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Steering the color of the *retro*-carotenoid rhodoxanthin by (*E*/*Z*)-isomeric ratios, controlled aggregation, and formulation technology

Roland Schex

Hochschule Geisenheim University

Steering the color of the *retro*-carotenoid rhodoxanthin by (*E/Z*)-isomeric ratios, controlled aggregation, and formulation technology

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"Colors are the smiles of nature"

Leigh Hunt

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PRELIMINARY REMARKS

The work presented in this doctoral thesis contains a collection of papers published in international peer-reviewed journals as listed in the following.

FULL PAPERS

1. RALLA T.*, SCHEX R.*, PORTA F., SCHÄFER C. (2021). From sugar to nutritional products – Product form development. *Chimia*, 75 (9), 766–771. *Both authors contributed equally.

2. SCHEX R., LIEB V.M., SCHÄFER C., SCHWEIGGERT R., STEINGASS C.B. (2021). Carotenoid profiles of red- and yellow-colored arils of cultivars of *Taxus baccata* L. and *Taxus* × *media* Rehder. *Phytochemistry*, 186, 112741.

3. SCHEX R., SCHWEIGGERT R., STEINGASS C.B. (2022). Atmospheric pressure chemical ionization mass spectrometry of *retro*-carotenoids. *Rapid Communications in Mass Spectrometry*, 36 (7), e9250.

4. SCHEX R., SCHWEIGGERT F., WÜSTENBERG B., BONRATH W., SCHÄFER C., SCHWEIGGERT R. (2020). Kinetic and thermodynamic study of the thermally induced (*E/Z*)-isomerization of the *retro*-carotenoid rhodoxanthin. *Journal of Agricultural and Food Chemistry*, 68 (18), 5259–5269.

5. SCHEX R., BONRATH W., SCHÄFER C., SCHWEIGGERT R. (2020). The impact of (*E/Z*)-isomerization and aggregation on the color of rhodoxanthin formulations for food and beverages. *Food Chemistry*, 332, 127370.

Parts of the work of this doctoral thesis were orally presented to the scientific community as listed below.

ORAL COMMUNICATIONS

1. SCHEX R.*, LIEB V.M., SCHÄFER C., SCHWEIGGERT R., STEINGASS C.B. (23 June 2021). Red- and yellow-colored *Taxus* arils: Natural sources of the exceptional *retro*-carotenoids rhodoxanthin and eschscholtzxanthin at high abundance. *1st Virtual International Conference on Carotenoids of the International Carotenoid Society*. *Presenting author.

2. SCHEX R.*, SCHWEIGGERT F., WÜSTENBERG B., BONRATH W., SCHÄFER C., SCHWEIGGERT R. (23 June 2021). Thermally induced (*E/Z*)-isomerization and controlled aggregation of rhodoxanthin to modulate the color of formulations for food and beverages. *1st Virtual International Conference on Carotenoids of the International Carotenoid Society*. *Presenting author.

3. SCHEX R. (6 February 2020). Introduction of the doctoral research project: Investigations into the (*E/Z*)-isomerization of carotenoids in common solvents and nutraceutical formulations. *Lecture seminars of the graduate school at the Hochschule Geisenheim University*, Geisenheim, Germany.

Further scientific contributions were generated during the period of elaboration of this doctoral thesis, being published in peer-reviewed journals and filed as patent applications. One earlier published paper is marked with a hashtag.

FULL PAPERS

LIEB V.M., SCHEX R., ESQUIVEL P., JIMENÉZ V.M., SCHMARR H.-G., CARLE R., STEINGASS C.B. (2019). Fatty acids and triacylglycerols in the mesocarp and kernel oils of maturing Costa Rican *Acrocomia aculeata* fruits. *NFS Journal*, 14–15, 6–13.

#SCHEX R., LIEB V.M., JIMENÉZ V.M., ESQUIVEL P., SCHWEIGGERT R.M., CARLE R., STEINGASS C.B. (2018). HPLC-DAD-APCI/ESI-MS^n analysis of carotenoids and α-tocopherol in Costa Rican *Acrocomia aculeata* fruits of varying maturity stages. *Food Research International*, 105, 645–653.

PATENT APPLICATIONS

RIDER S., SCHAEFER C., SCHEX R., VERLHAC V., ZWICK T. (2021). New feed additives of carotenoids. Patent application No. WO 2021/069733 A1.

SCHAEFER C., SCHEX R., TSEKOU C., ZWICK T. (2021). New feed additives of fat-soluble vitamins. Patent application No. WO 2021/069752 A1.

RIDER S., SCHAEFER C., SCHEX R., SCHLEGEL B., VERLHAC V., ZWICK T. (2021). New process for the manufacture of feed additives of carotenoids. Patent application No. WO 2021/069753 A1.

The experimental work presented in the CHAPTERS 2–5 of this thesis was mainly carried out at the R&D Formulation Center of DSM Nutritional Products (Kaiseraugst, Switzerland) and the Department of Beverage Research, Chair of Analysis and Technology of Plant-based Foods, Hochschule Geisenheim University (Geisenheim, Germany). Further analytical experiments were performed at the Institute of Food Science and Biotechnology, Chair of Food Material Science and Chair of Plant Foodstuff Technology and Analysis, University of Hohenheim (Stuttgart, Germany).

The contributions of co-authors to the papers presented in the CHAPTERS 1–5 are specified as follows.

Prof. Dr. habil. **Ralf Schweiggert** supervised the thesis and substantially contributed to the research concepts and the interpretation of results. He provided support throughout the entire period, proof-read the manuscripts presented in the CHAPTERS 2–5, and was responsible for all formal aspects as corresponding author of the CHAPTERS 4 and 5.

