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RESEARCH ON CAROTENOIDS

CAROTENOID SCIENCE

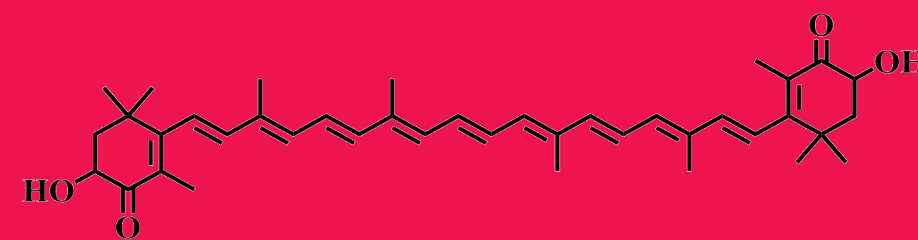
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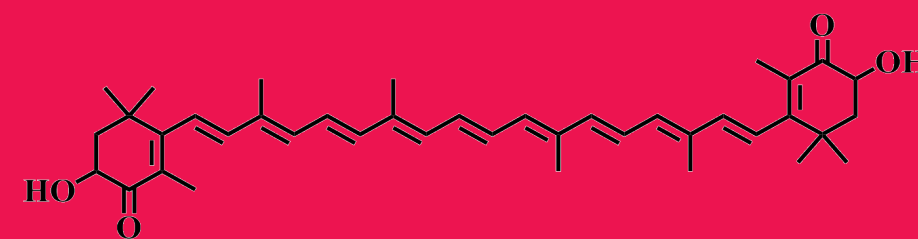
Abstracts of the Papers presented at The 19th International Symposium on Carotenoids



Toyama, Japan
9th-14th July, 2023



INTERNATIONAL CAROTENOID SOCIETY



JAPANESE SOCIETY FOR CAROTENOID RESEARCH(JSCR)
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PREFACE

In 1966, the first International Symposium on Carotenoids convened in Trondheim, Norway as a satellite meeting to the IUPAC Natural Products Symposium in Stockholm, Sweden. Little did we know that this inaugural gathering would ignite a series of symposia, spanning continents and decades, that would bring together the brightest minds in carotenoid research. We convened again every three years, exploring the boundaries of our understanding of these fascinating compounds in Las Cruces, New Mexico, USA (1969), Cluj-Napoca Romania (1972), Bern, Switzerland (1975), Madison, Wisconsin, USA (1978), Liverpool, UK (1981), Munich, Germany (1984), Boston, Massachusetts, USA (1987), Kyoto, Japan (1990), Trondheim, Norway (1993), Leiden, The Netherlands (1996), Cairns, Australia (1999), Hawaii, USA (2002), Edinburgh, UK (2005), Okinawa, Japan (2008), Utah, USA (2011), Krakow, Poland (2014), and Luzern, Switzerland (2017).

And now, we gather again in July 2023 in Toyama, Japan for the 19th International Symposium on Carotenoids. Despite the unforeseeable challenge of the COVID-19 pandemic, which caused the postponement of this event from its originally planned date in 2020 to the current year of 2023, we persisted with our shared goal. I commend each and every one of you for your dedication and commitment to making this symposium a reality. It is a testament to our collective resilience and determination that we are able to come together today to discuss and learn about the latest developments in the field of carotenoids and their vital role in promoting human health, nutrition, and so on.

Since 1966, the field of carotenoids has expanded greatly, branching out into many new areas of scientific study. Our approach has become more interdisciplinary, and our methods more advanced, thanks to wide-area collaborations and cutting-edge technology. I am thrilled that the Book of Abstracts for the Symposium has once again been published as a special issue of Carotenoid Science, the journal of the Japanese Society for Carotenoid Research. This practice not only serves as a valuable resource for Symposium participants, but also brings the work presented at the Symposium to the attention of a wider readership as an ISBN-listed publication.

The International Symposium on Carotenoids has been the official Symposium of The International Carotenoid Society, and I am grateful to the Society for their support during the preparation of the Symposium for the past 6 years. This Symposium marks the third time in 16 years that we have returned to Japan, since the fifteenth gathering held in Okinawa. Toyama has a rich Japanese history, fascinating culture, and delectable cuisine, and I hope that all participants will have the opportunity to enjoy both the scientific program and the surrounding environment throughout the week.

I am indebted to many people whose effort have helped us so much. I extend my sincerest thanks to Drs. Eiji Yamashita, Norihiko Misawa, and Michiaki Murakoshi for their invaluable contributions as Organizers. And finally, I would like to express my special appreciation to Dr. Chiasa Uragami and Ms. Kaya Kuruma for their exceptional work in compiling, organizing, and producing this Book of Abstracts.

Hideki Hashimoto

Symposium Chair of ISC2023-Toyama

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Contents

Preface	i
List of Sponsors	ii

Abstracts of Special Lecture, Invited Lectures and Oral Presentations

Special Lecture

SL

Richard Cogdell, Hashimoto Hideki	3
Carotenoids in photosynthesis: unlikely molecules?	

Session1: Genetics, Biosynthesis and Metabolism ~In memory of Prof. Brian H. Davies~

1P

Norihiko Misawa	6
<i>E. coli</i> as the tool of functional analysis for carotenogenic genes	

1ST

Gerhard Sandmann, Paul D. Fraser	7
Prof. Norihiko Misawa –A Pioneer in Carotenoid Molecular Biology	

1I-1

Morote Lucía, Ahrazem Oussama, Rubio-Moraga Ángela, Diretto Gianfranco, Frusciante Sarah, Aragonés Verónica, Darós José Antonio, Gómez-Gómez Lourdes	8
Crocins in plants: much more beyond saffron	

1I-2

Banh Anh Thi-Mai, Thiele Bjoern, Matsubara Shizue	9
Carotenoid turnover in leaves studied by ¹³ CO ₂ labeling and LC-MS	

1I-3

Rocheford Torbert	10
Biofortification breeding for higher levels of macular carotenoids and provitamin A carotenoids in maize grain: An update	

1O-1

Harbart Vanessa, Frede Katja, Fitzner Maria, Baldermann Susanne	11
Metabolic responses and regulation of carotenoid and flavonoid biosynthetic pathways in lettuce (<i>Lactuca sativa</i>) in protected cultivation	

1O-2

Tianhu Sun, Li Li	12
A novel regulatory mechanism to synchronize photosynthetic pigment synthesis in plants	

10-3

Rodrigo Maria J., Zacarias Lorenzo, Zacarias-Garcia Jaime, Tadeo Francisco J., Terol Javier, Talón Manuel 13

Color diversity in fruits of ancestral *Citrus* species: A comparative study of carotenoid metabolism during maturation

Session 2: New Trends of Carotenoids Science

2P

Robert Bruno 16

Carotenoids: the plant toolbox

2I-1

Erdman John 17

Is lutein ready for public health dietary recommendations?

2I-2

Dufossé Laurent 18

So many International Symposia on Carotenoids (19!!), so few industrially produced carotenoids... What are the problems?

2I-3

Rodriguez-Concepcion Manuel 19

Novel approaches to produce and store carotenoids in plant cells

2I-4

Tanumihardjo Sherry, Bwar Wai, Gannon Bryan, Palacios-Rojas Natalia 20

Bioefficacy of nixtamalized high β -cryptoxanthin biofortified maize and a novel β -cryptoxanthin supplement

2O-1

Umeno Daisuke, Ojima Takumi 21

Breaking Symmetry: laboratory evolution of carotenoid pathways to explore extra-natural space of carotenoid functions

2O-2

Lim Boram, Kim Minsoo, Kim Dahye, Jung Hyunuk, Yoo Hyebin, Koo Sangho 22

Conductance control in the carotenoid molecular wires by phenyl substituents

Session 3: Astaxanthin Symposium

3P

Naito Yuji 24

Interventions against gut frailty lead to healthy longevity

3I-1	Allah Nawaz, Yasuhiro Nishida, Tomonobu Kado, Muhammad Bilal, Shiho Fujisaka, Takashi Nakagawa, Kazuyuki Tobe	25
	Astaxanthin treatment improves endurance and ameliorates insulin resistance in the muscle of obese mice.	
3I-2	Aoi Wataru	26
	The potential role of astaxanthin in metabolic health and exercise performance	
3I-3	Mark JS Miller	27
	Why is astaxanthin a perfect dietary supplement?	
3I-4	Maoka Takashi	28
	Astaxanthin: Discovery, history, and distribution in nature	
3O-1	Autenrieth Caroline, Ghosh Robin	29
	A new modular expression platform for the high-level production of hydroxy- or ketocarotenoids in the purple photosynthetic bacterium <i>R. rubrum</i>	
 Session 4: Photochemistry, Photophysics and Photosynthesis		
~In memory of Prof. Yasushi Koyama~		
4P	Shen Jian-Ren	32
	Binding patterns and functions of fucoxanthin and related pigments in photosynthesis	
4I-1	Polívka Tomáš	33
	Relation between carotenoid structure and spectroscopy: Implications for function of carotenoids in photosynthesis	
4I-2	Kosumi Daisuke, Hashimoto Hideki	34
	Photophysics in natural and artificial carbonyl carotenoids	
4I-3	Dan-Hong Li, Hai-Dan Yao, Nami Yamano, Jian-Ping Zhang	36
	Chlorophyll excitation quenching dynamics of the light harvesting complex II from the marine green alga <i>Bryopsis corticulans</i>	

4O-1	Varotsis Constantinos, Tselios Charalambos, Andreou Charalambos, Koutsoupakis Constantinos, Ioannou Aristos	37
	Probing the fucoxanthin-chlorophyll <i>a/c</i> -binding proteins (FCPs) of the marine diatom <i>fragilariopsis sp</i> by resonance Raman, FTIR, and fluorescence spectroscopy	
4O-2	Šebelík Václav, Šimová Ivana, Polívka Tomáš	38
	Radical cation formation during the two-photon excitation of β -apo-8'-carotenal	
4O-3	Seki Soichiro, Nakaniwa Tetsuko, Pablo Castro-Hartmann, Kasim Sader, Akihiro Kawamoto, Hideaki Tanaka, Pu Qian, Genji Kurisu and Ritsuko Fujii	39
	Structures of siphonaxanthin and its acyl ester in marine green algal light-harvesting complex II	
4O-4	Kuznetsova Valentyna	40
	New insights into carotenoids higher electronic states dynamics	
Session 5: Chronic Disease Prevention and Treatment		
5P	Wang Xiang-Dong	42
	Mechanistic understanding of carotenoid benefits against chronic diseases	
5I-1	Amengual Jaume	43
	Tissue-specific expression of BCO1 and its implication in cardiometabolic disease	
5I-2	Sugawara Tatsuya, Manabe Yuki	44
	Potential of siphonaxanthin for disease control and prevention	
5I-3	M. Luisa Bonet, Andreu Palou, Jaume Amengual, Johannes von Lintig, Joan Ribot	45
	Early-life metabolic programming by micronutrients and food bioactives with special focus on preformed vitamin A and beta-carotene: insights from murine models	
5O-1	Darawshe Aya, Trachtenberg Aviram, Sharoni Yoav	46
	Mechanisms involved in the protective effect of carotenoids and estradiol in human skin cells under mitochondrial oxidative stress	

5O-2	Stringham Nicole, Green Marina, Roche Warren, Nolan John	47
	Lutein, zeaxanthin, and meso-zeaxanthin supplementation attenuates markers of systemic inflammation and oxidative cardiovascular processes	
5O-3	Nishino Hoyoku	48
	Carotenoids as health promoting natural gifts	
5O-4	Green Marina, Stringham Nicole, Nolan John	49
	Targeted nutrition with xanthophyll carotenoids and reduced cardiometabolic risk: A randomised double-blind placebo-controlled study	
Session 6: Carotenoid Metabolism and Function		
6P	Von Lintig Johannes, Bandara Sepalika, Thomas Linda, Ramkumar Srinivasagan, Golczak Marcin	52
	Molecular components affecting carotenoid homeostasis in mammalian cells and tissues	
6I-1	Kim Youn-Kyun, Giordano Elena, Acharya Pooja, Chattopadhyay Atrayee, Lin Xinhua, Hanna Nazeeh, Hussain Mahmood, Quadro Loredana	54
	Mechanisms of carotenoid transport across the maternal-fetal barrier	
6I-2	Redmond T. Michael, Uppal Sheetal, Dergunov Sergey, Gentleman Susan, Rogozin Igor, Pinhassik Eugene, Poliakov Eugenia	55
	Structural and functional role of cysteine palmitoylation in mammalian carotenoid cleavage dioxygenases	
6I-3	Solano Yasmeeen, Everett Michael, Dang Kelly, Abueg Jude, Kiser Philip	56
	Structure-function studies of a carotenoid isomeroxygenase reveal mechanistic diversity in the synthesis of 11- <i>cis</i> -retinoids for animal vision	
6I-4	Jessica Cooperstone	57
	Integrating multi-omics techniques towards understanding metabolism and the health effects of carotenoids	
6O-1	Naoki Takatani, Kana Sakimura, Fumiaki Beppu, Yumiko Yamano, Takashi Maoka, Masashi Hosokawa	58
	Identification and tissue distribution of fucoxanthin-derived novel metabolites	

6O-2

Miller Anthony, Amengual Jaume

59

Exploring the role of HDL in carotenoid efflux and delivery to tissues

Session 7: Chemistry and Analysis

~In memory of Prof. Takao Matsuno, Prof. Synnøve Liaaen Jensen and Prof. Kurt Bernhard~

7P

Richard Sliwka

62

Carotenoids – an account of arbitrary exclusion and subjective inclusion

7I-1

Maoka Takashi

63

My chemical and biochemical studies of natural carotenoids during 45 years

7I-2

Giuffrida Daniele, Zoccali Mariosimone, Dugo Paola, Mondello Luigi

64

Apocarotenoids determination in *Capsicum* species and biological fluids by supercritical fluid extraction-supercritical fluid chromatography-triple quadrupole/mass spectrometry

7I-3

Shindo Kazutoshi

65

Structural analyses of the compounds produced by acidic treatment of epoxycarotenoids (violaxanthin, 9-*cis*-violaxanthin, antheraxanthin, and 9-*cis*-antheraxanthin), and the antioxidant activities of epoxycarotenoids and the acid treatment products.

7O-1

Shi Gaosheng, Kim Dahye, Kim Yunji, Gu Lina, Kim Hyein, Koo Sangho

66

Rapid assembly of carotenoids of phenyl, phenolic, and acyl end groups with superior antioxidant activities

7O-2

Suzuki Ryuju, Yasuhara Kazuma, Deguchi Shigeru

67

Analysis of optical properties of carotenoid nanoparticles by considering molecular flexibility

7O-3

Czett Dalma, Böddi Katalin, Agócs Attila, Deli József, Nagy Veronika

68

Effect of the aggregation behaviour on the antioxidant capacity of carotenoids

7O-4

Focsan A. Ligia, Polyakov Nikolay. E., Gao Yunlong, Kispert Lowell D.

69

Chemistry of carotenoid radicals and complexes: A compilation of tens of years of research

Session 8: Plant Carotenoids

8P

Salim Al-Babili 72

Plant apocarotenoid formation: destruction for construction

8I-1

Stange Claudia, Quian-Ulloa Rocío, Munizaga Fabiana, Gonzalez-Calquin Christian 73

Illuminating carotenoid synthesis regulation in the carrot taproot grown underground

8I-2

Demurtas Olivia, Frusciante Sarah, Ferrante Paola, Sulli Maria, Mini Paola, Petrillo Maria Grazia, Diretto Gianfranco, Giuliano Giovanni 74

Dissection of the saffron crocin biosynthesis pathway and its biotechnological applications

8I-3

Sierra Julio, McQuinn Ryan, Agreda-Laguna Kenny Alejandra, Cordoba Elizabeth, Acevedo Leslie, Pogson Barry, León Patricia 75

Carotenoid derived signals and their impact in plant development

8I-4

Pogson Barry 76

Carotenoids, Apocarotenoid signals and plant development

8O-1

Valentina Metličar, Kranjc Krištof, Albreht Alen 77

Using renewable sources of xanthophylls to produce xanthophyll esters with improved physicochemical properties in a sustainable manner

Session 9: Food, Nutrition and Epidemiology

9I-1

Benítez-González Ana M., Stinco Carla M., Antonio J. Meléndez-Martínez 80

Increasing the bioaccessibility of carotenoids by cooking practices. Sustainability aspects

9I-2

Bohn Torsten, Iddir Mohammed 81

Dietary-derived factors influencing aspects of carotenoid bioavailability – focus on protein-interactions

9I-3

Böhm Volker, Faber Marius, Zerres Sarah, Stahl Wilhelm 82

Photoprotection with food rich in carotenoids

9O-1	Morifuji Masashi, Ichikawa Satomi, Miyayama Masami, Manabe Yuki, Sugawara Tatsuya	83
	Fermented milk containing high content of exopolysaccharide enhance dietary carotenoid bioavailability in humans.	
9O-2	Fitzpatrick Naomi, Chachay Veronique, Capra Sandra, Bowtell Joanna, Shore Angela, Jackman Sarah, Briskey David	84
	Validation of dietary lutein and zeaxanthin screeners in healthy Australian adults	
9O-3	Iddir Mohammed, Leners Bernadette, Pittois Denis, Guignard Cedric, Weber Bernard, Gantenbein Manon, Larondelle Yvan, Bohn Torsten	86
	Whey- and soy protein isolates added to a carrot-tomato juice alter carotenoid bioavailability in healthy adults	
Session 10: Carotenoids in the Eye and Brain		
10P	Johnson Elizabeth	88
	Lutein and cognitive function throughout the lifespan: an update.	
10I-1	Akira Obana, Ryo Asaoka, Yuko Gohto, Yuji Takayanagi, Aya Miura, Miho Nozue, Shigeki Muto, Werner Gellermann, Paul S. Bernstein, Mieko Nakamura	89
	Application of the measurement of skin carotenoids by refractive spectroscopy, Veggie Meter.	
10I-2	Hammond Billy, Harth Jacob, Renzi-Hammond Lisa	90
	Functional effects of macular pigment density on the central nervous system	
10I-3	Toomey Matthew	91
	The mechanisms and meaning of ketocarotenoid-based signals in animals	
10I-4	Nolan John	92
	Macular carotenoids: Keeping an eye on vision and beyond!	
10I-5	Bernstein Paul S., Addo Emmanuel K., Ruit Sanduk, Thapa Raba	93
	Noninvasive screening for vitamin A deficiency in high-risk populations using skin carotenoid measurements	

10O-1

Rodríguez Ana M., Martín-Chamorro Rocío A., Palou Andreu, Bonet M. Luisa, Ribot Joan 94

Brain regulation of gene expression by β -carotene and metformin: possible role in the control of energy metabolism and cognitive function

Session 11: Biotechnology and Industrial Production ~In memory of Prof. Chengfu Zhu~

11P

Fraser Paul, Nogueira Marilise, Almeida Juliana, Drapal Margit, Harriet Berry, Laura Perez-Fons, Eugenia Enfissi 96

Plant cell factories for industrial and nutritional carotenoids

11I-1

Barja Victoria, Nicholls Joshua, Calame Julie, Poudel Sujata, Llorente Briardo 97

Multi-subcellular engineering for carotenoid production

11I-2

Kuwahara Daichi 98

Industrial production of carotenoids using *Paraococcus carotinifaciens* and carotenoid applications for animal feed

11I-3

Sandmann Gerhard 99

Metabolic engineering of *Xanthophyllomyces dendrorhous* for the production of commercially interesting carotenoids and the generation of novel structures

11I-4

Hasunuma Tomohisa 100

Dynamic metabolomics approach and engineering biology for microbial carotenoid production

11O-1

Demurtas Olivia Costantina, Sulli Maria, Ferrante Paola, Mini Paola, Aprea 101

Giuseppe, Nicolìa Alessandro, Dall'Osto Luca, Bassi Roberto, Martí

Maricarmen, Aragonès Verónica, Daròs José-Antonio, Giuliano Giovanni

Nicotiana benthamiana as a cell factory for high value-added (apo)carotenoids

11O-2

Göttl Vanessa L., Peters-Wendisch Petra, Wendisch Volker F., Henke Nadja A. 102

Perturbing native gene expression with a CRISPRi-library and application of a genetically encoded biosensor to study carotenogenesis

11O-3

Furubayashi Maiko 103

Engineering novel retinal-related compounds through carotenoid pathway engineering and enzyme mutagenesis

Abstracts of Poster Presentations

P1-1	Torres-Montilla Salvador, Morelli Luca, Bombarely Aureliano, Rodriguez-Concepcion Manuel	107
	Transcriptional changes associated to the chloroplast-to-chromoplast transition in carotenoid-overaccumulating leaves.	
P1-2	Nagashima Aya, Ishihara Daichi, Araki Yui, Ando Hiroto, Miki Tashiro, Takumi Ojima, and Daisuke Umeno	108
	Promiscuous carotenoid pathways as indicators for intercellular proportion of IPP and DMAPP	
P1-3	Araki Yui, Ojima Takumi, Ando Hiroto, Umeno Daisuke	109
	Molecular breeding of C ₂₀ carotenoid pathways	
P1-4	Ishihara Daichi, Onozato Yumi, Minakata Katsuya, Iwasaki Miki, Kawai-noma Shigeko, Umeno Daisuke	110
	Directed evolution of high-titer non-mevalonate pathway	
P1-5	Ando Hiroto, Ojima Takumi, Umeno Daisuke	111
	Converting squalene synthase into carotenoid synthase for biosynthesis of minisqualene	
P1-6	Rodrigo Maria J., Rey Florencia, Lozano-Omeñaca Aurora, Zacarias Lorenzo	112
	Carotenoid metabolism during fruit maturation of Nadorcott, a highly pigmented mandarin	
P1-7	Tamaki Shun, Nomura Toshihisa, Ishikawa Marumi, Yamada Koji, Suzuki Kengo, Mochida Keiichi	113
	Pathway-scale genome editing provides a comprehensive understanding of carotenoid biosynthesis in microalga <i>Euglena gracilis</i>	
P1-8	Seki Soichiro, Yamano Yumiko, Oka Naohiro, Kamei Yasuhiro, Fujii Ritsuko	114
	Blue-to-green light regulates siphonaxanthin biosynthesis: Discovery of deoxysiphonaxanthin in an edible macrogreen alga, <i>Codium fragile</i>	
P1-9	Sugiyama Kenjiro, Takaichi Shinichi	115
	Two types of lycopene cyclases in cyanobacteria: CruA/CruP-type and CrtL-type (Overview)	
P1-10	Nayak Jwalit J., Cazzonelli Christopher I.	116
	Light-mediated isomerisation of acyclic <i>cis</i> -carotenes regulates metabolic feedback and nuclear gene expression in <i>Arabidopsis</i> .	

P1-11	Zheng Hui, Rodriguez-Concepcion Manuel, Lu Shan	117
	Transient overexpression of STAY-GREEN causes the formation of chromoplasts in dark-incubated leaves	
P2-1	Czett Dalma, Böddi Katalin, Nagy Veronika, Takátsy Anikó, József Deli, Paul Tone, György T. Balogh, Anna Vincze, Attila Agócs	118
	Synthesis and antioxidant capacity of carotenoid succinates and their melatonin conjugates	
P2-2	Polyakov Nikolay E., Focsan A. Ligia, Gao Yunlong, Kispert Lowell, D.	119
	Supramolecular delivery systems for carotenoids: EPR and optical studies	
P2-3	Pervin Mahfuza, Brenya Eric, Johnson Scott, Cazzonelli Christopher I.	120
	Mechanical stimulation contra-regulates carotenoid and ABA biosynthesis in leaves from naïve versus touch-acclimated Arabidopsis plants	
P3-1	Schmitt Ina, Meyer Florian, Henke Nadja A., Peters-Wendisch Petra, Kousoulaki Katherina, Zeytin-Schüning Sinem, Alberts-Hubatsch Hilke, Wendisch Volker F.	121
	From aquaculture to aquaculture: Production of the fish feed additive astaxanthin and other valuable carotenoids from aquaculture sidestream by <i>Corynebacterium glutamicum</i>	
P4-1	Miki Bando-Uotani, Vasco Joris, Yujiro Hama, Kyoko Shinzawa-Itoh, Natsuko Inoue-Kashino, Yasuhiro Kashino, Keisuke Kawakami, Koji Yonekura and Daisuke Kosumi	122
	Excitation energy transfer dynamics of carotenoids in the far-red light utilizing photosystem II from <i>Acaryochloris marina</i>	
P4-2	Nohara Taiki, Bando-Uotani Miki, Serikawa Hiroki, Kawakami Keisuke, Yonekura Koji, Kamiya Nobuo, Kosumi Daisuke	123
	Photosynthetic functions of carotenoids in photosystem I from cyanobacteria, as revealed by ultrafast spectroscopy	
P4-3	Hama Yujiro, Kosumi Daisuke	124
	Real-time observation of molecular vibrations of fucoxanthin by 10 fs pump-probe spectroscopic measurement	
P4-4	Yamamoto Hayata, Kida Masatoshi, Kawakami Keisuke, Uchida Hiroko, Murakami Akio, Kamiya Nobuo, Kosumi Daisuke	125
	Ultrafast energy transfer dynamics in photosynthetic antenna acpPC from dinoflagellate <i>Symbiodinium</i>	

P4-5	Papageorgiou Marios, Tselios Charalampos, Varotsis Constantinos, Pinakoulaki Eftychia	126
	Photoreduction of carotenoids and binding dynamics of the tetraheme-RC-bound cytochromes <i>c</i> and cytochrome <i>c</i> ₂ in <i>Roseobacter denitrificans</i> probed by resonance Raman spectroscopy	
P4-6	Kawakami Keisuke, Hamaguchi Tasuku, Shinzawa-Itoh Kyoko, Inoue-Kashino Natsuko, Kashino Yasuhiro, Yonekura Koji	127
	Identification of carotenoids in photosynthetic protein complexes based on structural analysis	
P4-7	Kojima Risa, Kida Masatoshi, Kosumi Daisuke, Oh-Oka Hirozo	128
	Analysis of the excited energy transfer from 4,4'-diaponeurosporene to bacteriochlorophyll- <i>g</i> in the photosynthetic reaction center of <i>Heliomicrobium modesticaldum</i>	
P4-8	Sei Ryuya, Horiuchi Kota, Uragami Chiasa, Hashimoto Hideki	129
	Temperature-dependent absorption spectra of carotenoids exploring the relationship between solvent-carotenoid interaction and 0-0 band width	
P4-9	Kishida Ikuya, Uragami Chiasa, Hashimoto Hideki, Cogdell Richard	130
	Photoprotective function evaluation of light-harvesting systems containing bacteriochlorophyll <i>b</i> in <i>Blastochloris viridis</i>	
P4-10	Tao Ruohan, Yumeka Kakuta, Chiasa Uragami, Hideki Hashimoto	131
	Decoding the molecular structures and functions of carotenoid radical species using resonance Raman spectroscopy and DFT calculations	
P4-11	Mitani Koki, Uragami Chiasa, Yukihiro Nao, Gardiner Alastair, Richard J. Cogdell and Hideki Hashimoto	132
	Triplet-triplet-type photoprotective pathway in the spheroidene-reconstituted LH1 complex	
P4-12	Echigo Mayu, Kota Horiuchi, Chiasa Uragami, Hideki Hashimoto	133
	Exploring the structure-function relationship of β -apo-8'-carotenal isomers: Insights into photosynthetic pigments from computational chemistry analysis	
P4-13	Morita Yuya, Uragami Chiasa, Hashimoto Hideki	134
	Replication of absorption spectrum of carotenoid in purple photosynthetic bacteria	
P4-14	Sago Yuma, Horiuchi Kota, Uragami Chiasa, Hashimoto Hideki	135
	Exploring the excited state properties of β -apo-8'-carotenal through steady-state fluorescence spectroscopy	

P4-15	Horiuchi Kota, Uragami Chiasa, Ruohan Tao, Kosumi Daisuke, J. Cogdell Richard, Hashimoto Hideki	136
	Excited-state dynamics of <i>cis</i> isomers of β -apo-8'-carotenal: Classification of rate constants and "inverse energy gap law"	
P4-16	Yamano Nami, Wang Peng, Zhang Jian-Ping	137
	Application of lipid-nanodisc to investigate the photoprotective function of carotenoids bound to photosynthetic light harvesting complex	
P4-17	Yoshida Kazuhiro, Seki Soichiro, Yamano Yumiko, Fujii Ritsuko	138
	Analysis of ICT state with fluorescence at low-temperature	
P5-1	Wu Wen-Bin, Tsai Yih-Jeng, Wu Pi-Hui	139
	Effects of lycopene and β -carotene on cytokine production and secretion in chronic rhinosinusitis without nasal polyps-derived nasal fibroblasts: differential anti-inflammatory activities of carotenoids in CRSsNP	
P5-2	Lee Jung Hyeun, Kim Yuri, Heo Seung Chul	140
	Anticancer effect of β -carotene in oxaliplatin-resistant colon cancer cell by regulating apoptosis and cancer stemness	
P5-3	Yeonsoo Oh, Hyeonju Yu, Kwang Ko, Yuri Kim	141
	An integrative transcriptomic analysis reveals the potential benefits of carotenoid for cancer therapy in female patients with colorectal cancer	
P5-4	Ribot Joan, Stojnić Bojan, Palou Andreu, Diretto Gianfranco, Bonet M. Luisa	142
	Effects of beta-carotene supplementation alone and in combination with metformin on tissue lipidomic profiling after high-fat diet feeding in mice	
P5-5	Ashino Hiromi, Yanai Yu, Sadamatsu Hisato, Mukai Katsuyuki, Oka K., Yao S., Nakajima Hiroshi, Iwase Katsuro	143
	Suppressive effects of fucoxanthin derived from a diatom, <i>Chaetoceros sp.</i> , on angiogenesis	
P5-6	Kwon Hyeji, Han Hyejin, Oh Yeonsoo, Kim Yuri, Kim Junghyun	144
	Anti-cancer effects of low-intensity exercise and genistein supplementation	
P6-1	Ikeda Chiaki, Manabe Yuki, Nami Tomonaga, Wada Tatsuya, Maoka Takashi, Sugawara Tatsuya	145
	Evaluation of inhibitory effect of halocynthiaxanthin on differentiation into pro-inflammatory macrophages and its gastrointestinal absorption	

P6-2	Betekhtin Alexander, Sala Katarzyna, Perez-Perez Renee, Kwasniewska Jolanta	146
	Buckwheat calli with different morphogenic potential as a unique system to study levels of both carotenoids and phenolic compounds	
P6-3	Bradley Madelyn, Hashida Megumi, Black Molly, Amengual Jaume, Erdman John	147
	Interaction of dietary lycopene with the ISX/SRB1 axis	
P6-4	Manabe Yuki, Maoka Takashi, Sugawara Tatsuya	148
	Dehydrogenation increases anti-inflammatory activity of siphonaxanthin	
P6-5	Göttl Vanessa L., Pucker Boas, Wendisch Volker F., Henke Nadja A.	149
	Screening of structurally distinct lycopene β -cyclases for cyclic C40 carotenoid production of β -carotene and astaxanthin in <i>Corynebacterium glutamicum</i>	
P6-6	Shukla Anushka, Bohn Torsten	150
	Exploring the impact of dietary fibers on carotenoid bioaccessibility and bioavailability	
P7-1	Giuffrida Daniele, Mertinez-Giron Jader, Osorio Coralía, Ordonez-Santos Luis Eduardo, Rigano Francesca, Cafarella Cinzia, Mondello Luigi	151
	Peach palm fruit (<i>Bactris gasipaes</i>) peel as a source of Provitamin A carotenoids	
P7-2	Ryo Asaka, Kazuki Ohshima, Shinji Kawasaki, Takashi Maoka, Chisato Tode, Zheng-Yu Wang-Otomo, Shinichi Takaichi	152
	Major carotenoids of meiothermus ruber are deinoxanthin glucoside esters, not meiothermoxanthin glucoside esters	
P7-3	Shinichi Takaichi	153
	Identification protocol of carotenoids	
P7-4	Focsan A. Ligia, Gao Yunlong, Polyakov Nikolay E., Kispert Lowell D.	154
	Hydrogen bonding and nonpolar environment applications to some rare carotenoids	
P7-5	Hoffmann Julian, Baldermann Susanne	155
	Structural elucidation of β -carotene oxygen copolymers by complementary analytical method	
P7-6	Línzembold Ildikó, Nagy Veronika, Agócs Attila, Deli József, Gulyás-Fekete Gergely	156
	Synthesis of phenolic acids esters of carotenoids	

P8-1	Parralejo-Sanz Sara, Gomez-Maqueo Andrea, Gloria Lobo, M. Pilar Cano	157
	Cactaceae as new carotenoid sources: Carotenoid characterization in <i>Opuntia</i> fruits and cladodes (<i>O. ficus-indica</i> , <i>O. dillenii</i> and <i>O. joconostle</i>)	
P8-2	Gómez-Maqueo Andrea, Parralejo-Sanz Sara, Gloria Lobo, Bandino Elisa, Cano M. Pilar	158
	Carotenoid and carotenoid ester deposition in plastids of <i>Pouteria lucuma</i> , <i>Persea americana</i> and <i>Diospyros kaki</i> and its effect on bioaccessibility	
P8-3	Molina-Márquez A., Hegemann P., Kelterborn S., Rengel R., Vigar J., León R.	159
	Comparative assessment of Cas9/sgRNA ribonucleoprotein complexes and herbicide treatment for PDS knock-out in chlorophyte microalgae	
P8-4	Homma Masato, Wakabayashi Takatoshi, Moriwaki Yoshitaka, Shiotani Nanami, Shigeta Takumi, Isobe Kazuki, Okazawa Atsushi, Ohta Daisaku, Terada Tohru, Shimizu Kentaro, Mizutani Masaharu, Takikawa Hirosato, Sugimoto Yukihiro	160
	Stereoselective orobanchol biosynthesis catalyzed by dirigent domain containing protein in tomato	
P8-5	Kwasniewska Jolanta, Betekhtin Alexander	161
	Buckwheat - a unique plant system to improve carotene production by using mutagenesis	
P8-6	Šimić Domagoj, Galić Vlatko, Jambrović Antun, Ledenčan Tatjana, Kljak Kristina, Buhiniček Ivica, Šarčević Hrvoje	162
	Relations among carotenoids and vitamin E compounds in a diverse inbred line collection of temperate maize	
P8-7	Takagi Tatsuyoshi, Hong Hung, Dillon Natalie, Alam Mobashwer, O'Hare Tim	163
	Identification of candidate genes affecting mango flesh colour and validation through GWAS	
P8-8	Kantoğlu K. Yaprak, İç Erhan, Özmen Dilan, Kantoğlu Ömer	164
	Changes in carotenoid and tocopherol contents of cherry tomato lines improved by radiation induced mutation breeding	
P8-9	Wakabayashi Takatoshi, Honma Masato, Takikawa Hirosato, Mizutani Masaharu, Sugimoto Yukihiro	165
	Conversion of carlactonoic acid to various strigolactones in plants	

P8-10	Mladenov Petko, Planchon Sébastien, Leclercq Céline, Renaut Jenny, Tomlekova Nasya	166
	Proteome profiling of sweet pepper genotypes (<i>Capsicum annuum</i> L.) with beneficial mutations	
P8-11	Chen Nan, Wu Fan, Peng Yu, Wen Xin	168
	Characterization of chromoplasts as natural food colorants and their stability under different storage conditions	
P8-12	Boba Aleksandra, Kochneva Yelyzaveta, Kulma Anna	169
	Role of apocarotenoids in the management of fusariosis in flax	
P8-13	Agarwal Rimjhim, Hong Hung, Hayward Alice, Harper Steve, O'Hare Tim	170
	Possible reasons for high zeaxanthin in orange capsicums	
P8-14	Ningrum Andriati, Sarifudin Achmat, Kumalasari Rima, Ekafitri Riyanti, Kristanti Dita, Setiaboma Raima	171
	In silico methodologies to improve antioxidant evaluation of carotenoids in soybean and protein hydrolysates from soybean dregs (Okara)	
P8-15	Mizuno Khoki, Ariumi Masashi, Miura Kenji, Takeda Satomi	173
	Modification of chloroplast pigment composition by overexpression of carotenoid biosynthesis enzyme genes	
P8-16	Julie Leroux, Ryan P. McQuinn, Thuy T. Truong, Barry J. Pogson	174
	Exploring mass spectrometry technologies to decipher metabolomic shifts during floral transition in <i>Arabidopsis thaliana</i>	
P9-1	Pintea Adela, Muntean Patricia, Gherasim Cristina, Bunea Andrea, Dulf Francisc V., Rugina Dumitrita, Focsan Monica	175
	Zeaxanthin loaded nanoemulsion systems – preparation, characterization and stability	
P9-2	Rugină Dumitrita, Gherasim Cristina, Nistor Mădălina, Muntean Patricia, Coman Cristina, Pintea Adela	176
	Bioaccessibility and cellular uptake of zeaxanthin from oil-in-water nanoemulsions	
P9-3	Prado-Cabrero Alfonso, Nolan John M.	177
	Low-tech production of astaxanthin-rich copepods: Towards a self-sustainable and decentralized food production system	

P9-4	Shi Hao, Pryor Colette, Green Marina, Power Tommy, Nolan John, Prado-Cabrero Alfonso	178
	Preparation, evaluation and bioavailability of a novel lutein-fortified yoghurt: Fortixan	
P9-5	Flynn Róisín, Prado Cabrero Alfonso, Green Marina, Nolan John	179
	β -carotene degradation in a sport isotonic beverage	
P9-6	Maeshima Nozomi, Kobayashi Manami	180
	Simultaneous determination of fat-soluble vitamins in beverages using a triple quadrupole mass spectrometer	
P9-7	Schmidt Mario, Fehn Simon, Tauber Svenja, Hopfhauer Sofia, Schwarzenbolz Uwe, Böhm Volker	181
	Impact of high pressure processing on lipophilic bioactive plant	
P9-8	Lietz Georg, Oxley Anthony, Goddard Alice	182
	Bioefficacy of β -cryptoxanthin in comparison to β -carotene using stable isotopes.	
P10-1	Power Tommy, Prado Cabrero Alfonso, Oakley Donna, Nolan John	183
	Assessment of dietary supplements containing lutein, zeaxanthin and meso-zeaxanthin and/or omega-3 fatty acids for compliance to label claim	
P10-2	Li Binxing, Chang Fu-Yen, Wan Zihe, Bernstein Paul S.	184
	Imaging macular carotenoids and their related proteins in the human retina with confocal resonance Raman microscopy	
P10-3	Fitzpatrick Naomi, Chachay Veronique, Shore Angela, Briskey David, Jackman Sarah, Capra Sandra, Bowtell Joanna	185
	Is electronic device use related to macular pigment optical density, dietary and plasma lutein and zeaxanthin in healthy Australian adults?	
P10-4	Sivaperuman Parimala, Gomez-Green Marina, Stringham James M., Roche Warren, Nolan John M.	186
	An evaluation of xanthophyll carotenoids, visual function and sport performance in an athlete population	
P10-5	Pap Ramóna, Pandur Edina, Jánosa Gergely, Horváth Adrienn, Sipos Katalin, Agócs Attila, Deli József	187
	Lutein and its metabolites modulate inflammation, oxidative stress, and lipid peroxidation at glutamate-induced neurotoxicity	

P10-6	Wysocky Cameron, Dandurand Jade, Hogan Kassidy, Beer Jenay, Hammond Billy, Renzi-Hammond Lisa	188
	Xanthophylls as moderators of cognitive functional improvement during a 6-month cognitive training intervention in older persons at risk for Alzheimer's disease or a related dementia	
P10-7	Addo Emmanuel Kofi, Susan Allman, Ranganathan Arunkumar, Joanna E. Gorka, Mohsen Sharifzadeh, Michael W. Varner, Paul S. Bernstein	189
	Infant ocular and systemic effects of prenatal carotenoid supplementation in the lutein and zeaxanthin in pregnancy (L-ZIP) trial	
P10-8	Arunkumar Ranganathan, Li Binxing, Addo Emmanuel, Chang Fu-Yen, Bernstein Paul	190
	β , β -carotene-9',10'-oxygenase 2 (Bco2-/-) mice are protected against oxygen-induced retinopathy (OIR) relative to wild-type (WT) mice	
P11-1	González J, Rengel R, Molina-Marquez Ana, Morillo David, Vigara Javier, León Rosa	191
	Carotenoid-rich microalgae extracts as antimicrobial agents against marine pathogens	
P11-2	Chino Tomoki, Saito Yohei, Takemoto Kayo, Kawai-Noma Shigeko, Seki Takahiro, Saito Kyoichi, Umeno Daisuke	192
P11-3	Grassino Maria, Batstone Damien, Hulsen Tim	193
	Resource recovery using mixed purple phototrophic bacteria for carotenoid production	
P11-4	Parralejo-Sanz Sara, Lara-Abia Sara, Lobo Gloria, Cano M. Pilar	194
	Formulation of a potential healthy ingredient using papaya carotenoid green extracts to improve carotenoids stability and bioaccessibility	

Abstracts of Luncheon and Sunset Seminars

Fuji Chemical Industries Co., Ltd.	197
Measuring Health: Future Considerations for Nutrition Research	
Panelists: Jennifer Lovejoy, Suzan Wopereis, John Nolan, Vincent Wood (Moderator), Karen Hecht (Moderator)	
OmniActive Health Technologies	199
Macular Carotenoids and the Eye-Brain Connection: A Panel Discussion on the Evolving Basic Science, Clinical Translation and Product Innovation Landscapes	
Panelists: Paul Bernstein, Rudarani Banik, Dan Stein, Deshanie Rai (Moderator)	

BASF SE

200

Why and how to fortify with carotenoids?

