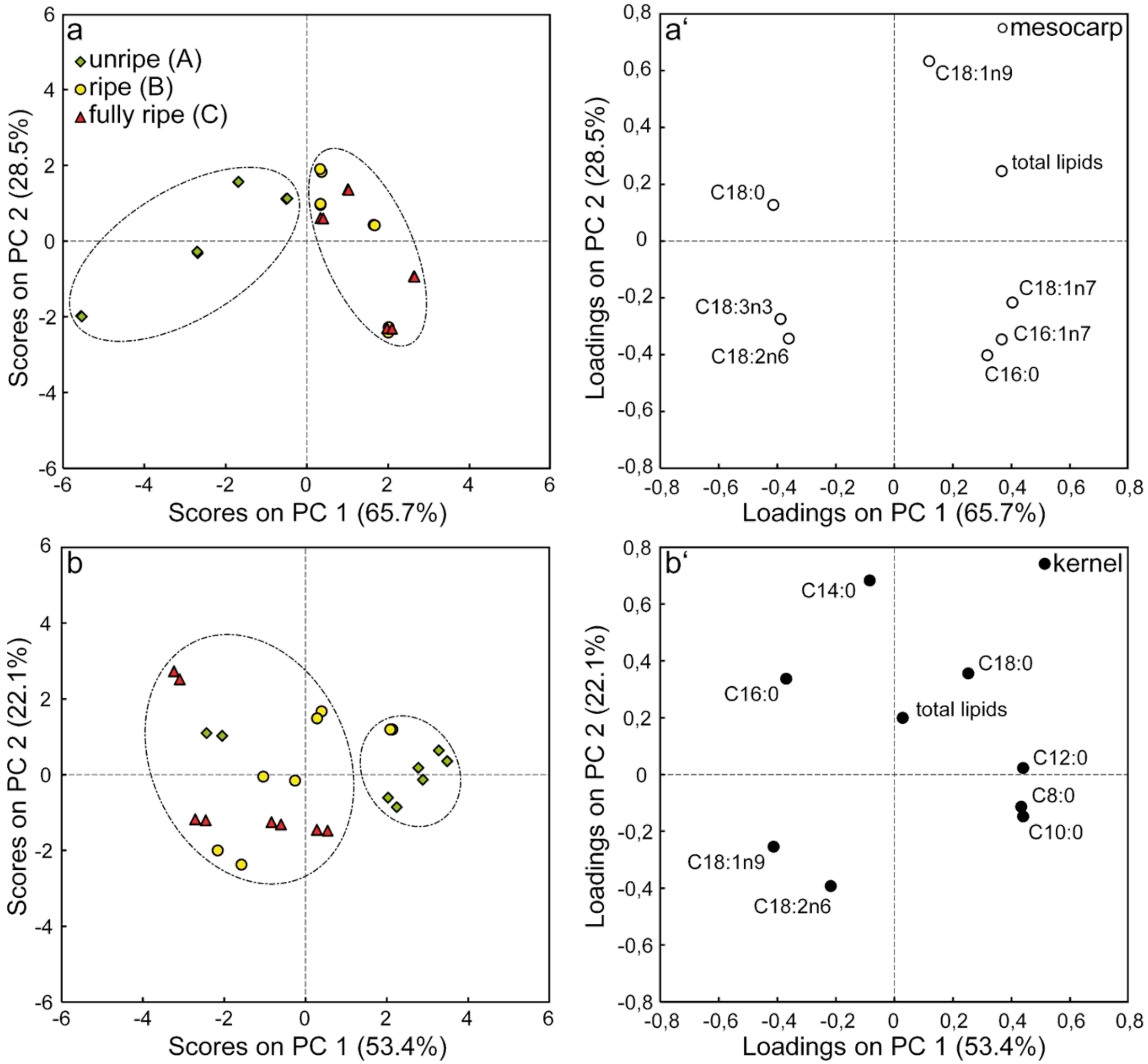
PROMOS scholarship
(VML)

Herzog Carl scholarship
(MK)

GC-FID profiling

Fatty acid profiles: *Acrocomia aculeata* maturity stages





Overview

1. Introduction
→ African oil palm: nutritional value and current situation
→ Substitutes: American oil palm, interspecific *Elaeis* hybrids, Macáuba palm

2. Identification: HPLC-DAD-APCI/ESI-MS^n
→ MS^n experiments
→ Isomerisation of reference standards

3. Quantitation: HPLC-DAD/FLD
→ Carotenoid and tocochromanol profiles

4. Summary and conclusions

Elaeis species

African oil palm (*E. guineensis* Jaqc.)

- Origin: subtropical and tropical Africa (Guinean Coast)
- Introduced to Malaysia and Indonesia as an ornamental plant in 1884
- Leading source for vegetable oil
- Production: industrial scale

E. guineensis

American oil palm (*E. oleifera* [Kunth] Cortés)

- Origin: tropical Central and South America
- Production: small to medium sized plantations

Hybrids (*E. guineensis* × *E. oleifera*)

E. oleifera ×
E. guineensis

E. oleifera

⇨ **Diversification of monocultures**

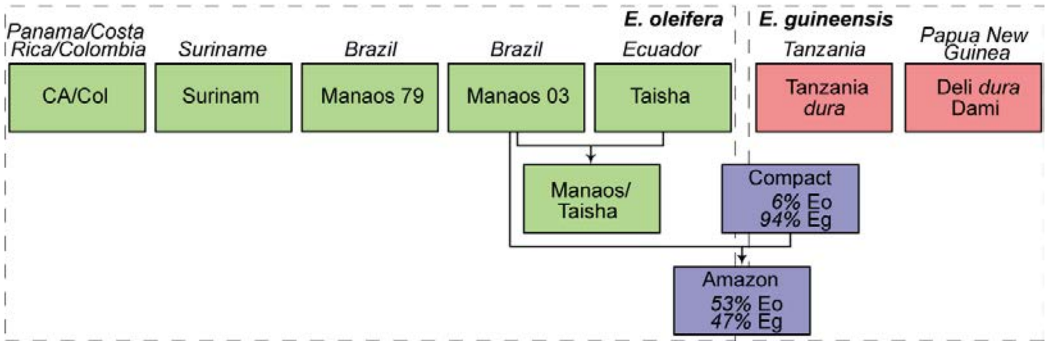
Aims & scopes

- Simultaneous extraction and analysis of tocochromanols and carotenoids
- Compound identification by HPLC-DAD-APCI/ESI-MS^n in lipid-rich matrices

Samples

Elaeis sp. (ASD Costa Rica)

- American oil palm (*E. oleifera*)
- African oil palm (*E.* guineensis)
- Interspecific hybrids



Acrocomia sp. (wild-growing plants)

- Costa Rica: Green Integrated Energies
- Paraguay: Dr. Hilger (University of Hohenheim)

Fig.Lieb et al., J. Agric. Food Chem. 2017 65(18) 3617.