Dr. **Christian Schäfer** conceptualized the review article presented in CHAPTER 1, wrote parts of its general introduction, proof-read the manuscript, and was responsible for all formal aspects as corresponding author (CHAPTER 1). He functioned as scientific advisor for defining concepts of the work content of the CHAPTERS 2, 4, and 5 and proof-read the respective manuscripts.

PD Dr. habil. **Werner Bonrath** provided scientific guidance regarding the experimental design and the interpretation of analytical results with special focus on the (*E/Z*)-isomerization of rhodoxanthin reported in the CHAPTERS 4 and 5, including the proof-reading of both respective manuscripts.

Dr. **Christof B. Steingaß** supervised the scientific design of the CHAPTERS 2 and 3 and particularly contributed to the respective experimental work using LC-MS analyses. He was responsible for all formal aspects as corresponding author of CHAPTER 2.

Dr. **Veronika M. Lieb** accessed and harvested *Taxus* aril samples (CHAPTERS 2 and 3). In addition, she provided scientific advice to design the research, supported the experimental work on LC-MS analyses, and proof-read the manuscript presented in CHAPTER 2.

Prof. Dr. **Franz Schweiggert** contributed to design the research presented in CHAPTER 4 and proof-read the respective manuscript. He provided training in MATLAB programming, critically reviewed the computational modeling, and supported all developments of mathematical models to describe the (*E/Z*)-isomerization of carotenoids (CHAPTER 4).

Dr. **Bettina Wüstenberg** supported the development of (*E/Z*)-isomerization models of carotenoids and the interpretation of kinetic and thermodynamic parameters obtained by computational modeling (CHAPTER 4). In addition, she proof-read the manuscript of CHAPTER 4.

Dr. **Theo Ralla** equally contributed to the review article presented in CHAPTER 1, therein being responsible for writing the sections about modified starches and pectin. Moreover, he wrote parts of the general introduction and proof-read the entire manuscript of the review article (CHAPTER 1).

Dr. **Fabiola Porta** supported the writing, drawing of Scheme 1, and proof-reading of the review article of CHAPTER 1.

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LIST OF ABBREVIATIONS

3D	three-dimensional
ΔG	Gibbs energy
ΔH_R	reaction enthalpy
ΔS	entropy
λ_i	eigenvalues
λ_{max}	UV/vis absorption maxima
$\overrightarrow{v_i}$	eigenvectors
abs.	absorption
ADIs	acceptable daily intakes
amu	atomic mass unit
APCI	atmospheric pressure chemical ionization
AU	absorption unit
BHT	butylhydroxytoluene
calc.	calculated
cDNA	complementary deoxyribonucleic acid
CID	collision induced dissociation
CIE-L*	lightness
CIE-a*	green to red axes (‘redness’)
CIE-b*	blue to yellow axes (‘yellowness’)
CIE-C*	chroma; color saturation
CIE-h°	color hue
CIE-ΔE	total color difference
CO_2	carbon dioxide
d_{32}	Sauter particle size diameter
D_{II}	absorbance at the main absorption maximum
D_{III}	absorbance at the absorption maximum at longest wavelength
D_B	absorbance at the ‘(*Z*)-peak’ (syn. ‘*cis*-peak’)
DAD	diode array detection
DSC	differential scanning calorimetry
E_a	activation energy
EDTA	ethylenediaminetetraacetic acid
EI	electron ionization
eq	equation
equ	equilibrium
ESI	electrospray ionization
EU	European Union
i.d.	inner diameter
F_{mod}	modeled data points
FAB	fast atom bombardment
fr. wt	fresh weight
HPLC	high performance liquid chromatography

HRMS	high-resolution mass spectrometry
IBS	irritable bowel syndrome
k	rate constant
k_0	pre-exponential factor (Arrhenius law)
K_{equ}	equilibrium constant
LC	liquid chromatography
MRSS	mean residual sum of squares
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MS^n	multi-stage mass spectrometry
n.d.	not detected
NMR	nuclear magnetic resonance
ODE	ordinary differential equation
OEHHA	Office of Environmental Health Hazard Assessment
OSA	octenyl succinic anhydride
o/w	oil-in-water
QTOF	quadrupole time-of-flight
R	universal gas constant
R^2	coefficient of determination
Rdx	rhodoxanthin
sh	shoulder
t	time point
T	temperature
*t*BME	*tert*-butyl methyl ether
T_g	glass transition temperature
t_R	retention time
tr.	traces
UHPLC	ultra-high performance liquid chromatography
XRPD	X-ray powder diffraction

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CHAPTER 5

Table 1 Relative concentrations of rhodoxanthin (*E/Z*)-isomers after isothermal heating at 60 °C for 0, 45, 90, and 150 min in acetone and the respective CIE-L*a*b* color values at a total rhodoxanthin concentration of 4.7 mg/L. 148

Table 2 CIE-L*a*b* color values of rhodoxanthin (*E/Z*)-isomer mixtures in pure and aqueous acetone at 25, 50, and 75% water content (*v/v*) at a total rhodoxanthin concentration of 3.3 mg/L and mean shares of (all-*E*)-configured isomers of 73, 35 and 20%. 154

Table S1. CIE-L*a*b* color values and total color differences (ΔE) of rhodoxanthin (*E/Z*)-isomer mixtures in pure acetone at total rhodoxanthin concentrations of 0.9–9.4 mg/L and shares of (all-*E*)-configured isomers of 75, 44, 30, and 23%. 163