Panelists: Ute Obermueller-Jevic, Wolf Pelletier, Cosima Hirschberg, Christian Koepsel

Abbott Nutrition

201

Lutein as a modulator of oxidative stress: implications to health and disease

Panelist: Elizabeth J. Johnson

DSM Nutritional Products

202

Importance of Lutein throughout life stages

Panelists: Paul Bernstein, Lisa Renzi, Christian Schaefer

*Special Lecture, Invited Lectures
and
Oral Presentations*

Carotenoids in photosynthesis: unlikely molecules?

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If you were to write a job description for a pigment to be used in photosynthesis, then carotenoids would almost certainly not meet the set criteria. Yet they have proved to be very effective at both accessory light harvesting and photoprotection. In this lecture I will describe how a combination of structural biology, ultrafast time-resolved spectroscopy and theory have combined to show, in spite of their apparently inappropriate photochemical properties, carotenoids are able to fulfil their photosynthetic roles.

Using mainly examples from Purple Photosynthetic Bacteria I will describe the structure, organisation and function of carotenoids in both reaction centres and light harvesting complexes. I will show how progress in this field has followed developments in both structural biology and laser physics over the past forty years.

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Session 1:
Genetics, Biosynthesis and Metabolism
~ In memory of Prof. Brian H. Davies ~

***E. coli* as the tool of functional analysis for carotenogenic genes**Norihiko Misawa**Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi-shi, Ishikawa 921-8836, Japan.*

Carotenoids are biosynthesized in all photosynthetic prokaryotes that contain photosynthetic bacteria and cyanobacteria, in all photosynthetic eukaryotes including algae and land plants, and further in some non-photosynthetic bacteria, archaea, and fungi. Nowadays, carotenoid biosynthetic pathways are sufficiently elucidated at gene levels in bacteria, fungi, and higher plants. Also, in pathway engineering for isoprenoid (terpene) production, carotenoids have been one of the most studied targets. However, in 1988 when the author started carotenoid research, no carotenoid biosynthesis genes were identified. It was because carotenogenic enzymes are easily inactivated when extracted from their organism sources, indicating that their purification and subsequent cloning of the corresponding genes were infeasible or difficult. On the other hand, natural products-chemistry of carotenoids had advanced a great deal. Thus, those days, carotenoid biosynthetic pathways had been proposed based only on the chemical structures of carotenoids without findings on relevant enzymes and genes.

Escherichia coli naturally cannot biosynthesize carotenoids, and is amenable to gene manipulation. The first meeting of carotenoid biosynthesis genes and *E. coli* was the cloning of a 12.4-kb carotenogenic gene cluster from *Erwinia herbicola* (reclassified as *Pantoea agglomerans*) in *E. coli*, resulting in yellow pigmentation [1], significantly indicating that these genes were functionally expressed in *E. coli*. And then, studies on carotenoid biosynthesis at the gene level broke out through use of *E. coli* as the tool of functional analysis for carotenogenic genes, which was also triggered to rapid advance on carotenoid research in biochemistry, molecular biology, and pathway engineering. **Figure 1** shows examples of the functions of carotenoid biosynthesis genes from plants, algae, and bacteria, which have been identified with the *E. coli* functional analysis system up to the present [2-4].

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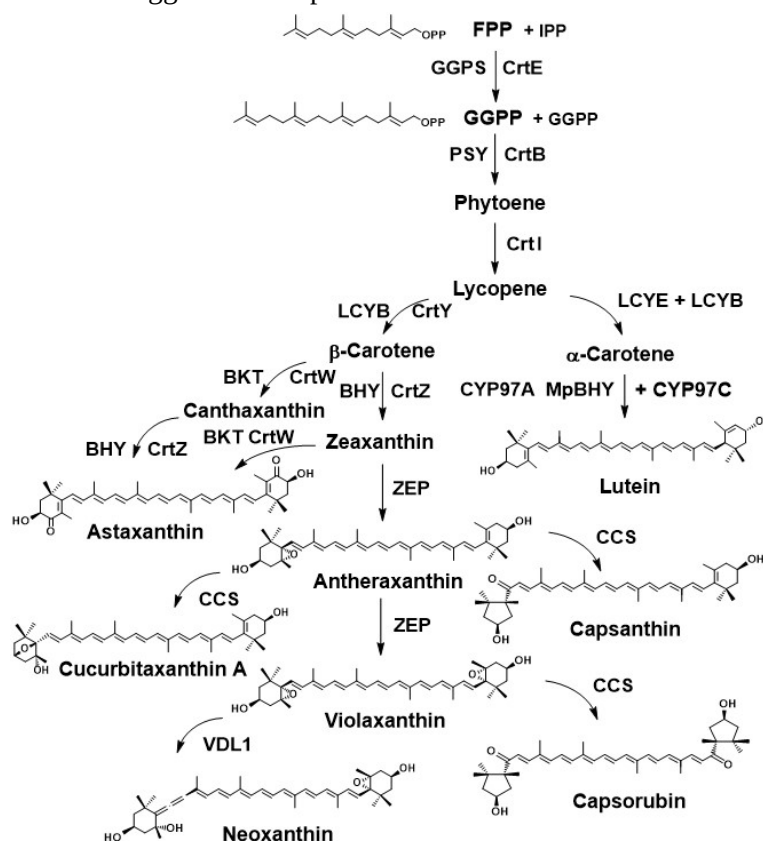


Figure 1. Carotenoid biosynthetic pathway of land plants and algae, whose carotenogenic genes were functionally expressed in *E. coli*. Crts are derived from bacteria.

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Prof. Norihiko Misawa –A Pioneer in Carotenoid Molecular Biology

Gerhard Sandmann: **From Erwinia towards yellow cyclamen and beyond**

Paul D. Fraser: **From yellow to red, elucidation of astaxanthin formation at Kirin Brewery**

These presentations highlight the scientific career of Prof. Misawa working both in industry and academia. He pioneered the cloning and functional identification of genes from different carotenoid biosynthesis pathways. This overview commemorates his PhD work, his great research achievements, while visiting Konstanz University as a post-doc and illustrates his time with the Kirin company, developing bio-based metabolic engineering prototypes for the production of valuable carotenoids. Finally, it concludes with his appointment to Ishikawa Prefectural University

Crocins in plants: much more beyond saffron

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1. Introduction

One of the most commercially valuable apocarotenoid pigments, crocetin, is obtained from saffron stigmas and gardenia fruits. Crocetin is present in these tissues as glucosylated isoforms known as crocins. Economically, crocins rank first among modern natural color additives. In *Crocus* species, CCD2 drives zeaxanthin catabolism at positions 7,8;7',8' yielding crocetin-dialdehyde, further transformed into crocins. Besides CCD2, inside the CCD4 subfamily, specific CCD4 enzymes from *Buddjela davidii* and gardenia act on carotenoids at positions 7,8;7',8' to generate as well crocins. *Verbascum* is a widespread genus of the family Scrophulariaceae, which comprises among 300 to 325 Eurasiatic species. Many of them have been used to treat a wide range of pathologies including pulmonary problems, asthma, spasmodic coughs, inflammatory diseases, diarrhoea, migraine headaches and malaria. In this work, we determine the apocarotenoid content in the flowers of two *Verbascum* species and identified four CCD enzymes with high homology to the enzymes previously characterized in *B. davidii* [1].

2. Experimental

Apocarotenoid and carotenoid analyses, enzymatic assays in bacteria and plants were done as previously described [2].

3. Results and Discussion

The levels of carotenoid and apocarotenoids were analyzed in the petals of two *Verbascum* species. In both cases the crocins profile was similar to the one observed in saffron. Transcriptome analyses allowed the identification of CCD4 homologs to the enzymes previously identified in *Buddleja* and catalyzing the production of crocins. These enzymes were tested for activity in bacteria and *Nicotiana benthamiana* plants, using a virus-driven system. Metabolic analyses of bacteria extracts expressing these enzymes and infected tissues demonstrated the activity of the CCD4 enzymes for the production of crocins using β -carotene and zeaxanthin as substrates. Accumulations of 0.19% of crocins in leaf dry weight were reached in only two weeks using the recombinant virus that expressed VgCCD4.1, as the amount previously produced using the enzyme from saffron. The identification of these enzymes with a broad substrate spectrum, opens new possibilities for their commercial application.

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Carotenoid turnover in leaves studied by $^{13}\text{C}\text{O}_2$ labeling and LC-MS

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1. Introduction

Carotenoids are accessory pigments of photosynthesis that play vital roles in photoprotection by enhancing thermal energy dissipation and scavenging reactive oxygen species (ROS). Chemical ROS scavenging results in oxidative cleavage of carotenoids, as has been shown for β -carotene under high light (HL) and low temperature stress [1]. Notably, β -carotene and chlorophyll *a* undergo continuous degradation and replacement even in non-stressful light conditions [2]. Although previous studies with radioactive $^{14}\text{C}\text{O}_2$ labeling revealed turnover of these pigments in *Arabidopsis* leaves [2, 3], we still lack quantitative information on the turnover. Furthermore, ^{14}C incorporation could not be detected for xanthophylls while their pool size was substantially increasing during HL exposure [2]. In the present study we therefore sought to estimate the turnover rates of different carotenoids and figure out the source of stress-induced xanthophyll accumulation.

2. Experimental

To perform long-term labeling with stable $^{13}\text{C}\text{O}_2$, we constructed a chamber (Figure 1) which allowed monitoring of CO_2 concentration, light intensity, air temperature, relative humidity and overpressure [4]. Following $^{13}\text{C}\text{O}_2$ labeling of different durations, leaves were harvested from *Arabidopsis* plants and leaf area was quickly determined by image analysis. Pigments were extracted from the leaves to assess carotenoid concentrations and isotopolog compositions by LC-MS [4, 5].



Figure 1. Long-term $^{13}\text{C}\text{O}_2$ labeling chamber with *Arabidopsis* plants inside

3. Results and Discussion

We did mass peak assignment of violaxanthin (Figure 2) in addition to lutein and β -carotene reported before [4, 5]. Our $^{13}\text{C}\text{O}_2$ labeling and LC-MS methods produced highly reproducible results, thus enabling the turnover analysis in steady-state conditions. The results from time course experiments indicated distinct turnover rates for different carotenoids, with the highest value for β -carotene followed by lutein and then violaxanthin. The HL-induced accumulation shifted the balance between ^{13}C -labeled and non-labeled isotopologs of xanthophylls, which could be largely explained by *de novo* synthesis of these pigments using newly fixed carbon. Whilst β -carotene is the precursor of zeaxanthin in the biosynthetic pathway, pool size enlargement of the xanthophyll-cycle pigments does not seem to rely on β -carotene molecules bound in reaction centers and core antenna complexes.

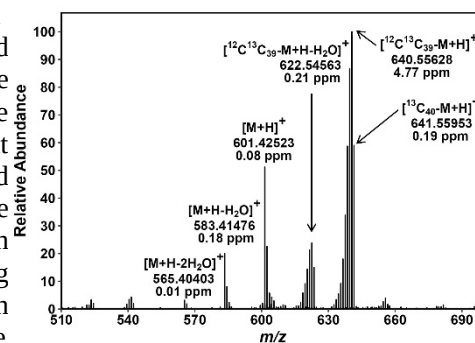


Figure 2. High-resolution mass spectrum of violaxanthin extracted from ^{13}C -labeled *Arabidopsis* leaves

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Biofortification Breeding for Higher Levels of Macular Carotenoids and ProVitamin A Carotenoids in Maize Grain: An Update

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Introduction and Summary

This presentation will provide an update and summary of genetics and biofortification efforts designed to increase levels in maize grain of provitamin A carotenoids for Sub-Saharan Africa and levels of the macular carotenoids lutein and zeaxanthin for USA. Considerable progress has been made for identifying genes associated with levels of carotenoids in maize, with 11 biosynthetic genes controlling most of the variation. Progress has also been made in identifying genes associated with yellow and orange color in maize grain, important for acceptance of biofortified corn grain in Africa. Considerable progress has been made for selecting for higher proVA carotenoids in Africa through marker assisted selection of the genes phytoene synthase, lycopene epsilon cyclase, and beta-carotene hydroxylase. In the USA, HPLC based phenotypic selection has been very effective in breeding for higher levels of lutein and zeaxanthin in parallel with selection for higher grain yields, using subtropical orange germplasm crossed with temperate yellow germplasm derived from former plant variety protected inbreds.

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Metabolic responses and regulation of carotenoid and flavonoid biosynthetic pathways in lettuce (*Lactuca sativa*) in protected cultivation

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1. Introduction

In 2019, an area of more than 5 million ha was used for protected agriculture worldwide. Protected cultivation systems including polytunnels can be one approach to increasing productivity and yields as well as improving the nutritional quality of crops. An important aspect of crop production in protected cultivation is the choice of covering material. Greenhouse covers can be used to control temperature and light regimes, in particular, to trigger adaptations in the metabolic response of plants and hence, provide also approaches to boost secondary metabolites such as carotenoids in vegetables. However, little is known how horticultural crops regulate the biosynthesis, accumulation, and degradation of carotenoids and flavonoids in protected cultivation. The aim of this study was to advance our understanding of the metabolic regulation of carotenoid and flavonoid biosynthetic pathways in lettuce.

2. Experimental

To gain insights into regulatory mechanisms we grew lettuce (*Lactuca sativa* var *capitata* L., cultivar ‘Veronique’) without or under polytunnels, testing two different covering materials. Climatic conditions were monitored during the experiments, and carotenoid and flavonoid profiles as well as the phytohormone (ABA) content were determined by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Promising target gene candidates encoding key biosynthetic enzymes of both metabolic pathways (PSY, CCD4 and CHS) as well as light-dependent transcription factors (HY5, PIF1, UVR8, OR and OR-like) were analyzed by RT-qPCR. The metabolic flux of the upper carotenoid pathway was determined using the bleaching herbicide norflurazon [1].

3. Results and Discussion

We found that polytunnel cultivation of lettuce leads to higher total and individual carotenoids and in lower overall flavonoid glycoside contents, mainly related to different spectral properties of the light (quality and intensity) as well as higher temperatures due to the covering material. Whereas the transcripts of flavonoid-related biosynthetic enzymes were associated with flavonoid content in lettuce, the regulation of carotenoid metabolism is more complex. PIF1 transcripts are associated with biosynthetically active genes, suggesting an involvement of phytochromes. Transcripts of UVB photoreceptor UVR8 were lower in lettuce grown under polytunnels, but did not show an association with carotenoid content. Overall, the higher carotenoid content seems more likely to be the result of higher metabolic flux. Additionally, the study demonstrates that different mechanisms, at both the transcriptional (flavonoids) and posttranscriptional (carotenoids) levels, influence the accumulation of bioactive compounds in lettuce. The inverse relationship between flavonoid and carotenoid contents is possibly caused by physicochemical mechanisms rather than a shared transcriptional signaling pathway [1].

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A novel regulatory mechanism to synchronize photosynthetic pigment synthesis in plants

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Optimal photosynthesis in plants requires coordinating the biosynthesis of two groups of photosynthetic pigments, carotenoids and chlorophylls. However, how these two biosynthesis pathways are coordinated, particularly at posttranslational level to allow rapid control, remains largely unknown. We discovered that the DnaJE1 ORANGE (OR) family chaperone proteins, which are highly conserved in the plant kingdom, are the common regulators of both pathways by posttranslationally regulating the first committed enzyme in each pathway. OR family proteins are the master regulators of phytoene synthase to control carotenoid biosynthesis in chloroplasts. They also physically interact with magnesium chelatase subunit I to regulate chlorophyll biosynthesis. OR proteins concurrently regulate phytoene synthase and magnesium chelatase subunit I enzyme protein stability and activity. Loss of the *Or* family genes hinders both carotenoid and chlorophyll biosynthesis, limits light-harvesting complex assembly, impairs thylakoid grana stacking in chloroplasts, and reducing photosynthesis efficiency. Overexpression of *Or* safeguards photosynthetic pigment biosynthesis and enhances plant thermotolerance. Our findings establish a conserved mechanism of green plants to coordinate carotenoid and chlorophyll photosynthetic pigment biosynthesis and provide a potential genetic target to generate climate-resilient crops.

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Color diversity in fruits of ancestral *Citrus* species: A comparative study of carotenoid metabolism during maturation

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1. Introduction

Citrus is one of the most important fruit-tree crops worldwide. Citrus fruits exhibit a wide range of color diversity with large differences in carotenoids content and composition. However, the molecular basis of this genetic trait has not been fully elucidated. In recent years, the exhaustive sequencing of *Citrus* genomes has revealed that most of the commercial citrus varieties are genetic admixtures derived from interspecific hybridization from the five ancestral species: papeda micrantha (*C. micrantha*), kumquat (*C. japonica*) citron (*C. medica*), pummelo or shaddock (*C. maxima*) and mandarin (*C. reticulata*) [1]. Interestingly, fruit of micrantha remain pale-green during ripening, those of citron and pummelo are yellow, whereas ancestral mandarin and kumquat are the only species displaying orange pigmentation (Figure 1). To better understanding the origin of fruit color diversity among the genus *Citrus*, the objective of this work has been to perform a comprehensive analysis of carotenoid composition and a comparative genomic and transcriptomic analysis of selected genes of chlorophyll and carotenoids metabolism in fruit of the five ancestral species.

2. Experimental

Fruits of Chandler pummelo (*C. maxima*), Sun Chu Sha Kat mandarin (*C. reticulata*), Diamante citron (*C. medica*), Nagami kumquat (*C. japonica*) and micrantha (*C. micrantha*) were harvested periodically at different ripening stages from the Citrus Germplasm bank at IVIA (Moncada, Valencia, Spain) and Botanical Garden (University of Valencia) (Figure 1). The peel of the fruits was collected, and pigments were extracted and analyzed as described [2]. Gene expression was performed essentially as described [2].

3. Results and Discussion

Important differences in both carotenoid content and composition were detected in the peel of fruit of the five ancestral species after the breaker stage. The diversification of fruit coloration was not related to total carotenoids content being more likely related to an early loss of chlorophylls, and the accumulation of specific β , β -xanthophylls and derived apocarotenoids. Transcriptomic results revealed a myriad of patterns in which the differential expression of carotenoid-cleavage dioxygenases, lycopene-cyclases and other genes involved in xanthophylls esterification appears to be correlated with the singular carotenoid profiling of the fruit of the different species. An integrated analysis of the allelic diversity of key genes of the carotenoid pathway with transcriptomic and metabolic results in the ancestral citrus species will be presented and discussed.

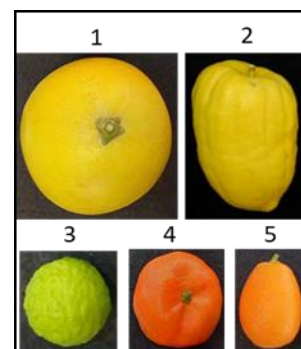


Figure 1. Representative mature fruit of pummelo (1), citron (2), micrantha (3), mandarin (4) and kumquat (5). Pictures are not to scale.

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Session 2:
New Trends of Carotenoid Science

Carotenoids: the plant toolboxBruno Robert**Institute of Integrative Biology of the Cell, CEA Sclay, 91191 Gif sur Yvette Cedex,*

Carotenoid molecules intervene at several essential levels in the life of plants, and can be found in their tissues, from the roots to the flowers. They are essential constituent of the light-harvesting system, and also play a (photo)protective roles at the level of the photosynthesis apparatus and the chloroplast membranes. They confer colour to fruits and flowers, and some of their volatile breakdown products confer to these organs their scent. Finally, apocarotenoid constitute essential plant hormones involved stress response, plant development and signaling. The ensemble of functions of carotenoids and carotenoid-derived molecules in plant will be presented and summarized during the conference.

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Is Lutein Ready for Public Health Dietary Recommendations?

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There are a number of essential and non-essential food components in foods that we consume. Epidemiologic findings and clinical data support the health benefits for some non-essential food components – often referred to as bioactives. In most countries there are well-established processes in place to make science-based dietary recommendations for essential nutrients. However, only a few countries have a mechanism for setting numerical intake recommendations for qualified bioactive food components. For example, China and South Korea have established intake ranges for lutein. This presentation will review the DRI framework for establishing requirements for essential nutrients for North America. Then, the potential of development of public health recommendations for bioactives will be explored and the benefits and challenges of making recommendations will be reviewed. The biological concentration of lutein in the macula of the eye, and the fact that macular degeneration is the primary cause of blindness in persons over 65 years of age provides a compelling rationale for evaluating the relationship of dietary lutein and reduced risk of age-related macular degeneration (AMD). We previously providing an argument for considering dietary intake recommendations for lutein for reduction of risk of AMD [1] utilizing nine criteria [2]. We concluded that based upon the published literature at that time that lutein was ready for consideration for intake recommendations. Recent scientific support, published in the last few years, will be reviewed. Finally, suggestions for frameworks that might be considered by authoritative policy-making bodies when evaluating the efficacy and safety of proposed bioactives will be discussed. Providing dietary guidance for lutein would encourage consumption of lutein-rich foods and perhaps reduce the risk of AMD, among other health benefits.

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So many International Symposia on Carotenoids (19!!), so few industrially produced carotenoids... What are the problems?

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1. Is there a general compromise on the list of carotenoids produced on a multi-ton scale?

β -carotene (chemical synthesis, *Blakeslea trispora* -fungus), lutein (marigold), canthaxanthin (chemical synthesis), astaxanthin (*Haematococcus pluvialis* -microalgae), lycopene (tomato extract, chemical synthesis, *Blakeslea trispora* -fungus), zeaxanthin, etc.

2. What are the obstacles for large-scale production? How to remove them to produce more carotenoids?

Increasing chemophobia from general population. Slow progresses from molecular biology, it took decades to achieve high carotenoid concentrations in biomass (>30-40 g carotenoid/kg dried biomass) or in fermenters (39.5 g/L β -carotene using *Yarrowia lipolytica*) [1].

Not so many big world players, BASF, DSM, Allied Biotech, LycoRed, Algatechnologies, etc. As a whole, they are not doing very well, slow progress in the number of carotenoids launched on the market, these last decades. This slows down the benefits that humanity could have with carotenoids. Progress in industrial aryl carotenoid production? (isorenieratene and hydroxyl derivatives, EP1539984A2 patent in 2003 by Du Pont de Nemours). Progress in industrial rhodoxanthin production (US3624105A patent in 1976 by Hoffmann La Roche; WO2019016384A1 patent in 2019 by DSM)? As few examples...

A significant step forward would be to create a non-profit fund financed by the largest groups, which would be able to encourage the large-scale production of new carotenoids (so many to choose among 1100), with free-licenses on patents. Access to the market is also a huge step, very costly, with FDA, EFSA, SFA clearances and approvals.

3. Do we need pure carotenoids or mixtures? or both?

Mixed carotenoids from *Paracoccus carotinifaciens* (adonixanthin 33.2% of total carotenoids, 3'-Hydroxyechinenone 14.6%, astaxanthin 11.5%, canthaxanthin 9.5%, adonirubin 9.4%, + 6 more), from palm oil (β - carotene, α -carotene, lycopene, + 10 more) [2], from *Rhodotorula glutinis* (β -carotene, torulene and torularhodin), etc. Technical and health properties better monitored with pure carotenoids?

4. Carotenoids from GMOs, social acceptability may vary from one part of the world to another

Novel genetic engineering technologies are being investigated to improve productivity and reduce the cost of harvesting carotenoids extracted from or excreted by various living organisms. However, high resource efficiency and low costs alone are no guarantee that carotenoids from GMOs will find their way into the market. Technologies must be accepted by the public to become valuable for society [3].

[1] Removal of lycopene substrate inhibition enables high carotenoid productivity in *Yarrowia lipolytica*. Ma, Y., Liu, N., Greisen, P., Huang, S., Stephanopoulos, G., *Nature Communications* **2022**, 13(1), 572 | <https://doi.org/10.1038/s41467-022-28277-w>

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Novel approaches to produce and store carotenoids in plant cellsManuel Rodriguez-Concepcion**Institute for Plant Molecular and Cell Biology (IBMCP), CSIC-UPV, 46022 Valencia, Spain*

Biofortification of foodstuffs with health-promoting metabolites such as carotenoids is a powerful tool to fight against hidden hunger and unhealthy eating habits. One of the last frontiers in plant carotenoid biotechnology is enriching plant leaves with carotenoids, as the key role of these pigments for photosynthesis and photoprotection in chloroplasts normally prevents major manipulations. In the last few years, major advances have been made in our understanding of how plant cells make and store carotenoids in chloroplasts and chromoplasts, the plastid types in which carotenoids accumulate at highest levels [1]. Novel molecular mechanisms regulating the levels and activity of carotenoid biosynthetic enzymes have been unveiled [2,3] and new strategies are now available to promote the differentiation of leaf chloroplasts into artificial chromoplasts with specialized subplastidial structures that provide an optimal environment for these enzymes and for the sequestration of their products [4,5]. An alternative to producing carotenoids in their natural cell compartment - the plastid - is moving the production and storage of carotenoids to extraplastidial locations [6]. Our results show that combination of these strategies boosts the accumulation but also the bioaccessibility of health-promoting carotenoids in plant tissues that have been recalcitrant to biofortification such as leaves.

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Bioefficacy of Nixtamalized High β -Cryptoxanthin Biofortified Maize and a Novel β -Cryptoxanthin Supplement

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1. Introduction

β -Cryptoxanthin (β CX) is an interesting carotenoid because it is a xanthophyll and possesses vitamin A (VA) activity (**Figure 1**). In an animal model, our research group has demonstrated that β CX from supplements and food sources had similar VA bioefficacy to β -carotene despite having half of the theoretical yield [1, 2]. Consuming β CX instead of preformed VA in food and supplements may improve bone health and mitigate hypervitaminosis A. We have traditionally bred high provitamin A orange maize with significant amounts of β CX through biofortification to improve its health profile. Maize is an important staple crop, and it is processed for consumption in many different ways. Nixtamalization is an ancient technique that involves soaking the grain in lime (calcium hydroxide). This processing improves the bioavailability of B vitamins. The interactions of food processing and micronutrients are important areas of research to optimize nutritional status.

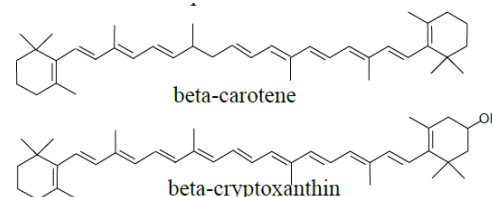


Figure 1. Chemical structures of all-trans- β -carotene and β -cryptoxanthin

2. Experimental

Two studies evaluated the bioefficacy of β CX from biofortified nixtamalized and non-nixtamalized maize and a novel β CX supplement using male Mongolian gerbils. After 3 weeks VA depletion, baseline ($n = 6$) was established and remaining gerbils ($n = 66$) were divided into Study 1, which consisted of nixtamalized, whole grain, and refined maize ($n = 10/\text{group}$) with similar theoretical VA; and Study 2, which included a novel β CX supplement, VA-free maize (negative control), and VA oil dose (positive control) ($n = 12/\text{group}$). High performance liquid chromatography was used to quantify retinol, retinyl esters, β -carotene, and β CX in serum, liver, spleen, kidneys, brain, eyes, and lungs.

3. Results and Discussion

All maize and β CX treatment groups maintained baseline VA concentrations in all tissues except brain ($P < 0.0001$). In both Studies 1 and 2, total liver VA and VA concentration ($\mu\text{mol/g}$) did not differ among treatment groups but were higher than those of the negative control group ($0.044 \pm 0.029 \mu\text{mol/g}$, $0.119 \pm 0.073 \mu\text{mol/liver}$; $P < 0.001$). Liver β CX concentration ($\mu\text{mol/g}$) for the degermed (0.0062 ± 0.0017), nixtamalized (0.0055 ± 0.0021), whole grain (0.0062 ± 0.0023), and β CX supplement (0.0072 ± 0.0025) groups did not differ among the groups but differed from baseline and control groups ($P < 0.0001$). A novel β CX supplement performed as well as a VA oil dose in maintaining VA status based on liver VA concentration. The gerbils were able to maintain or increase VA concentrations in the eye, but not the brain, when compared with baseline.

Despite lime and heat treatment, bioefficacy of β CX from nixtamalized maize was not different from whole-grain or degermed maize. Data from children in Zambia revealed that consuming whole grain and restricting preformed VA intake improved vitamin B6 status. Introducing nixtamalization in maize consuming countries may also improve B vitamin status without compromising provitamin A bioefficacy.

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Breaking Symmetry: laboratory evolution of carotenoid pathways to explore extra-natural space of carotenoid functions

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1. Introduction

Studies on the physical properties of organically synthesized artificial carotenoids have shown that gradients in molecular polarity or electron affinity along the molecular backbone often dramatically enhance the energy transfer efficiency or nonlinear optical materials. However, such asymmetric carotenoids are difficult to biosynthesize in general, because all carotenoids are biosynthesized via symmetric precursors, phytoene or 4,4'-diapophytoene. Many carotenoid desaturases and decorating enzymes are known to be locally-specific in substrate specificity, and it is nearly impossible for them to distinguish between the two ends of the symmetric precursors. Therefore, carotenoid pathways reconstituted in heterologous cells is destined to yield symmetric carotenoids with identical ends as main products. This fact has also greatly limited the variation in the optical properties of carotenoid pigments accessible to by synthetic biologists. In this talk, I will present an engineering strategy that breaks this skeletal symmetry of carotenoid, and report on the successful biosynthesis of a number of carotenoids with various optical properties that neither nature nor synthetic biologists have never been able to access.

2. Experimental

By combining evolutionary tuning of the size distribution of prenylpyrophosphate in *E. coli* cells and introducing asymmetry inside the reaction pocket of the carotenoid backbone synthase CrtM, we have established a pathway for biosynthesis of numbers of asymmetric phytoenes consisting of two building blocks with different sizes. The novel "phytoene routes" we established this way include: C₁₅+C₂₀, C₁₅+C₂₅, C₁₅+C₃₀, C₁₅+C₃₅, C₂₀+C₂₅, C₂₀+C₃₀, C₂₀+C₃₅, C₂₅+C₃₀, C₁₀+C₁₅, and C₅+C₁₀. Additional and systematic expression of carotenoid desaturases, cyclases, ketorases, hydroxylases, glycosylases, and glycosylases revealed that each of these carotenogenic enzymes has unique size selectivity.

3. Results and Discussion

As a result, we have succeeded in establishing selective routes to numbers of novel carotenoids with unique photochemical/ physical properties. In this talk, we would introduce numbers of one-sided desaturated carotenoids, one-side tethered carotenoids with polar groups, carotenoids with very rare chromophores (c.d.b. = 4 and 6), non-natural carotenoids with fluorescent properties (c.d.b.=5), solvent-extractable carbon-saving "mini"-carotenoids, selectively biosynthesized in *Escherichia coli*.

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Conductance control in the carotenoid molecular wires by phenyl substituents

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1. Introduction

Carotenoids are characterized by the conjugated polyene chain, which endows their energy transport property in photosynthesis as well as antioxidant activity in biological system. Delocalization of π -electrons in the polyene chain provides the carotenoids with electron-conducting ability as organic molecular wires. We were interested in controlling the conductance of carotenoid molecular wires by introducing phenyl substituents of diverse electronic natures in various positions along the polyene chain (**Figure 1**). The conductance of carotenoid molecular wires can be measured by STM break junction method utilizing gold-sulfur interactions [1]. *para*-Methylthiophenyl end group was utilized as the anchoring unit to a gold plate as well as to a gold tip in STM. Carotenoid wires with X-substituted (OMe, Me, H, Br or Cl) phenyl groups were synthesized, which were located in various positions at 9,9' for **1**, 10,10' for **2** [2], 13,13' for **3** [1], and 9,9',13,13' for **4** [3] in the polyene chain.

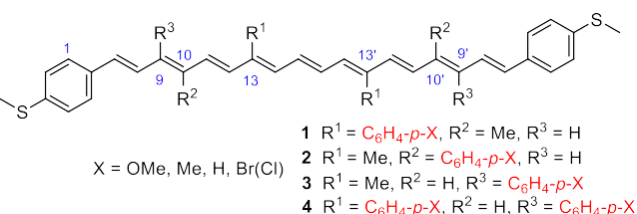


Figure 1. Chemical structure of carotenoid molecular wires with variable conductance by phenyl substituents.

2. Experimental

Four carotenoid wires (X = OMe, Me, H, Br or Cl) of all-*trans*-configuration were synthesized in each series of **1**, **3**, and **4**, and only one case (X = H) was demonstrated for **2**, which gave 9,9'-*cis* configurations. All the synthesized carotenoids were confirmed by NMR, IR, and High-Resolution Mass Spectroscopies. The conductance of carotenoid wires was measured by STM break junction method in diluted (0.5 μ M) mesitylene solution. The conductance histogram is demonstrated in **Figure 2** for 9,9'-diphenylcarotene series **1**.