Elaeis samples

CA/Col 03 (Costa Rica/Panama/Colombia) *E. oleifera*

Manaos/Taisha 12 (Brazil/Ecuador) *E. oleifera*

Taisha 04 (Ecuador) *E. oleifera*

Deli Dami 08 (Papua New Guinea) *E. guineensis*

Surinam 79 (Suriname) *E. oleifera*

Tanzania 06 (Tanzania) *E. guineensis*

Manaos 03 (Brazil) *E. oleifera*

Compact 97 back-cross of hybrid

Manaos 79 (Brazil) *E. oleifera*

Amazon 12 hybrid

Photographs and Figure taken
from MSc-thesis Kerfers, 2016

HPLC-DAD-APCI/ESI-MS^n analysis

Method development: *Elaeis* sp.

Isolation: tocochromanols & carotenes

1. Freeze-dried mesocarp*

⇩ + $CaCO_3$

2. Extraction: MeOH/EAc/LP (3×), LP (2×)

⇩

3. Saponification: 4 mL 10% KOH (in MeOH) + 40 mg ascorbic acid

⇩ Stirring 3 h, room Temp.

4. Washing and LLE: hexane/EAc 85/15 *v/v*

⇩

5. Evaporation to dryness

⇩

6. Dissolution in MeOH/*t*BME 1/1 *v/v*

* Autoclaved mimicking industrial practice: 70 min, 140 °C
Inactivation of endogeneous lipases/microorganisms

HPLC analysis

	System 1: *Elaeis*
Column (C30)	250×4.6 mm i.d., 3 µm
Eluent A	91:5:4 MeOH/*t*BME/H_2O
Eluent B	8:90:2 MeOH/*t*BME/H_2O
Column Temp.	23 °C
Flow rate	0.42 mL/min
Injection volume	10 µL
Total run time	55 min

Recoveries

β-carotene 101%
α-tocopherol 95%

HPLC-DAD-APCI/ESI-MS^n analysis

HPLC-DAD-FLD: *Elaeis* sp.

HPLC-DAD-FLD analysis: tocochromanols and carotenes in the mesocarp of *Elaeis* sp. fruits

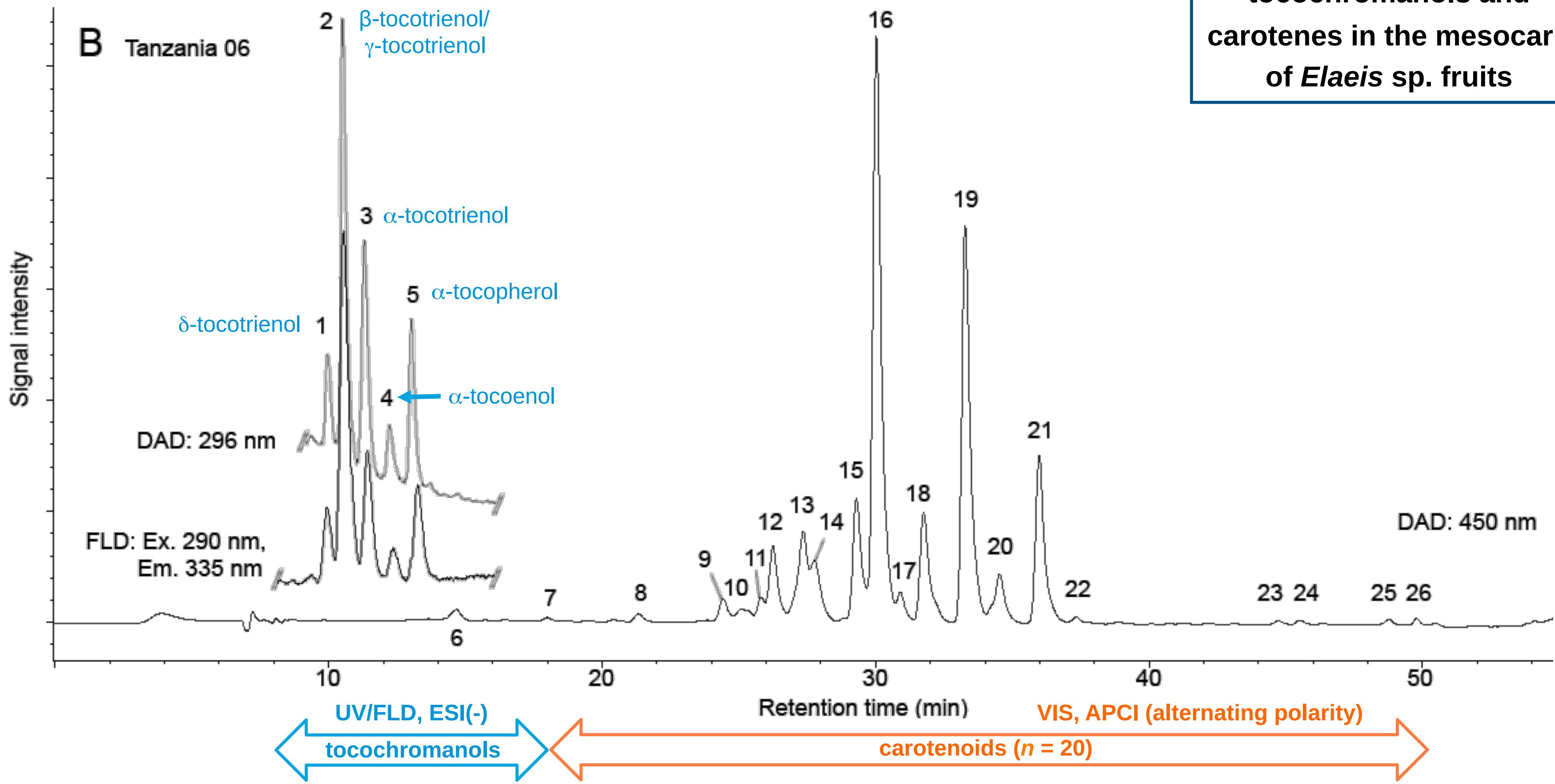
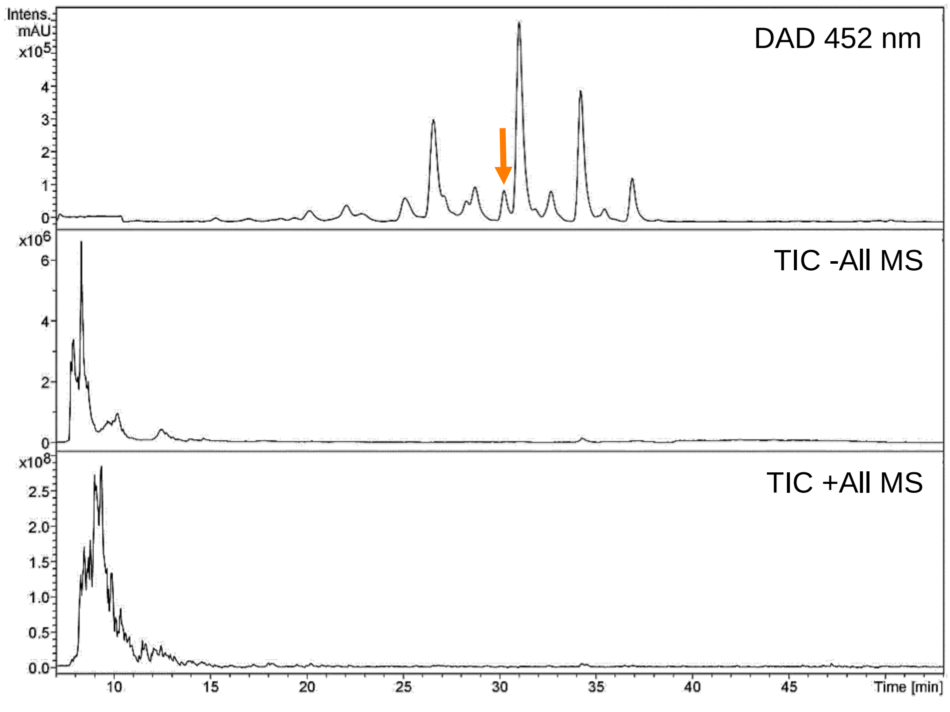