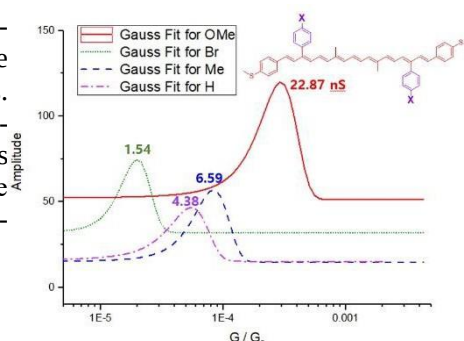


Figure 2. Gaussian fit to the conductance histogram of 9,9'-diphenyl carotenoids **1** with substituent X (OMe, Br, Me, H) by the repeated break-junction method.

3. Results and Discussion

Electron-rich phenyl substituents render higher conductance value to the carotenoid wires, and 9,9'-position is slightly more effective than 13,13'-position in controlling the carotenoid conductance. Highest conductance values were observed for 9,9',13,13'-tetraphenyl case. We also demonstrated all-*E*-carotene with 9,9',13,13'-tetramethyl substitution is almost twice more conducting than its 9-*cis* isomer [4].

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Session 3:
Astaxanthin Symposium

Interventions against gut frailty lead to healthy longevityYuji Naito**Department of Human Immunology and Nutrition Science Kyoto Prefectural University of Medicine*

“Gut frailty” is named in the sense of frailty of gastrointestinal function. The important point is that gut frailty may be an exacerbating factor of various diseases, a cause of chronic inflammation, and a precursor to systemic frailty. In particular, constipation is an important factor for gut frailty, and it has been shown that constipation patients have significantly lower survival rates after 10 and 15 years than those without constipation. Furthermore, chronic constipation is reported to increase the risk of developing chronic kidney disease, acute myocardial infarction, and Parkinson's disease. A cross-sectional survey also revealed that constipation was more frequent in the frailty group than in the non-frailty group. A weakened intestinal barrier mechanism likely contributes to gut frailty. Hyposecretion of mucus is thought to be an extremely early pathological condition. There are many factors involved in mucus reduction, such as environmental factors such as high-fat diets and microplastics, drugs, and dysbiosis of the intestinal flora. The antioxidant action of astaxanthin is well known, but in this lecture, I would like to explain its effects on the intestinal flora and the intestinal environment. I would like to introduce the concept of gut frailty, understanding its pathogenesis, and the importance of maintaining the intestinal barrier function by the gut microbiota.

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Astaxanthin treatment improves endurance and ameliorates insulin resistance in the muscle of obese mice.

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1. Introduction

Astaxanthin (AX), a natural antioxidant, has been shown to ameliorate insulin resistance in animals. Carotenoids are widely known for their antioxidant activities, and it has been shown that AX, a xanthophyll carotenoid, possesses a particularly high antioxidant capacity, as it has more conjugated double bonds than many other carotenoids. AX has also been demonstrated to ameliorate hepatic insulin resistance in obese animals and partially improve metabolic parameters in obese humans. However, no systematic investigation has been conducted yet to clarify whether AX improves the insulin sensitivity in skeletal muscle of obese mice.

2. Methods

We used 6-week-old male C57BL/6J mice fed with normal chow (NC) or NC supplemented with AX (NC+AX) and HFD or HFD supplemented with AX (24 weeks). We assessed effect of AX on various parameter including insulin sensitivity, glucose uptake, inflammation, kinase signalling, gene expression and mitochondrial function in muscle.

3. Results

We demonstrated that AX activated AMPK in the muscle and upregulated the expressions of a transcriptional coactivator and transcriptional factors, thereby inducing mitochondrial remodeling, including increased mitochondrial oxidative phosphorylation and FFA metabolism. Furthermore, AX treatment increased the endurance and attenuated glucose intolerance in HFD mice. Thus, we identified AX as a potent exercise mimetic. Both *in vivo* and *in vitro* studies revealed that AX administration enhanced AMPK activation in the skeletal muscle, along with upregulation of the expressions of the genes associated with metabolic and mitochondrial oxy-phosphorylation, independently of its antioxidant activities. A hyperinsulinemic-euglycemic clamp study also revealed that AX improved glucose metabolism by improving glucose incorporation into peripheral target tissues, such as the skeletal muscle, rather than by suppressing gluconeogenesis in the liver of the HFD mice; this was considered to be attributable to activation of the AMPK pathway in the skeletal muscle, leading to enhanced mitochondrial activity and FFA utilization in the HFD mice.

4. Conclusion

We concluded that AX treatment stimulated mitochondrial biogenesis and significantly ameliorated insulin resistance through activation of AMPK pathway in the skeletal muscle.

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The Potential Role of Astaxanthin in Metabolic Health and Exercise Performance

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Metabolic improvement has an important role in prevention of several non-communicable diseases, including type 2 diabetes, obesity, cardiovascular diseases, and cancer. With development of oxidative stress research, the benefit of dietary antioxidant combined with exercise training for preventive diseases and physical performance has been debated for over 20 years. The specific actions of antioxidant may be responsible for structure of each compound and physical properties of subjects such as sex, age, and individual performance. Astaxanthin is a unique antioxidant that contributes to metabolic regulation. Dietary astaxanthin causes upregulation of key metabolic modulators, e.g. proliferator-activated receptor gamma coactivator 1-alpha and AMP-activated kinase in skeletal muscle, leading to activation of mitochondrial aerobic metabolism. The muscle metabolic improvement can contribute to weight management and preventive fatigue during exercise. Growing evidence has shown that skeletal muscle regulates whole-body metabolic homeostasis by communicating with systemic organs via neural and endocrine routes. Skeletal muscle secretes several bioactive factors, which referred to the myokine. In contrast, various fluid factors derived from other organs affect the metabolic function in skeletal muscle. Here, I would provide the possibilities of dietary astaxanthin with advancements in metabolic and secretory functions of skeletal muscle and a perspective on its future implications.

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Why is astaxanthin a perfect dietary supplement?

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MBA Kaiviti Consulting, LLC

1. Introduction

Natural products are fundamentally linked to health and wellness, creating a state of balance whilst being alert for threats and disruptions. Dietary sources are often adequate, but for astaxanthin (ASX) dietary intake is never enough. Supplementation is the only answer.

2. Structure

The unique structure of astaxanthin facilitates the formation cross-membrane bridges, where the polar ends are embedded in the protein layers and the aliphatic chain crossing the lipid layer. Astaxanthin's structure is solely suited for this critical positioning. The lipophilic nature of ASX allows it to cross Blood Brain and Retinal Barriers that allows for specific brain and vision benefits.

3. Function

There are many actions of astaxanthin that are difficult to replicate with other natural products, either in breadth, potency or specificity. Firstly, it is a pre-eminent free radical scavenger, and here this action is pure. In other words, while it functions to quench the unpaired electron that constitutes a free radical, ASX in of itself does not become a free radical. This potent action means that ASX is very effective at limiting oxidative stress across many sources e.g., UV radiation (eyes, skin), mitochondrial dysfunction or inflammation. ASX modifies gene expression. This epigenetic action restores balance to a wide range of genes involved in tissue repair (activates) and inflammation (suppresses). ASX has positive actions on metabolism, limiting lactic acid accumulation and promoting endurance. How else can salmon make their remarkable journeys up rivers to spawn?

Finally, ASX has truly desirable actions as a pre-eminent mitochondrial nutrient, where it limits the oxidative stress resulting from dysfunctional, leaking mitochondria, whilst taking off-line these damaged mitochondria (mitophagy) and replacing them with new healthy ones. Critical actions that make astaxanthin an essential nutrient for healthy aging.

4. Conclusion

Astaxanthin has diverse and unique health-promoting actions, but unfortunately dietary sources are woefully inadequate. All things considered astaxanthin is the perfect dietary supplement.

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Astaxanthin: Discovery, History, and Distribution in Nature

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Astaxanthin, a fat-soluble xanthophyll carotenoids, is ubiquitous in nature. Throughout human history, astaxanthin has only been recognized as the characteristic red or pink/orange color found in certain organisms. However, due to its unique biological activity, it has the potential to be a solution to diverse global environmental and social issues that threaten life on earth, thereby achieving significant industrial growth. Unfortunately, almost 90 years after the discovery of this pigment, the story of its discovery and knowledge of its structure is somewhat fading in contrast to the great attention it has received. What organisms does astaxanthin exist in and are they exactly the same? The answer is often ignored. Therefore, this symposium will provide a comprehensive introduction to the distribution of astaxanthin in nature, its structural differences, and biological significance.

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A new modular expression platform for the high-level production of hydroxy- or ketocarotenoids in the purple photosynthetic bacterium *R. rubrum*

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1. Introduction

A bacterial platform for the production of industrially interesting plant carotenoids is still of great interest, and to date, apart from *Escherichia coli*, which will produce carotenoids but does not tolerate them well, no universal bacterial production strain has been described. Photosynthetic bacteria, such as purple bacteria or cyanobacteria, which Nature has optimized to produce carotenoids, seem a natural choice for the construction of a universal carotenoid platform. Here, we present advances we have made in engineering the purple bacterium, *Rhodospirillum rubrum* to produce plant carotenoids semi-aerobically, using the "anaerobic, photosynthetic" metabolic potential of the organism which is induced under these conditions. In particular, we show that the introduction of a new designer expression plasmid, which allows semi-aerobically induced *crtD*-promoter-driven expression of hydroxy- and ketocarotenoid genes from *Paracoccus* sp., allows high-level expression of oxygenated plant carotenoids, even under semi-aerobic conditions.

2. Experimental

Hydroxy- and ketocarotenoid genes (*crtZ* and *crtW*, respectively [1]) from a *Paracoccus* sp. were commercially synthesized using an *R. rubrum*-specific codon usage, and then cloned into our novel *crtD*-driven modular expression vector, pRK4GH2 (manuscript submitted), to yield the plasmids pRKCrtW and pRKCrtZ, respectively. The plasmids were (separately) introduced into a β -carotene-producing strain of *R. rubrum* by triparental conjugation, then isolated as pure strains. The carotenoids of the highly coloured pure strains were characterized by both UHPLC, as well as spectroscopy.

3. Results and Discussion

The characterization of the CrtW- and CrtZ-expression strains confirms that our approach to developing a general carotenoid-producing strain of *R. rubrum* is promising (results will be presented).

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Session 4:
Photochemistry, Phoyophysics and
Photosynthesis
~ In memory of Prof. Yasushi Koyama ~

Binding patterns and functions of fucoxanthin and related pigments in photosynthesis

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Fucoxanthin (Fx) is a group of carotenoids that has conjugated carbonyl groups in their polyene backbones, and thus has a high ability to harvest light in the blue-green region. They bind to light-harvesting proteins together with chlorophyll (Chl) *c*, which constitute the major light-harvesting proteins (LHCs) called FCPs (fucoxanthin Chl *c*-binding proteins) in diatoms and related brown algae. FCPs harvest light energy and transfer them to the reaction center (RC) of photosystem I and II (PSI and PSII), where charge separation and electron transfer reactions occur. Owing to the high ability of light-harvesting in the blue-green region of FCPs, they constitute one of the major reasons for the success of diatoms in water, which alone contribute to 40% of the total primary productivity in the ocean, or 20% in the whole earth.

In order to reveal the binding pattern and functions of Fx and Chl *c* in FCPs, we purified a homo-dimer of an FCP from a marine pennate diatom *Phaeodactylum tricornutum*, crystallized it, and solved its crystal structure. The results showed that while the overall structure of the FCP is more or less similar to a typical LHCII protein, it binds 7 Chls *a*, 2 Chls *c* and 7 Fxs [1], which is less in Chls but much more in Fxs compared to a typical LHCII subunit. In this structure, each Fx is surrounded by one or more Chls in a close distance, suggesting an efficient energy transfer from Fxs to Chls and vice versa. The 7 Fxs were found to be located in different hydrophobic/hydrophilic environments, suggesting their different light-harvesting properties due to the large solvent effect seen in the conjugated carbonyl groups, which will cause a large redshift in the absorption upon an increase in the polarity of the solvent.

As FCPs are associated with PSII or PSI in vivo to act as the LHCs, we solved a structure of PSII-FCPII supercomplex from a centric diatom *Chaetoceros gracilis* by cryo-electron microscopy (cryo-EM) [2, 3]. The results showed that each PSII core is associated with one strongly bound and one moderately bound tetrameric FCPs, and three FCP monomers. The strongly associated FCP tetramer is bound to CP47 directly, and can transfer the energy directly to RC, whereas the moderately associated FCP tetramer is bound to the CP43 side through two FCP monomers, and transfer energy indirectly to RC through these two monomers. Further analysis showed that FCP trimers and dimers are also present and associated with the PSII core in different diatoms. In addition, FCPs also function as LHCs to PSI, and we have solved the structure of PSI-FCPI by cryo-EM analyses [4, 5]. This structure shows that each PSI core can bind up to 24 FCP monomers, which are arranged in three layers surrounding the PSI core, and different numbers of FCPI monomers can exist in the different PSI-FCPI supercomplex. Whether the different numbers of FCPI in different PSI-FCPI supercomplex exist in vivo, or it is an artifact due to detergent solubilization, is not clear at present, but the results suggested that FCP subunits are much more complicated in both the numbers and association patterns than those of LHCs seen in green algae and higher plants.

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Relation between carotenoid structure and spectroscopy: Implications for function of carotenoids in photosynthesis

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1. Introduction

Carotenoid photophysics is largely determined by structural features of a particular carotenoid, the number of conjugated C=C bonds being the most prominent factor. Yet, extension of the conjugation to various groups which may have different orientation in respect to the conjugation backbone led to the concept of the effective conjugation length, a parameter that can be tuned by small changes in carotenoid structure. Consequently, carotenoid photophysics (and therefore also their function in photosynthesis) can be modified by specific binding pockets in photosynthetic proteins. There is accumulating evidence that it is just such tuning that enables some carotenoids to play both light-harvesting and photoprotective role in photosynthesis.

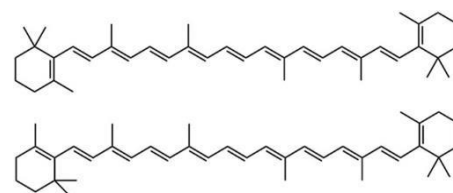


Figure 1. Two conformational variants of β -carotene.

2. Experimental

All time-resolved data were collected using a femtosecond spectrometer using tunable excitation pulses of ~ 100 fs duration and broadband (400–1100 nm) probe pulses detected by either grating or prism spectrometer with CCD camera detection.

3. Results and Discussion

Using a model system in solution, we show that small structural change can significantly alter both the S₁ lifetime and S₁ energy of a carotenoid. Then, for specific high-light induced photosynthetic proteins (Hlips and ELIP) we demonstrate that twisting of the end ring of either β -carotene or zeaxanthin induce down-shift of the S₁ energy, opening efficient quenching channel between the excited chlorophyll and carotenoid S₁ state. By modification of the protein, this quenching channel can be switched on or off [1]. Similar structural change of canthaxanthin allows for efficient quenching of phycobilisomes by the orange carotenoid protein [2].

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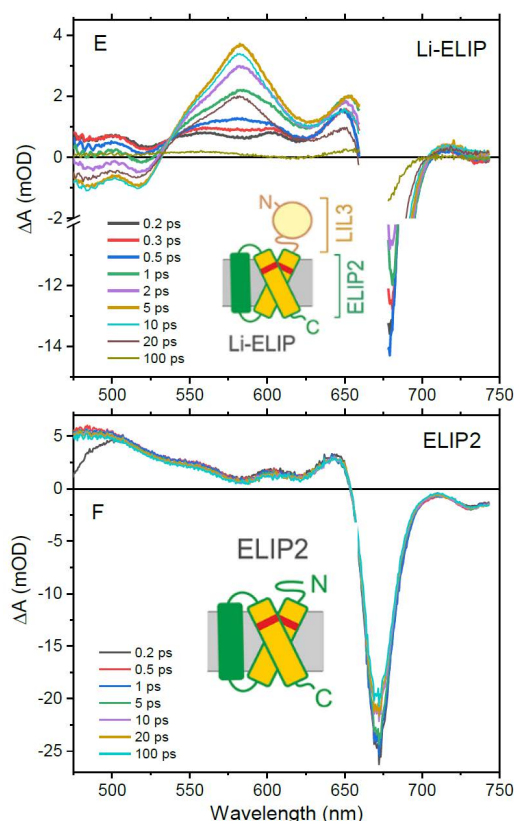


Figure 2. Femtosecond time-resolved absorption spectra of Li-ELIP and ELIP proteins excited at 670 nm. The carotenoid signal, which signals efficient quenching in Li-ELIP is absent in ELIP.

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Controlling Intramolecular Charge-Transfer in Carbonyl Carotenoids for Efficient Energy Transfer in Photosynthetic Antennas

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Carotenoids (Cars) are the lifeblood of natural photosynthesis, serving as essential pigments for both light-harvesting and photoprotection. These molecules absorb light in the blue-green range of the spectrum and rapidly transfer it to chlorophyll in photosynthetic antennas [1]. The excited states of Cars are characterized by a three-electronic level system (S_0 , S_1 , and S_2) [2]. The optically allowed S_2 state (designated as 1^1B^+ assuming C_{2h} symmetry of the polyene backbone) is produced via a one-photon transition from the S_0 ground state (1^1A^+), and promptly decays to the lower-lying S_1 state (2^1A^+) in just 100 femtoseconds. Conversely, relaxation from S_1 to S_0 is comparatively slower (picoseconds to nanoseconds), while the S_1 - S_0 transition is optically forbidden [2]. Due to the optically forbidden character, energy transfer from the Car S_1 state to chlorophyll is often inefficient [1]. However, Cars that contain a carbonyl group in their polyene backbone generate the intramolecular charge-transfer (ICT) state below S_1 in polar environments, resulting in the strongly coupled S_1 /ICT state depicted in **Figure 1** [3]. As a result, the S_0 - S_1 /ICT transition dipole moment of carbonyl Cars increases due to their charge-transfer character, thereby enabling efficient energy transfer from carbonyl Cars to chlorophyll *a* via S_1 /ICT in photosynthetic antennas [4, 5].

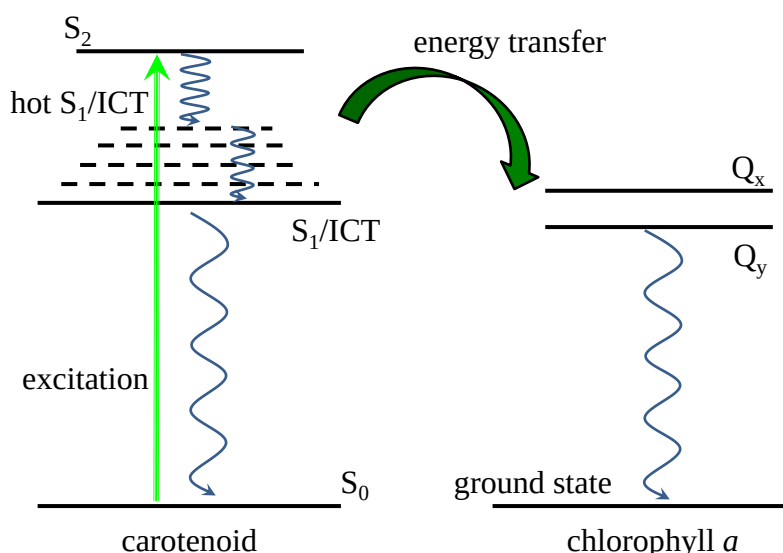


Figure 1: Schematic representation of excitation energy transfer dynamics from carotenoid to chlorophyll *a* for photosynthetic antenna containing carbonyl carotenoids.

Our groundbreaking investigations have demonstrated that through organic chemistry, we have the power to precisely control the intramolecular charge-transfer (ICT) character of carbonyl carotenoids [6-8]. As a testament to the incredible potential of our work, the reconstitution of these carotenoids into purple bacterial light-harvesting 1 (LH1) complexes achieved an unprecedented energy transfer efficiency of ~80%, outperforming that of wild-type LH1 [9]. Now, our focus shifts to exploring the ICT character of carbonyl Cars from both natural and artificial systems, dissecting how it correlates with the length and chemical structure of the conjugated polyene chain (**Figure2**). Here, we present recent, awe-inspiring examples of the ultrafast dynamics of excited states in carbonyl Cars.

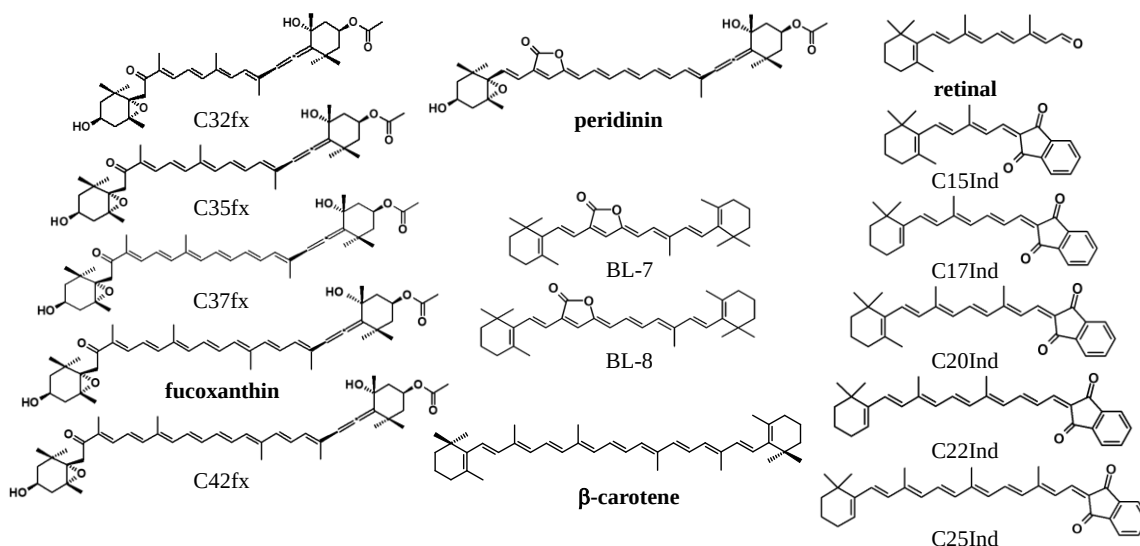


Figure 2: Chemical structures of natural and artificial carotenoids.

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Chlorophyll excitation quenching dynamics of the light harvesting complex II from the marine green alga *Bryopsis corticulans*

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1. Introduction

In oxygenic photosynthesis, the light harvesting complex II (LHCII) functions as light harvesting and photoprotection. Regarding photoprotection, LHCII can quench the excessive chlorophyll singlet excitation ($^1\text{Chl}^*$) before transferring the energy to the photosystem II (PSII) reaction center, a process which is referred to as energy-dependent quenching (qE), the fastest-response component among various mechanisms of non-photochemical quenching (NPQ). To date, the detailed qE-mechanism remains contentious, on which the following major views have been put forward: (i) The incoherent transfer of $^1\text{Chl}^*$ energy to the lowest-lying singlet excited state ($S_1(2A_g^-)$) of carotenoid (Car), (ii) the ultrafast Chl-Car charge transfer (CT) reaction followed by rapid charge recombination (CR), (iii) the formation of an excitation trap state from Car-Chl excitonic coupling despite the recent challenge from two-photon excitation studies, and (iv) the Chl-Chl CT and subsequent CR reaction. Note that the first three proposed mechanisms involve significant Car-Chl interactions, and that the LHCII in qE state is assumed to take a conformation that is promotive for Chl-to-Car singlet excitation energy transfer (EET) or Chl-Car CT reactions.[1]

The LHCII of a green alga *Bryopsis corticulans* (B-LHCII) contains siphonein and siphonaxathin as Car. Since their $S_1(2A_g^-)$ states lie substantially higher than the Q_y state of Chl *a*, the Chl *a*(Q_y)-to-Car(S_1) EET is unfeasible. To understand the qE mechanism of algal photosynthesis, we carried out time-resolved spectroscopic studies on the excitation dynamics of B-LHCII with reference to that of spinach LHCII (S-LHCII).

2. Experimental

B-LHCII and S-LHCII were prepared in trimeric and aggregated forms corresponding to the LH and the qE states, respectively. Their excited state dynamics were investigated comparatively with time-resolved absorption spectroscopy in a broad spectral range (400 ~ 950 nm) and with time-resolved fluorescence spectroscopy under low excitation photon fluence at different temperatures.

3. Results and Discussion

For both kinds of LHCII, red-light excitation at Chl Q_y -band, but not Car-band, induced transient absorption features resembling the characteristic spectroelectrochemical spectra of Chl $a^{\cdot+}$ and Chl $b^{\cdot+}$, indicating the ultrafast photogeneration of Chl-Chl CT species; Absolute fluorescence quantum yield and $^3\text{Car}^*$ declined whereas CT species increased upon prolonging excitation wavelength, showing positive correlation of $^1\text{Chl}^*$ deactivation with Chl-Chl CT formation. Importantly, the ultrafast Car-to-Chl EET process was confirmed for B-LHCII. Upon lowering temperature, the characteristic 680 nm fluorescence of aggregated B-LHCII showed emission extending over 690 ~ 780 nm with pronounced blue shift, lifetime prolongation and intensity boost, which is ascribed to the Chl-Chl CT state. The red-light induction of Chl-Chl CT species is considered as a general occurrence for LHCII, and the Car-independent deactivation of $^1\text{Chl}^*$ is proposed as a feasible qE mechanism for B-LHCII.[2]

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Probing the Fucoxanthin-Chlorophyll *a/c*-binding proteins (FCPs) of the Marine Diatom *Fragilariopsis sp* by resonance Raman, FTIR, and Fluorescence spectroscopy

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1. Introduction

Marine Diatoms are involved in major photosynthetic biochemical cycles, in oxygenic photosynthesis and carbon fixation. They contain the light-harvesting pigment systems Fucoxanthin-Chlorophyll *a/c*-binding proteins (FCPs) to collect light energy in the blue-green region that is also available under water, and transfer the trapped energy to the reaction centers where the primary electron transfer reactions convert energy into an electrochemical gradient. The photoacclimation strategy of species growing under variable light conditions enables the efficient regulation of photosystem structures to the amount of absorbed energy.

2. Experimental

To obtain the biophysical properties of the Fucoxanthin-Chlorophyll *a/c*-binding proteins (FCPs) of the Marine Diatom *Fragilariopsis sp* we have applied resonance Raman, FTIR, and Fluorescence spectroscopy [1].

3. Results and Discussion

We report resonance Raman, Fluorescence-excitation and FTIR spectra of the light-harvesting Fucoxanthin-Chlorophyll *a/c*-binding proteins (FCPs) of the Marine Diatom *Fragilariopsis sp*. The Raman shifts in the ¹⁵N-isotope enriched diatom provide the first spectroscopic evidence for the characterization of the C_a-N marker bands, and thus, of the penta- and hexacoordinated states of chlorophylls *a/c* in the FCPs. Under 405 and 442 nm Raman excitations, all the marker bands of Chl *a/c* are observed, and the isotope-based assignments provide new information concerning the structure of the Chls *a/c* in the FCPs and their interactions with the protein environment. In addition to the ¹³C=O of Chl *a/c* observed in the Raman spectra, the FTIR spectra revealed the acetyl, ester, and keto C=O of Chl *a/c*. The fluorescence-excitation spectra of FCP at pH 8 and 4.9 showed a reversible 447 to 452 nm transition of Chl *c* and from the pH-dependent absorption spectra a pK_a=5.4 attributed to the deprotonation/protonation of the 17-acrylate group was determined. The observed decrease in the energy transfer efficiency from Chl *c* to Chl *a* under acidic conditions is coupled to the protonation of the 17-acrylate moiety. The implications of these results with respect to the effect of the pH-recognized sites, to the switch of the FCPs from light-harvesting to energy-quenching state at acidic pH conditions, are discussed.

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Radical cation formation during the two-photon excitation of β -Apo-8'-carotenal

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1. Introduction

The one-photon forbidden nature of the first excited state (S_1) of carotenoids is known since a long time ago. In the presented study, we have examined the carotenoid β -Apo-8'-carotenal after two-photon excitation, which is the only possibility to observe the spectral features of this dark state without additional events caused by the depopulation from higher energetic states. In **Figure 1**, the chemical structure together with the steady-state absorption spectra of the sample in the used solvents is shown. Compared to our earlier experiments, we used the prism spectrometer enabling to observe both the visible and NIR spectral regions in a single experiment.

2. Experimental

Our primary laser system produces ~ 100 fs pulses at 1 kHz repetition rate, centered at 800 nm. The excitation wavelength in the 1200-1500 nm range was generated in an optical parametric amplifier, while the white light supercontinuum was produced in a sapphire plate by focusing a 1300 nm beam generated in a home-build NOPA. The polarization between excitation and probe was set parallel. The intensity at the sample position was kept at about 10^{16} photons/pulse cm^2 . β -Apo-8'-carotenal was purchased from Sigma-Aldrich ($\geq 96\%$, HPLC), and was dissolved in *n*-hexane and methanol to OD $\sim 0.75/\text{mm}$.

3. Results and Discussion

In the visible part of the spectra, the typical transient absorption signals were present. For the sample in hexane, we observed the S_1 -SN transition with a lifetime of about 26 ps. For the methanol-dissolved sample, the S_1 -SN lifetime was shortened to ~ 6 ps, and the ICT state signatures appeared, in agreement with the previous experiments obtained after one-photon excitation [1].

Among the features observed in the visible spectral region, we have also observed a strong peak with a high energy shoulder in the NIR, especially for β -Apo-8'-carotenal in methanol (**Figure 2**). For the sample dissolved in hexane, the NIR peak was also detected, however, it was much weaker and its shoulder observed in methanol was missing. In both solvents, the NIR peak exhibited dynamical blueshift and narrowing within a few ps. Due to its spectral shape, we suggest that the NIR signal is caused by the carotenoid cation.

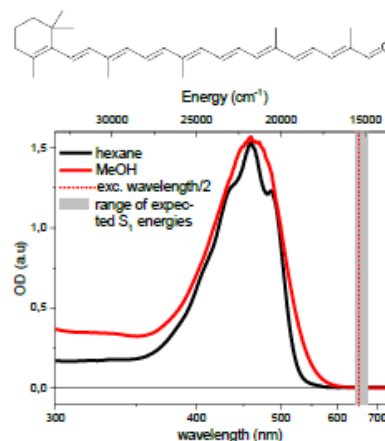


Figure 1. Chemical structure of β -Apo-8'-carotenal (top). Steady-state absorption spectra (bottom) with the expected S_1 energy and the excitation wavelength divided by two.

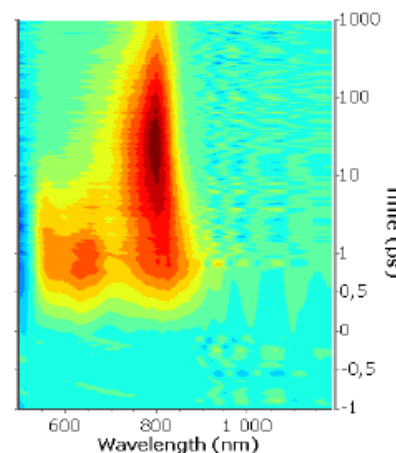


Figure 2. 2D plot of the femtosecond time-resolved absorption data of β -Apo-8'-carotenal in methanol at room temperature.

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Structures of siphonaxanthin and its acyl ester in marine green algal light-harvesting complex II

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1. Introduction

Carotenoids in light-harvesting complex II (LHCII) perform the dual role of light-harvesting and excess-energy quenching, enabling photosynthesis in a wide range of light conditions. Siphonaxanthin (Sx)–Sx ester (Sn)–chlorophyll (Chl) *a/b*-binding protein (SCP) is an LHCII of siphonous green algae that absorbs blue-green light and efficiently utilizes it for photosynthesis [1]. The characteristic blue-green absorption is attributable to carbonyl carotenoids. However, the chemical structures of the chromophores of Sx and Sn are identical, there are no clue which one contributes to this absorption band. Here, we determined the high-resolution structure of SCP using Cryo-EM [2]. Structural evidence for a mechanism that absorbs blue-green light is discussed.

2. Experimental

Codium fragile (KU-0654) was cultivated in our laboratory. SCP was solubilized by β -DDM, isolated by sucrose density gradient (SDG), and purified using a series of anion-exchange chromatography, SDG and gel filtration. Cryo-EM images were acquired with Falcon 4 and processed with cryoSPARC2 and RELION-3.1, resulting in a final global resolution of 2.78 Å.

3. Results and Discussion

The binding sites of Sx and Sn are clearly determined to be SxL2 and SnL1 sites, respectively. In the former site, Sx faces a polar surface, and the backbone of the conjugated system is distorted. In addition, Sx accepts two hydrogen bonds: between the 8-conjugated carbonyl group and a cysteine residue and between the 19-hydroxy group and a peptide carbonyl (Fig. 1). These features suggest that the Sx is responsible for the green absorption in SCP. Also, these hydrogen bonds can enhance the ICT characters, resulting in efficient energy transfer in SCP.

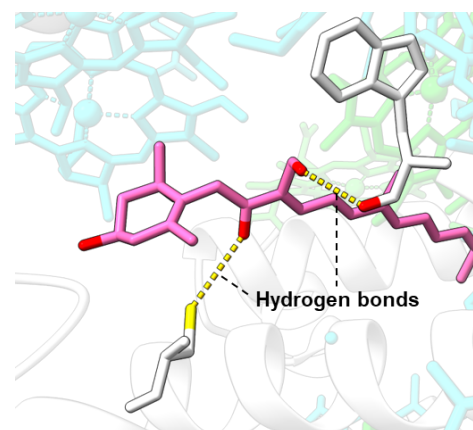


Figure 1. The 3D structure of siphonaxanthin and its hydrogen bonds formation in SCP.

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New insights into carotenoids higher electronic states dynamics

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Introduction

Carotenoids are important pigments in photosynthesis, with diverse structures and functions. Their excited states have unique properties and several dark states have been identified, including the intramolecular charge transfer (ICT) state and the S^* state. The S_1 and S_2 states are well-known and have been extensively studied, but the nature of the upper state (S_{UV}) and the S_N state remain less understood (**Figure 1**). The S_{UV} state has been identified in the 250-350 nm spectral region, and its excitation leads to excess energy indicated by an increase of the S^* signal. The excitation of the S_2 state leads to a fast relaxation of the S_1 state through the intersystem crossing. And the main transient absorption signal follows the S_1 - S_N transition. The S_N state can not be accessed from the ground state and detected in absorption spectra due to the factors that determine allowed transitions. The comparison of the estimated energies of S_N and S_{UV} states shows a difference of 3000 cm^{-1} . Here we aimed to investigate the differences in relaxation dynamics of those two upper states for two prototypical carotenoids, β -carotene and astaxanthin.

Experimental

We have carried out two sets of experiments. The monitoring relaxation dynamics of the S_{UV} state after tuning the excitation wavelength to the UV spectral region and the S_N state by using multipulse excitation. While the pump-probe experiment observes the difference in the absorption signal after the excitation by the first pulse, the second excitation pulse is resonant with the S_1 - S_N transition that drives the excited state population into a higher excited state.

Results and Discussion

In **Figures 2a** and **2b**, transient absorption spectra obtained after UV excitation (blue) is compared with pump-repump (PrP) spectra (red). The S_1 - S_N bands are normalized to the same number of excited molecules. Both spectra are presented at delay times corresponding to 1 ps after excitation of the upper excited state, to allow direct comparison. For astaxanthin, the different response to two excitations is obvious. While the relaxation of the S_N state populated by repumping the S_1 state generates essentially the same S_1 - S_N spectrum, except for a slight increase in the ICT amplitude, the S_1 - S_N spectrum obtained after UV excitation is different. The UV excitation of astaxanthin led to a broader S_1 - S_N spectrum with increased amplitude in the blue shoulder of the S_1 - S_N band, which is characteristic of the S^* signal, indicating the generation of specific conformations in the S_1 state. In contrast, no extra signal in the S^* region was observed for β -carotene after both UV excitation and relaxation of the S_N state after repump, but a longer cooling time of the S_1 state was observed after UV excitation. Nevertheless, the S_1 state decay remained unchanged for both carotenoids, and the kinetics taken at the maximum of the S_1 - S_N signal obtained after UV or S_2 excitation were nearly identical. Based on the analysis of data obtained at two experiments, we have concluded that the S_{UV} and S_N states are not vibrational states at the same potential surface, but different electronic excited states.

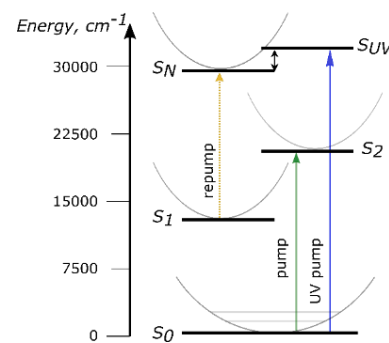


Figure 1. Schematic representation of the tested experimental model of the carotenoid excited state manifold.

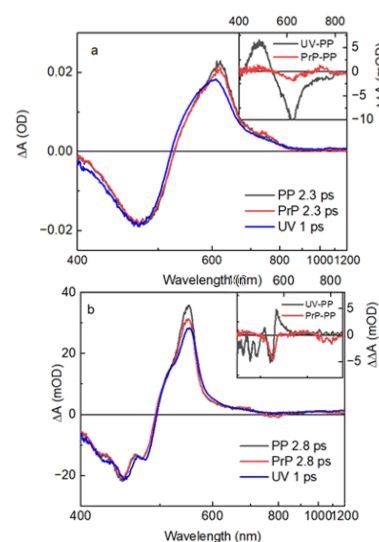


Figure 2. Transient absorption spectra of astaxanthin (a) and β -carotene (b) samples measured in methanol and n-hexane respectively.

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Session 5:
Chronic Disease Prevention and
Treatment

Mechanistic Understanding of Carotenoid Benefits against Chronic DiseasesXiang-Dong Wang*

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Keywords: β -Cryptoxanthin, Lycopene, Chronic diseases, Animal models

Epidemiologic studies have shown that dietary intake patterns with increased consumption of fruits and vegetables rich in carotenoids and, thus, increased serum levels of carotenoids have been associated with decreased development of several chronic diseases. While the initial impetus for studying the benefits of carotenoids in chronic disease prevention was their antioxidant capacity and pro-vitamin A activity, significant advances have been made in the understanding of the actions of carotenoids and their biologic active metabolites with regards to other mechanisms. The use of appropriate animal models are the justifiable approaches to resolve mechanistic issues regarding protective effects of carotenoids at specific organs and tissue sites. This presentation will focus on two common carotenoids, β - cryptoxanthin and lycopene, as promising preventive agents compounds against disease development and progression. The biological functions of β -cryptoxanthin and lycopene are mediated, partially via their oxidative metabolites, through their effects on key molecular targeting events, such as p53, sirtuin 1 and PPARs signaling pathways will be highlighted. Recent studies that the protective effects of β - cryptoxanthin and lycopene are related to regulating carotenoid cleavage enzymes (β -carotene-15, 15'-oxygenase and β -carotene-9', 10'-oxygenase), gut microbiome, cholesterol metabolism, and energy metabolism in animal models offered new opportunities to further our understanding of common and distinct mechanisms that involve carotenoids in chronic disease prevention. As our understanding of carotenoid metabolism, molecular biologic properties, and their interactions with genetic and epigenetic factors improves, greater insight will be achieved into the role and application of carotenoids for personalized recommendations to prevent chronic diseases.

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Tissue-specific Expression of BCO1 and its Implication in Cardiometabolic Disease

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1. Introduction

Dietary carotenoids such as β -carotene accumulate in human tissues and plasma. However, wild-type mice are “great carotenoid cleavers,” failing to store carotenoids even after chronic feeding strategies. We can overcome this limitation by feeding carotenoids to mice lacking one or both carotenoid oxygenases BCO1 and BCO2. Our previous studies showed that wild-type mice fed β -carotene, but not BCO1-deficient mice, display (1) a reduction in obesity, (2) improvement of plasma lipid profile, (3) a delay in atherosclerosis development, and (4) an acceleration in atherosclerosis healing.

2. Hypothesis

Our current studies aim to examine whether β -carotene accumulated in tissues can reproduce the positive effects of our dietary interventions with β -carotene.

3. Methods/Results

First, we show that we can reproduce the antiobesogenic effect of β -carotene in wild-type mice in BCO1-deficient mice fed β -carotene and infused with an expression vector encoding BCO1. We observed that the degradation of β -carotene stored in adipocytes results in the production of retinoic acid and a reduction in adipose tissue size. In our second study, we over-expressed BCO1 in the liver of BCO1/LDLR double KO mice fed β -carotene. BCO1-injected mice showed a reduction in plasma cholesterol and atherosclerosis.

4. Conclusions

These data highlight the potential of β -carotene accumulated in human tissues as a local source of vitamin A/retinoic acid, which could have significant implications in preventing and treating cardiometabolic diseases.

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Potential of siphonaxanthin for disease control and prevention

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1. Introduction

Algae are a potential renewable resource in the marine environment that are used as a culinary item in East Asia and excellent sources of nutritional and bioactive compounds such as carotenoids, dietary fiber, amino acids, essential fatty acids, vitamins, and minerals, which contribute to several beneficial effects in human health. Algal carotenoids are of particular interest as nutraceuticals, because they are structurally and functionally different from those found in land plants and animals. We have focused on the utilization of functional food components in unutilized biological resources and food processing residues, and have found that siphonaxanthin has various biological activities such as anti-angiogenic, anti-obesity, and anti-inflammatory effects which can contribute to disease control and prevention. Siphonaxanthin (**Figure 1**) is a specific keto-carotenoid of green algae, which helps in absorbing available green and blue green light under water. In edible green algae such as *Codium fragile*, *Caulerpa lentillifera*, and *Umbraulva japonica*, siphonaxanthin content is approximately 0.03%–0.1% of the dry weight.

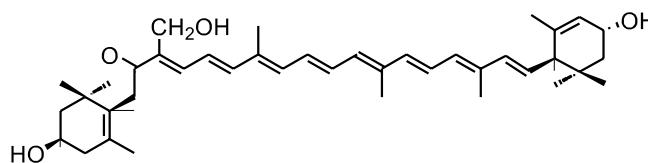


Figure 1. Chemical structure of siphonaxanthin

2. Disease prevention potential of siphonaxanthin

Siphonaxanthin could be potentially useful as a chemopreventive and/or chemotherapeutic agent. We have demonstrated that siphonaxanthin potently suppresses the viability of human leukemia HL-60 cells via induction of apoptosis [1] and possesses significant anti-angiogenic activity via down-regulation of signal transduction by fibroblast growth factor receptor-1 in vascular endothelial cells [2]. In addition, dietary siphonaxanthin shows the anti-obesity [3] and protective effects against lipotoxicity [4] in model mice. These findings support preventive effect of dietary siphonaxanthin on dyslipidemia and lifestyle diseases. Siphonaxanthin also exhibits the unique anti-inflammatory properties [5-7]. C8-keto structures of siphonaxanthin could be crucial for its inhibitory effect on the activation of nuclear factor-kappa B and interferon regulatory factors, respectively. Interestingly, siphonaxanthin supplementation can prevent skin photoaging such as wrinkling and skin barrier disruption in UVA-irradiated mice. In this case, detectable amounts of siphonaxanthin and its metabolites were accumulated in the dermis and epidermis. We also have evaluated the mechanism of intestinal absorption and metabolic fate of dietary siphonaxanthin [8]. Two dihydrometabolites and a tetrahydrometabolite were identified as metabolites from siphonaxanthin in cultured cells and experimental animals.

3. Conclusions and future perspectives

We found that siphonaxanthin has strong potential as a nutraceutical candidate among marine carotenoids. Our findings open up new avenues for future research on the advanced utilization of siphonaxanthin as a bioactive substance, although further investigation, especially clinical studies, are needed.

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Early-life metabolic programming by micronutrients and food bioactives with special focus on preformed vitamin A and beta-carotene: insights from murine models

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1. Introduction

Early-life nutrition can influence metabolic health and susceptibility to develop obesity later in life via epigenetic and other mechanisms targeting the development of tissues, anatomic structures, and homeostatic systems/circuitries relevant to the control of energy balance and storage. A majority of experimental studies in nutritional programming have focused on early-life exposures to gross dietary challenges such as caloric restriction, changes in macronutrient composition, or micronutrient insufficiencies, and generally during the gestational (fetal) period. The possible beneficial programming activity of mild micronutrient/bioactive supplements received during lactation has been less studied. The activity in this sense of preformed vitamin A (retinol or retinyl ester (RE)) and beta-carotene (BC) is the focus of the present research. The lactation period is a critical developmental window that offers many possibilities for preventive or corrective interventions to ensure optimal programming.

2. Experimental

We have studied the metabolic programming effects of mild supplements of preformed vitamin A and BC during lactation using different study designs and murine models. These include direct oral administration to suckling pups born from dams fed regular diet or an obesogenic Western diet (WD) rich in fat and sucrose from preconception and during gestation and lactation (WD dams), as well as *Isx*^{-/-} and *Bco1*^{-/-} mouse dams, used to increase maternal vitamin A or BC supply to the newborn, respectively. The offspring was analyzed at weaning (or soon thereafter) and in adulthood after challenging (or not) the animals with an obesogenic diet.

3. Results and Discussion

In our initial studies (in rats), direct supplementation of a ~3-fold excess of vitamin A as RE during the suckling period exacerbated hyperplastic WAT expansion in response to subsequent high-fat diet (HFD) feeding and led to changes in WAT at weaning consistent with the retention of an increased proliferation capacity of adipocytes that were not reproduced by supplementation as BC [1,2,3]. However, our recent studies showed that, in mice born from WD dams, the same RE supplementation (3x) during the suckling period counteracted the increase in adiposity, glycemia, and insulin resistance induced by exposure to a WD diet from conception and throughout life. Further, increasing maternal vitamin A (retinol) supply through milk (~2.5-fold) afforded resistance to HFD-induced increases in body weight, adiposity, and blood lipids, and favored a healthier hyperplastic (over hypertrophic) WAT expansion on an HFD in the *Isx*^{+/-} offspring. Preliminary results in the *Bco1*^{-/-} model suggest that BC supply through milk may program resistance to dietary obesity by mechanisms independent of its conversion to retinoids selectively in the female offspring. Thus, our recent results: (i) confirm that mild excess vitamin A during lactation has programming effects affecting the response to later obesogenic diet feeding; (ii) point to different programming outcomes when preformed vitamin A is administered directly to the pups or through maternal milk, and to offspring of dams fed a regular diet or an unbalanced obesogenic diet; and (iii) are suggestive of possible programming effects of BC independent of BCO1 cleavage.

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Mechanisms involved in the protective effect of carotenoids and estradiol in human skin cells under mitochondrial oxidative stress

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1. Introduction

Skin ageing is influenced by several factors including environmental exposure and hormonal changes. Reactive oxygen species (ROS), which mediate many of the effects of these factors, can be formed by extrinsic factors, such as sun exposure, or can result from mitochondrial dysfunction as occurs during ageing. The aim of the current study was to examine the damage to dermal fibroblasts by mitochondrially generated ROS due to chemically induced mitochondrial dysfunction, and then, to examine the protective effect of tomato carotenoids and estradiol and to study the mechanisms of the damage and the protection.

2. Experimental

Rotenone, a complex I inhibitor, was used to cause mitochondrial dysfunction in human dermal fibroblasts and its effects on mitochondrial and cytosolic ROS levels, cell death, apoptosis, MMP1 and pro-collagen secretion were determined as markers of skin damage.

3. Results and Discussion

Rotenone increased mitochondrial and cytosolic ROS. This was followed by increased transcriptional activities of the antioxidant response element/Nrf2 (ARE/Nrf2), NFκB and AP-1 transcription systems, and by apoptotic cell death, and MMP1 secretion and decreased collagen secretion. Pretreatment with carotenoid rich tomato extracts or with estradiol, reduced mitochondrial and cytosolic ROS levels and MMP1 secretion and increased cell number and collagen secretion. These effects can be partially explained by inhibiting caspase 3 activity and by cooperatively increasing ARE/Nrf2 activity, which leads to upregulation of antioxidant proteins such as NQO-1 and Trxr1. This was accompanied by decreased expression of MAPK2/3 and decreased activity of NFκB and AP-1 transcriptional activities. To determine if activation of ARE/Nrf2 is a mechanism for cell protection, this transcription system was inhibited using the Nrf2 inhibitors, ML385 and ochratoxin A (OTA). The inhibition of ARE/Nrf2 prevented the protective effects of the carotenoids and estradiol, by diminishing their effect to reduce cytosolic ROS. This study indicates that carotenoids and estradiol protect skin cells from damage caused by mitochondria generated ROS and thus, may delay skin aging and improve skin health.

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Lutein, Zeaxanthin, and Meso-zeaxanthin Supplementation Attenuates Markers of Systemic Inflammation and Oxidative Cardiovascular Processes

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1. Introduction

In the human body, systemic inflammation and oxidation is a primary contributor to poor physiologic function and development of diseases, such as atherosclerosis within the cardiovascular system [1]. Indeed, oxidative stress is one of the most important risk factors in the pathogenesis of atherosclerosis [2]. Mechanistically, the oxidation of low-density lipoprotein (LDL) particles within the vascular endothelium has been hypothesized to be the initial event in the formation of atherosclerotic plaques [3], therefore it is likely that increased antioxidant concentration within the blood would interrupt this process. It has been demonstrated that supplementation with the antioxidative macular xanthophylls (lutein [L], zeaxanthin [Z], and meso-zeaxanthin [MZ]), can attenuate the inflammatory response within the human body [4]. Therefore, we hypothesized that supplementation with L, Z, and MZ would have a systemic anti-inflammatory effect (i.e., a reduction in inflammatory cytokines: IL-6, IL-1 β , and TNF- α) and that markers of cardiovascular oxidative processes (i.e., OxLDL) would be reduced.

2. Experimental

The Carotenoid-Omega Availability Study (COAST) [5] was a 6 month, double-blind, block-randomized placebo-controlled study; 81 healthy individuals between 18 and 65 years old participated. In this intervention study, participants were randomly allocated to receive the active intervention containing L (10mg) + MZ (10 mg) + Z (2 mg) or placebo (containing sunflower oil). Serum concentrations of carotenoids (assessed by HPLC), inflammatory cytokines (IL-6, IL-1 β , TNF- α) and oxidative LDL (measured via solid-phase sandwich ELISA) at baseline and at 6-months.

3. Results and Discussion

Serum concentrations of L, Z, and MZ significantly increased compared to placebo ($p < 0.05$). Comparison of change in inflammatory cytokine levels over the supplementation period between active and placebo revealed statistically significant reductions in IL-1 β ($p < 0.001$) and TNF- α ($p = 0.003$) with very large to large effect sizes of $d = 1.095$ (95% CI = (0.489, 1.694)) and $d = 0.890$ (95% CI = (0.293, 1.482)), respectively. Additionally, a statistically significant difference was observed for change in OxLDL between placebo and active groups ($p = 0.009$), with a large effect size of $d = 0.820$ (95% CI = (0.206, 1.429)). Our data show that L, Z, and MZ supplementation achieves a decrease in OxLDL and reduction in IL-1 β and TNF- α , that together suggest these carotenoids are acting systemically to attenuate oxidative products and inflammation before they can contribute to atherosclerotic plaque formation. Given that cardiovascular disease is globally the leading cause of death [6] these findings have the potential to be very impactful and clinically relevant for much of the population.

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Carotenoids, as Health Promoting Natural Gifts

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Carotenoids have multi-functions, including health promoting activity. At first, we have investigated cancer-preventive function of carotenoids, such as alfa-carotene, lycopene, fucoxanthine, et al. However, we have quickly realized that carotenoids have much more wide variety of functions for health promotion. In this presentation, I would like to discuss the future possibility of carotenoids, as health promoting natural gifts.

Targeted Nutrition with Xanthophyll Carotenoids and Reduced Cardiometabolic Risk: A Randomised Double-Blind Placebo-Controlled Study

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1. Introduction

The xanthophyll carotenoids (XC) lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ), may have a role in the management of cardiometabolic diseases (CMD), such as cardiovascular disease, diabetes, and stroke. Here we examine whether supplementation with XC have different effects within subgroups of risk to develop a CMD.

2. Experimental

In this subgroup analysis of the Carotenoid- Omega Study (COAST), 79 participants randomized to receive L + Z + MZ or placebo, were further stratified by CMD risk factors: no-risk (Group 1- no risk factors, n = 31) and high- risk (Group 2- two or more risk factors, n = 38). Serum concentrations of L, Z, and MZ, inflammatory biomarkers of CMD (IL-6, IL-1 β , TNF- α , and oxidative-LDL [oxLDL]), lipid profile, and skin carotenoid score were analysed at baseline and at 6-months.

3. Results and Discussion

Baseline mean ($\pm$ SD) serum concentrations of L and Z and skin carotenoid score were significantly higher in the no-risk group, 0.227 ± 0.112 $\mu\text{mol/L}$, 0.083 ± 0.030 $\mu\text{mol/L}$, and $41,477 \pm 13,436$, respectively, compared to the high risk group, 0.172 ± 0.094 $\mu\text{mol/L}$, 0.067 ± 0.024 $\mu\text{mol/L}$, and $31,265 \pm 11,010$, respectively, ($p = 0.036$, $p = 0.012$, and $p = 0.002$); whereas, inflammatory biomarkers were higher in the high-risk group (IL-6 and TNF- α , $p = 0.026$ and $p = 0.014$). Supplementation with L, Z, and MZ reduced markers of inflammation in both the no-risk and high-risk groups compared to placebo ($p < 0.05$ for all). However, a larger effect was observed in the high-risk group in reducing IL-1 β and OxLDL ($t = 5.2$, $p < 0.001$ and $t = 3.28$, $p = 0.004$) compared to the no-risk group. Our study demonstrates that apparently healthy individuals with risk factors for CMD have low levels of carotenoids in blood and skin. Supplementation with XCs in this group has a greater effect in decreasing biomarkers of cardiometabolic inflammation compared to the no-risk group. These findings suggest that targeted carotenoid interventions may potentially decrease the rate of progression to a CMD and should therefore be considered as part of a preventive strategy for CMD.

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Session 6:
Carotenoid Metabolism and Function

Molecular components affecting carotenoid homeostasis in mammalian cells and tissues

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1. Introduction

Low carotenoid status has been associated with a number of chronic disease states such as cardiovascular disease, cognitive impairments, and age-related macular degeneration (AMD) [1]. In the past decades, genes encoding proteins of carotenoid metabolism have been identified, including class B scavenger receptors for cellular carotenoid uptake [2, 3], gram-domain containing (GRAMD1) proteins for intracellular carotenoid transport [4], and β -carotene-oxygenase 1 and 2 (BCO1 and 2) for carotenoid conversion to apocarotenoids [2, 5]. A coordinated interplay of these proteins constitutes the metabolic basis of mammalian carotenoid metabolism and tissue homeostasis. However, knowledge about carotenoid metabolism is still incomplete and there are large species-specific variations in this metabolism in vertebrates. Many vertebrates, including humans, absorb carotenoids intact and accumulate them in specific patterns in tissues [6]. However, mice the most common model for biomedical research do not accumulate the pigments in significant amounts.

2. Experimental

To obtain essential new insights into carotenoid metabolism, we established test systems to characterize the biochemical activities of SR-B1, GRAMD1a-c, and BCO2. We generated knockout mouse models to study the contribution of the different genes to carotenoid metabolism and tissue homeostasis. Using gene and protein expression analysis, histology, and HPLC and MS-analyses of carotenoids, we analyzed how the different genotypes affect tissue carotenoid homeostasis and composition.

3. Results and Discussion

We observed that BCO2 expression in enterocytes of the intestine serves as the gatekeeper for zeaxanthin absorption and body distribution in mice. We further identified the transcription factor ISX as an important modulator of the intestinal activity of SR-B1. Zeaxanthin absorption was concentration-dependent and the jejunum was the major place of absorption. In mouse tissues, zeaxanthin underwent oxidation into ϵ,ϵ -3,3'-carotene-dione enantiomers. The ratio of parent and oxidized zeaxanthin was variable between tissues. We also showed that GRAMD1C modified zeaxanthin absorption in mice. Finally, we established an albino *Isx*^{-/-}/*Bco2*^{-/-} DKO mouse. This mouse model displays hypercarotenemia (Figure 1) as humans when subjected to high dose supplementation and accumulates carotenoids in many tissues including the eyes. Albino DKO mice are a resourceful animal model to characterize the physiological roles and benefits of the pigments in mammals.

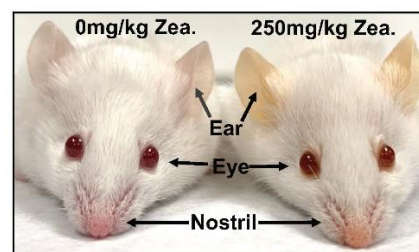


Figure 1. Photograph of albino *Isx*^{-/-}/*Bco2*^{-/-} mice on 0 mg/kg zeaxanthin (left) or 250 mg/kg zeaxanthin (right) diet. The yellow skin phenotype was observed in the ears, eyelids, and nostrils of *Isx*^{-/-}/*Bco2*^{-/-} mice on a 250 mg/kg zeaxanthin diet.

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Mechanisms of carotenoid transport across the maternal-fetal barrier

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β-carotene (BC) supports normal embryonic development as it serves as a source of vitamin A within the developing tissues (1). Mechanisms of maternal-fetal transport of intact BC are unclear. Our previous studies implicate lipoprotein biosynthesis in BC transfer across the placenta, involving microsomal triglyceride transfer protein (MTP), the master regulator of intracellular assembly and secretion of apoB-lipoproteins (2). Recent data suggest that a temporal shift in the generation of retinoic acid (RA) vs. apo10-carotenal (Apo10al) from BC optimizes placenta lipoprotein synthesis and likely transfer of BC to the fetus. Moreover, our findings implicate the intestinal homeobox transcription factor (ISX), also expressed in placenta, as a potential direct target of the above-mentioned transcriptional regulation upstream of *Mtp*. Ongoing studies aim at proving this hypothesis. Finally, if the placenta transports lipids and fat-soluble vitamins within lipoprotein particles has been discussed over many decades, especially regarding the directionality of such transport and the size of the particles. Here we will present novel data about the localization of the molecular machinery to produce lipoproteins in human and mouse placenta, and the identification of HDL-like particles into the media of human placenta explant cultures. These data overall support the possibility that indeed the regulation of MTP by BC might have physiological significance.

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Structural and functional role of cysteine palmitoylation in mammalian carotenoid cleavage dioxygenases

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1. Introduction

Mammalian/vertebrate carotenoid cleavage dioxygenase (CCD) superfamily members are comprised of three closely related proteins, β -carotene dioxygenase 1 (BCO1), β -carotene dioxygenase 2 (BCO2), and RPE65 retinol isomerase (RPE65), sharing ~40% identity but with divergent functions. Despite this, these CCDs share common structural features/motifs, including the -PDPCCK- motif. The cysteine (C) residue contained can undergo a palmitoylation post-transcriptional modification (PTM). At least 2 of these CCDs (RPE65 and BCO2) are peripheral membrane proteins but lack defined membrane domains. We propose that this PTM is a key structural/functional feature of vertebrate CCDs playing a major role in membrane association and/or access to substrate of these proteins.

2. Materials and Methods

Various biochemical and biophysical techniques were used to assess palmitoylation and structural and functional features of BCO2 and RPE65. Xanthophyll-carrying large unilamellar vesicles (LUVs) were prepared by hydration of phospholipids with carotenoids and using limonene as a “sherpa” molecule, followed by extrusion and extraction of limonene yielding LUVs of narrow size distribution to deliver xanthophylls to various cultured cells (COS7 and ARPE-19).

3. Results

We have shown that RPE65 is palmitoylated at C112 and that this PTM is absent in the presence of active LRAT + all-*trans* retinol, i.e., RPE65's all-*trans* retinyl ester substrate. Also, in the absence of its β -carotene substrate, the paralogous C111 of BCO2 is palmitoylated. However, in the presence of β -carotene, BCO2 loses its palmitoylation and, also, shows partial re-localization from mitochondria to nuclei. As BCO2 also cleaves xanthophylls, we made novel non-disruptive (i.e., no detergents) to deliver xanthophylls to cells, using limonene to assist solubilization of xanthophylls in bilayers of appropriately sized phospholipids. We found that when treated with lutein, BCO2 is depalmitoylated with partial concentration-dependent nuclear re-localization. Higher concentrations of zeaxanthin and violaxanthin treatment also induce depalmitoylation and protein translocation to a lesser degree. This suggests that loss of palmitoylation upon substrate binding promotes BCO2 shuttling to the nucleus. Furthermore, C112 palmitoylation of RPE65 has an additional important structural/functional role. We find that the region aa107–125 of RPE65 containing the -PDPCCK- motif, but that is absent from all crystal structures of RPE65, is essential for binding of RPE65 to smooth endoplasmic reticulum (ER) membranes. When a synthetic peptide of aa107–125 interacted with membrane mimics there was a transition from unstructured loop to a structured amphipathic α -helix. However, palmitoylation of C112 in this peptide causes the transition to α -helix to be automatic. Furthermore, aa107–125 peptide alone targets heterologous proteins to the ER. We are investigating the paralogous region of BCO2 to determine a potential role in membrane targeting. Interestingly, we did not detect palmitoylation of BCO1.

4. Conclusions

Both RPE65 and BCO2 can be palmitoylated, but this PTM is lost in presence of their substrate. In RPE65, palmitoylation is important for sER binding and potential access to substrate. In BCO2, it may be required for mitochondrial localization. The translocation of BCO2 into the nucleus in the presence of β -carotene and various xanthophylls suggests this as a possible mechanism for transport of carotenoids and/or carotenoid cleavage products to the nucleus to affect transcriptional regulation, such as activation of cellular oxidative stress response genes. These and other aspects of CCD palmitoylation may be important to our understanding of retinoid/carotenoid metabolism and these are currently under investigation.

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Structure-function studies of a carotenoid isomeroxygenase reveal mechanistic diversity in the synthesis of 11-*cis*-retinoids for animal vision

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1. Introduction

Animal vision starts with a photochemical event where an opsin-linked 11-*cis*-retinaldehyde chromophore absorbs a photon resulting in its isomerization to an all-*trans* configuration. The 11-*cis*-retinaldehyde (visual chromophore) required for animal vision is derived from dietary carotenoid precursors containing multiple potentially cleavable double bonds⁸⁻¹⁰. Also, 11-*cis*-retinaldehyde is a high energy isomer that constitutes an inconspicuous percentage of total retinaldehyde at thermal equilibrium¹¹. These factors necessitate the involvement of enzymes to ensure the fidelity of retinaldehyde formation from carotenoids and its specific isomerization to the 11-*cis* configuration. Iron-dependent, membrane-associated enzymes belonging to the carotenoid cleavage dioxygenase (CCD) superfamily are pivotal in this conversion process⁸. Animals require two enzymes (BCO1 and RPE65) to produce 11-*cis*-retinaldehyde from carotenoid precursors whereas insects use a single enzyme called NinaB [1]. The mechanism of NinaB isomeroxygenase activity and its relationship to the isomerase activity found in RPE65 are unclear.



Figure 1. Crystal structure of NinaB. The substrate-binding pocket is shown in surface representation.

2. Experimental

Well expressing NinaB enzymes were identified through ortholog screening. Activity analyses were performed using HPLC. A NinaB ortholog was crystallized and its structure determined to 1.95 Å resolution. Structure-guided mutagenesis studies were carried out to identify the site of isomerization within the NinaB substrate-binding cleft.

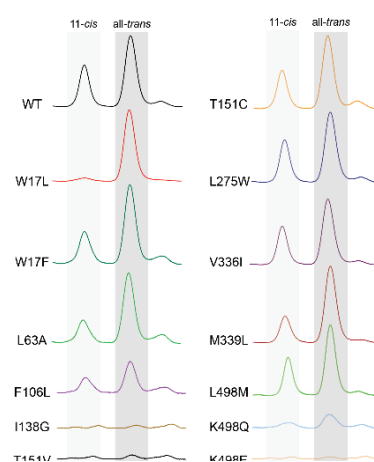


Figure 2. A mutagenesis scan reveals residues critical for NinaB isomerase activity.

3. Results and Discussion

The NinaB enzymes we studied could be purified in native form with high yield and displayed robust carotenoid isomeroxygenase activity. A high-resolution structure of NinaB revealed the special molecular architecture of its substrate binding cleft that allows bicyclic carotenoid cleavage as well as the details of its membrane-interacting structural elements. Our structure-guided mutagenesis approach pinpointed the site responsible for isomerase activity within the expansive substrate-binding cleft of NinaB. Comparison to the site of isomerization known for RPE65 [2] revealed mechanistic diversity in the evolution of CCDs for production of visual chromophores.

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Integrating multi-omics techniques towards understanding metabolism and the health effects of carotenoids

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Many studies have found associations between diets rich in carotenoids and a decreased risk for chronic diseases. There has been a long-standing hypothesis that non-provitamin A metabolites of carotenoids may be contributing to this effect, though these apocarotenoids and apolycopenoids have been elusive in human plasma. These observations have led our group to work to use novel and multi-omic approaches in both animal studies and human samples, in the goal of understanding carotenoid metabolism, which I plan to discuss at ICS 2023.

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Identification and tissue distribution of fucoxanthin-derived novel metabolites

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1. Introduction

Marine carotenoid fucoxanthin (Fx), which is expected to have various nutraceutical functions such as preventing non-alcoholic steatohepatitis [1], is converted to fucoxanthinol (FxOH) in intestinal tract and then absorbed in body. FxOH is further metabolized to amarouciaxanthin A (AmxA) and paracentrone [2]. In recent years, identification of carotenoid fatty acid esters in mammals has attracted attention to various metabolisms of carotenoids. However, there is no information on the conversion of Fx-derived metabolites to fatty acid esters. In this study, we aimed to identify novel ester-type metabolites and clarify their distribution in tissues of Fx-fed mice.

2. Experimental

Five-week-old C57BL/6J male mice (n=7) were fed AIN-93G diet containing 0.2% Fx for 1 week. After extraction of total lipids from the tissues and serum by Folch method, Fx metabolites were quantified by HPLC. Hydrolysis of total lipids extracted from liver and adipose tissue was performed using cholesterol esterase. Carotenoid fatty acid esters were prepared by esterification reaction of FxOH or AmxA with fatty acid chlorides.

3. Results and Discussion

Several unidentified peaks were observed in total lipids extracted from liver and adipose tissue of Fx-fed mice, as well as Fx-derived metabolites such as FxOH and AmxA. These peaks were purified by HPLC and subjected to enzymatic hydrolysis. The unidentified peaks disappeared and new peaks with retention times corresponding to FxOH and AmxA appeared, suggesting that the unidentified peaks may be esterified derivatives of FxOH and AmxA. Then, carotenoid fatty acid esters were prepared from free-form FxOH or AmxA and fatty acid chlorides. Comparison of HPLC chromatograms with the synthesized carotenoid fatty acid esters and total lipids found that the unidentified peaks were carotenoid fatty acid monoesters in which fatty acids such as palmitic acid and oleic acid were bound to FxOH and AmxA. Interestingly, the monoesters were abundantly accumulated in liver and adipose tissue, whereas the contents of those esters in small intestine, kidney, skeletal muscle, and serum were low. Furthermore, the composition of accumulated FxOH and AmxA fatty acid esters differed between liver and adipose tissue. Therefore, it was suggested that FxOH and AmxA are mainly transported as free forms to each tissue via the bloodstream and then partly converted to fatty acid esters, especially in the liver and adipose tissue [3].

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Exploring the Role of HDL in Carotenoid Efflux and Delivery to Tissues

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1. Introduction

The high-density lipoprotein (HDL) eliminates excess cholesterol accumulated in tissues by transporting it to the liver. HDL also transports carotenoids, and previous data suggest that HDL can deliver carotenoids to tissues. The overall objective of this study is to examine whether HDL can mediate the efflux of carotenoids from tissues. We hypothesized that, as it occurs with cholesterol, HDL transports carotenoids from tissues to the liver.

2. Methods

To examine the role of HDL in carotenoid distribution, we cross-bred β -carotene oxygenase 1-deficient (*Bco1*^{-/-}) mice, which mediates the cleavage of β -carotene to vitamin A, with mice over-expressing (*ApoA1*^{tg/tg}) apolipoprotein A I (ApoA-I), the main protein component in HDL. We fed our mice a standard diet containing 50 mg/kg β -carotene for four or ten weeks. The concentration of β -carotene in plasma and tissues was measured by HPLC, and plasma ApoA-I levels were quantified by Western blot.

To determine the effect of HDL on carotenoid efflux from tissues, we fed *Bco1*^{-/-} mice a standard diet containing 50 mg/kg β -carotene for four weeks. After this period, we infused them with either isolated human HDL or the same volume of saline. Carotenoids in tissues and plasma were collected for HPLC analyses and circulating ApoA-I levels were measured by Western blot.

3. Results and Discussion

Bco1^{-/-}*ApoA1*^{tg/tg} mice presented less β -carotene in the blood, adipose tissue, and eyes than *Bco1*^{-/-} mice, while hepatic β -carotene levels followed the opposite trend. We did not observe changes in food intake or fecal carotenoid elimination. HDL infusions depleted plasma and adipose tissue β -carotene pools and increased hepatic β -carotene stores.

4. Conclusion

Overall, these data suggest that HDL promotes carotenoid efflux from tissues, which opens new avenues in our understanding of the role of lipoproteins in the biodistribution of carotenoids in mammals.

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Session 7:

Chemistry and Analysis

*~In memory of Prof. Takao Matsuno, Prof. Synnøve Liaaen
Jensen and Prof. Kurt Bernhard~*

Carotenoids – an account of arbitrary exclusion and subjective inclusionRichard Sliwka**Department of Chemistry, Norwegian University of Science and Technology, 7491 Trondheim, Norway***1. Introduction**

A carotenoid is defined by a combination of rules, habits, customs, conventions and directives. These various regulations are confusing and discriminatory. Some non-polyene compounds received the status of carotenoids, several true carotenoids were denied this classification.

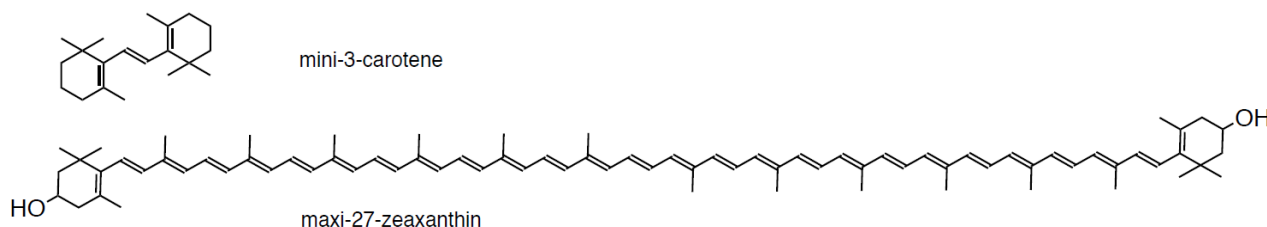
Regardless of rules, canonical carotenoids were defined by authoritative personalities as “naturally occurring”. The distinction between “naturally occurring” and “others” has played a key role in forming the perception of carotenoids. The “natural” label has become the criterion in portraying the “otherness” as reason to keep “unnaturally occurring carotenoids” outside the Carotenoid Handbook and the Carotenoid database. [1] The entrenched positions of “natural” and “unnatural” has become a central part of the carotenoid identity. Consequently, it has been difficult to locate the shortest and the longest carotenoid. Individual minimizing (mini-3-carotene) [2] and a subjective maximization limit (Kuhn postulate) [3] has so far prevented to define the boundaries of carotenoids.

Carotenoid and polyene chemists were in former years awarded the Nobel prize: Fischer 1930, Karrer 1937, Kuhn 1938, Wittig 1979). The more recently synthesized polyenes by Nobel laureates Lehn 1987, Heeger 2000 and Schrock 2005 were ignored by the carotenoid community.

It is time to extend the element limit of carotenoids beyond C, H, O. It is high time to unite “naturally” and “unnaturally occurring” carotenoids under the canopy of polyenes.

2. Presentation

The lecture exemplifies the exclusion of carotenoids and the inclusion of non-carotenoids in representative compilations; the need for a change in rules and nomenclature is evident. The lecture shows that innovative syntheses to canonical carotenoids can be replaced by using well-known syntheses to produce innovative carotenoids. Several of non-canonical carotenoids will be mentioned *e.g.* the invisible, the wires, the compass, the tree, the infinitene etc. The potential of carotenoids as lipogenic and proteinogenic compounds are illustrated; the use of carotenoids as visual tracers is exemplified.



Unrecognized boundaries of carotenoids: mini-3-carotene [2], maxi-27-zeaxanthin [4]

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My Chemical and Biochemical Studies of Natural Carotenoids During 45 Years

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Since 1978, I have studied natural carotenoids from chemical and biochemical points of view. During this time, I developed analysis, isolation and structural determination methods of natural carotenoids and determined structures of more than 150 kinds of natural carotenoids in microorganisms, algae, plants and animals and also revealed formation mechanism and function of these carotenoids from chemical points of view. In this symposium, I will review my natural carotenoid studies during 45 years.

1. Developments of analysis, isolation, and structural determination methods for natural carotenoids using HPLC, LC/MS, MS/MS and NMR [1]. Database creation of NMR, LC/MS and MS/MS data of natural carotenoids [1].
2. Structural elucidation of more than 150 kinds of natural carotenoids in microorganisms, algae, plants and animals [2]
3. Food chain studies of animal carotenoids [3-6].
Metabolism of fucoxanthin, peridinin, diatoxanthin etc. in marine animals by food chain studies [3-5].
Food chain and chemical ecological studies of carotenoids in dragonfly etc. [4,6].
4. Elucidation of function of natural carotenoids from chemical points of view. Secondary metabolites of carotenoids in plants and these functions [4]
Elucidation of uptake mechanism of active oxygen species carotenoids from chemical studies [4].