Figure taken from MSc-thesis Kerfers, 2016

HPLC-DAD-APCI-MS^n analysis

APCI-MS^n: MS1 experiments *Elaeis* sp.

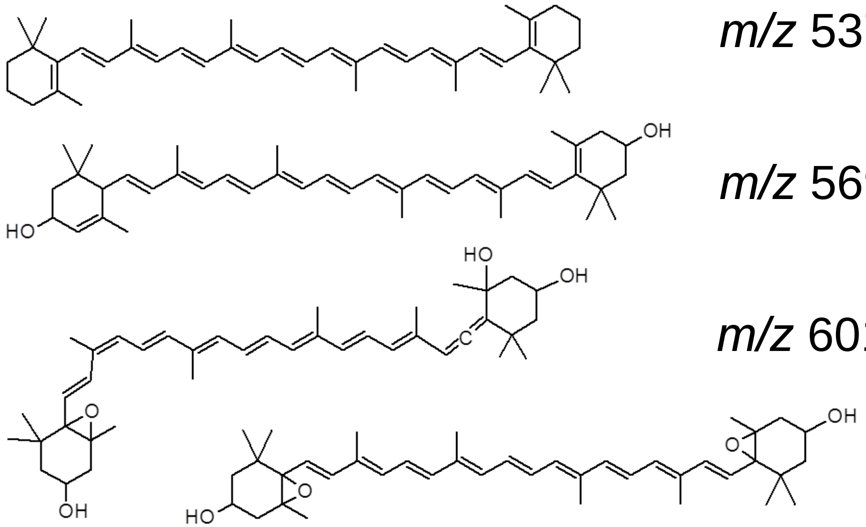
MS1 TIC oil palm (*Elaeis* sp.)



Example No. 15

- Compound assignment difficult
- EIC chromatograms of characteristic *m/z*
  → visualization of **related carotenoids**

Examples: m/z ratios



m/z 537

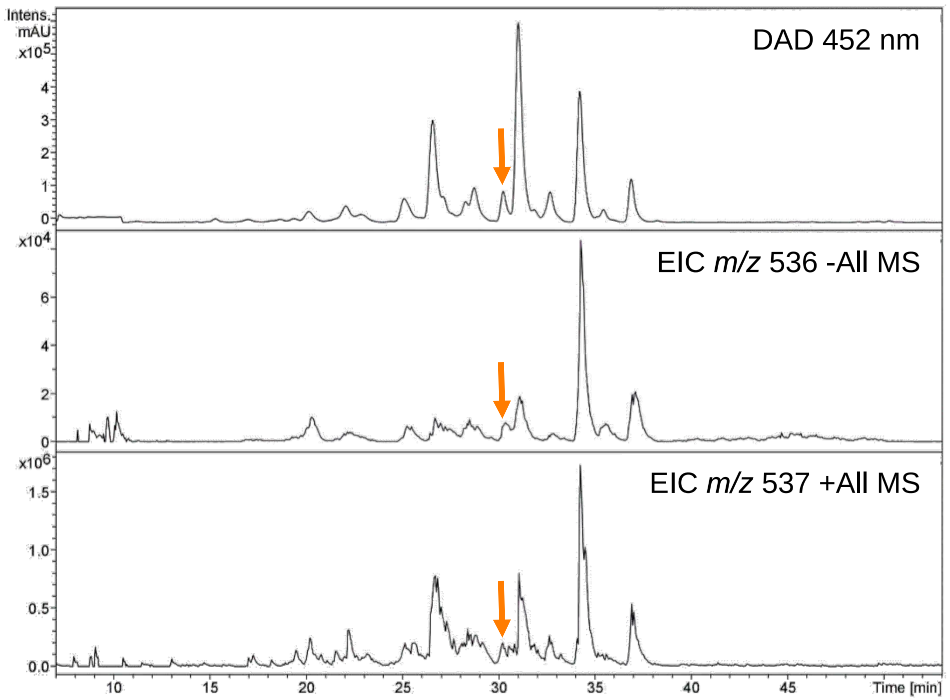
m/z 569 (551)