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Apocarotenoids determination in *Capsicum* species and biological fluids by supercritical fluid extraction-supercritical fluid chromatography-triple quadrupole/mass spectrometry

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1. Introduction

Carotenoids oxidative and enzymatic cleavage products called apocarotenoids are very important bioactive molecules in plants with different functions, such as regulation of development; their importance is also emerging in mammals where their role on gene expression and modulation of ligand-activated nuclear receptors has been outlined. Relatively few studies are available in the literature on the apocarotenoids occurrence in food samples and biological fluids, which were mainly based on liquid chromatographic approaches, with relatively long analytical times and considerable organic solvent consumption. Although recently supercritical fluid chromatography (SFC) coupled to mass spectrometry has gained attention as a green, fast and useful technology applied to the carotenoids analysis only very few recent papers are available in the literature on the direct online extraction and determination of apocarotenoids, by a supercritical fluid extraction-supercritical fluid chromatography-mass spectrometry (SFE-SFC-MS) methodology [1].

2. Experimental

Samples were ground by using the ultra-turrax T 25 and homogenized with an adsorbent powder “Miyazaki Hydro-Protect,” and 100 mg of the mixture were placed in the extraction vessel in the supercritical fluid extraction (SFE) unit. Biological fluids were directly analysed, without any sample preparation step. All applications were carried out on an Nexera-UC online SFE-SFC-QqQ MS (Shimadzu) system.

3. Results and Discussion

This presentation will focus on the first apocarotenoids profiling, including apocarotenoid fatty acids esters, in 17 different *Capsicum* varieties, using an online supercritical fluid extraction-supercritical fluid chromatography with triple quadrupole mass spectrometry detection (SFE-SFC-QqQ/MS) system. Moreover, the same methodology has also been applied for the first time to the apocarotenoids determination in biological fluids. The developed system could be used as a fast screening tool for the apocarotenoids detection in food, food products and biological fluids, providing important information on the occurrence of these metabolites which could be used also as a food authenticity parameter and could be correlated to an healthy state.

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Structural Analyses of the Compounds Produced by Acidic Treatment of Epoxycarotenoids (Violaxanthin, 9-*cis*-Violaxanthin, Antheraxanthin, and 9-*cis*-Antheraxanthin), and the Antioxidant Activities of Epoxycarotenoids and the Acid Treatment Products.

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1. Introduction

Some acylated epoxycarotenoids are contained in fruits a lot, while their changes after ingestions and the antioxidant activities of them have not been studied so far mainly due to their unstable physical properties.

In this study, we prepared pure major epoxycarotenoids (violaxanthin, 9-*cis*-violaxanthin, antheraxanthin, and 9-*cis*-antheraxanthin) (Fig. 1) from mango, permission fruits, and nable, and performed acid treatment on them to understand their changes in stomach.

We also examined lipid peroxidation inhibitory (inhibition of rat brain homogenate peroxidation that was initiated by Fe^{2+} - Fe^{3+} - O_2 complex and progressed by lipid peroxides derived radicals) and $^1\text{O}_2$ quenching (inhibition of methylene blue-sensitized linoleic acid photo-oxidation) activities of epoxycarotenoids and their acid treatment products.

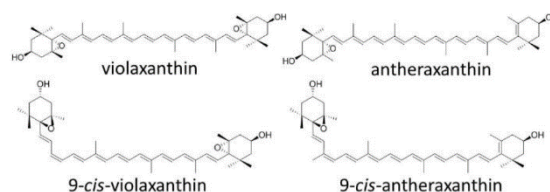


Fig. 1 The structures of epoxycarotenoids

2. Results and Discussions

To obtain the acid conversion products of violaxanthin and 9-*cis*-violaxanthin, each carotenoid was dissolved in 0.1 M HCl in 50% EtOH, and stirred for 10 min at room temperature. The solution was partitioned between EtOAc and H_2O , and EtOAc layers containing the converted products were analysed by silica gel HPLC (Cosmosil 5SL) using hexane: EtOAc (1:1) as the solvent. For both violaxanthin and 9-*cis*-violaxanthin, HPLC analysis showed three peaks with similar UV-Vis absorbance at 17.0 min, 20.5 min, and 23.5 min, and the peak area ratio was approximately 4:6:1. The three peaks derived from violaxanthin and 9-*cis*-violaxanthin were proved to be identical by co-chromatography of the acid conversion products of violaxanthin and 9-*cis*-violaxanthin (the identities of the three peaks were further substantiated by ^1H NMR spectra). The peaks at 17.0 min, 20.5 min, and 23.5 min were identified to be (8*S*,8'*S*)-auroxanthin, (8*R*,8'*S*)-auroxanthin, and (8*R*,8'*R*)-auroxanthin, respectively. (Fig. 2 (a)).

Similarly, the products of antheraxanthin and 9-*cis*-antheraxanthin after acid conversion were analysed by silica gel HPLC. The products from each carotenoid were confirmed to be same compounds ((8*R*)-mutatoxanthin and (8*S*)- mutatoxanthin (3:2)) (Fig.2 (b)).

We measured the lipid peroxidation inhibitory and $^1\text{O}_2$ quenching activities of epoxycarotenoids and their acid treatment products. They showed potent lipid peroxidation inhibitory activities (IC_{50} 0.43 – 2.1 μM) (β -carotene IC_{50} 72 μM) and moderate $^1\text{O}_2$ quenching activities (IC_{50} 9.8 – 100 μM) (astaxanthin IC_{50} 1.2 μM).

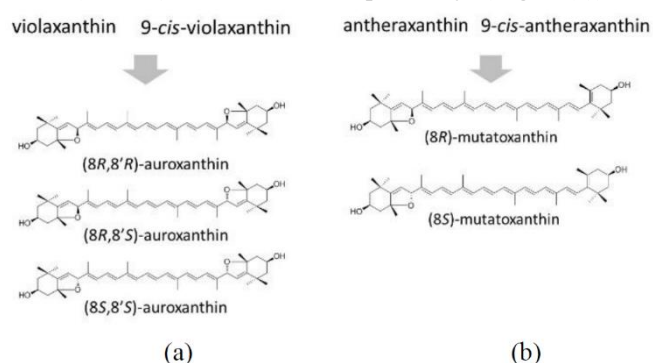


Fig. 2. Acid treatment products of violaxanthin and 9-*cis*-violaxanthin (a). and antheraxanthin and 9-*cis*-antheraxanthin (b).

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Rapid assembly of carotenoids of phenyl, phenolic, and acyl end groups with superior antioxidant activities

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1. Introduction

Carotenoids are important natural pigments showing orange to red colors due to extensive conjugation of C=C bonds, which endows their photosynthetic energy transport and antioxidant activities. Reactive oxygen species (singlet oxygen, oxy radical, superoxide radical etc.) are easily quenched by the polyene chain of carotenoids, which produce the stabilized conjugated carbon radicals. The antioxidant activity of carotenoids would be exhibited not only by the polyene chain, but also by the end groups. We were interested in revealing the synergistic end group effects on their antioxidant activities. We synthesized the carotenoids with different end groups of diversely substituted phenyl **1** [1], phenolic **2** and **3** [2], and acyl **4** [3] (**Figure 1**) and compared their antioxidant activities. The key intermediate structures **A** and **B** were efficiently prepared to warrant the rapid assembly of the carotenoid libraries.

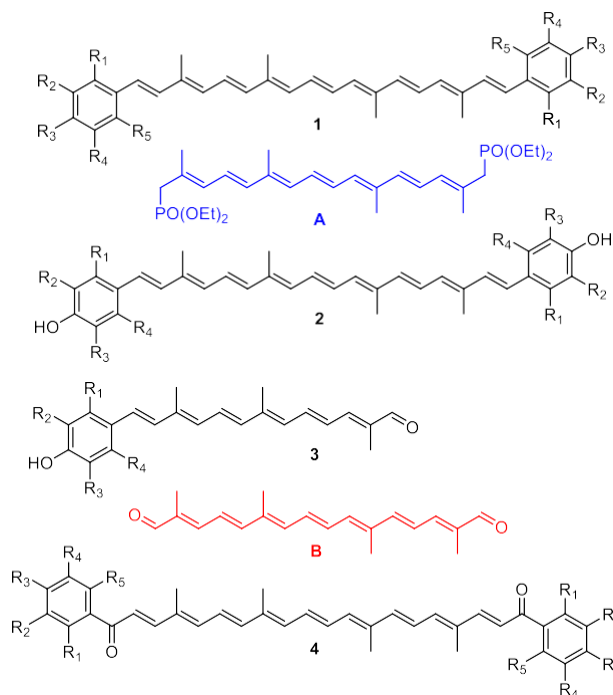


Figure 1. Carotenoid structures of phenyl **1**, phenolic **2** and **3**, and acyl **4** end groups and the intermediates **A** and **B** required for rapid assembly.

2. Experimental

All the intermediates and the carotenoid products were identified by NMR, IR, and High-Resolution Mass Spectroscopies. The antioxidant activity of carotenoids was measured by DPPH, ABTS, and superoxide radical scavenging assays with natural carotenes such as β -carotene, lycopene, and canthaxanthin as positive controls. DFT calculations were also carried out to give the optimal structural information and HOMO and LUMO energy levels of the newly synthesized carotenoids.

3. Results and Discussion

Eighteen carotenoids with phenyl end groups were synthesized, and the *E/Z* configuration of the polyene chain depends on the substitution pattern of the phenyl groups. Carotenoids with electron-rich, multi-substituted phenyl groups show better antioxidant activities than β -carotene [1]. Four phenolic carotenes **2** and five phenolic apo-12'-carotenals **3** were prepared. Apo-12'-carotenals **3** without ortho substituents exhibited superior antioxidant activities to their corresponding symmetrical carotenes **2** as well as β -carotene [2]. Fifteen carotenoids with acyl end groups were synthesized, and exhibited 105~151% better superoxide radical scavenging activity than β -carotene [3].

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Analysis of optical properties of carotenoid nanoparticles by considering molecular flexibility

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1. Introduction

Certain vegetables and fruits produce carotenoid compounds during their ripening process, which give them vivid yellow, orange, or reddish colors. The optical properties of carotenoids are of practical importance in the food industry as they are widely used as natural pigments in various food products. Unlike typical organic pigments, which have a rigid, aromatic-based molecular structure, carotenoids have flexible molecular structures derived from polyene skeletons (**Figure 1a**: β -carotene is one of the carotenoids), suggesting carotenoid molecules are easily distorted in response to stress and change their properties. In this paper, we show that molecular distortion plays an important role in the optical properties of carotenoid-based nanoparticles (**Figure 1b**).¹

2. Results and Discussion

Nanoparticles of four carotenoids (β -carotene, lycopene, astaxanthin, and lutein) were obtained with < 100 nm in diameter by reprecipitation from their solutions in tetrahydrofuran. Transmission electron microscopy, electron diffraction analyses, and powder X-ray diffraction measurements revealed that obtained nanoparticles comprised a crystalline and an amorphous domain (**Figure 1c**).² It is known that the peak position of the Raman band assigned to the C=C stretching vibration (ν_1) of the carotenoid polyene chain shows a good correlation with the effective π -conjugation length.³ Detailed Raman spectroscopic analysis showed that the crystalline domain of the nanoparticles contained less-distorted molecules with a fully extended effective π -conjugation length, whereas the amorphous domain contained distorted molecules with a short effective π -conjugation length (**Figure 1c**).² We also found a good correlation between the absorption spectra and effective π -conjugation lengths of the carotenoids, indicating that molecular distortion strongly affects their solid-state properties. Our results unveil that actively controlling molecular distortion in flexible molecules can potentially provide new materials.

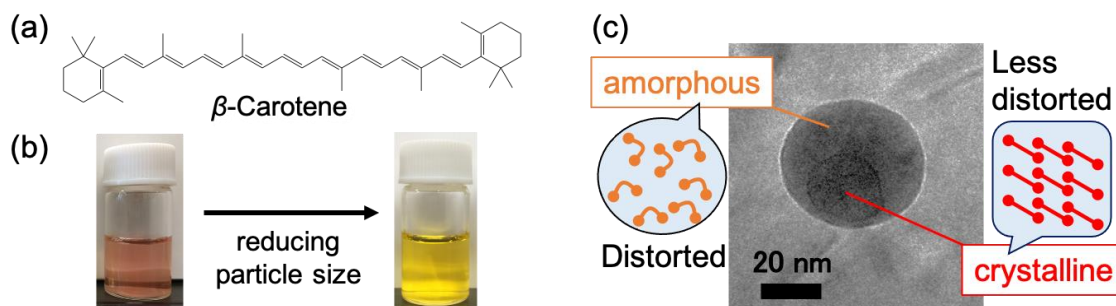


Figure 1. (a) Chemical structure of β -carotene, (b) β -carotene particles dispersion that change color as particle size reduction, and (c) β -carotene nanoparticle taken by a cryo-transmission electron microscope

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Effect of the aggregation behaviour on the antioxidant capacity of carotenoidsDalma Czett¹, Katalin Böddi¹, Attila Agócs¹, József Deli^{1,2}, Veronika Nagy^{1*}¹Department of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary;²Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Rókus u. 2, H-7624 Pécs, Hungary**1. Introduction**

Lipophilic substances such as carotenoids show a tendency to aggregate in aqueous medium. Aggregation changes the physical and chemical properties of individual carotenoid molecules, such as light absorption, reactivity, effective size, and solubility [1]. To estimate the antioxidant properties of carotenoids, trolox-equivalent antioxidant capacity (TEAC) values are routinely determined with ABTS[•] solutions [2]. As this reagent is a salt, it is applied in aqueous solutions to which the carotenoids are added in organic solvents. Depending on the solubility of individual carotenoids, various organic solvents are used and there is no standard procedure for the TEAC determination. Here we present a systematic investigation on the solvent and concentration dependence of TEAC measurements of 8 carotenoids, as well as the relationship between the aggregation and TEAC values.

2. Experimental

Carotenoids or trolox were dissolved in dimethyl sulfoxide (DMSO) or tetrahydrofuran (THF) to get stock solutions which were diluted with ethanol, distilled water or phosphate buffer saline (PBS) for the ABTS[•] reactions, in which the decrease in ABTS[•] concentration were followed by spectrophotometric measurements. The percentage inhibition of absorbance values were plotted against the final concentration of the antioxidants, the slopes of the curves were compared with that for trolox, TEAC values were calculated as the ratio of the slopes for the antioxidant and for trolox. For the aggregation studies carotenoid solutions were prepared the same way and were examined by UV-vis and dynamic light scattering (DLS) spectroscopies.

3. Results and Discussion

The percentage inhibition of absorbance values generally gave linear relationship with the concentration of the carotenoids, but in certain cases some fell below the straight line. By examining the UV-vis and DLS spectra of the carotenoid solutions we found that in these specific cases an exceptional aggregation happened yielding bulky clumps (**Figure 1**). Interestingly, the large aggregates were formed at a particular concentration, typically in 2.5 or 3.37 μ M almost for all the investigated carotenoids, but in higher or lower concentrations the size of the aggregates were much smaller.

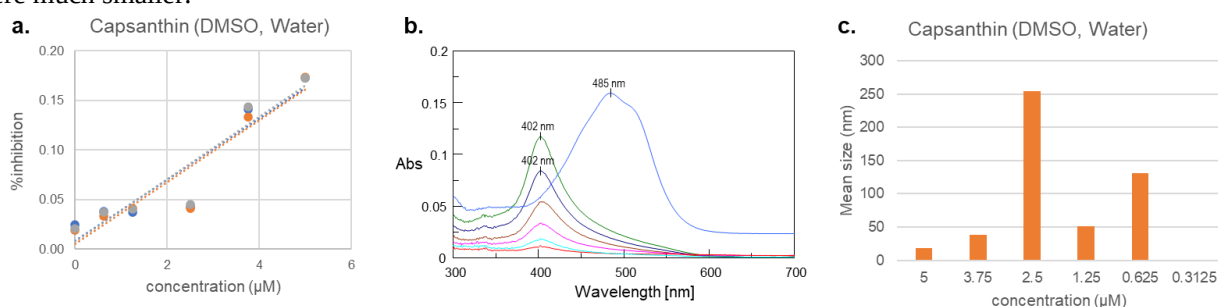


Figure 1. a. Percentage inhibition in ABTS[•] reaction, b. UV-vis spectra, c. Hydrodynamic diameters by DLS

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Chemistry of carotenoid radicals and complexes: A compilation of tens of years of research

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1. Introduction

The continuous support from the U. S. Department of Energy from 1986 until 2014 for carotenoid research at The University of Alabama enabled properties and structures of carotenoid radicals to be established. In a collaborative effort, we are still investigating these properties today [1], and the most representative results have been recently compiled in a book entitled "Chemistry of carotenoid radicals and complexes" [2]. Among the numerous physiochemical techniques that have been employed in Prof. Kispert's group since 1986 in the studies of oxidation/reduction reactions of carotenoids are the following: electrochemical measurements such as cyclic voltammetry (CV) with DigiSim simulation and Osteryoung square wave voltammetry (OSWV), optical spectroscopy (UV-Vis, near IR), optical spectroscopy of carotenoid solutions treated with chemical oxidants, electron paramagnetic spectroscopy (EPR), simultaneous electrochemical and EPR (SEEPR) detection, continuous wave and pulsed electron nuclear double resonance (ENDOR), and density functional theory (DFT) calculations.

2. Results and Discussion

While the results of some methods simply confirm those of others, in general, each method addresses different aspects of the redox behavior of carotenoids or of the physical properties of the transient, positively charged ($\text{Car}^{\bullet+}$, Car^{2+} and $\# \text{Car}^+$) and neutral ($\# \text{Car}^\bullet$) intermediates formed upon the initial electron transfer. We will focus on radical species $\text{Car}^{\bullet+}$ (radical cations) and $\# \text{Car}^\bullet$ (neutral radicals) detected in solution by electrochemical measurements (**Figure 1**), EPR and ENDOR measurements in conjunction with DFT calculations (example of Davies ENDOR spectrum in **Figure 2**), and the implications of these results in photoprotection and formation of carotenoid complexes. Such studies on carotenoid radicals are essential to understand some of the complex functions of the ubiquitous carotenoids.

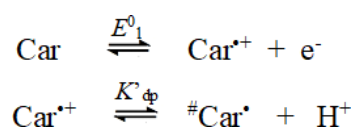


Figure 1. Radical species detected electrochemically in solution

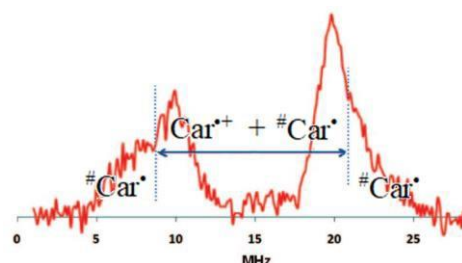


Figure 2. Radical species detected by pulsed ENDOR on solid support

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[2] A. L. Focsan, L. D. Kispert, Chemistry of carotenoid radicals and complexes, under contract with World Scientific Publishing Co. Pte. Ltd

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Session 8:
Plant Carotenoids

Plant Apocarotenoid Formation: Destruction for Construction

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Carotenoids are an essential structural and functional component of the photosynthetic apparatus, contributing to the harvesting of light energy and protecting other molecules from photo-oxidative destruction. However, the conjugated double bond system of these long chain isoprenoids, which enables them to fulfill their diverse biological functions, is also the reason for their instability. Indeed, carotenoids are continuously oxidized through attack of ROS and degraded into smaller molecules carrying different oxygen containing groups, which are generally called apocarotenoids. However, several of these degradation products are not just a catabolic trash, but are bioactive metabolites involved in sensing and mediating of cellular responses. Moreover, plants have multiple types of carotenoid cleavage dioxygenases (CCDs), which belong to an ubiquitous family of enzymes catalyzing the oxidative cleavage of carotenoids, and have much more diverse carotenoid “catabolic” processes, compared to animals and microorganisms. Indeed, plant CCDs mediate the formation of plant hormones, such as abscisic acid and strigolactones, different growth regulators, including xaxinone, and pigments and aroma of ecological importance. In other words, the cleavage of carotenoids is the first step in different “anabolic” pathways yielding regulatory metabolites that affect almost all aspects in plant’s life [1]. In this talk, I will summarize our knowledge about apocarotenoid formation and briefly describe the function of plant apocarotenoids. This will be followed by an update on our recent results on new functions of these metabolites and on alternative routes for abscisic acid [2, 3] and crocetin biosynthesis [4].

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Illuminating carotenoid synthesis regulation in the carrot taproot grown underground

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Light stimulates both plastid development and the biosynthesis of carotenoids during plant photomorphogenesis and fruit ripening. White light (high Red/FarRed) activates photoreceptors (PHYB-PHYE) and molecular factors for carotenoid and chlorophyll biosynthesis are induced thereof. In roots, only a fraction of low R/FR light (considered as shade) reaches this tissue, which is not required for carotenoid synthesis, but it is essential for root development. However, the orange taproot of *Daucus carota* (carrot) grown underground synthesizes large amounts of carotenoids in chromoplasts. When carrot plants are grown under high R/FR and high intensity ($165 \mu\text{mol m}^{-2} \text{s}^{-1}$) respect to low light intensities ($54 \mu\text{mol m}^{-2} \text{s}^{-1}$), the carrot taproot develops earlier without affecting carotenoid content. This suggest that higher intensities of white light promotes a better plant fitness, which induces the development of the carrot taproot.

However, when carrot root is grown directly under white light, the expression of key carotenogenic genes and total carotenoid levels decrease and the root develop chloroplasts, similar to normal roots exposed to light. To find genes that regulate carotenoid synthesis in the carrot taproot, we performed a RNA-seq between root grown in WL (R/L) and underground (R/D). Interestingly, genes that are activated under low R/FR (shade) such as *PHYA*, *PAR1* and *PIF3* were upregulated in the underground root and down-regulated in light. *PHYA* is active and stable under low R/FR were it promotes photomorphogenesis and carotenoid synthesis. *DcPHYA* exhibits 73% amino acid identity with *AtPHYA*. *Nicotiana tabacum* overexpressing *DcPHYA*, present reduced hypocotyl length and reduced expression of *NtPEPCK* and *NtPAR1* under FR, suggesting that *DcPHYA* is functional. Carrot transgenic lines with over 80% reduced relative expression of *DcPHYA* present between 65%-80% reduction in carotenoid content as well as in the relative expression of carotenogenic genes. These results suggest that *DcPHYA* is functional and participates in carotenoid synthesis in the carrot taproot grown underground.

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Dissection of the saffron crocin biosynthesis pathway and its biotechnological applications

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Crocins are glycosylated apocarotenoid pigments synthesized by few plant taxa (*Crocus*, *Buddleja*, *Gardenia*). They have multiple roles in the prevention of chronic disease and their chemical synthesis is not possible, due to the abundance of chiral centers. We have characterized the crocin biosynthetic pathway and compartmentation in *Crocus sativus*. Synthesis proceeds through a cleavage step by a plastid-localized CCD2, followed by dehydrogenation by an ER-localized ALDH3, glycosylation by one or more cytoplasmic UGTs, and vacuolar transport by ABCC-type transporters. We are using a variety of heterologous expression systems (tobacco, tomato, *N. benthamiana*, yeast) with different carotenoid complements, in order to study the substrate specificity of the various enzymes, and optimize crocin production.

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Carotenoid derived signals and their impact in plant development

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Plastids are essential metabolic hubs and important environmental sensors for plants. Over the years it has become clear that diverse retrograde signals emerging from plastids regulate plant development and fitness by modulating nuclear gene expression. In particular in the past years many of these signals have been shown to derive from carotenoids and carotenoid-derived molecules. The characterization of the *Arabidopsis* carotenoid biosynthetic mutant *clb5* showed that the accumulation and cleavage of diverse zeta-carotenoids (referred as ACS1), results in alterations of fundamental aspects of the plant developmental program in both leaves and roots. Accumulation of ACS1 affects the expression of hundreds of nuclear genes in comparison to the previous biosynthetic step (PDS) mutant, and some of their direct targets included the transcriptional repression of more than 50% of the nuclear genes encoding for chlororibosomal proteins resulting in inhibition of the overall plastid translation. The plastid translation inhibition initiates a second retrograde GUN1-dependent signaling cascade, that parallels the one observed with plastid translation inhibitors and that results in leaf and roots developmental defects. One of the major components of the ACS1 signaling cascade is light that our findings supports is required for the synthesis of the ACS1 apocarotenoid signals. The defects caused by ACS1 accumulation extends to alterations in SAM and RAM meristem identity and progression, as observed with the analyses of the expression pattern of different meristem markers. We demonstrate that this regulation translates into leaf, flower and root developmental defects demonstrating a general role of ACS1 in critical plant developmental decisions. Finally, the elucidation of the role of ACS1 in floral development exposes a novel layer of regulation from plastids that is conserved in plants such as tomato. advance in understanding the mechanisms participating in organelle retrograde signaling and open novel avenues into the mechanisms that modulate plant development.

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Carotenoids, Apocarotenoid signals and plant development

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Abstract

A current frontier in carotenoid biosynthesis and function is to gain insights into the regulation of apocarotenoid biosynthesis and how they function as development signals, collectively referred to as apocarotenoid signals (ACS). Herein, we sought to explore how regulation of the multi-enzyme poly-*cis*-transformation of 15 *cis*-phytoene to all *trans*- lycopene, which evolved from the single enzymatic reaction in bacteria, provides a unique means to regulate various aspects of plant development. As a result, a novel link between carotenoid biosynthesis and metabolism and the regulation of FM identity and floral organ development has emerged. This seminar will provide an overview of our recent work into the detection of novel apocarotenoids and other metabolites, and, the discovery of new functions for apocarotenoids as signalling molecules that regulate the differentiation of stem cells, floral organ identity and leaf morphology.

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Using renewable sources of xanthophylls to produce xanthophyll esters with improved physicochemical properties in a sustainable mannerValentina Metličar,¹ Krištof Kranjc,² Alen Albreht^{1*}¹*National Institute of Chemistry, Department of Analytical Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia*²*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia*

Xanthophylls, the oxygenated relatives of carotenes, are well known for their many beneficial effects on human and animal health. As a consequence, they have many industrial applications, including food supplements. However, the majority of them suffer from one major drawback – chemical instability, especially in the presence of either oxidants, acids, and metals or when exposed to light or increased temperatures. Moreover, xanthophylls are often acquired (extracted from plants) by non-sustainable means, leaving behind a significant carbon footprint.

Here, we present a green technology for the production of xanthophyll esters as a means to increase their chemical stability and enable their use as improved food supplement ingredients with potentially high added value. These esters (55 in total) were synthesized using β -pinene as a bio-derived solvent at ambient temperature and pressure with excellent selectivity (median 97%) and finally isolated in high yields (median 81%) and with high purities (median 97%). The production of tailored xanthophyll esters can also be produced within a solvent-free reaction, further contributing to environmental sustainability. The use of food waste and invasive alien plant species, such as Japanese knotweed, not only helps to preserve biodiversity but these materials also represent a renewable and practically unlimited source of natural pigments.

Finally, 13 structurally and electronically distinct model lutein diesters were subjected to a forced degradation study and the relationships between their structure and their degradation rate were systematically determined. The forced degradation data show that the stability of a particular lutein diester may depend to a large extent on the type of exposure (elevated temperature, light, oxidant, or acidic environment). Not every esterification attempt led to an increase in stability, but three branched products – lutein di(2,2-dimethylpropanoate), lutein di(2-methylpropanoate) and lutein di(3-methylbutanoate) – proved particularly important as they were as much as 1.5 – 21 times more stable compared to free lutein, regardless of the type of the stress condition applied. We also show the intricate effect that the matrix of the Japanese knotweed has on pigment degradation kinetics, which could not be easily predicted. Thus, our proposed valorization strategy is certainly feasible, but the esterification approach should be tailored to the intended use of a particular lutein diester.

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Session 9:
Food, Nutrition and Epidemiology

Increasing the bioaccessibility of carotenoids by cooking practices. Sustainability aspects

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1. Introduction

Industrial and culinary practices are known to lead to carotenoid losses, although there is strong evidence that, depending on the conditions, they can also increase considerably their release from plastids, cells and eventually their potential bioavailability. For instance, the size reduction of the matrix and changes in plastids as a result of industrial treatments are known to have an impact on the bioaccessibility of carotenoids. However, the processing conditions (for instance intensity and time) need to be controlled as if they are too severe the losses of carotenoids cannot be compensated by their increased release from the matrix [1,2]. Besides, overcooking leads to an unnecessary energy expenditure. In this work the effect of different cooking practices in the levels and bioaccessibility of tomato and carrot carotenoids in relation to energy expenditure is evaluated.

2. Experimental

Tomato and carrot samples matched in size were subjected to different cooking practices (steaming, water cooking, microwaving, baking) at different potencies and lengths of time. Carotenoids bioaccessibilities were obtained following the INFOGEST static *in vitro* digestion methodology followed by micelle isolation and extraction [3]. The carotenoid bioaccessible content, that is, the amount of carotenoid incorporated into micelles from a certain food amount serving was also assessed. Energy consumption was calculated as Kwh.

3. Results and Discussion

Our results clearly show that, as already known, cooking practices can lead to dramatic increases of potential bioavailability of carotenoids. As an example a 8.90-fold increase in the carrot total carotenoid bioaccessible content was observed as a result of baking. Interestingly baking was not the most efficient practice in terms of energy expenditure in increasing CBC- Thus, when considering the CBC per Kwh consumed, microwaving proved to be the best cooking technique. The results of this study add up to other investigations on the effect of cooking on carotenoid bioavailability by pinpointing the importance of considering energy consumption in order to contribute to more sustainable food systems.

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Dietary-derived factors influencing aspects of carotenoid bioavailability – focus on protein-interactions

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1. Introduction

Dietary intake of carotenoids and their circulating plasma concentrations have been related to a reduced risk of chronic non-communicable diseases such as cardiovascular ones and diabetes. However, due to their lipophilicity, their absorption is usually low and quite variable. While it is known that a minimum amount of lipids are required to facilitate optimal absorption, other factors, such as dietary fiber, divalent minerals and also proteins have been studied far less. This overview strives to highlight some of these understudied aspects, focusing on dietary proteins.

2. Experimental

A sequence of studies was conducted to investigate effects of frequently consumed dietary proteins on relevant aspects of carotenoid bioavailability including bioaccessibility, cellular uptake, and bioavailability in humans, and findings are discussed together with complementary relevant literature.

3. Results and Discussion

Whey protein isolate (WPI), gelatin, sodium caseinate and soy protein isolate (SPI) received most attention regarding their interactions with carotenoids. In a [first in vitro study](#), WPI modulated beta-carotene bioaccessibility depending on digestive conditions. While high simulated peristalsis and a low amounts of co-digested lipids resulted in positive effects on beta-carotene bioaccessibility (ca. 20% increase), high amounts of oil, lower bile salt and pancreatine concentrations produced negative effects. Co-digested proteins increased the bioaccessibility of individual carotenes, i.e. lycopene and beta-carotene (by almost 2- fold), while individual xanthophylls such as lutein were [reduced in their bioaccessibility](#) to 50%. Similarly findings were observed for carotenoid-rich food matrices (tomato juice, carrot juice and spinach), studying both bioaccessibility and [cellular uptake](#). Soluble and well digestible proteins also resulted in more positive effects on carotenoid bioaccessibility/cellular uptake. In vitro findings were confirmed in a [human postprandial crossover trial](#) (n=24 participants), in which a well soluble and digestible protein (WPI, ca. 30 g) vs. a less soluble/digestible one (SPI, ca. 30 g) improved by about 1/3 the absorption of individual and total carotenoids from a tomato/carrot juice as studied by responses in the plasma-triacylglycerol rich fraction.

4. Conclusions

Taken together, proteins can act as a double-edged sword that could either increase or impede (Figure 1) aspects important for carotenoid bioavailability. Well digestible and soluble proteins have the potential to significantly increase the bioavailability of these health-associated pigments, likely via fostering lipid droplet processing into mixed micelles and fostering their stability. Results could be relevant for the food industry, for vegetarians and for persons with gastro-intestinal disorders.

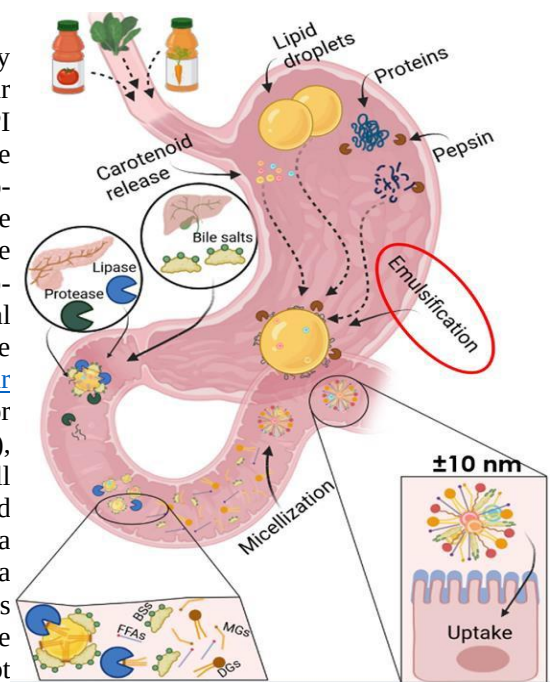


Figure 1. Interaction of proteins, lipid droplets and mixed micelles during digestion related to aspects of carotenoid bioavailability. FFA=free fatty acids, MGs=monoacylglycerides, DGs=diacylglycerides, BSs=bile salts

Photoprotection with food rich in carotenoidsVolker Böhm^{1*}, Marius Faber¹, Sarah Zerres², Wilhelm Stahl²¹ Institute of Nutritional Sciences, Friedrich Schiller University Jena, Dornburger Str. 25-29, 07743 Jena, Germany.² Institute of Biochemistry and Molecular Biology I, Faculty of Medicine, Heinrich-Heine-University Düsseldorf, P.O. Box 101007, 40001 Düsseldorf, Germany.**1. Introduction**

Carotenoids are photoprotective compounds quenching excited state molecules and scavenge light-induced ROS [1]. The xanthophyll lutein (blue light filter) is part of the macula lutea, the yellow spot in the eye, and there is evidence that the risk for age-related macula degeneration (AMD) increases with decreasing optical density of the macular pigment. Thus, lutein intake has been attributed to the protection of the macula [2]. Besides the eye, the human skin is another target tissue for adverse effects of UV light which has been associated with the induction and promotion of skin cancer. Light penetrates the skin and interferes with different biomolecules including the DNA. Photooxidative processes lead to the formation of reactive oxygen species (ROS), damaging molecules and cell structures. Thus, antioxidant carotenoids likely contribute to dermal photoprotection [1]. Fruits and vegetables are suitable sources of various carotenoids, ingested with the diet at levels of several mg per day [1].

2. Experimental

A randomized, double blind, placebo controlled human intervention pilot study (10 weeks (w): 2 w run-in, 4 w intervention, 4 w washout) was conducted with 20 patients with non-exudative AMD. One group (n=10) received 50 mL/d of a beverage with oleaginous kale extract, the other group (n=10) 50 mL/d of a placebo beverage [3].

As UV radiation induces DNA damage (e.g. formation of cyclobutane pyrimidine dimers (CPDs)), several experiments were carried out to test protective effects of carotenoids. After UV radiation of calf thymus DNA, CPDs were detected by immunoassay. For detection of specific CPDs, a LC-MS/MS method was optimized. The formation of “dark dimers” was also tested in cell culture. In addition, the UV-protection potential of carotenoids was investigated in a liposome model (unpublished data).

4. Results and Discussion

Four weeks of intervention (oleaginous kale extract beverage) with 10 mg lutein and 3 mg zeaxanthin per day led to significantly increased contents of lutein and zeaxanthin in blood plasma. Three out of four parameters for macular pigment optical density (MPOD) investigated significantly increased. During the washout period, the xanthophyll contents in plasma as well as the MPOD parameters significantly decreased. Thus, a continuous uptake is necessary to avoid a decrease in MPOD [3].

Although CPDs were shown in UV-radiated DNA by immunoassay, the optimized LC-MS/MS method was not sensitive enough to detect the photoproducts. However, a direct and delayed formation of CPDs was shown in fibroblasts. This indicates that in addition to the photochemical CPD generation a delayed chemical process is operative forming so called “dark dimers”. Photoprotective properties of lutein and β -carotene were shown by the inhibition of lipid peroxidation, as demonstrated in a liposomal model.

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Fermented milk containing high content of exopolysaccharide enhance dietary carotenoid bioavailability in humans.

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1. Introduction

Dietary carotenoids have beneficial health effects because of their antioxidant and anti-inflammatory properties. In humans, the bioavailability of β -carotene from carrots or spinach ranges from 3–16%. Therefore, it is important to increase the bioavailability of dietary carotenoids in order to maximize their potential for health effects. The aim of this study was to evaluate the effect of fermented milk containing high content of exopolysaccharide on the bioavailability of dietary carotenoids in humans.

2. Experimental

Three studies were conducted using a randomized, crossover method. The study subjects (N=16/study) consumed a test drink on two separate days with a two-week washout period. Subjects consumed a vegetable (carrot, tomato, or spinach) drink alone or with a fermented milk. Fermented milk was produced by incubating *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1251 and *Streptococcus thermophilus* OLS3290 with 10% nonfat skimmed milk powder. Blood samples were collected before the test drink was consumed (0 h) and again 2, 4, 6, 8 h after ingestion. Aliquots of fresh plasma from each blood sample were processed to isolate the triacylglycerol-rich lipoprotein (TRL fraction) because the postprandial carotenoid content in the TRL fraction of plasma is elevated. Carotenoid concentrations in the all and TRL fraction of plasma were quantified using a HPLC.