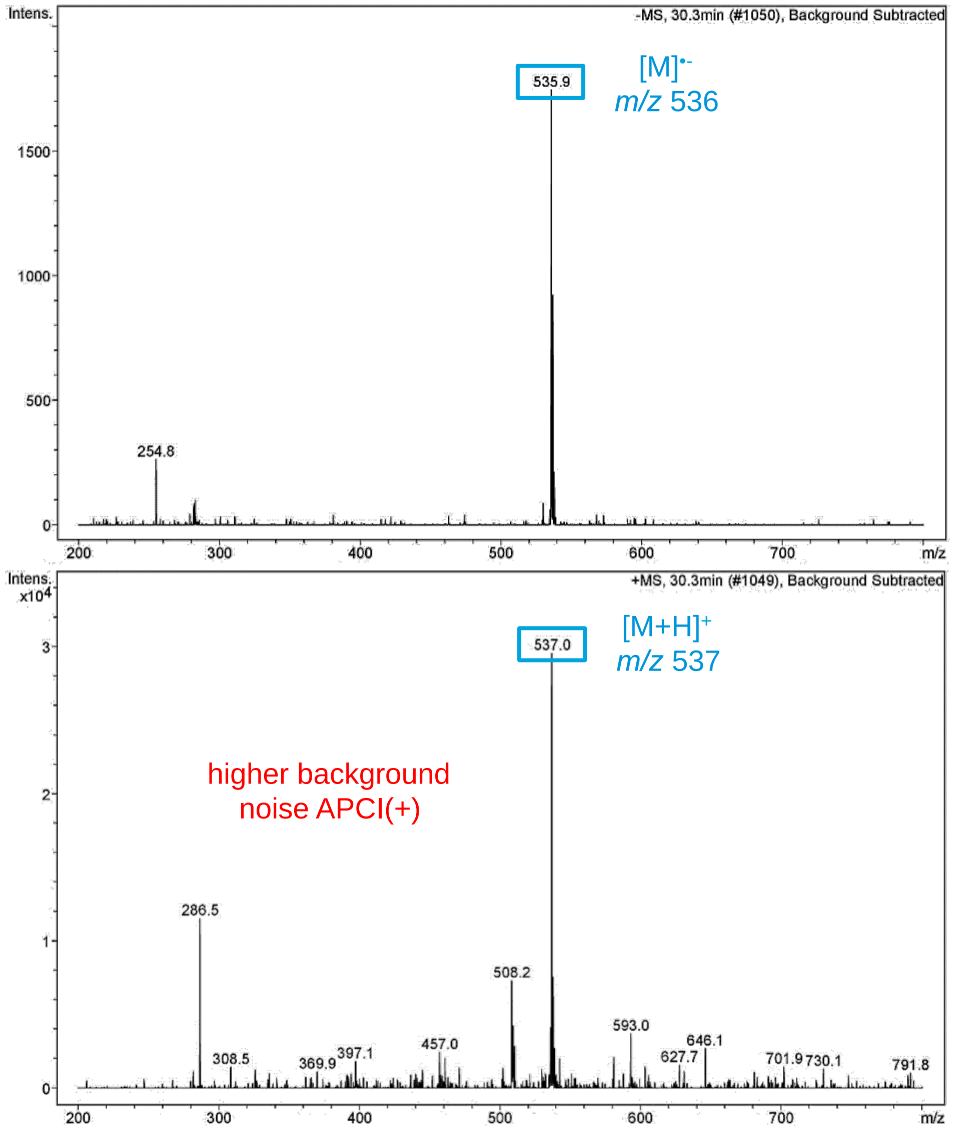
m/z 601

HPLC-DAD-APCI-MS^n analysis

APCI-MS^n: MS1 experiments *Elaeis* sp.

MS1 EIC oil palm (*Elaeis* sp.)



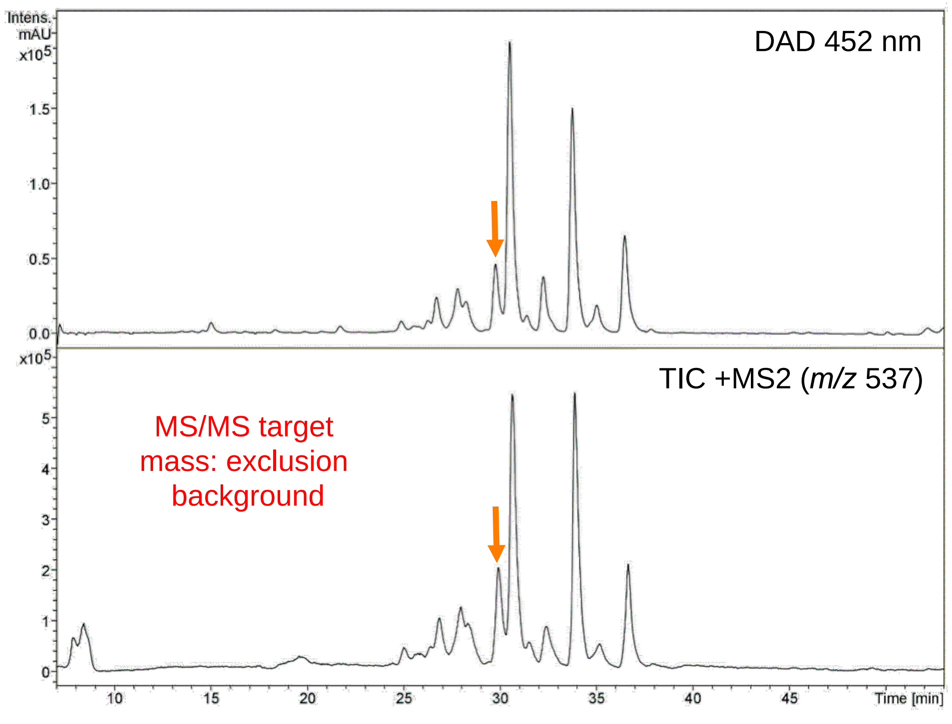


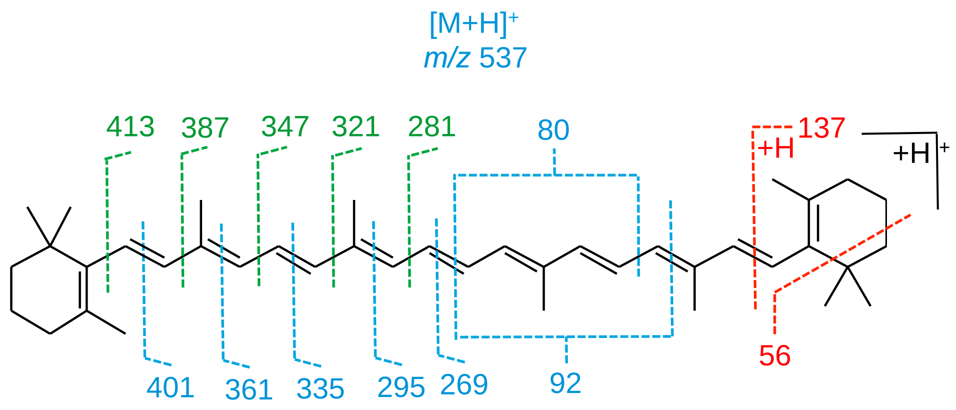
⇨ **Molecular mass**

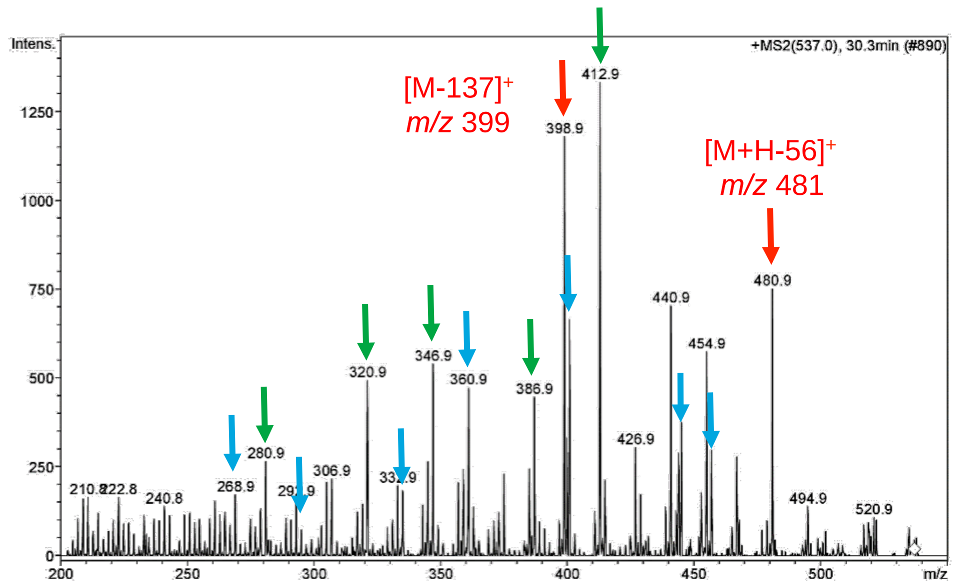
HPLC-DAD-APCI-MS^{n} analysis

APCI-MS^{n}: MS/MS fragmentations *Elaeis* sp.

MS/MS oil palm (*Elaeis* sp.)







APcI(+)-MS/MS experiments

⇨ **Indicative mass fragments**

HPLC-DAD-APCI-MS^n analysis

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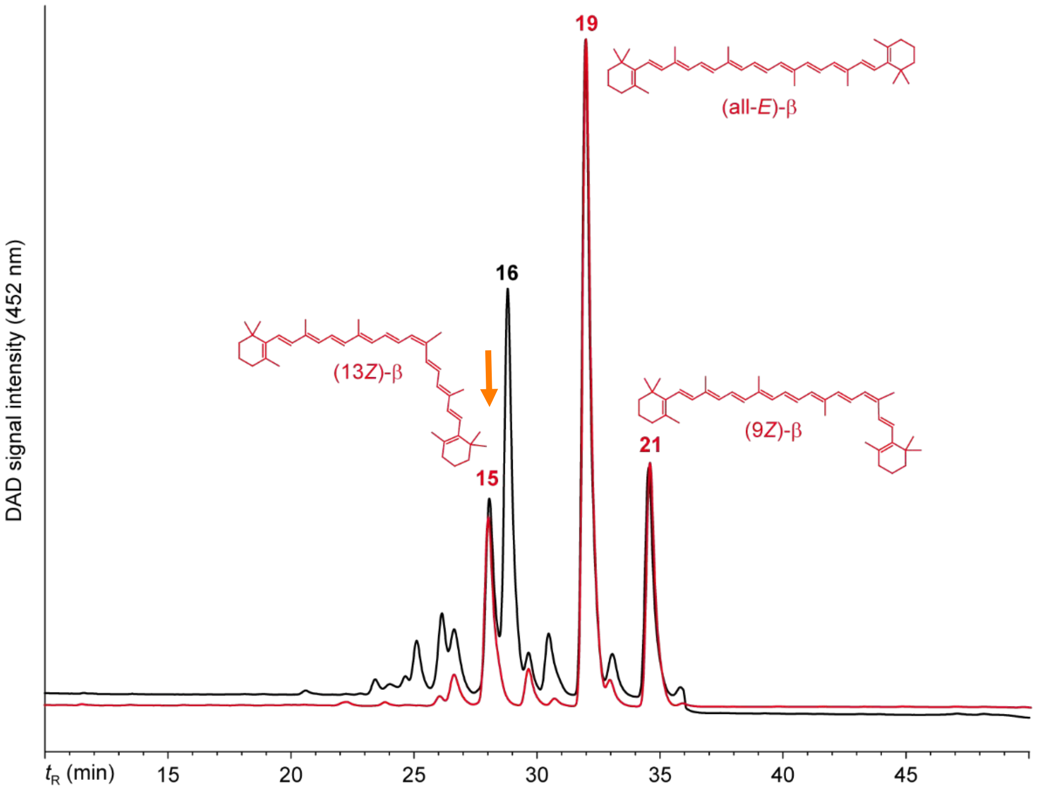
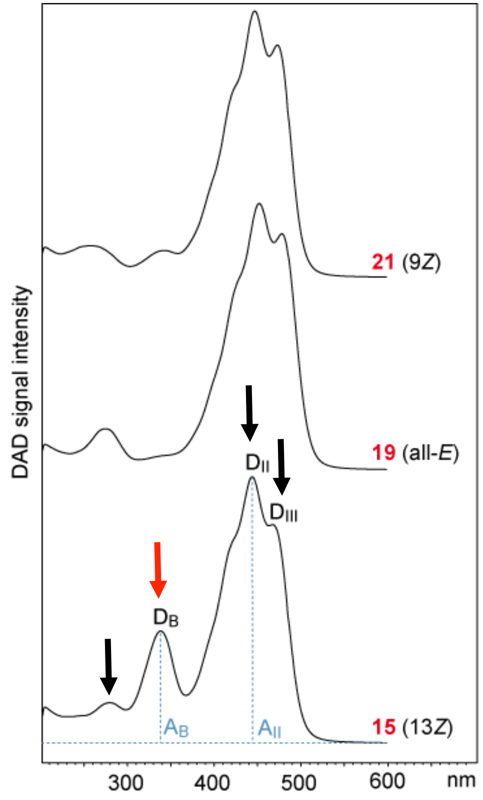
UNIVERSITÄT HOHENHEIM

Isomerisation of reference standards: *Elaeis* sp.