3. Results

A significantly higher iAUC_{0-8 h} for β -carotene, α -carotene was observed in the TRL fraction of plasma when carrot + fermented milk was consumed compared with carrot alone (β -carotene: 1.8-fold change). For lycopene, the iAUC_{0-8 h} of the TRL fraction of plasma was significantly larger in the group that consumed tomato combined with fermented milk versus the groups that consumed tomato alone (6.5-fold change). The iAUC for lutein in all plasma fractions was significantly higher for the group that consumed spinach + fermented milk compared to the spinach alone group (Figure 1).

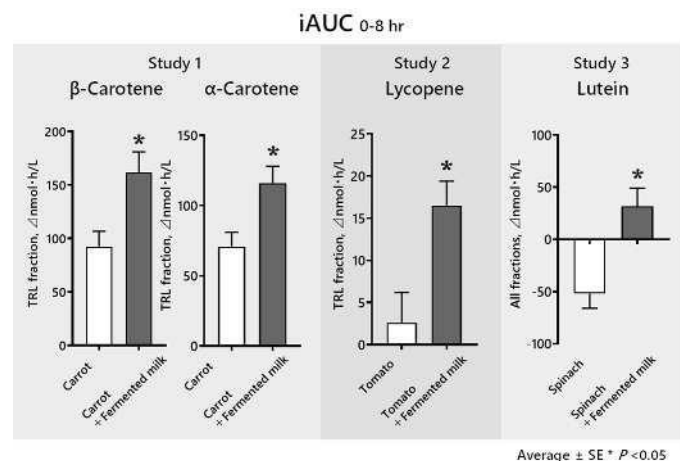


Figure 1 iAUC_{0-8 h} for plasma carotenoids

4. Conclusion

Co-ingestion of vegetables and fermented milk significantly increased dietary carotenoid bioavailability in humans. These findings may provide new methods to increase dietary carotenoid bioavailability [1].

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Validation of dietary lutein and zeaxanthin screeners in healthy Australian adults

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1. Introduction

Habitual dietary lutein and zeaxanthin (L/Z) intake may reduce risk and severity of age-related macular degeneration. Habitual intake is a significant confounding variable when investigating change in L/Z macular concentration in L/Z intervention studies (supplemental or food based). [1] Yet, comprehensive L/Z specific screeners are currently not available. This study aimed to validate two purposely developed screeners in healthy Australian adults.

2. Methods

Two screeners with 91 food items were developed, with recall timeframe of a month or a week. Over four weeks, participants completed eight 24-hour diet recalls, four weekly screeners and two monthly screeners. The United States Department of Agriculture food composition tables were used to estimate foods contributing to total dietary L/Z intake. [2]. A Bland-Altman plot analysis and Spearman's rank order correlation tests were performed, to compare the L/Z intake reported by the three tools (expressed as mean daily intake in milligrams). [3] Referring to intervention dosages previously reported, the limits of agreement for validity were set at ± 0.25 mg/day L/Z. [1] Statistical significance set at $p < 0.05$.

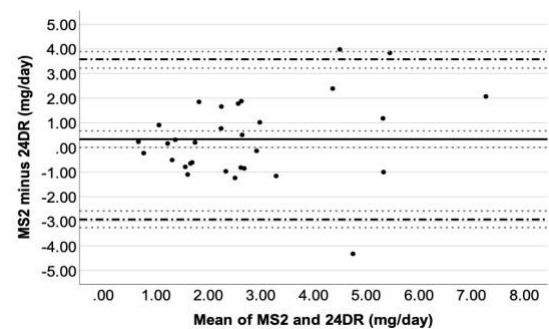


Figure 1. Bland-Altman plot comparing daily lutein and zeaxanthin intake captured by 24-hour diet recall (24DR) or monthly screener (MS2)

3. Results

Fifty-seven participants (median age 27 years and 73% female) reported a median (25th -75th percentile) daily of L/Z intake of 2.4 (1.6–3.1) mg/day from the 24-hour diet recalls, 2.7 (1.7–3.5) mg/day from the monthly screener, and 2.8 (2.1–4.3) mg/day from the weekly screeners. Significant correlations with 24-hour diet recall intake were $r = 0.7$ for the weekly screeners and $r = 0.58$ for the monthly screener. The Bland-Altman plot indicated the screeners were not valid with 95% limits of agreement > 0.25 mg/day. The monthly screener mean difference was 0.33 mg/day and 95% limits of agreement between -2.91 mg/day and 3.58 mg/day L/Z (see Figure 1 for Bland-Altman plot). Baby spinach, broccoli, and pumpkin provided the greatest proportion of L/Z intake and contribution to differences in total L/Z intake between the three tools.

4. Discussion and conclusions

The Bland-Altman plot analyses indicated the screeners were not valid. Prior investigations of food frequency questionnaire validity for capture of L/Z or antioxidants have relied upon correlational statistics and returned poor outcomes. [4] Despite poor agreement in the Bland-Altman plots in this study the tools were moderately correlated, this outcome suggests antioxidant questionnaire validation studies solely reliant on correlational statistics to determine validity should be interpreted with caution. Moderate to high L/Z containing foods contributed to poor validity of the screeners through mis-estimation and missed capture of foods. Future studies should consider an alternate dietary intake method, such as a 3-day diet diary, to perform relative validity against the screeners.

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Whey- and Soy Protein Isolates Added to a Carrot-Tomato Juice Alter Carotenoid Bioavailability in Healthy Adults

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1. Introduction

Carotenoids are bioactive secondary plant metabolites, presumably with health beneficial effects for humans. Owing to their rather low water solubility requiring emulsification prior to their potential absorption, carotenoids are of low and varying bioavailability, depending on host and dietary factors, including the presence of dietary fiber, and lipids. While our *in vitro* findings suggested that proteins can differentially affect carotenoid bioaccessibility during gastro-intestinal (GI) digestion, not much is known on the *in vivo* influence of these macromolecules on carotenoid absorption efficiency. For this purpose, we conducted a human trial to confirm that proteins, specifically whey- and soy-protein isolates (WPI/SPI) impact postprandial carotenoid bioavailability from plant food items rich in carotenoids.

2. Experimental

The human trial consisted of a randomized, postprandial crossover trial with 24 healthy adults (n = 12 males, n = 12 females), receiving the test meal that consisted of a mixture of 350 g of tomato and carrot juice (1:1, v/v, approx. 32 mg total carotenoids, mostly lycopene and β -carotene) that contained or not 30 g of either

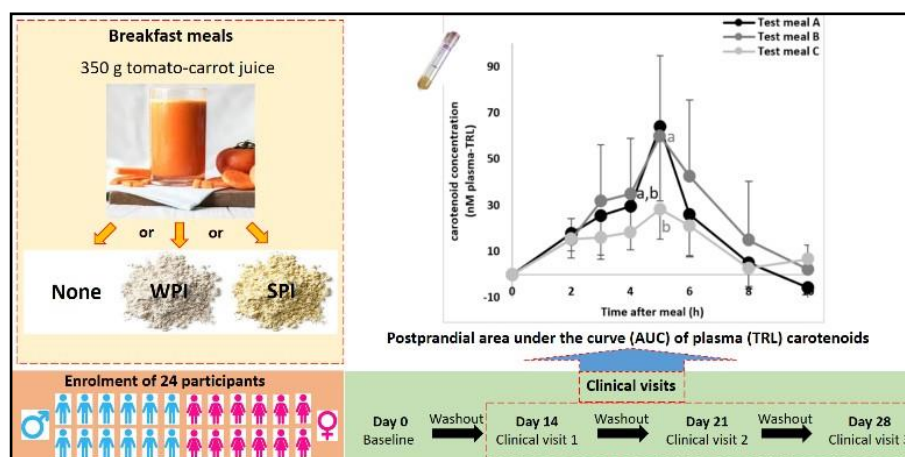


Figure 1. Main steps of the study procedure, including recruitment, clinical visits, sample processing, postprandial experiments and carotenoid analysis.

SPI or WPI (equivalent to ca. 50% of the recommended dietary allowance, RDA $\approx$ 60 g/d).

Absorption kinetics of carotenoids and triacylglycerols were evaluated via the triacylglycerol-rich lipoprotein (TRL) fraction response, at timed intervals up to 10 h after test meal intake, on three occasions separated by 1 week (see **Figure 1** for the main steps of the study procedure).

4. Results and Discussion

The estimated area under the curve (AUC) for WPI significantly increased by 45% vs. the control, and by 57% vs. SPI. In males, significant differences were also found for phytoene, phytofluene, α -carotene, and β -carotene in the presence of WPI vs. control. Cmax for total carotenoids was significantly increased by WPI (by approx. 15%), while significantly lowered by SPI (by approx. 25%) vs. the control. The results suggest that a well digestible protein such as WPI could be beneficial for carotenoid bioavailability, whereas a less digestible one such as SPI may result in hampered availability. A positive effect on generally well-absorbed colorless carotenoids, i.e. phytoene and phytofluene was also recognized. This is, to our knowledge, the first study finding effects of proteins on carotenoid absorption in humans.

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Session 10:
Carotenoids in the Eye and Brain

Lutein and cognitive function throughout the lifespan: an update.

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Keywords: lutein, cognition, lifespan

It has been fifteen years since dietary of lutein was first recognized as having a potential role in cognitive function in older adults [1]. Since then, evidence has accumulated to support a role for lutein in cognitive function throughout the lifespan. Among the dietary carotenoids, lutein is selectively taken up into brain tissue. In part, this may be due to the presence of a specific binding protein [2] and provides biological rationale for its importance. Several research groups have reported significant associations between macular pigment optical density, a non-invasive biomarker of lutein in neural tissue [3], and cognitive measures in various age groups [4].

Additionally, interventions studies in older adults report improvements in cognitive function [4]. Fewer studies have been conducted in children. However, recent prospective cohort studies suggest that dietary lutein intake may also have cognitive implications during pregnancy and in childhood. In this study, early childhood intake of lutein was positively associated with receptive vocabulary and mid-childhood executive function [5]. Additionally, it was reported that higher maternal lutein intake during pregnancy was associated with better offspring verbal intelligence and behavior in mid-childhood [6]. This observation provides evidence of neurodevelopmental benefit of lutein intake. Little is known regarding the mechanisms by which lutein functions in brain tissue. Future research directions include an understanding of factors involved in lutein uptake into neural tissue as well as further characterization of the relationships between lutein and other nutrients of interest to cognitive health. Lutein intake in American adults is low (~1–2 mg/d) and tends to be even lower in early life (~300–500 µg/d). As with many aspects of diet, low levels in childhood could start a cascade that amplifies with age. Although the precise mechanisms by which lutein may be influencing neural health across the lifespan remain to be investigated, efforts may be warranted to establish recommended intakes for this dietary bioactive given the sum of the evidence to date.

Acknowledgements: Abbott Nutrition, DSM Nutritional, Kemin Foods, NIH EY-11600, NIH EY-14800, NIH UH3 OD023286, NIH/NICHD RO1 HD34568, USDA ARS

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Application of the measurement of skin carotenoids by refractive spectroscopy, Veggie Meter

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1. Introduction

Macular pigment (MP) consisting of three carotenoids, lutein, zeaxanthin, and meso-zeaxanthin, works for preventing eye diseases caused by photooxidative damage, such as age-related macular degeneration (AMD). Since the main source of carotenoids is green and yellow vegetables, much intake of vegetables as well as fish oil and supplement taking is recommended. Reflection spectroscopy, Veggie Meter (VM), can measure skin carotenoid (SC) levels non-invasively, and VM can quantify the intake of vegetable and fruit. We investigated SC levels in patients in our ophthalmology clinic and health examination center and analyzed the association between SC levels and AMD, cardiovascular disease, and cancer history.

2. Methods

SC and MP levels were measured by VM and autofluorescence spectroscopies (Spectralis) in 985 and 150 patients, respectively. The risk for cardiometabolic disease and disease history including AMD was estimated by scoring of blood pressure, low density lipoprotein, hemoglobin A1c, and alanine aminotransferase, and the association between SC levels and risk scores was analyzed in 2917 participants who received health examinations.

3. Results and Discussion

The median of SC levels was 320. SC levels showed large individual differences, but was higher in adult women than in adult men, and also higher in those who took lutein supplements. People with high BMI, metabolic syndrome and smokers had significantly lower VM scores than their counterparts [1] [2]. SC levels weakly correlated with total MP volume within 9° eccentricity ($r=0.22$, $p=0.006$). SC levels equal to 419 or more (high SC category) showed a significantly lower odds ratio of around 0.2 than VM score less than 270. VM score from 270 to 419 showed insignificantly low odds ratios of around 0.5. High SC category showed significantly lower odds ratios for use of antihypertensive agents, hypolipidemic agents, oral diabetes drugs and insulin, and history of cancer in non-smokers and insignificant lower odds ratio for AMD. Measurement of SC levels can be used for dietary education to prevent chronic diseases including AMD. SC levels of 419 or more induced significantly lower risk for cardiometabolic disease and cancer history. This cut-off value can be used as a target value in dietary education using VM.

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Functional effects of macular pigment density on the central nervous systemBilly R. Hammond^{1*}, Jacob B. Harth¹, and Lisa Renzi-Hammond²¹ Vision Sciences Laboratory, University of Georgia; Athens, GA, USA.²Institute of Gerontology, College of Public Health, University of Georgia; Athens, GA, USA**1. Introduction**

Macular pigment (MP) is known to influence the central nervous system (CNS) through both optical and physiological mechanisms. To assess optical effects, two experiments were conducted. In the first, motion perception was assessed by measuring movement thresholds to a broad-band white light target. These thresholds were measured in the fovea where MP is dense and in the parafovea where MP density is optically minimal. Optical effects of absorbing high-energy visible (HEV) light were also assessed by measuring neural correlates of functional vision while subjects were photostressed using varying patterns of filtered light. We also assessed physiological changes by measuring the relation between MP optical density at baseline and the magnitude of cognitive change achieved after six months of a cognitive training intervention (piano training) in older participants with memory loss.

2. Experimental

Optical effects. MP density was measured using heterochromatic flicker photometry. Motion thresholds were measured based on the movement of a bright white (xenon) visual target. The subject's head was stabilized and fixed in place and the visual target was moved using a highly-regulated computer-controlled translation slide. Subjects were exposed to the light stimulus with one-second exposures while the light was moved at varying precise speeds. The slowest speed where the subject could still identify movement was determined as the motion threshold. In the second fMRI experiment, subjects were evaluated while they were conducting a challenging visual task (judging gap direction in Landolt Cs that randomly varied in size) and exposed to bright glaring light that emulated natural sunlight. Five conditions were measured: four lighting conditions created using filters that attenuated the entire visible spectrum and a step filter that absorbed only the highest-energy visible (violet).

Physiological effects. The CNS Vital Signs computerized test platform was used as part of a larger neuropsychological test battery to measure cognitive function in multiple cognitive domains (with change in the composite of visual and verbal memory as the primary outcome), as well as global function (computed as the neurocognitive index). Tests were conducted at baseline and after 6-months of engagement with a music learning cognitive training intervention.

3. Results and Discussion

Optical effects: Based on our neuroimaging assessment, (using a $p < 0.01$ directional criterion not corrected for aggregated error) we found, as expected, that as light attenuation increases, the brain activity that accompanied the challenging visual task also increased. This effect was most significant in the violet-filtering condition (especially for the older subjects) despite only reducing the very highest energy portion of the photostressor (i.e., much less light attenuation than each of the other filters). This result shows the strategic advantage of the very selective short-wave filtering profile of carotenoids. In addition to protective effects (such as reducing photo-oxidation) selective filtering can increase the neural activity associated with functional vision with minimal effects on overall spectral sensitivity. The effects of MP on motion processing will also be discussed.

Physiological effects: Participants with higher MP optical density at baseline experienced greater improvements in global cognitive function ($rs = 0.47$, $p = 0.18$) and composite memory ($rs = 0.50$, $p = 0.010$) after 6-months of a cognitive training intervention.

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The mechanisms and meaning of ketocarotenoid-based signals in animals

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1. Introduction

Brilliant red plumes, fins, and patches are distinctive features of many animals and important signals in mating and aggressive encounters. Often these traits are produced through the accumulation of C4- ketocarotenoid pigments that are metabolized from common dietary precursors (e.g. lutein, β -carotene). It is hypothesized that these red traits have evolved to reveal information about an individual and their expression is linked to core physiological processes. However, the mechanisms of C4-ketocarotenoid carotenoid metabolism in animals are complex and incompletely understood.

2. Experimental

To reveal the mechanisms of ketocarotenoid metabolism, my collaborators and I have used comparative genomics of animals with discrete red-to-yellow color polymorphisms and gene expression analyses of ketocarotenoid accumulating tissues to identify candidate genes. We then screen these candidates for carotenoid modifying function with heterologous expression in cell culture systems and HPLC pigment analyses.

3. Results and Discussion

We have found, in birds, that the cytochrome P450 enzyme, CYP2J19, and a short-chain dehydrogenase, BDH1L, sequentially oxidize common dietary carotenoids to C4-ketocarotenoids (e.g. zeaxanthin to astaxanthin). This two-step mechanism explains not only the origin of ketocarotenoids but the enigmatic presence of ϵ,ϵ -carotenoids in the feathers of many bird species. We have identified a similar pair of enzymes, cyp2ae2 and bdh1a, in fish (*Danio albolineatus*), with identical catalytic activity and demonstrate through genetic knockout that these enzymes are necessary for the expression of red fin coloration. Finally, we investigated the subcellular localization of these enzymes and will discuss implications for our understanding of the information content and evolution of brilliant red traits.

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Macular Carotenoids: Keeping an Eye on Vision and Beyond!John Nolan^{1*}¹*Nutrition Research Centre Ireland (NRCI), School of Health Sciences, South East Technological University (SETU), Carriganore House, X91K236 Waterford, Ireland.*

The macular carotenoids lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ) selectively accumulate in the central retina (macula lutea), where they are collectively referred to as macular pigment (MP). The anatomic (central and pre-receptorial location), biochemical (antioxidant and anti-inflammatory) and optical (short-wavelength [blue] light-filtering) properties of the macular carotenoids make these ideal candidates to enhance vision and protect against retinal tissue diseases, including the world's leading cause of age-related blindness (age-related macular degeneration, AMD). Indeed, the vision-related benefits that these nutrients offer human health has been confirmed by our group via the ERC-funded (grant number; 281096) CREST study, which demonstrated significant efficacy of these nutrients for patients with AMD,[1] and for patients free of retinal disease.[2] More recently, we also have been able to demonstrate the benefits of using these carotenoid micronutrients and in combination with the omega-3 fatty acids (DHA and EPA) and vitamin E for enhancing brain function for the general population[3], for patients with mild cognitive impairment[4], and for patients with early- to moderate-stage Alzheimer's disease.[5] Moreover, our work via the COAST trial has identified how to enhance the bioavailability of the carotenoids via supplementation with Z and MZ diacetates in a micromicellar formulation, which significantly reduce biomarkers of inflammation (IL-1 β , TF- α , and oxidised LDL). In summary, and building upon over two decades of research with these nutrients, we are now in a unique position to examine further their mechanisms of action, with a view to new applications for human health.

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Noninvasive Screening for Vitamin A Deficiency in High-Risk Populations Using Skin Carotenoid Measurements

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1. Introduction

Vitamin A deficiency (VAD) is an ongoing public health concern among children and pregnant women of Nepal despite robust national efforts to screen and treat this vision- and life-threatening condition. We evaluated skin carotenoid scores measured with the Veggie Meter® as a rapid, non-invasive screening tool for VAD in Nepali children and pregnant women [1].

2. Experimental

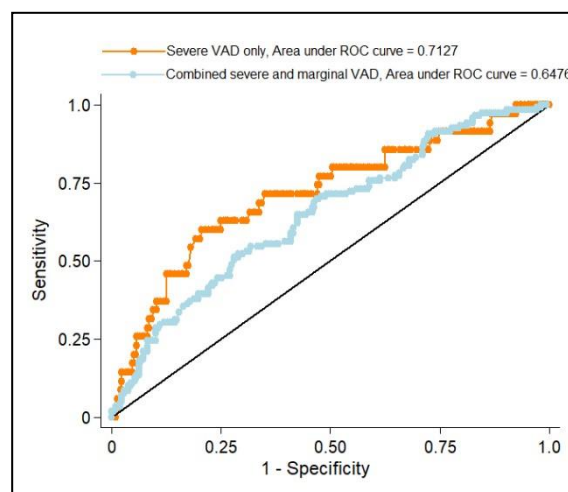
This comparative cross-sectional study enrolled 164 pregnant women and 168 children (aged 8 to 12 years) from public hospitals in three distinct outlying ecological regions of Nepal (Terai, Hill, and Mountain). The primary outcome assessed whether skin carotenoid status could be a biomarker for VAD. We determined skin carotenoid scores with the Veggie Meter® and compared them with serum retinol and total carotenoid concentrations assessed by high-performance liquid chromatography (HPLC). Correlation analysis was used to determine bivariate associations between serum retinol and total carotenoid concentrations and Veggie Meter® assessed skin carotenoid status. Receiver operating characteristics (ROC) curves were determined and a p-value <0.05 was considered statistically significant.

3. Results

We found that 8.5% of pregnant women and 13.0% of children in this study had severe VAD (serum retinol <200 ng/ml). There were significant correlations between skin carotenoid scores with serum retinol and total carotenoid concentrations among pregnant women and children ($r = 0.253$ to 0.530 , $p \leq 0.001$). The Veggie Meter® detected severe VAD with 57.1% sensitivity and 82.7% specificity in pregnant women and 61.9% sensitivity and 75.9% specificity in children.

4. Conclusions

Although sensitivity and specificity were moderate for detecting VAD with the Veggie Meter®, skin carotenoid assessment using this rapid, non-invasive portable device could still be valuable for high-risk VAD screening in Nepal and similar developing countries with limited access to laboratory measurement of serum vitamin A concentrations.



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Brain regulation of gene expression by β -carotene and metformin: possible role in the control of energy metabolism and cognitive function

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1. Introduction

High-fat diets have extensively been associated with obesity and type II diabetes mellitus (T2DM), related to energy metabolism and cognitive impairment. β -carotene (BC) (**Figure 1A**) is a common carotenoid that has been suggested to have different health-promoting effects, including protection against obesity and T2DM. Nevertheless, although different studies have started to establish the possible connection between BC and protection against cognitive degenerative diseases, little is known about BC impact on specific brain centres and cognitive function.

We studied how BC treatment in a murine model exposed to a high-fat diet (HFD) affects the regulation of two key brain centres controlling energy metabolism and cognitive function, i.e. the hypothalamus and the hippocampus. We compared its effects with the treatment with a common T2DM drug, metformin (Met) (**Figure 1B**), which was also administrated alone or in combination with BC.

2. Experimental

The experimental design is summarised in **Figure 2**. Briefly, we established four groups of male Black 6 mice submitted to a HFD and treated with BC, Met, both or vehicle, compared to a control (normal fat diet) group with the vehicle. Gene expression of key genes for the control of energy metabolism and cognitive function was analysed (by RT-qPCR) in the hypothalamus and the hippocampus.

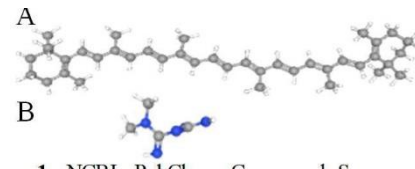


Figure 1. NCBI. PubChem Compound Summary (A) CID 5280489, Beta-Carotene. <https://pubchem.ncbi.nlm.nih.gov/compound/Beta-Carotene>, (B) CID 4091, Metformin. <https://pubchem.ncbi.nlm.nih.gov/compound/Metformin>. Accessed Apr. 13, 2023.

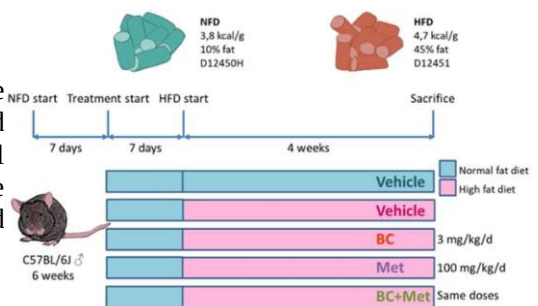


Figure 2. Experimental design

We found that BC treatment regulated the expression of genes related to feeding and energy expenditure control and cognitive function. Outstanding results in the hypothalamus showed that BC treatment reduced *Prkaa2* (AMPK) expression, while Met increased it. The AMPK mRNA reduction by BC treatment might be in accordance with previous results of more active brown adipose tissue appearance (i.e. higher thermogenic capacity) in the same model [1]. In the hippocampus, we can highlight the upregulation of the expression of *Creb*, *Nrf2*, *Trkb* and *App*, associated with proper synaptic function and signalling, with some additive effects of Met treatment. In conclusion, BC treatment, alone or in combination with Met, impacts the expression of key genes involved in energy metabolism and cognitive function in the brain. The results highlight the interest in more research to establish the functional consequences of these effects and the potential use of BC as a coadjuvant for the protection against high-fat feeding-associated disorders.

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Session 11:
Biotechnology and Industrial Production
~In memory of Prof. Chengfu Zhu~

Plant cell factories for industrial and nutritional carotenoids

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Chemical refining is a highly efficient process that has driven industrialisation and globalisation. Carotenoid production using chemical synthesis has traditionally been the method of choice. However, dwindling fuel reserves and climatic fluctuation, are now imposing key societal and economic challenges to health and welfare provision, agriculture, manufacturing, and energy. This has resulted in the development of sustainable production platforms for carotenoids. At scale plants are one of the best biobased modes of production. In this presentation the development of Solanaceae crops, particularly tomato for the production of the carotenoid's lycopene, beta-carotene and xanthophylls will be described using both metabolic engineering and introgression approaches from natural variation. Ketocarotenoids will be of particular focus showing industrial feasibility in aquaculture and as poultry feeds will be demonstrated [1]. Fundamentally, the multi-omic characterisation of these varieties has illustrated the metabolic re-programming required to obtain high level production [2]. In addition, the sequestration mechanisms available to store carotenoids elucidated through multiplex CRISPR approaches to elucidate the modification of plastids, plastoglobuli formation and esterification will be described [3].

[1]. Nogueira, M., Enfissi, E.M.A., Martínez Valenzuela, M.A., Menard, G.N., Driller, R.L., Eastmond, P.J., Schuch, W., Sandmann, G and Fraser, P.D. (2017). Engineering of tomato for the sustainable production of ketocarotenoids and its evaluation in aquaculture feed. PNAS, 114, 10876-1088, 10.1073/pnas.1708349114.

[2]. Nogueira, M., Enfissi, E.M.A., Price, E.J., Menard, G.N., Venter, E., Eastmond, P. J., Bar, E., Lewinsohn, E. and Fraser, P.D. Ketocarotenoid production in tomato triggers metabolic reprogramming and cellular adaptation: The quest for homeostasis? bioRxiv 2023. 10.1101/2023.01.09.523254.

[3]. Almeida, J. Perez-Fons, L., Drapal, M., Liew, K., and Fraser, P.D. The *FIBRILLIN* multigene family in tomato, their roles in plastoglobuli structure and metabolism. bioRxiv 2023, 10.1101/2023.01.05.522848

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Multi-subcellular engineering for carotenoid production

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To date, bioengineering strategies for producing compounds of interest have primarily focused on engineering single subcellular compartments. These approaches have a common inherent constraint underpinned by the fact that the production potential of any given subcellular compartment is limited; and ultimately restrained by factors such as the content of metabolic precursors, the presence of competing pathways, and the stability of the desired compound in that compartment.

We hypothesize that parallel production of a compound of interest in multiple subcellular compartments will result in higher content of such a compound than if produced in a single compartment due to additive exploitation of biosynthetic capacities of multiple compartments. To test this hypothesis, we are engineering microorganisms and plants to simultaneously produce carotenoid compounds in different subcellular compartments.

We expect the project will provide conceptual and technical advances and a new general bioengineering principle applicable to the enhanced production of many compounds of interest.

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Industrial production of carotenoids using *Paracoccus carotinifaciens* and carotenoid applications for animal feed

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1. Industrial production of carotenoids using *Paracoccus carotinifaciens*

Paracoccus carotinifaciens (Fig.1) is an aerobic Gram-negative bacterium that was isolated from soil early in the 1990s[1]. It mainly produces (3S,3S')-astaxanthin, which is in free form without esterification. To achieve the commercial production of astaxanthin, we conducted mutation breeding and optimized media components, culture conditions, and manufacturing processes. Selective breeding has been performed using classical mutation methods using chemical treatment and UV irradiation. Genetic engineering technology hasn't been used because consumers have preferred non-genetically modified products. The astaxanthin production medium consists of carbon and nitrogen, inorganic salts, vitamins, and amino acids. The optimal concentration of each ingredient has been determined by bacterial growth and astaxanthin productivity. As a result, its productivity has increased over 500 times more than that in original conditions, and we successfully started the industrial production of astaxanthin in 2000s.[2]

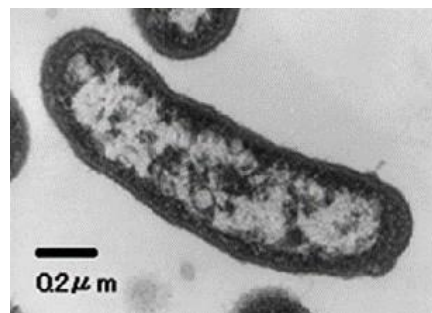


Fig.1 *Paracoccus carotinifaciens* sources

P. carotinifaciens is cultured under optimized conditions and internally accumulates more than 10 kinds of carotenoids, such as adonirubin, canthaxanthin, and adonixanthin. The carotenoid composition can be controlled by the concentration of dissolved oxygen (DO) in the culture. Lower DO increases canthaxanthin and adonirubin accumulations, whereas higher DO leads to adonixanthin productivity (Fig. 2). These phenomena are brought by the activities of 2 conversion enzymes, CrtW and CrtZ. In addition, through the mutation breeding process, we could find some strains which has no or less functional CrtW or CrtZ and can produce only specific carotenoid, such as zeaxanthin or canthaxanthin. Hence, *P. carotinifaciens* are suitable for producing desired several kinds of carotenoid.

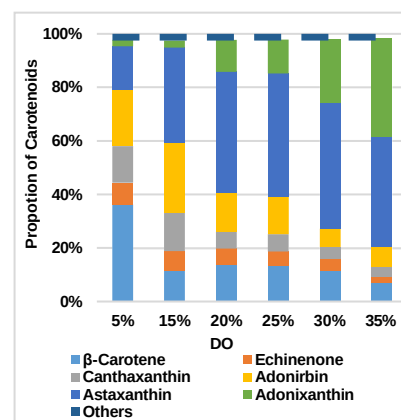


Fig.2 Carotenoid composition on each DO conc.

2. Carotenoid applications for animal feed

Dehydrated *P. carotinifaciens* is sold under the name Panaferd® as pigment for fish, crustaceans and egg yolk. Since almost all carotenoids included in Panaferd® are transferred to animals, Panaferd® imparts natural pigmentation, which is favored by consumers. We also explored that Panaferd® has additional effect on improving productivity, releasing stress, and increasing immunity of animals. Panaferd® is not only a source for pigmentation, but a multi-functional feed additive.

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Metabolic engineering of *Xanthophyllomyces dendrorhous* for the production of commercially interesting carotenoids and the generation of novel structures

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1. The Organism

Xanthophyllomyces dendrorhous is a yeast-like growing Basidiomycete with a diploid cell cycle. Its red color originates from the accumulation of astaxanthin indicating the presence of a carotenoid storage system. In contrast to other organisms, its established biosynthesis pathway is catalyzed by only three gene products.

2. Molecular Genetic Potential

Xanthophyllomyces is efficiently transformable. Multiple plasmids with different selection markers are available for integration into the ribosomal DNA of the genome. Alternatively, homologous recombination for gene integration works and is used for gene knock-out and knock-in. Due to the diploid nature of *Xanthophyllomyces*, the transformants are heterozygous. Homozygous lines can be selected after mitotic recombination or initiation of a sexual cycle. Then, the resulting transformants are persistently stable in the absence of selection pressure.

3. Achievements

The three goals of engineering the carotenoid metabolism of *Xanthophyllomyces* to increase the pathway capabilities were: further enhancement of astaxanthin synthesis, accumulation of pathway intermediates, and formation of novel carotenoid structures by combinations of non-native genes. In each case, metabolic engineering was carried out with a chemically generated high-yield astaxanthin mutant. Astaxanthin formation was increased to 9 mg/g dw by over-expression of the genes encoding 3-hydroxymethyl-3-glutaryl coenzyme A reductase, geranylgeranyl pyrophosphate synthase, phytoene synthase/lycopene cyclase, and astaxanthin synthase. For accumulation of phytoene (10 mg/g dw), the phytoene desaturase gene was inactivated and extra copies of the pathway genes integrated as mentioned above [2]. A zeaxanthin producing transformant with 5.2 mg/g dw was engineered by pathway extension at the stage of β -carotene with a bacterial hydroxylase gene [3]. Finally by combined insertion of bacterial genes into the β -carotene background, it was possible to establish a pathway to 2,3,2',3'-tetrahydroxy-4,4'-diketo- β -carotene and 2,3,2',3'-tetrahydroxy-4-monoketo- β -carotene [4]. Both carotenoids could be chemically reduced to the corresponding hexa and penta hydroxy derivatives. By use of the gene of a non-conventional prenyl methyl transferase, novel C41-C43 carotenoids have been generated [5].

4. Prospects

Xanthophyllomyces is a versatile host for carotenoid pathway engineering. This makes this yeast a promising system for bio-based carotenoid synthesis with great potential for the production of industrially interesting carotenoids. Furthermore, gene combinations enable the tailoring of novel carotenoid structures of potential biotechnological interest.

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[5] C. Takagi, M. Abe, Y. Kaneko, A. Sasaki, A. Ito, Y. Sakemi, T. Maoka, J. Breitenbach, G. Sandmann, K. Shindo, *Tetrahedron Letters* **61** (2020), 152633.

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Dynamic metabolomics approach and engineering biology for microbial carotenoid production

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To meet the increasing worldwide demand for carotenoids, stable and cost-effective carotenoid production technologies using microorganisms are needed. Improved carotenoid productivity is desired for commercialization, which could be realized by engineering valuable strains via synthetic biology approaches [1]. Microalgae and cyanobacteria produce biomass photosynthetically and some of the strains are able to grow rapidly compared with terrestrial plants. Algae have the advantage of not requiring the arable land required to grow terrestrial plants, and using marine species avoids freshwater use. Accordingly, microalgae become chassis strains for carotenoid production.

The emerging field of synthetic biology aims to reverse engineer the inventions of nature for the purpose of reconstructing artificial biological systems. More importantly, a design, build, test, and learn (DBTL) cycle (**Figure 1**) is accelerating synthetic biology research and is highly compatible with metabolomics data to further expand bioproduction capability [2]. ¹³CO₂ feeding with capillary electrophoresis (CE) coupled to MS/MS has been used for the dynamic metabolic profiling of metabolites involved in photosynthesis. We have developed cyanobacteria that produce high levels of astaxanthin and green algae that produce lutein, and analyzed their dynamic metabolomes [3].

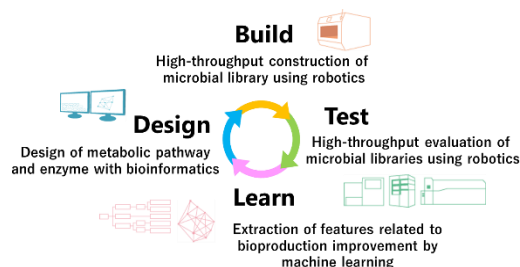


Figure 1. Conceptual diagram of DBTL

A genetically engineered strain of *Synechococcus* sp. PCC 7002, a fast-growing marine cyanobacterium, harboring the β -carotene hydroxylase and β -carotene ketolase derived from the marine bacterium *Brevundimonas* sp. SD212 showed an astaxanthin content of 3 mg/g dry cell weight and astaxanthin productivity of 3.35 mg/L/day, which were comparable to the values obtained using *Haematococcus pluvialis*. Metabolic profiling revealed the astaxanthin producing cells highly accumulated 1-deoxy-D-xylulose 5-phosphate, which is the gateway metabolite in the MEP pathway. Dynamic metabolic profiling showed the metabolic turnover of 3-phosphoglycerate, sedoheptulose 7-phosphate, fructose 6-phosphate, and phosphoenolpyruvate was accelerated in astaxanthin producing cells. Thus, activation of photosynthetic central metabolism in astaxanthin-producing cells appears to compensate for the low β -carotene availability as a light-harvesting pigment, likely via its conversion. In lipid-rich mutant *Chlamydomonas* sp. KOR1, disruption of an isoamylase-type starch debranching enzyme was found to enhance carbohydrate degradation and repartitioning carbon resources into lipid and carotenoid. Both lutein (2.64 mg/g-DCW) and β -carotene (1.27 mg/g-DCW) content increased 1.3 times by disruption.

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***Nicotiana benthamiana* as a cell factory for high value-added (apo)carotenoids**

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1. Introduction

Nicotiana benthamiana is a promising cell factory system for the production of biopharmaceuticals and small molecules [1]. We have taken a genome editing approach, combined with agroinfiltration and virus-mediated overexpression, for the production of zeaxanthin and its cleavage products, crocins, high value apocarotenoids that accumulate in saffron (*Crocus sativus*) stigmas.