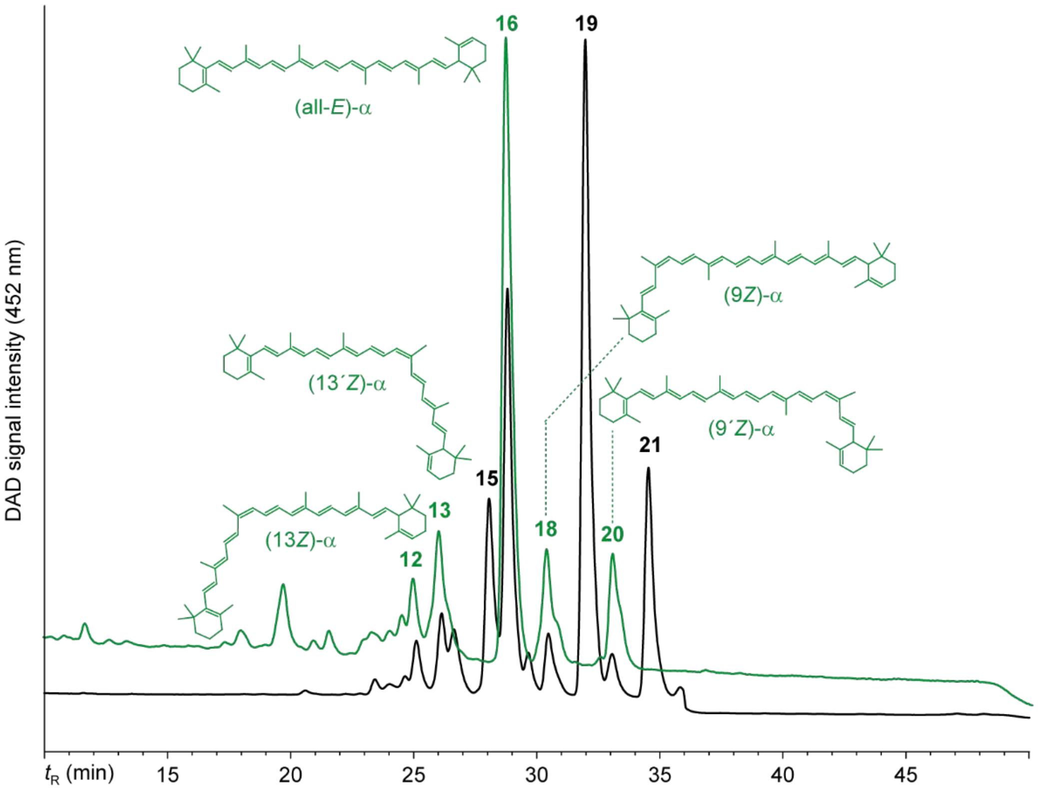
UV/Vis spectra

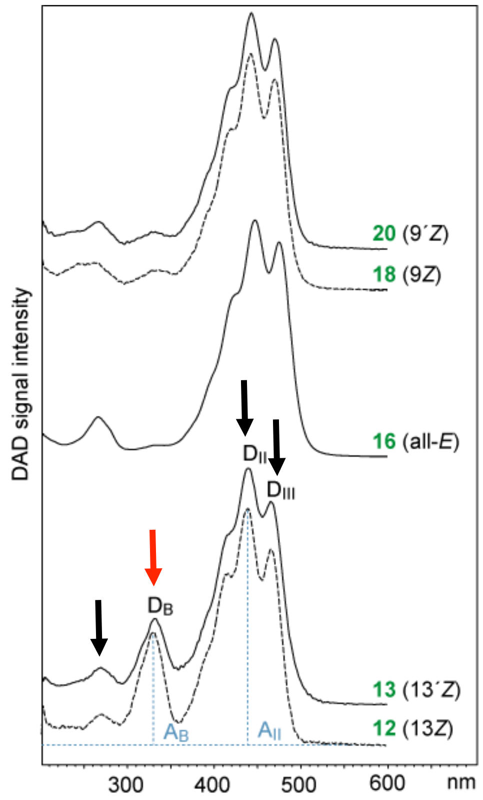
- VIS-maxima: D_{II} and D_{III}
- UV-maxima: D_B (near UV-maxima, "*cis*-peak") and short wavelength maxima
- Intensity ratio A_B/A_{II} and A_{III}/A_{II}

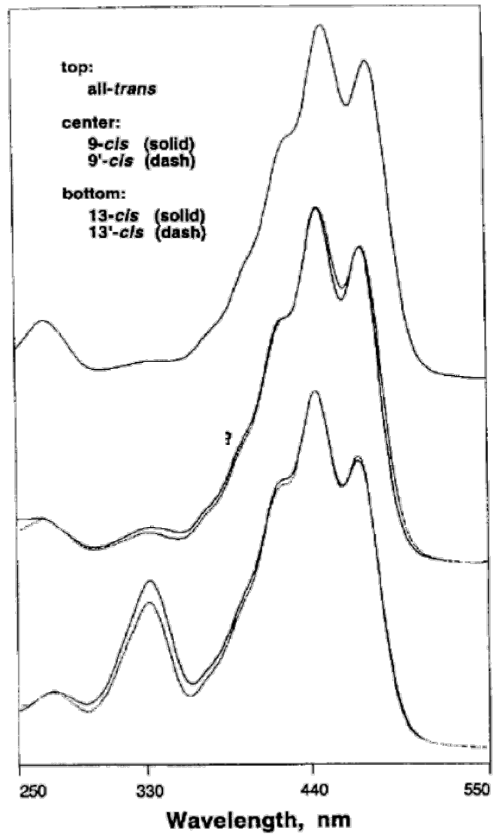
HPLC-DAD-APCI-MS^n analysis

UNIVERSITY OF HOHENHEIM

Isomerisation of reference standards: *Elaeis* sp.







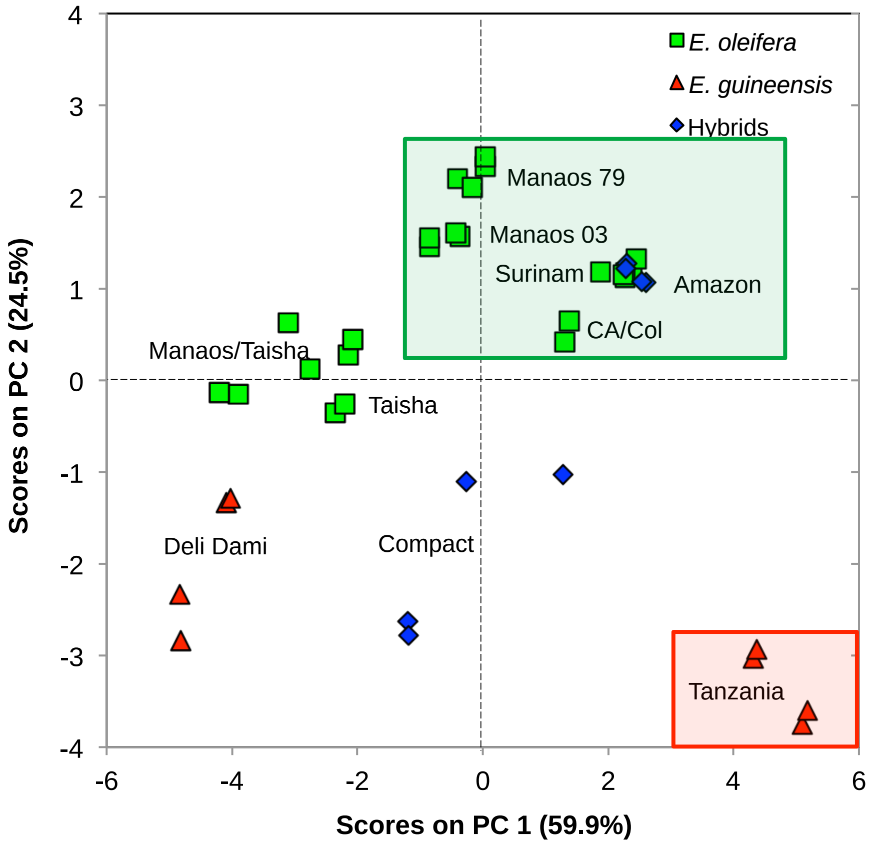
Emenhiser *et al.*,
J. Chromatogr. A 1996 719(2) 333