The normal leaf xanthophyll complement is composed by lutein, violaxanthin, antheraxanthin and neoxanthin. Knocking out both paralogous genes lycopene epsilon cyclase (LCY) and zeaxanthin epoxidase (ZEP), we expected to produce an accumulation of the xanthophyll zeaxanthin that is the preferred substrate of the *Crocus sativus* enzyme CsCCD2, catalyzing the first dedicated step for crocins production in saffron [2].

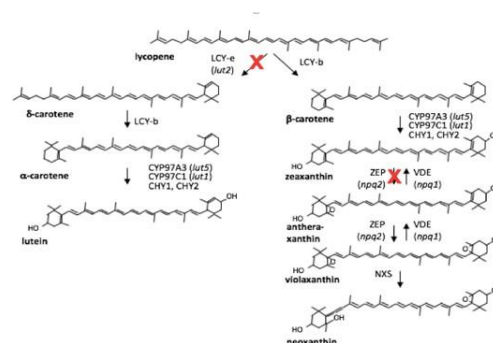


Figure 1 Carotenoid pathway in plants

2. Experimental

To obtain *N. benthamiana* plants accumulating zeaxanthin, the two the paralogous genes of lycopene epsilon cyclase (LCYe1-LCYe2; NbV6.1trP33255-NbV6.1trP19906) and zeaxanthin epoxidase (ZEP1-ZEP2: NbV6.1trP61527-NbV6.1trP32819) were knocked-out (KO) by CRISPR/Cas9 mutagenesis. Selection of triple *lcy1 lcy2 zep2* (HZ-9 line) and quadruple *lcy1 lcy2 zep1 zep2* (HZ-11 line) KO plants were performed by ICE (<https://ice.synthego.com/#/>) and by LC-MS. *N. benthamiana* plants accumulating zeaxanthin were used in agroinfiltration and virus-mediated overexpression (tobacco etch virus, TEV) of CsCCD2 for crocin production. Analysis to study photosynthetic activity of the edited lines were also carried out.

3. Results and Discussion

Triple *lcy1 lcy2 zep2* and quadruple *lcy1 lcy2 zep1 zep2* KO plants contained up to 450 µg/g DW of zeaxanthin as the only xanthophyll. In spite of their partial ABA deficiency and alteration in several photosynthetic parameters, such as a reduction of Fmax, the mutants were viable and could be used for the transient overexpression - using both agroinfiltration with *Agrobacterium tumefaciens* and TEV infection - of the saffron CsCCD2, which catalyzes the first dedicated step in crocin biosynthesis. Over-expression of CsCCD2 in zeaxanthin accumulating plants using a TEV vector led to higher crocin levels than in the WT (up to 400 µg/g DW).

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Perturbing native gene expression with a CRISPRi-library and application of a genetically encoded biosensor to study carotenogenesis

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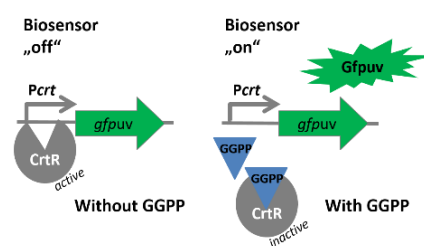
1. Introduction

Corynebacterium glutamicum was discovered more than 60 years ago in Japan as a natural L-glutamate producer. Its industrial potential to produce secreted compounds is exploited with more than 5 million tons per year of amino acids. In addition, *C. glutamicum* harbors the potential for carotenoid production as it accumulates the rare C50 carotenoid decaprenoxanthin in its membranes. Metabolic engineering demonstrated that this organism is a suitable production host for various C40 and C50 carotenoids (e.g. β -carotene, astaxanthin, BABR, Sarcinaxanthin, etc.). The methods presented here, namely a CRISPRi-library and a biosensor give insights into carotenoid biosynthesis and facilitate development of new metabolic engineering strategies.

2. Experimental

In this work, first a CRISPRi-library was established in *C. glutamicum* for targeted gene knock down of 75 genes from glycolysis, pentose phosphate pathway, tricarboxylic acid cycle, carotenoid biosynthesis as well as further regulatory genes [1]. The plasmid-based CRISPRi system comprising a dCas9 and a sgRNA was tested in decaprenoxanthin producing wildtype of *C. glutamicum*. The library screening was performed in a microcultivation system (biolector) for growth characterization, followed by carotenoid extraction and quantification by HPLC.

Second, a biosensor system for the detection of the carotenoid precursor geranylgeranyl pyrophosphate was established based on the genetically encoded transcriptional regulator of carotenogenesis CrtR [2]. Plasmid-based transcriptional fusion of the carotenogenic promoter (*PcrtE*) to the fluorescence reporter gene *gfpUV* was tested as an easy read-out system by flow cytometry for detection of the analyte GGPP *in vivo* in *C. glutamicum*.



3. Results and Discussion

The CRISPRi-library screening resulted in the identification of 14 new targets for metabolic engineering for elevated carotenoid production. These insights could be transferred into metabolic engineering strategies, namely genomic deletions of *pgi*, *sugR* and *gapA* for stable and an improved decaprenoxanthin production. The chromosomally encoded biosensor in combination with the fluorescence reporter plasmid was able to detect GGPP in metabolically engineered strains of *C. glutamicum*. The correlation of the intracellular GGPP concentration of up to 4 mM with the GFP fluorescence signal was demonstrated by LC-MS and flow cytometry, respectively. The two presented methods, a genetically encoded biosensor and the screening of a CRISPRi-library have the potential to investigate carotenoid biosynthesis and production in a wide range of organisms.

Figure 1. CrtR-based biosensor system for detection of GGPP.

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[2] N. A. Henke, S. Austermeier, I. L. Grothaus, S. Götker, M. Persicke, P. Peters-Wendisch, V. F. Wendisch, *Int. Jour. Mol. Sci* 21 (2020), 5482

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Engineering Novel Retinal-Related Compounds through Carotenoid Pathway Engineering and Enzyme Mutagenesis

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1. Introduction

Retinal is a crucial molecule for vision and light sensing in both prokaryotic and eukaryotic organisms. Its biosynthesis is well-characterized in nature and involves the conversion of a specific carotenoid, β -carotene, to all- trans-retinal via the action of the carotenoid cleavage oxygenase enzyme. Retinal is also a key component of microbial rhodopsins, light-activated membrane proteins that are widespread in bacteria, archaea, and eukaryotes. Despite the essential role of retinal in these processes, generating non-natural and novel retinal-related compounds using genetic engineering techniques remains a challenging task. However, such novel retinal-related compounds could have significant implications for biotechnology and medicine. In this study, I engineered an *E. coli* to produce non-natural and novel retinal-related compounds by creating diverse carotenoid pathway constructs and carotenoid cleavage dioxygenases with altered substrate specificity.

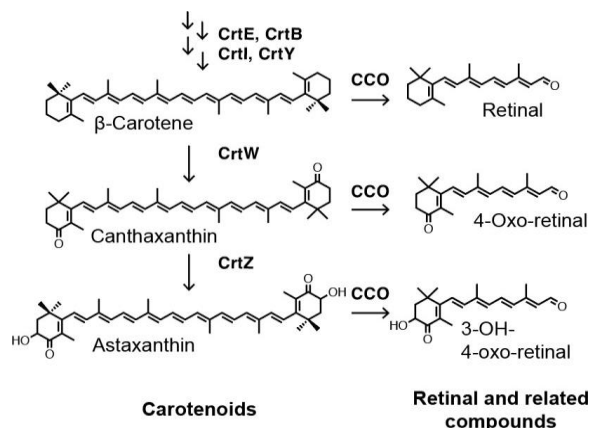


Figure 1. Engineered pathway for carotenoids, retinal and related compound constructed in this study.

2. Experimental

A diverse set of carotenoid pathway operons were constructed in *E. coli* and these were co-expressed with carotenoid cleavage oxygenase enzymes. The cells were cultivated for 2 days, and the retinal-related compound were extracted using dodecane overlay. Furthermore, specific enzyme mutations were introduced to enlarge the cavity and observed an increase in the production of new retinal structures. LC-PDA-MS was used to identify and characterize the novel retinal-related compounds.

3. Results and Discussion

By co-expressing diverse carotenoid pathway enzymes and carotenoid cleavage oxygenase, I was able to detect multiple non-natural and novel retinal-related compounds. This result indicates that this approach could potentially produce a vast array of novel retinal-related compounds. Furthermore, I found that by introducing specific enzyme mutations, I could increase the production ratio of new retinal structures. This suggests that enzyme engineering could further improve the diversity and yield of novel retinal-related compounds.

Overall, this study provides a new approach for the engineering of retinal biosynthesis and expands the toolbox for the synthesis of non-natural and novel retinal-related compounds. The generation of novel retinal-related compounds has significant implications for biotechnology and medicine, as they could potentially be used as novel biomaterials or therapeutics. Further studies are required to fully characterize and understand the potential applications of these novel retinal-related compounds.

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Poster Presentations

P1-1

Transcriptional changes associated to the chloroplast-to-chromoplast transition in carotenoid-overaccumulating leaves.

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1. Introduction

Transient expression of a bacterial *crtB* gene encoding phytoene synthase is able to induce chromoplastogenesis in leaves due to a massive production of phytoene and downstream carotenoids [1]. This artificial system provides a simpler way to investigate the chloroplast-to-chromoplast differentiation process, detached from other ripening- or flowering-related events observed in natural systems. The differentiation process occurs in two phases: (I) loss of chloroplast identity followed by (II) change of plastid ultrastructure [1]. At the end of phase II, artificial chromoplasts with abundant plastoglobules and membrane stacks are formed [1,2]. Molecular events taking place during the differentiation process are unknown.

2. Experimental

Samples at different time-points covering the two phases of the *crtB*-triggered chloroplast-to-chromoplast conversion in *Nicotiana benthamiana* leaves were used for RNA-seq analysis of transcriptomic changes associated with this process.

3. Results and Discussion

Two main waves of transcriptional changes were detected: one at the beginning of the process (soon before the first accumulation of phytoene is detected) and a second one after photosynthesis is lost. Genes encoding carotenoid biosynthetic enzymes downstream of phytoene only show increased expression at the end of the differentiation process, while those encoding enzymes of the MEP pathway (which supplies the precursors of carotenoids and related plastidial isoprenoids) show an early peak of induction. Similarly, jasmonic acid (JA) biosynthetic genes induce their expression early during the transition, whereas JA content only increases at the end of the process. Results exploring the biological relevance of some of the identified transcriptional changes will be presented.

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Promiscuous carotenoid pathways as indicators for intercellular proportion of IPP and DMAPP

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1. Introduction

Most natural carotenoids are tetraterpenoids (C₄₀), which are condensates of two molecules of dimethyl diphosphate (DMAPP) and six molecules of isopentenyl diphosphate (IPP) (IPP/DMAPP = 0.66), while some bacteria add two units of DMAPP on lycopene yielding various C₅₀ carotenoids (IPP/DMAPP = 0.6). [1]. In contrast, C₅₀ pathway we established by laboratory evolution [2] are consist of 8 molecules of IPPs for 2 molecules of DMAPPs (IPP/DMAPP = 0.8). To achieve efficient microbial production of these carotenoids, feeding ratio of DMAPP and IPP needs to be optimized to each carotenoid to be produced. However, DMAPP and IPP are structurally similar, and they are difficult to distinguished even with using HPLC. The goal of this study was to develop a simple method to score the intercellular DMAPP/IPP ratio (Figure 1).

2. Experimental

A size mutant pairs of prenyltransferase and carotenoid synthase (CrtM) were co-expressed in a combinatorial manner to create carotenoid synthesis pathways with low size selectivity. We introduced these pathways into *Escherichia coli* strains and analyzed the size distribution of resultant carotenoids produced by these pathways using HPLC, under various environmental factors that can affect the intercellular DMAPP/IPP ratio.

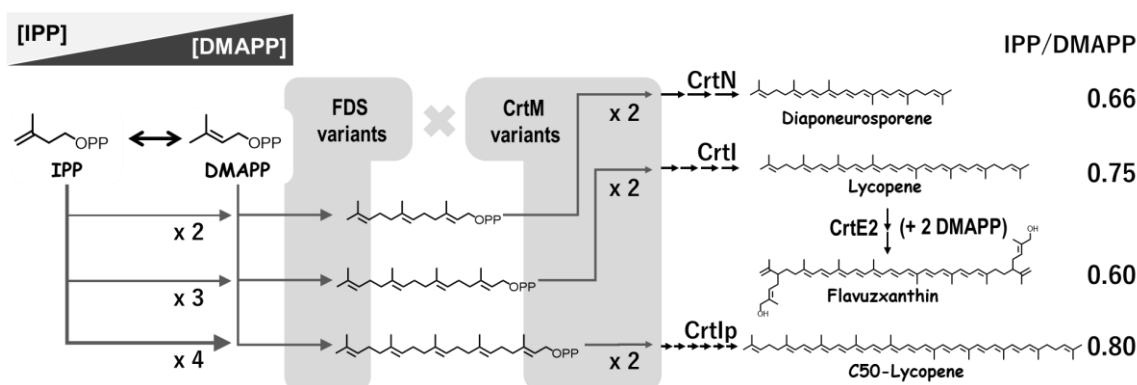


Figure 1. Size distribution of promiscuous carotenoid pathway depending on DMAPP/IPP ratio.

3. Results and Discussion

Promiscuous CrtM mutants can use a variety of intracellularly fed prenylpyrophosphates (C_xPP, X= 5, 10, 15, 20, 25, 30, 35...etc.) as substrates, and their product size distribution reflects the cellular composition of C_xPP. Whereas prenylpyrophosphates are made by the stepwise addition of IPPs (elongation units) to DMAPP (starter unit). We demonstrate that the size distribution of the carotenoid shifted toward the smaller ones as the intracellular DMAPP-to-IPP ratio increased. Taking advantage of this observation, one can conveniently monitor the change of IPP/DMAPP ratio and can establish high-throughput search for environmental and genetic factors that can possibly affect the IPP/DMAPP ratio inside cells.

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P1-3

Molecular breeding of C₂₀ carotenoid pathwaysYui Araki¹, Takumi Ojima², Hiroto Ando¹, and Daisuke Umeno^{1*}¹ Department of Applied Chemistry,

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1. Introduction

Because carotenoids are highly hydrophobic and have large molecular weights and low volatility, all carotenoids produced intracellularly accumulate in the plasma membrane [1]. Therefore, efficient biosynthesis of carotenoids ends up with overloading the membrane capacity. This is in sharp contrast to the bioproduction of volatile terpenoids, which can be continuously extracted to dodecane phase simply added to the culture medium. In this study, we attempted to modify the size specificity of CrtM, a C₃₀-carotenoid synthase from *Staphylococcus aureus*, to establish a biosynthetic pathway for carotenoid pigments with smaller molecular weights (**Figure 1**).

2. Experimental

We selected two residues in the substrate pocket of CrtM, especially around the floor of the S2 site that accommodates the prenyl acceptor substrate in condensation reaction, and replaced them with various types of amino acids, especially those with bulky side chains. We introduced the resultant CrtM mutants into *Escherichia coli*, cultured them under various precursor supply conditions, and performed HPLC analysis of carotenoid fractions.

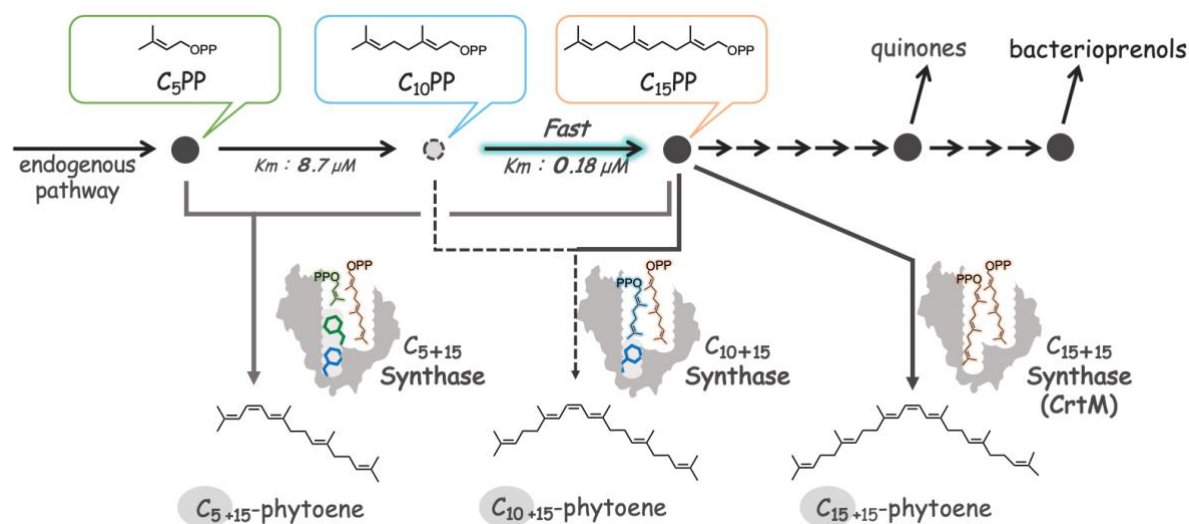


Figure 1. Reengineering CrtM for the production of C₂₅/C₂₀-phytoenes.

3. Results and Discussion

E. coli accumulates significant amount of farnesyl diphosphate (C₁₅PP) and dimethylallylpyrophosphate (C₅PP), while it accumulates negligible amount of geranyl pyrophosphate (C₁₀PP). However, through additional expression of C₁₀PP synthase and combinatorial testing of various size-specific mutations, a robust biosynthetic route to C₂₅ phytoene was established. Interestingly, the C₂₅-carotenoid could be extracted without disrupting/lysis of the cells, simply by adding a small amount (10 vol %) of dodecane to the medium. This result contrasts with the fact that C₃₀-carotenoid remains in the cell upon dodecane treatment. The biosynthesis of C₂₅-carotenoids requires an equal molar ratio of C₁₀PP as C₁₅PP, and the feeding of C₁₀PP should be the bottleneck of this pathway. We therefore constructed the other pathway for C₂₀ carotenoids by hetero-condensation of C₅PP and C₁₅PP, both of which are abundant in the cell, by reengineering the upstream pathway for massive supply of DMAPP and by selective reduction of S2 sites.

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Converting squalene synthase into carotenoid synthase for biosynthesis of minisqualene

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1. Introduction

Squalene (SQ) is a triterpene (C₃₀) synthesized by the condensation reaction of two farnesyl diphosphates (C₁₅PPs). There has been strong demands to reduce the molecular weight of SQ in order to improve its fuel/oil properties, but nobody has come up with the direct screening for minisqualene (mini-SQ). We took advantage of the fact that SQ synthase (SQS) is evolutionarily related to carotenoid synthase (CarS); we first introduce a mutation that confer carotenoid synthase (CarS) activity to SQS [1]. Once converted into CarS-like enzyme, size specificity of SQS can be easily screened by co-expressing carotenoid desaturases. We attempted to find size-shifting mutations of SQS in carotenoid biosynthetic context, followed by reverting it back to SQS.

2. Experimental

When V325D is introduced into SQS from *Thermocynechococcus*, it becomes a carotenoid synthase with almost no loss of activity [1] (**Fig. 1a**). The structural gene of C₃₀CarS was subjected to error-prone PCR to create a library, and size-specificity mutants were searched for by a fluorescent screening system (Ojima *et al.*, unpublished method) (**Fig. 1b**). From the obtained mutant (C₂₅CarS), V325D, which was initially introduced, was reverted (**Fig. 1c**) and introduced into *E. coli* capable of C₁₀PP synthesis for HPLC analysis.

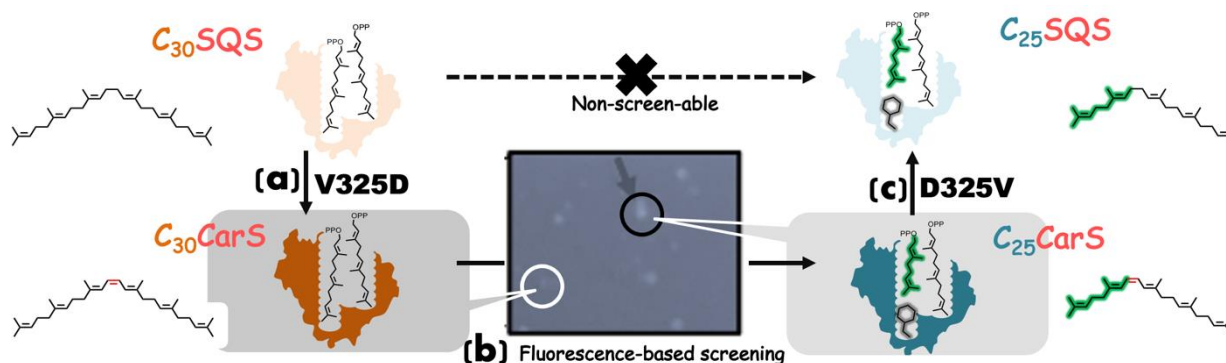


Figure 1 Three-step evolution of minisqualene synthase.

3. Results and Discussion

Wild-type SQS yields SQ as a sole product, while SQS_{V325D} dehydrosqualene (DSQ) predominantly (3,516 µg/g-DCW) with trace, if not zero, of SQ. DSQ is a C₃₀-type phytoene (diapophytoene) and the cognate substrate for carotene desaturase such as Staphylococcal CrtN. When mutation library of SQS_{V325D} was co-expressed with CrtI and IspA_{S80F} (C₁₀PP synthase) in *E. coli*, small fraction (~0.18%) of the transformants exhibited fluorescent colonies. These fluorescent colonies were isolated, and some of the variants were found to accumulate 286 µg/g-DCW of C₂₅-carotenoids (condensation product of C₁₀PP and C₁₅PP). The SQS-to-CarS mutation V325D was removed from these C₂₅-Car producing mutants of SQS. The resultant SQS mutant accumulated 36.8 µg/g-DCW of C₂₅-Squalene.

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P1-6

Carotenoid metabolism during fruit maturation of Nadorcott, a highly pigmented mandarin

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1. Introduction

Mandarins comprise a heterogeneous group of *Citrus* fruit displaying significant variability in pigmentation, and in the carotenoid content among the different species and varieties [1]. The biosynthesis and regulation of carotenoids accumulation in *Citrus* have been extensively studied, but the molecular basis determining differences in pigmentation and carotenoids among mandarins is not well understood. Nadorcott (syn. Afourer) is a mandarin variety recently incorporated in the international citrus market highly appreciated by the late harvesting, well-balanced ratio sugars/acids and rich flavor [1]. Moreover, a distinctive feature of Nadorcott fruit is the high pigmentation showing a deep reddish-orange rind and an intense-colored pulp. The aim of this work has been to characterize the carotenoid profile and the transcriptional changes of the carotenoid metabolism genes in the peel and pulp of Nadorcott fruit during maturation and compared with those occurring in Clementine mandarin fruit, a reference variety with less pigmented flavedo and pulp.

2. Experimental

Fruit of Nadorcott (*C. reticulata*) (N) and Clementina de Nules (*C. clementina*) (C) mandarins were harvested from the Citrus germoplasm bank (IVIA, Valencia) at breaker (b) and mature (m) stage (**Figure 1**). The carotenoid profile was determined in the flavedo and pulp by HPLC-DAD [2]. The expression of carotenoid metabolism genes was analyzed by RTqPCR [2] in the same samples used for pigments analysis.



Figure 1. Fruit of Nadorcott and Clementine mandarin used in this study.

3. Results and Discussion

The total carotenoid contents in the fruit tissues of N mandarin were between 2- and 4-times higher than in C at both ripening stages. The main carotenoid in the flavedo was violaxanthin followed by β -citraurin and β -cryptoxanthin (BCX), reaching contents between 3 to 5-times higher in N than in C. BCX was the main carotenoid in the pulp of both varieties, with concentrations in mature fruits of 2.6 $\mu\text{g/g}$ FW in C and 16 $\mu\text{g/g}$ FW in N fruit. The content of other carotenoids were also greater in the pulp of N than in C, and the ratio of BCX/violaxanthin was 2-fold higher in the mature pulp of N. Transcriptomic analysis showed that most of the biosynthetic genes analyzed were more highly expressed in the peel of N than in C mandarins, except for the functional allele a of lycopene β -cyclase2. The differences in the expression of carotenoid biosynthetic genes in the pulp were mainly restricted to the breaker stage, and the relative levels of upstream genes were higher in N, but the lycopene β -cyclase2 was reduced. Overall, results suggest that the high pigmentation of N mandarins is due to elevated carotenoids content, particularly BCX in the pulp, that appears to be associated with a higher expression of most upstream biosynthetic genes of the pathway at the breaker stage.

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P1-7

Pathway-scale genome editing provides a comprehensive understanding of carotenoid biosynthesis in microalga *Euglena gracilis*

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1. Introduction

Carotenoids are essential for photosynthesis and phototaxis in microalgae. The secondary endosymbiotic alga *Euglena gracilis* has unique carotenoids, such as diadinoxanthin and diatoxanthin, that are absent in primary endosymbiotic algae chlorophytes [1]. However, the carotenoid biosynthetic pathway in *E. gracilis* has not yet been comprehensively understood at the genetic level. This study generated a series of carotenoid biosynthesis-related gene mutants of *E. gracilis* using CRISPR-Cas9 genome editing and investigated the contribution of each gene to carotenoid biosynthesis.

2. Experimental

The genome editing targets were 8 genes (*crtE*, *crtB*, *crtP1*, *crtP2*, *Z-ISO*, *crtQ*, *LCY*, and *CYP97H1*) characterized previously and 8 genes (*crtISO1*, *crtISO2*, *CYP97F2*, *ZEP1*, *ZEP2*, *ZEP3*, *VDE1*, and *VDE2*) identified from a transcriptome dataset (Figure 1). A complex of single guide RNA and Cas9 protein was introduced into *E. gracilis* cells by electroporation to isolate genome editing mutants. The contents and compositions of carotenoids extracted from *E. gracilis* were analyzed using LC/MS with the assistance of Dr. Takashi Maoka (Research Institute for Production Development).

3. Results and Discussion

After heterotrophic culture, the mutants of 6 genes (*crtE*, *crtB*, *crtP2*, *crtQ*, *LCY*, and *CYP97H1*) were colorless, indicating the requirement of these genes for carotenoid biosynthesis and chloroplast development. The mutants of 2 genes (*CYP97F2* and *ZEP1*) were mixtures of green and colorless cells, suggesting a partial carotenoid deficiency in these mutants. The mutants of 8 genes (*crtP1*, *Z-ISO*, *crtISO1*, *crtISO2*, *ZEP2*, *ZEP3*, *VDE1*, and *VDE2*) displayed no observable changes at least under experimental conditions in this study. The carotenoid analysis detected only lycopene in the *LCY* mutants, confirming *LCY* is the sole lycopene cyclase in *E. gracilis*. Zeaxanthin was detected in β -carotene hydroxylase *CYP97H1* single mutants, but not in *CYP97H1* *CYP97F2* double mutants. In *E. gracilis* lacking non-heme β -carotene hydroxylase, it was demonstrated that two CYP97 enzymes were functionally redundant and *CYP97H1* was the dominant β -carotene hydroxylase [2].

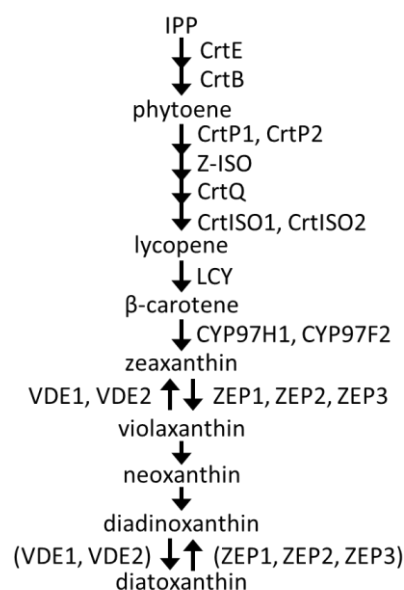


Figure 1. Predicted carotenoid biosynthetic pathway in *E. gracilis*

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Blue-to-green light regulates siphonaxanthin biosynthesis: Discovery of deoxysiphonaxanthin in an edible macrogreen alga, *Codium fragile*

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1. Introduction

Siphonaxanthin (Sx) is a carbonyl carotenoid found bound to the major light-harvesting complexes of a macrogreen alga, *Codium fragile* and absorbs blue-green light for photosynthesis. Sx is believed to be biosynthesized from lutein (L) via loroxanthin (Lo) [1], but the Lo-to-Sx conversion has never been observed yet. Raniello et al., suggested strong light causes Sx-to-L conversion based on observations for naturally occurring algal bodies [2]. In this study, to identify the specific light condition to induce such an interconversion, we traced the pigment composition of *C. fragile* cultivated under several light conditions.

2. Experimental

C. fragile (Ku-0654, Ku-MACC) was pre-cultivated in a filamentous form under dim white light at 21 °C, and was exposed to different light regimes: (1) blue, green and red LEDs at 50, 100, 200 PPFD for 7 days, and (2) 10 monochromatic lights between 425 to 700 nm at 100 PPFD for 5 days. In addition, photosynthetic proteins were prepared from *C. fragile* exposed under blue LED at 200 PPFD. Pigment composition was determined using HPLC [3].

3. Results and Discussion

We observed a decrease in Sx level after exposure to blue-green light, with concomitant accumulation of an unidentified carotenoid. By comparing ¹H-NMR and other optical spectra with the stereoselectively synthesized authentic dS [4], its absolute structure was determined to be 19-deoxysiphonaxanthin (dS) as shown in Figure 1. Thus, we concluded that dS is a direct precursor of Sx and blue-green light hampers dS-to-Sx conversion. We also determined that dS is accumulated exclusively in the photosynthetic light-harvesting complex. Plausible mechanism of the carotenoid interconversion and significance of dS in a diversified Sx-containing species will be discussed.

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[3] S. Seki, Y. Yamano, N. Oka, Y. Kamei, R. Fujii, *FEBS Lett.* **596** (2022) 1544-1555.

[4] Y. Yamano, M. Tanabe, A. Shimada, A. Wada, *Marine Drugs*, **20** (2022) 658.

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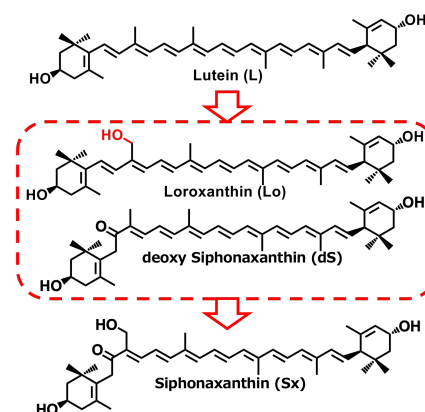


Figure 1. Chemical structure of lutein, loroxanthin, deoxy siphonaxanthin, and lutein with a plausible biosynthetic pathway.

Two Types of Lycopene Cyclases in Cyanobacteria: CruA/CruP-Type and CrtL-Type (Overview)

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1. Introduction

Cyanobacteria are considered to originate from photosynthetic bacteria, and to have given rise to plant chloroplasts through evolution and symbiosis. Cyanobacteria synthesize many kinds of carotenoids, which are known to play roles in light-harvesting, photoprotection, assembly of pigment–protein complexes in phototrophs, and stabilization of biological membranes [1]. Most cyanobacteria contain β -carotene and its derivatives such as zeaxanthin, echinenone, and nostoxanthin, and also contain unique carotenoid glycosides such as myxol fucoside and ketomyxol chinovoside [2]. These carotenoids are cyclized at one or both ends of lycopene. This cyclization reaction is catalyzed by lycopene cyclases. In this review, we summarize the cyclization of lycopene by the different types of lycopene cyclases in cyanobacteria [1].

2. Lycopene cyclase in cyanobacteria

To date, only seven lycopene cyclases from five species of cyanobacteria have been functionally confirmed. CrtL-type lycopene cyclase (homologous to bacterial CrtY and plant LCY) in *Synechococcus elongatus* PCC 7942 was the first functionally identified using transformed *E. coli* [3]. Two CrtL-type lycopene cyclases, CrtL-b and CrtL-e, in *Prochlorococcus marinus* MED4 were functionally identified using lycopene producing *E. coli*. [4]. Two lycopene cyclases, CruA (homologous to green sulfur bacterial CruA) and a paralog known as CruP in *Synechococcus* sp. PCC 7002 were shown to have lycopene cyclase activity by using lycopene producing *E. coli* [5]. However, Bradbury *et al.* was not able to replicate the lycopene cyclase activity of CruP. Consequently, physiological roles of the cyanobacterial CruP paralogs remain unclear [6]. Recently, Sugiyama *et al.* have reported that CruA in *Arthrospira platensis* NIES-39 has lycopene cyclase activity, whereas CruP does not, by using lycopene producing *E. coli* [7]. On the other hands, Xiong *et al.* have found that CruA in *Synechocystis* sp. PCC 6803 has lycopene cyclase activity and requires a bound chlorophyll *a* for activation, but the function of the bound chlorophyll *a* is unknown [8]. The homologous genes of *cruA* and *cruP* are widely distributed in the genomes of some cyanobacteria species; however, there are only a few reports that showed the lycopene cyclase activity in these gene products. Further studies are needed to elucidate the different properties.

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P1-10

Light-mediated isomerisation of acyclic *cis*-carotenes regulates metabolic feedback and nuclear gene expression in Arabidopsis.

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1. Introduction

A change in light quality and quantity can alter the rate of *cis/trans* photoisomerisation of *cis*-carotenes when CRTSIO is non-functional (1). During photomorphogenesis, plants absorb photons from the light spectrum and regulate different chemical and physiological processes during photomorphogenesis (2). For instance, blue light (BL) regulates photomorphogenesis and promotes stomata opening in plants, while red light (RL) seems to be more effective in improving the net photosynthetic rate (3, 4). This study aimed to; 1) assess the impact of blue vs red light on modulating prolycopene photoisomerisation, 2) correlate the kinetics of carotenoid accumulation with carotenoid gene regulation to determine key rate-limiting steps in the pathway, and 3) determine if prolycopene can behave like a yet-to-be-identified unidirectional photoswitch to sense light spectral changes and coordinate metabolic feedback through the α/β branch in the pathway. We hypothesised that blue wavelengths of light could switch *on* the efficiency of prolycopene photoisomerisation and modulate feedback regulation over nuclear gene expression.

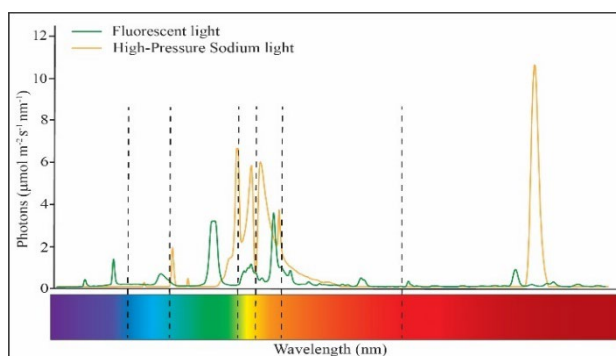


Figure 1. Overlaying of the two light spectra shows the shortage of blue light in HPSL light compared to FL.

2. Design

LED lights emitting blue or red wavelengths were used to assess the efficiency of prolycopene photoisomerisation. Two light sources generating different wavelength spectrums were identified. The Fluorescent Light (FL, yet less RL) had a major emission peak within the blue wavelength region that was absent in the High-Pressure Sodium Light (HPSL; 69% less BL) (Figure 1). Etiolated seedlings were exposed to FL and/or HPSL in two phases; 1) early stages of photomorphogenesis (constant FL for 5, 15, 30, 60 mins), and 2) prolonged photomorphogenesis (both FL and HPSL for 12, 24, 48, and 72 hrs of constant light). Carotenoid accumulation and gene expression were quantified in etiolated and de-etiolated seedlings.

3. Result and Discussion

Etiolated *ccr2* seedlings accumulated acyclic *cis*-carotenes and exhibited nuclear feedback regulation evident by altered expression of *LHCB* (2.6-3.3 folds), ϵ *LCY* (1.3-1.9 folds) and *ZDS* (0.4-2.7-folds). Following de-etiolation, *ccr2* seedlings exposed to FL exhibited rapid photoisomerisation of prolycopene within 5 mins leading to a reduction in acyclic *cis*-carotenes, accumulation of cyclic carotenoids that was further enhanced after 60 mins. The reduced expression of *PSY*, *ZISO*, β *OHASE1*, and *VDE* in *ccr2* correlated with the biosynthesis of pro-lycopene/neurosporene, tri-*cis*- ζ -carotene and/or neurosporene-isomers, evidencing a rapid feedback mechanism triggered by yet-to-be identified ACS. Hierarchical clustering of the carotenoid pigment profiles and gene expressions in *ccr2* seedlings grown under the HPSL throughout a time course revealed that majority of genes such as *LHCB*, β *LCY* and ϵ *LCY* were subject to feedback regulation and linked with the levels of *cis*-carotenes as well as β -branch cyclic carotenoids. We conclude that the quality of light, particularly within the blue wavelength spectrum, can rate-limit prolycopene photoisomerisation. We propose that prolycopene behaves like a unidirectional chemical photoswitch modulating carotenoid and apocarotenoid signals that feedback to rate-limit the expression of genes involved in the biosynthesis of β -branch carotenoids.