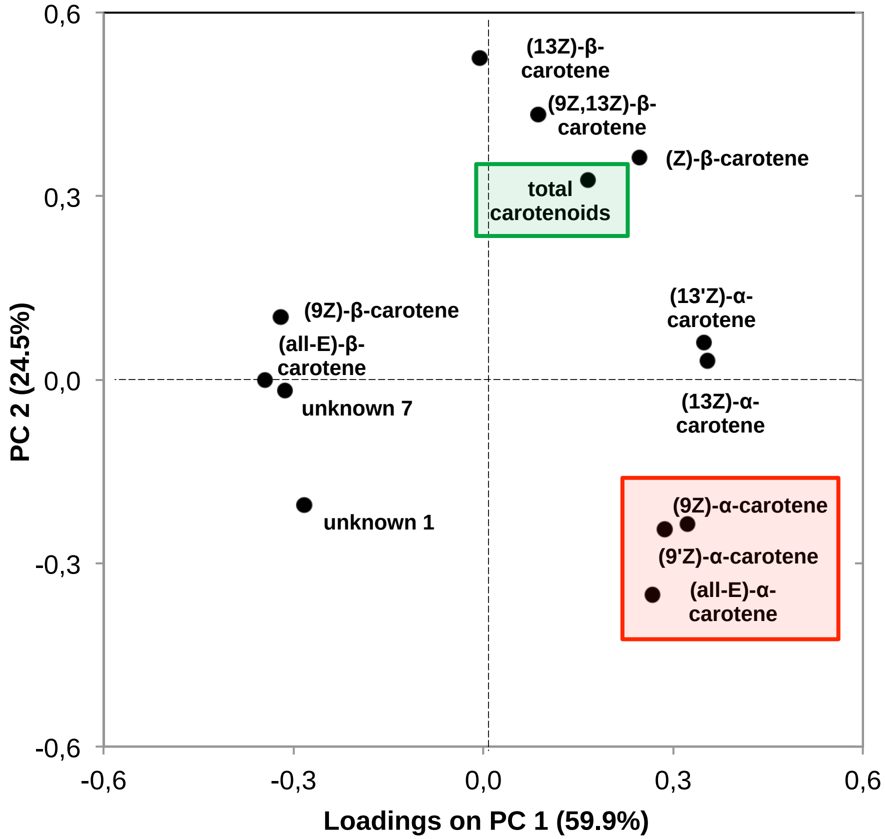
UV/Vis spectra: literature data

- NMR spectroscopy (geometrical isomers)
- Elution order C_{30} stationary phase

HPLC-DAD-FLD profiling

Carotenoid profiles: *Elaeis* sp.





PCA: total carotenoids (µg/g oil) and relative contribution of individual pigments (%)

- ✓ Similar qualitative carotenoid composition (exception: Tanzania)
- ✓ Differing total concentrations (236-3,527 µg/g oil)
- ✓ Mean retinol activity equivalents (RAE): **~60 µg RAE/g oil** (data not shown)
- Recommended dietary allowance (RDA): ~800 µg RAE/d → **~13 g palm oil**

HPLC-DAD-FLD profiling

Carotenoid profiles: *Elaeis* sp.

	E. oleifera						*E. guineensis*		*Hybrid*		All
	CA/Col 03	Taisha 04	Surinam 79	Manaos 03	Manaos 79	Manaos/Taisha 12	Deli Dami 08	Tanzania 06	Compact 97	Amazon 12	Min.-max.
Total β-car. [a]	1308 ± 42c	569 ± 19e	2324 ± 0a	505 ± 21ef	1799 ± 150b	188 ± 21g	251 ± 24g	325 ± 71fg	222 ± 82g	896 ± 10d	164-2324
Total α-car. [a]	601 ± 25b	172 ± 1ef	1084 ± 23a	157 ± 13ef	572 ± 46bc	52 ± 6f	65 ± 9f	299 ± 98de	105 ± 34f	438 ± 23cd	48-1100
Others [a]	53 ± 5b	35 ± 3c	101 ± 4a	23 ± 1cd	67 ± 5b	15 ± 0d	16 ± 1d	28 ± 9cd	15 ± 1d	30 ± 2cd	14-104
Total car. [a]	1961 ± 73c	776 ± 15e	3509 ± 26a	686 ± 35ef	2439 ± 201b	254 ± 27g	332 ± 32fg	652 ± 178ef	342 ± 118fg	1364 ± 31d	236-3527
RAE [ab]	96 ± 2c	40 ± 1e	172 ± 1a	36 ± 2ef	126 ± 10b	14 ± 1g	19 ± 2fg	29 ± 7efg	17 ± 7fg	67 ± 1d	12-173
Isomers (%)	49 ± 2a	44 ± 1ab	48 ± 1ab	47 ± 1ab	48 ± 0ab	40 ± 1bc	34 ± 1c	45 ± 1ab	41 ± 6abc	48 ± 0ab	33-50

[a] Concentrations in (μg/g oil)
[b] Retinoic acid equivalents (RAE) calculated according to the Institute of Medicine (1 μg β-carotene = 1 μg retinol)

Total carotenoids

- **α- and β-carotenes: 94–98% (β-carotene > α-carotene. Exception: Tanzania 06)**
- Broad intra- and interspecific variance
- *E. oleifera* (Surinam, Ca/Col, Manaos 79) and backcross hybrid Amazon 12

Mean retinol activity equivalents (RAE): ~60 μg RAE/g oil (Institute of Medicine, 2001)

- 5-fold higher than carrots (leafy vegetables: 19-fold, tomatoes: 121-fold)
- Recommended dietary allowance (RDA) of ~800 μg RAE/d → **~13 g palm oil**

HPLC-DAD-FLD profiling

UNIVERSITY OF HOHENHEIM

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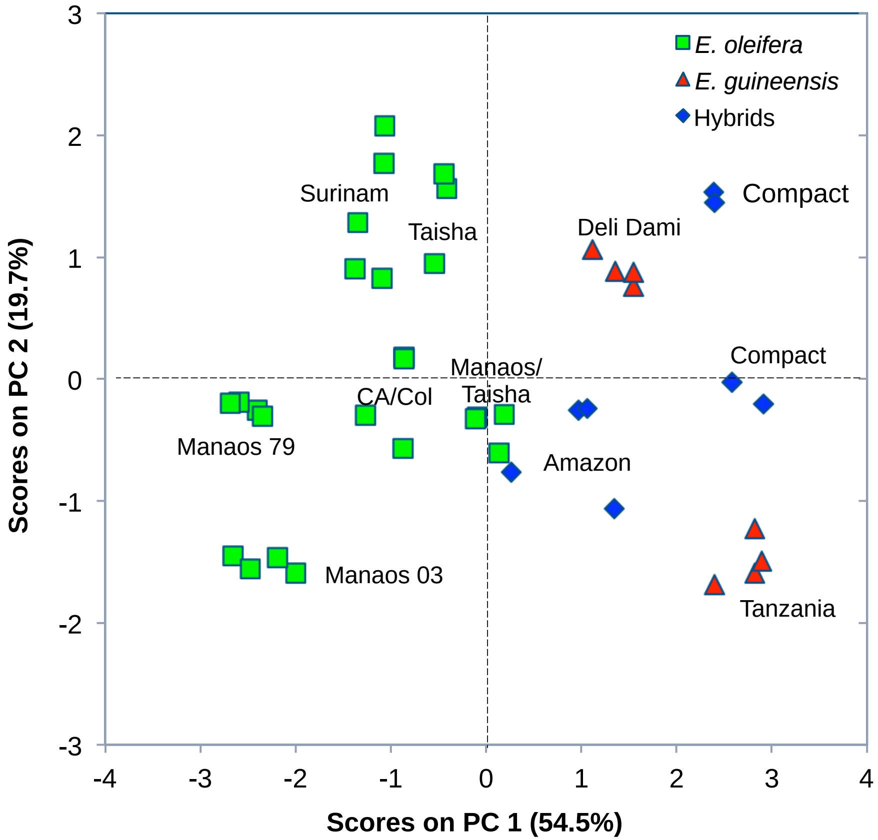
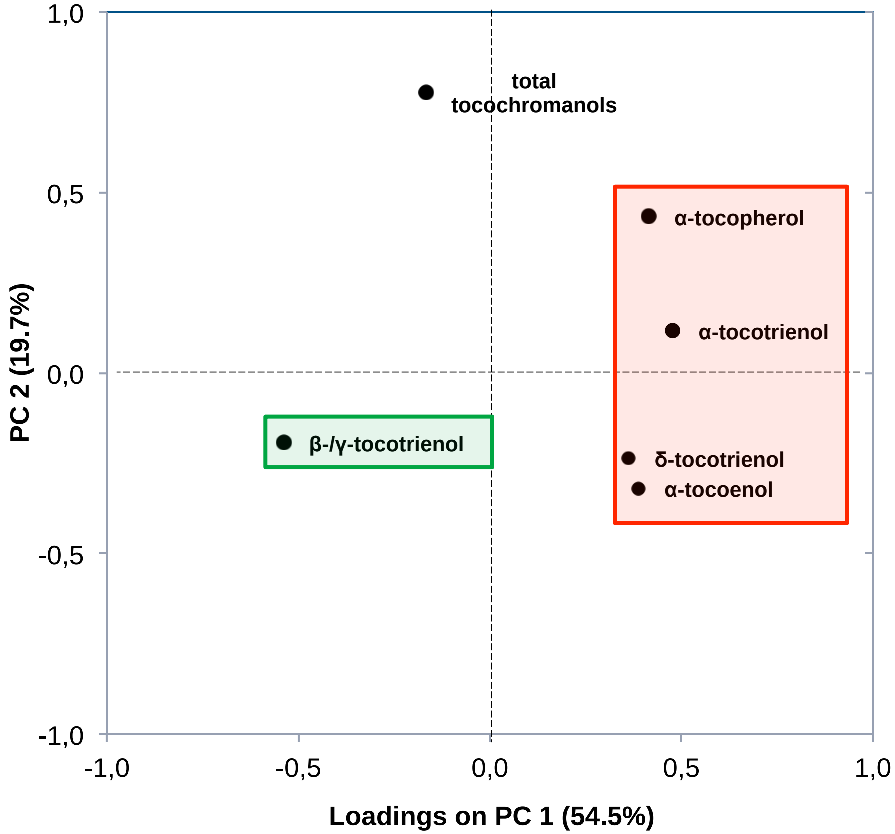
Tocochromanol profiles: *Elaeis* sp.





PCA: total tocochromanols (µg/g oil) and relative contribution of individual compounds (%)

- **Tocochromanol profiles**

✓ β-/γ-tocotrienol (52–83%): *E. oleifera* and hybrids

✓ α-tocochromanol homologues (52–65%): *E. guineensis*

HPLC-DAD-APCI-MS^n analysis

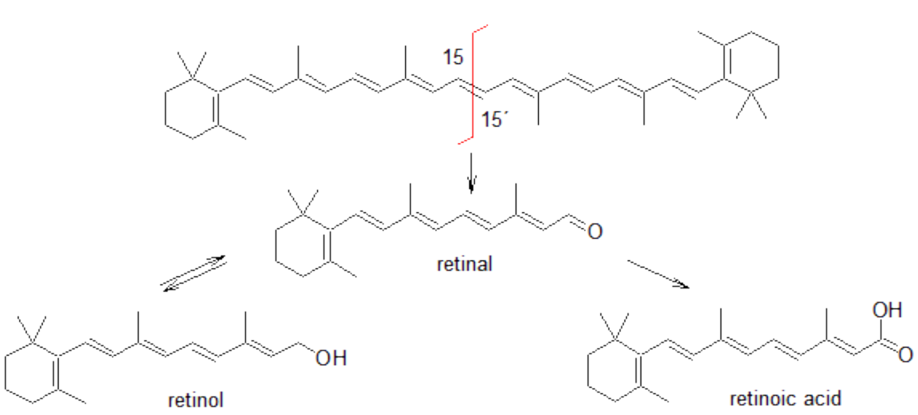
Why is proper chromatographic separation such an important issue ?

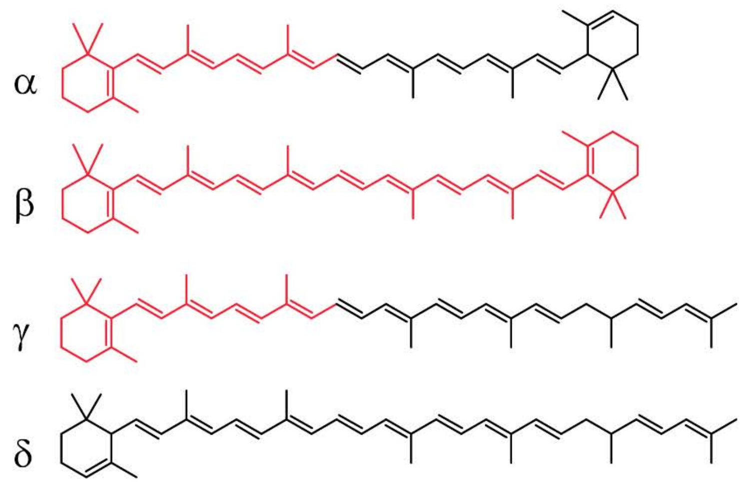
- **Optimized chromatographic separation (peak resolution α)**

✓ Clear UV/VIS and mass spectral information: **compound assignment**

✓ Proper peak integration: **quantitation**

- **Provitamin A estimation (RAE): β-ring**





✓ **Structural isomers:** e.g., α-/β-carotene, γ-/δ-carotene

✓ **Geometrical isomers:** e.g., efficiency of β-carotene conversion into vitamin A: 53 and 38% for (13*Z*)- and (9*Z*)-isomers, respectively (Zechmeister, 1962)

HPLC-DAD-APCI-MS^n analysis

Provitamin A activity

Table 3.2: Relative activities (%) of selected provitamin A carotenoids

Provitamin	Relative activity (%)
(all-*E*)-β-carotene	100[a]
(13*Z*)-β-carotene	53[b]
(*Z*)-β-carotene	38[b]
(all-*E*)-α-carotene	50-54[a]
(*Z*)-α-carotene (13Z?)	16[b]
(*Z*)-α-carotene (9Z?)	13[b]
(*Z*)-γ-carotene*	19[b]

[a] relative activities taken from Bauernfeind (1972)

[b] relative activities taken from Zechmeister (1949)

* used for calculation of γ-carotene isomers

Summary

Lipophilic antioxidants in palm fruits

	Elaeis sp.	*Acrocomia aculeata* (Costa Rica)
Detected compounds	n = 26	n = 26
Carotenes	α- and β-carotene	phytoene, phytofluene, β-carotene
Xanthophylls	n.d.	antheraxanthin, β-cryptoxanthin, lutein, luteoxanthin, neoxanthin, violaxanthin, zeaxanthin
Tocochromanols	δ-tocotrienol, β-/γ-tocotrienol, α-tocotrienol, α-tocoenol, α-tocopherol (n = 6)	α-tocopherol (n = 1)