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Transient overexpression of *STAY-GREEN* causes the formation of chromoplasts in dark-incubated leaves

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1. Introduction

Plastids are plant cell organelles that play different functions. In green tissues such as leaves, chloroplasts are in charge of photosynthesis, whereas in non-green tissues other plastid types differentiate to store lipids (e.g. seed elaioplasts), starch (e.g. root amyloplasts) or carotenoids (e.g. fruit and flower chromoplasts). During leaf senescence, chloroplasts differentiate into chlorophyll-lacking gerontoplasts. Chlorophyll removal during leaf senescence is carried out by several enzymes. Among them, *STAY-GREEN* (*SGR*) is a Mg-dechelatase which catalyses the first step of chlorophyll degradation. Here we show that transient expression of *SGR* in non-illuminated *Nicotiana benthamiana* leaves results in the formation of orange leaf sectors containing plastids that are different from gerontoplasts but similar to chromoplasts despite carotenoid production is not induced.

2. Experimental

Leaves of 4- to 6-wk-old *N. benthamiana* plants were infiltrated as described previously [1]. Some leaves were covered in aluminium foil while attached to the plant to keep them in darkness. Transmission electron microscopy and HPLC-based pigment quantification were performed at 4 or 5 days after agroinfiltration as previously described [1].

3. Results and Discussion

We transient overexpressed genes for *SGR* and other enzymes involved in chlorophyll degradation (such as chlorophyllases) in *N. benthamiana* leaves and then incubated them either in the light or in the dark. Light-incubated leaves turned yellow whereas only those overexpressing *SGR* developed a brilliant orange color in the dark. Plastid ultrastructure analysis of these orange *SGR* leaf sections showed the formation of plastids in which the photosynthetic thylakoids in chloroplasts have been fully decomposed and typical chromoplast membrane structures developed, distinguishing them from gerontoplasts. Pigment analysis indicated that chlorophylls were degraded but most carotenoids were retained when *SGR* was overexpressed in darkness. Previous work showed that enhanced production of carotenoids in *N. benthamiana* leaves was sufficient to differentiate chloroplasts into chromoplasts [2]. Here we provide another example of artificial leaf chromoplastogenesis that does not require an activation of carotenoid synthesis but it is associated to removing chlorophylls from chloroplasts. These two processes are different to natural chromoplast differentiation that occurs during pepper or tomato fruit ripening, when chlorophyll degradation mediated by *SGR* is coordinated with activated carotenoid production. Our results suggest that chromoplasts are carotenoid-enriched plastids that can be formed by multiple, independent pathways.

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Synthesis and antioxidant capacity of carotenoid succinates and their melatonin conjugates

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1. Introduction

Carotenoids are known as effective antioxidants with elevated hydrophobic character and as an important part of human nutrition. They can incorporate into cell membranes and can play a role in the inhibition of oxidative stress. [1]

2. Experimental

Carotenoid succinates were synthesized from hydroxy carotenoids and were coupled to a commercially available derivative of melatonin via amide bond for producing more powerful antioxidants. The coupling reactions produced conjugates in acceptable to good yields. The antioxidant behavior was measured with ABTS and FRAP methods for the carotenoids, the carotenoid succinates, and the conjugates with melatonin. The stock solutions were prepared with DMSO and THF and were diluted with PBS.

3. Results and Discussion

Succinylation increased the water solubility of the carotenoids, while the conjugation with melatonin resulted in more lipophilic derivatives. The conjugates showed self-assembly in aqueous medium and yielded relatively stable colloidal solutions in phosphate-buffered saline. A strong dependence on the quality of the solvent was observed. In the case of lutein and 8'-apo- β -carotenal, succinylation and further coupling with melatonin resulted in considerable increase of TEAC values. Derivatives of β -cryptoxanthin showed similar TEAC as the parent carotenoid from THF, while from DMSO stock solution the activity of β -cryptoxanthin succinate decreased, however, its melatonin conjugate increased significantly. When comparing the FRAP values, those of the melatonin conjugates of lutein and capsanthin were outliers, surpassing all the native carotenoids. The succinates of all carotenoids had similar FRAP values, the only exception was the 8'-apo- β -carotenol succinate with a 1.5–2 times more elevated value. [2]

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Supramolecular delivery systems for carotenoids: EPR and optical studies

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1. Introduction

Incorporation of carotenoids in supramolecular carriers can significantly change their physicochemical and biological properties, as well as their therapeutic potential. Nanoencapsulation of carotenoids is an effective strategy to improve their stability towards heat, light, oxygen, metal ions and processing conditions, as well as to increase water solubility. Additionally, we have shown that nanoencapsulation can change the oxidation potentials of carotenoids, their absorption spectra, and ability to form J- and H-type self-aggregates. Here we will describe two delivery systems for carotenoids, polysaccharide arabinogalactan (AG) and saponin glycyrrhizin or glycyrrhizic acid (GA), studied by EPR and optical techniques [1,2].

2. Results and Discussion

For example, noncovalent binding of carotenoids with AG or GA enhanced their water solubility more than 1000-fold as well as oxidation stability. Such inclusion complexes of carotenoids with AG have shown enhanced photostability by a factor of 10 in water solutions, and a significant decrease by a factor of 20 in the oxidation rate by iron ions and reactive oxygen species in solution. The complexation with GA affected the oxidation potentials resulting in increased antioxidant activity of selected carotenoids. Also, such inclusion complexes prevented aggregation of xanthophyll carotenoids in aqueous solutions. H-aggregation of xanthophyll carotenoids detectable as 100 nm blue shift in the optical absorption maximum significantly changes their photophysics by increasing the quantum yield of reactive excited triplet state and reduces the scavenging ability of these carotenoids towards ROS. In addition, a remarkable increase in the quantum yield and the lifetime of the charge-separated states of the carotenoid radical cations in the supramolecular “hosts” of AG and GA has been detected. Some rare carotenoids with oxygen containing functional groups are suggested for incorporation into similar delivery systems [3].

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P2-3

Mechanical stimulation contra-regulates carotenoid and ABA biosynthesis in leaves from naïve versus touch-acclimated Arabidopsis plants

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1. Introduction

The chloroplast serves as an environmental stress sensor modulating the biosynthesis of carotenoid-derived signals and phytohormones (e.g., Absciscic acid; ABA) that regulate nuclear gene expression and maintain cellular homeostasis during stress acclimation. The biosynthesis of jasmonic acid (JA) starts within the chloroplast, and its accumulation can facilitate thigmomorphogenesis a dwarf-like phenotype caused by prolonged mechanically induced stress (MIS) [1]. Here we investigated interactions between carotenoid, ABA, and JA in leaves from naïve and touch-acclimated plants following touch stimulation by; 1) assessing plant morphology, cell density, and chloroplast fluorescence, 2) measuring metabolite levels, and 3) quantifying gene expression (**Figure 1**).

2. Experimentation

Arabidopsis wild type plants (Col-0 accession) were grown under controlled growth conditions for ~3-4 weeks. Ten-day old seedlings were stimulated by MIS using a soft paint brush for single touch (naïve) and/or 7 days (touch-acclimated). Rosette morphologies and anatomical changes (10 to 28d old plant) were observed by ImageJ software and confocal microscopy respectively. To investigate the MIS effect on chloroplast derived substances, chlorophyll, carotenoid levels were quantified using Agilent HPLC system [2], JA and ABA were quantified using triple quadrupole Mass-spectrometer and gene expression were measured using the Roche light cycler system (**Figure 2**).

3. Results and discussion

Prolonged MIS (7 days) to juvenile Arabidopsis seedlings promoted a smaller rosette with shorter petioles, phenotypes reminiscent of thigmomorphogenesis herein referred to as a 'touch-acclimated' plant. Touch-acclimated transition leaves showed a 33% reduction in mesophyll cell size and number, greater stomatal and chloroplast density, and increased chlorophyll fluorescence. Rosette leaves from the touch-acclimated plants contained 69 % (3-fold) higher basal level of carotenoid and chlorophyll pigments. A single touch stimulation event caused a transient increase by ~ 38 % (~2-fold) in foliar carotenoid levels in naïve but not touch-acclimated foliar tissues. Mature older transition leaves from aging plants required additional daily touch stimulation events to transiently increase pigment biosynthesis revealing that touch-acclimated plants were desensitised. Intriguingly, a single touch event increased ABA by 3-fold in naïve leaves, yet in touch-acclimated leaves ABA levels were suppressed by 3-fold revealing that prolonged MIS contra-regulates ABA accumulation. Touch stimulation increased expression of *9-CIS EPOXYCAROTENOID DIOXYGENASE 3* (*NCED3*; 9.8-fold) and *NCED5* (2.5-fold) in naïve but not touch-acclimated foliar tissues. The contra-regulation of ABA levels in naïve (upregulated) versus touch-acclimated (down-regulated) plant leaves can play a critical role during the early stages of mechanical signal transduction and stress priming phase, respectively to facilitate a somatic memory and stress acclimation.

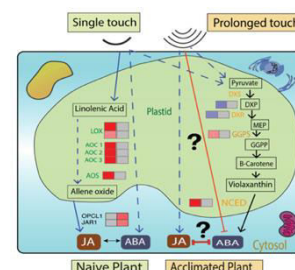


Figure 1. Schematic model showing how touch stimulation regulates chloroplast derived substrates in naïve and touch-acclimated plant tissues.

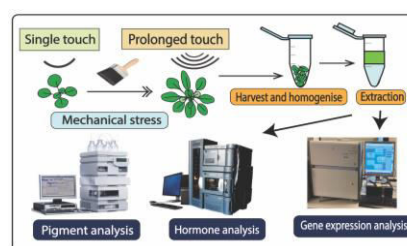


Figure 2. Overview of the experiment design was tested by comparing MIS effect on Arabidopsis rosette phenotypic and metabolic performance.

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P3-1

From aquaculture to aquaculture: Production of the fish feed additive astaxanthin and other valuable carotenoids from aquaculture sidestream by *Corynebacterium glutamicum*

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1. Introduction

Turning biotechnological production chains into sustainable, circular processes offers the possibility to contribute to climate protection and global waste reduction. Aquaculture generates nutritious sidestreams with residual fish feed or fish excrements that may be used for bacterial fermentation processes. *Corynebacterium glutamicum* is used in the biotechnological industry for the fermentative production of food and feed amino acids in the million-ton-scale [1]. This bacterium grows on a number of carbon sources and has been engineered to utilize several alternative growth substrates to enable a flexible feedstock concept [2]. *C. glutamicum* naturally synthesizes the yellow C50 carotenoid decaprenoxanthin and was engineered for production of several other isoprenoids [3], including the valuable, red C40 carotenoid astaxanthin [4] that exhibits strong antioxidative and ROS quenching properties. Mainly due to its colour, this ketocarotenoid is used as a feed supplement in the salmonoid and shrimp aquaculture.

2. Experimental

Cultures of *C. glutamicum* were supplemented with a pre-processed aquaculture sidestream (AQ) to evaluate its use as a growth medium component. Ingredients of the standard minimal salts medium for *C. glutamicum* (CGXII) were substituted by AQ and a new growth medium based on AQ was developed (CGAQ). The influence of AQ supplementation and CGAQ on carotenoid production was studied for several production strains. Astaxanthin was produced in large scale fermentations and used as feed supplement in a feeding trial with rainbow trout.

3. Results and Discussion

We demonstrated that AQ did not inhibit growth of *C. glutamicum* and could in fact replace a number of CGXII components. Furthermore, we showed that CGXII supplemented with AQ enhanced production of astaxanthin. This AQ improved astaxanthin production could be scaled-up to 2 L bioreactor fermentations. Moreover, this strategy was transferrable, and we showed that supplementation of AQ also improved production of native and other non-native carotenoids. In a feeding trial with rainbow trout, we could show that astaxanthin from *C. glutamicum* lead to a flesh colouration which is comparable to other astaxanthin sources. Thus, we have gained a proof-of-principle for production of astaxanthin from aquaculture sidestreams for use as feed additive in aquaculture, e.g. of salmonids or shrimp. We will discuss the potential and limitations of this aquaculture-based approach to circular economy.

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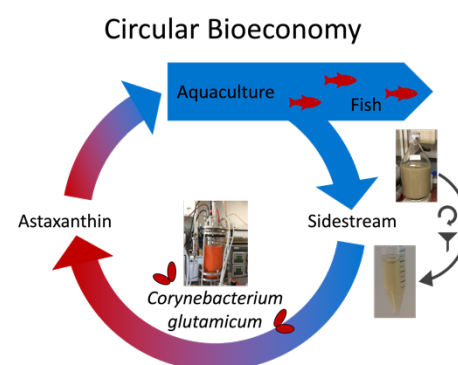


Figure 1. Astaxanthin production as an example for circular Bioeconomy

Excitation energy transfer dynamics of carotenoids in the far-red light utilizing photosystem II from *Acaryochloris marina*

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Natural photosynthetic organisms capture sunlight energy and transfer it to reaction centers (RC) where charge separation takes place to gain electro-chemical potentials. Photosystem II (PSII) is the photosynthetic pigment-protein complex consisting of core antenna and RC and mediates light-induced transmembrane electron transfer [1]. The high-resolution crystal structure revealed that the PSII monomer from a cyanobacterium *Thermosynechococcus* (*T.*) *vulcanus* contains photosynthetic chromophores of 35 chlorophyll *a* (Chl *a*), 10 β -carotene, and 1 β -cryptoxanthin molecules [2]. PSII from a cyanobacterium *Acharyochloris marina* (*A. marina*) has an ability utilizing far-red light due to presence of chlorophyll *d* (Chl *d*) [3]. Further, *A. marina* PSII (AmPSII) contains α -carotene instead of β -carotene. Energy transfer from β -carotene to Chl *a* in PSII has been reported to be inefficient because of the energy tuning between β -carotene and Chl *a* [4]. The conjugation length of α -carotene is shorter than that of β -carotene as shown in Fig.(A) resulting in the higher excitation energy of α -carotene. Then, the Q_y energy of Chl *d* is lower than that of Chl *a* (Fig.(B)). This situation implies that the light-energy absorbed by carotenoids is efficiently transferred to Chl *d* in AmPSII.

In the present study, we performed 100 fs pump-probe spectroscopies with different excitation wavelength on the isolated PSII-CBP (Chlorophyll Binding Protein) complex from *A. marina*, to clarify the detailed energy transfer dynamics from α -carotene to Chl *d*. Figure (B) compares the steady-state absorption spectra of PSII from *T. vulcanus* and AmPSII-CBP from *A. marina* at 140 K. The 0-0 vibronic transition from the S₀ ground state to the optically allowed S₂ state of α -carotene bound to AmPSII-CBP was observed at 493 nm. It has been reported that the absorption band of β -carotene whose molecular orientation is parallel to the membrane plane in PSII is red-shifted [5]. Thus, we selected the excitation wavelength as 495 and 515 nm. The detailed energy transfer dynamics from α -carotene to Chl *d* and its excitation wavelength dependency will be discussed.

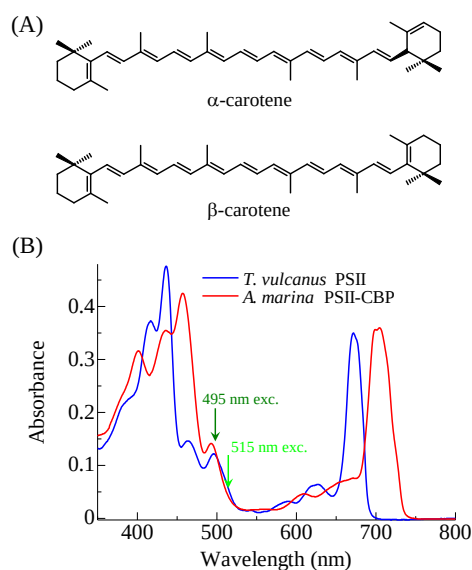


Figure: (A) Chemical structures of α -carotene and β -carotene. (B) The steady-state absorption spectra of PSII from *T. vulcanus* and *A. marina* at 140 K. Arrows indicate the excitation wavelengths.

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P4-2

Photosynthetic functions of carotenoids in photosystem I from cyanobacteria, as revealed by ultrafast spectroscopy

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Natural photosynthetic organisms capture sunlight energy and transfer it to reaction centers (RC) where charge separation takes place to gain electro-chemical potentials. Photosystem I (PS I) is the photosynthetic pigment-protein complex consisting of core antenna and RC and mediates light-induced transmembrane electron transfer [1]. The high-resolution crystal structure revealed that the PS I monomer from cyanobacterium *Thermosynechococcus* (*T.* *elongates*) contains photosynthetic chromophores of 96 Chlorophyll *a* (Chl *a*) molecules and 22 β -carotene (β -car) molecules [2]. One of the spectroscopic interests of PSI is a presence of red-chlorophylls (Chl) with strong interactions between Chl molecules. The red-Chls localize the excitation in PSI core antennas and are located closer to PSI-RC. Therefore, the red-Chls may play certain roles to concentrate the excitation near the RC, realizing efficient energy transfer from core antenna to RC in PSI. Further, it has been reported that the presence of red-Chl in PSI realizes the efficient energy transfer from β -car to Chl *a* [3]. In the present study, we performed 100 fs pump-probe spectroscopies with different excitation wavelength on the isolated PSI-trimer from a thermophilic cyanobacterium, *T. vulcanus*, to clarify the detailed energy transfer dynamics of β -car to Chl *a*.

Figure (A) shows the steady-state absorption spectra of PSI-trimer at 140 and 296 K. The absorption bands around 680 nm are attributed to the Q_y transition of Chl *a*. These bands extend above 700 nm due to the presence of red-Chl. Figure (B) shows the photo-induced absorption spectra of PSI recorded at 1.0 ps after excitation into the β -car S_2 absorption band at 500 and 520 nm. The positive absorbance changes, assigned to the S_1 transient absorption of β -car, appeared around 560 nm. On the other hand, the Chl *a* bleaching bands observed were around 700 nm, suggesting the energy transfer from β -car to Chl *a*. Interestingly, the β -car bleaching above 520 nm depends strongly on the excitation wavelength. The results suggest the different excitation energies of β -car depending on protein binding sites in PSI. The detailed energy transfer dynamics in PSI-trimer will be discussed in terms of the presence of the red-Chl.

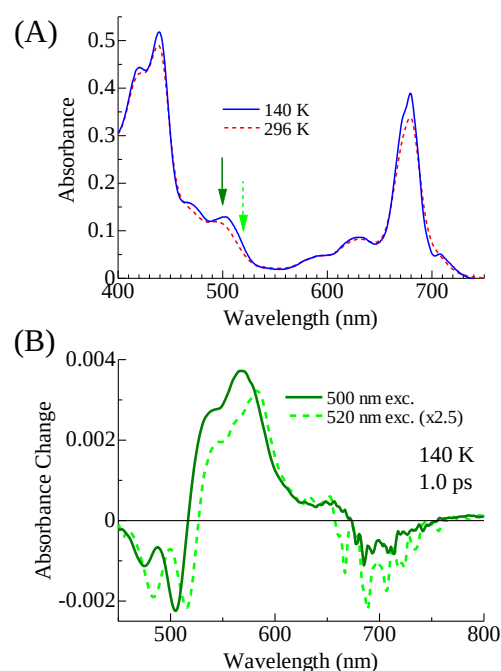


Figure: (A) The steady-state absorption spectra of PSI-trimer at 140 and 296 K. Arrows indicate the excitation wavelengths. (B) The photo-induced absorption spectra of PSI-trimer at 140 K excited at 500 and 520 nm.

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P4-3

Real-time observation of molecular vibrations of fucoxanthin by 10 fs pump-probe spectroscopic measurement

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Carbonyl carotenoids are common in marine algae and they participate in photosynthetic light-harvesting [1]. Characterization of the photophysical properties of carbonyl carotenoids is necessary to understand the highly efficient nature of energy transduction in the light-harvesting systems of marine organisms. The S_0 ground state of all-*trans*-carotenoids is denoted as $1^1A_g^-$, assuming C_{2h} symmetry of its polyene backbone. Strong absorption bands in the visible region are attributed to a transition from S_0 to the S_2 ($1^1B_u^+$) state. A one-photon transition from S_0 to the lowest-lying excited singlet state, S_1 ($2^1A_g^-$), is optically forbidden because S_0 and S_1 have the same symmetry. Ultrafast spectroscopic studies of carbonyl carotenoids have revealed several unique features that distinguish them from carotenoids without carbonyl groups. These include the dependence of the S_1 lifetime on solvent polarity which has been accounted for on the basis of an intramolecular charge transfer (ICT) state located near S_1 [2]. However, it has not yet been clarified whether S_1 and the ICT state are strongly coupled or distinct electronic states.

When relaxation events following electronic excitation are considered, electron-molecular vibration interaction is a key issue. It is now possible to observe real-time oscillations of molecular vibrations under impulsive excitation. Since the dephasing of coherent oscillations can reflect the interaction between the surrounding environment and electronic excitations, this dephasing provides important information when discussing the relaxation processes. In the present work, we performed 10 fs pump-probe measurement on fucoxanthin in solvents, since the dynamics of the electronic excitations as well as the coherent oscillations can be monitored simultaneously.

Home-built noncollinear optical parametric amplifier (NOPA) whose pulse width is about 8 fs was utilized as pump/probe pulses. Fig. (A) shows the steady-state absorption spectrum of fucoxanthin in methanol and the output spectrum of NOPA. Figs. (B) and (C) respectively present the time-wavelength 2D counter plot and the kinetic traces at 600 nm. Fig. (D) exhibits the fast Fourier transform (FFT) power spectrum of the coherent oscillation shown in the inset in Fig. (C). The detailed excited state dynamics will be discussed in terms of electron-molecular vibration interaction and its participation of the generation of the ICT state.

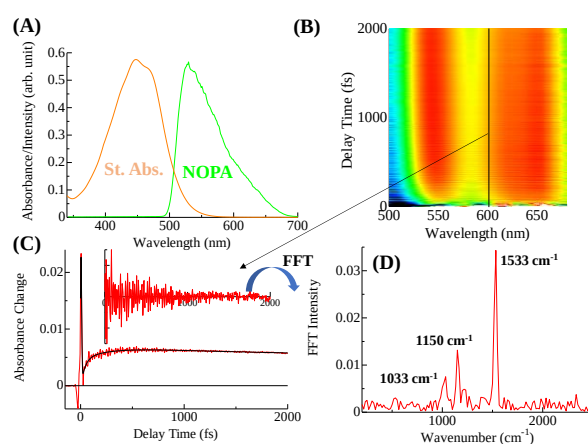


Figure: (A) Steady-state absorption of fucoxanthin in methanol and NOPA spectra. (B) Time-wavelength 2D counter plot of 10 fs pump-probe data. (C) Kinetic trace of the photo-induced absorption detected at 600 nm. The thin solid line is the best fit curves. Inset denote the coherent oscillation obtained by subtracting the slowly varying background. (D) FFT spectrum of the coherent oscillation detected at 600 nm.

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P4-4

Ultrafast energy transfer dynamics in photosynthetic antenna acpPC from dinoflagellate *Symbiodinium*

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Photosynthetic antenna apparatus efficiently capture sunlight and transfer the energy to reaction centers. Chlorophyll *a/c*-peridinin protein complex (acpPC) is an integral membrane complex from dinoflagellate [1]. The acpPC complex contains major carotenoid peridinin (per) and minor carotenoid diadinoxanthin (ddx) or diatoxanthin (dtx). It has been reported that acpPC shares high similarity to fucoxanthin Chl *a/c* protein (FCP) complex from diatom [2]. Per and fucoxanthin exhibit spectral characteristics attributed to an intramolecular charge transfer (ICT) state located nearby the S₁ state that arises in polar environments due to the presence of the carbonyl group in its polyene backbone, leading to the generation of a strongly coupled S₁/ICT state [3]. Consequently, per and fucoxanthin efficiently transfer the absorbed energy to nearby Chl *a* via S₁/ICT state [4]. On the other hand, ddx/dtx is one of the xanthophylls cycle carotenoids that play a photoprotective function by dissipating excitation energy as heat. In this study, we isolated the two different fractions of functional acpPC from dinoflagellate *Symbiodinium* sp. strain SGCH-03 [5], and performed femtosecond pump-probe spectroscopy to clarify functions of carotenoids.

Figure (A) presents the steady-state absorption spectra of the acpPC fractions (fr.1 and fr.2). The absorption spectra show the different carotenoid composition of fr.1 and fr.2 i.e., fr.1 contains much per while fr.2 contains much ddx. Femtosecond pump-probe spectroscopic measurements were performed on the two acpPC fractions to clarify energy transfer dynamic from carotenoids to Chl *a*. Figure (B) shows the photo-induced absorption spectra of fr.1 and fr.2 taken at 1.0 ps after selective excitation to ddx/dtx and per at 490 and 540 nm, respectively. The positive absorbance changes due to the carotenoid transient absorption and the negative signals of the Chl *a* bleaching appears in the all spectra. In the spectrum of fr.1 excited at 490 nm, a sharp transient absorption band was observed around 550 nm, assigned to ddx/dtx, while the Chl *a* bleaching was suppressed, suggesting an inefficient energy transfer from ddx/dtx to Chl *a*. In other cases, an efficient per→Chl *a* energy transfer was observed.

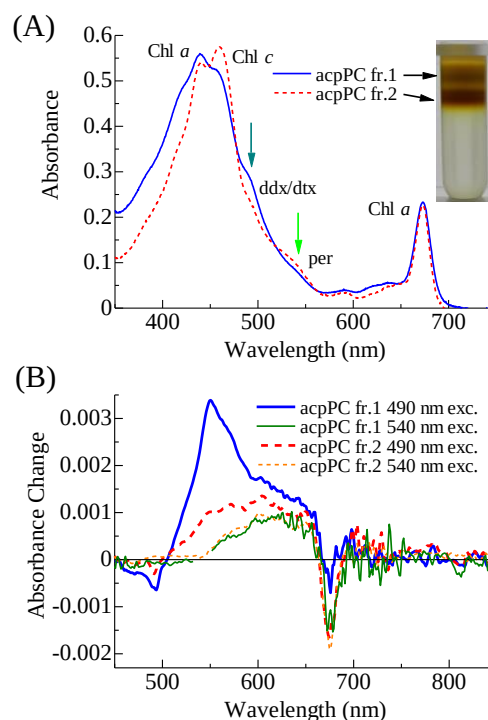


Figure: (A) Steady-state absorption spectra of the two different acpPC fractions. Arrows indicate the excitation wavelengths for fs pump-probe measurements. (B) Photo-induced absorption spectra of the different acpPC fractions excited at 490 and 540 nm.

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Photoreduction of carotenoids and binding dynamics of the tetraheme-RC-bound cytochromes *c* and cytochrome *c*₂ in *Roseobacter denitrificans* probed by resonance Raman spectroscopy

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1. Introduction

In *Roseobacter denitrificans*, the light harvesting complexes (LH1-RC) and the peripheral antenna (LH2) that transfers the absorbed energy to LH1-RC, have been investigated for elucidating the energy transfer efficiency. The LH1 complex consists of 16 heterodimeric protein subunits, which form a ring around the RC, two bacteriochlorophyll *a* (BChl*a*) molecules and a carotenoid molecule. Monomeric BChl*a* molecules absorb near 800 nm whereas dimeric BChl*a* near 850 nm. The LH2 complex display eight or nine cyclically arranged α,β -heterodimers, three bacteriochlorophyll *a* (BChl) molecules and one to two carotenoid molecules.

2. Experimental

To probe the photodynamics of the carotenoids and binding dynamics of the tetraheme-RC-bound cytochromes *c* and cytochrome *c*₂ in *Roseobacter denitrificans* we applied resonance Raman spectroscopy.

3. Results and Discussion

In this work, we extend our previous work on the photoreduction of carotenoids in *Roseobacter denitrificans* and present studies on the wild-type membranes of *R. denitrificans* isolated from semi-aerobic dark-grown cells [1]. The pH dependence of LH2 complex revealed that in the 8 to 7 pH transition there is an intensity decrease of the 806 nm transition and from the pH transition from 7 to 5 a 3 nm red shift to 809 nm and a further decrease in the intensity. The resonance Raman results indicate the presence of a five-coordinated cyt *c* Fe³⁺ and upon reduction it is converted to a high spin five-coordinated Fe(II) that is subject to ligand binding of CN in the oxidized- and to CO in the reduced-form. In the isolated form there are two cyt *c* in the reduced Fe²⁺ form and there is a reversible photoreduction mechanism for at least one cytochrome *c*. The photophysical properties of cytochromes *c* are of profound importance because they are involved in the formation of an active for electron transfer cyt-*c*₂: tetraheme bound cytochrome *c*:RC complex. Based on the frequencies of ν_{11} we can make conclusions on the ligation of the heme *c*. The observed bands of BChl*a* and Crt remain spectators in all events. As a whole, our investigation provides the first full resonance Raman spectroscopic characterization of the membrane bound cytochromes *c*, the carotenoids and BChl*a* present in *R. denitrificans*. We suggest that the membrane-surface binding pocket of cyt *c*₂ is responsible for both the exogenous ligand binding and reversible photoreduction behaviour and may provide a heme axial ligand environment distinct from any other photosynthetic bacteria characterized to date.

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P4-6

Identification of carotenoids in photosynthetic protein complexes based on structural analysis

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Photosynthetic organisms, such as plants and algae, absorb solar energy and convert it into chemical energy by utilizing numerous pigments found in the photosynthetic membrane protein complexes (photosystems I and II; PSI and PSII) and then undergo electron transfer reactions. The carotenoids present in these protein complexes not only transfer energy to chlorophyll (Chl), but also possess photoprotective functions, whereby excitation energy is converted into heat and excess excitation energy is dissipated. The structure of these protein complexes has been actively analyzed in various species to elucidate the arrangement and functions of the pigments in the photosynthetic proteins. In this conference, we will introduce and discuss the composition and arrangement of carotenoids in PSI and PSII of different species through structures analyzed using X-ray crystallography and cryo-electron microscopy (cryo-EM).

The PSII from *Thermosynechococcus vulcanus* (*T. vulcanus*), a thermophilic cyanobacterium, mainly contains β -carotene (β -Car). In 2011, we identified one β -cryptoxanthin (β -Crx) at the periphery of the *T. vulcanus* PSII by X-ray crystallography [1]. Recent cryo-EM analyses have identified one β -Crx in PSII from several cyanobacteria [2,3]. Additionally, *Acaryochloris marina*, a cyanobacterium, uses Chl *d* to absorb near-infrared light for photochemical reactions, whereas many oxygen-evolving photosynthetic organisms utilize Chl *a* for their photochemical reactions. Recently, we analyzed the *A. marina* PSI from at 2.6 Å resolution using cryo-EM and discovered a unique electron transfer mechanism involving Chl *d* and pheophytin *a* (Pheo *a*) [4]. The *A. marina* PSI contained a large number of carotenoids, and their arrangement was similar to the *T. vulcanus* PSII; however, fine rearrangements were observed due to alteration in the surrounding amino acid residues. Furthermore, pigment analysis revealed that unlike β -Cars, α -carotenes (α -Cars) were identified in the *A. marina* PSI. Moreover, one zeaxanthin (Zea) was found in the *A. marina* PSI.

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Analysis of the excited energy transfer from 4,4'-diaponeurosporene to bacteriochlorophyll-g in the photosynthetic reaction center of *Heliumicrobium modesticaldum*

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1. Introduction

Heliobacteria are photosynthetic bacteria and have type 1 reaction center (RC) which is the same as photosystem I. The RC of heliobacteria (hRC) contains 54 Bacteriochlorophyll (BChl) gs, 4 BChl g's, two 8¹-hydroxychlorophyll *a*_F (Chl *a*) and two 4,4'-diaponeurosporene (Car), which are all arranged along with a completely symmetrical C₂ axis (see **Figure 1**) [1]. Among these pigments, two BChl g's (special pair of dimer, P800), two BChl gs (Acc) and two Chl *a* (primary electron acceptor, A₀), which work as electron transfer cofactors where initial charge separation occurs. The other pigments function as light harvesting antenna pigments. Although the kinetics of excitation energy transfer between BChl gs in hRC have been analyzed previously [2], those between Cars and BChl gs have not been yet. In the present study, we observed the profiles of excitation energy transfer around Cars in the hRC with femtosecond pump-probe spectroscopy.

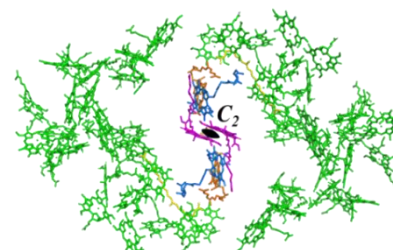


Figure 1. Arrangement of pigments in hRC (bottom view, P800 side)

2. Experiment

The hRC samples were purified from *Heliumicrobium modesticaldum* according to the method previously described [2]. In order to observe the charge recombination process between P800⁺ and A₀⁻, a strong reducing agent, dithionite, was added to the samples. Femtosecond pump-probe measurements were performed using excitation at 490 nm, 770 nm and 820 nm at 140 K.

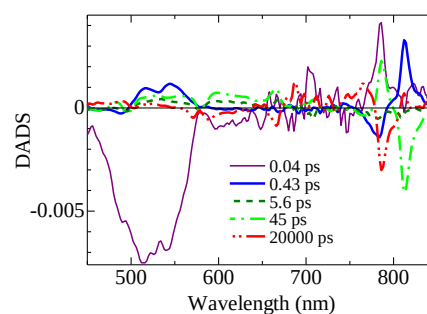


Figure 2. DADS obtained with five components as indicated.

3. Results and Discussion

Figure 2 shows the decay-associated difference spectra (DADS) in the wavelength region from 450 nm to 850 nm with 490-nm excitation by a global fit analysis. It revealed that the efficient excitation energy transfer from Car to the nearby BChl g's pigment pool (B_{md}787) with a time constant (τ) of 0.04 ps, and subsequently from B_{md}787 to the longest-wavelength BChl g's pigment pool (B_{md}812, red-BChl g) with $\tau = 0.43$ ps. The photochromic-band shifts of Cars were also observed at 1 ps after excitation at 820 nm and 770 nm. It suggested that the red-BChl gs would exist near Cars.

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Temperature-Dependent Absorption Spectra of Carotenoids: Exploring the Relationship between Solvent-Carotenoid Interaction and 0-0 Band Width

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1. Introduction

Carotenoids are integral to the primary processes of photosynthesis, playing a vital role in capturing light energy and efficiently transferring it to chlorophyll [1]. To express their function, carotenoids bind to pigment-protein complexes, resulting in a red shift of the absorption maximum wavelength and a sharper absorption band compared to their behavior in solution. This phenomenon highlights the powerful interplay between the carotenoid molecule and its surrounding environment, ultimately contributing to the remarkable efficiency of the photosynthetic process.

2. Experimental

In this study, we investigate the temperature-dependent behavior of several carotenoids, including β -carotene and β -apo-8'-carotenal, when dissolved in *n*-hexane, acetone, and methanol, by examining their steady-state absorption spectra from room temperature to low temperature. While previous studies have explored the absorption spectra of β -carotene and β -apo-8'-carotenal at room temperature and 80 K, none have reported on their response to stepwise temperature changes. Thus, we conducted measurements in 10°C decrements from 20°C (room temperature) to -90°C (the melting point of each solvent) in order to gain a comprehensive understanding of their temperature dependence.

3. Results and Discussion

As the temperature decreased, the absorption spectra of the carotenoids exhibited a remarkable shift towards longer wavelengths and a sharper, more distinct vibrational structure. These observations prompted us to employ Gaussian fitting to determine the precise width of the 0-0 band. In our ongoing analysis, we will delve deeper into the complex interplay between the solvent and carotenoid, shedding light on the intricate relationship between temperature and the 0-0 band width.

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P4-9

Photoprotective function evaluation of light-harvesting systems containing bacteriochlorophyll *b* in *Blastochloris viridis*

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1. Introduction

Photosynthetic bacteria have been actively used as research models to understand the primary processes of photosynthesis due to their relatively simple photosystems and ease of biochemical sample preparation. While many studies have investigated the light-harvesting (LH) systems containing bacteriochlorophyll (Bchl) *a*, few have focused on the LH systems containing Bchl *b* [1,2]. The photosystem from the purple photosynthetic bacterium *Blastochloris (Blc.) viridis* is composed solely of the LH1-RC complex, in which the LH1 pigment-protein complex surrounds the reaction center (RC). It contains two species of carotenoid pigments (1,2-dihydroneurosporene and 1,2-dihydrolycopene) and Bchl *b* dimers [1], which exhibit a Q_y absorption band in the far near-infrared region (1010 nm), longer than that of the Bchl *a*-containing LH1 complex. Purple photosynthetic bacteria produce reactive oxygen species that can destroy living organisms when exposed to excess light energy. However, the LH1-RC complex prevents cell death through the photoprotective mechanism, which dissipates the excess light energy as heat to the surroundings. In this study, we isolated the LH1-RC complex from *Blc. viridis* and evaluated its photoprotective function. This finding is expected to contribute to the development of robust artificial photosynthetic systems, especially for the effective usage of light energy in the far near-infrared spectral region.

2. Experimental

Photosynthetically grown cells of *Blc. viridis* were harvested and subjected to French press to obtain photosynthetic membranes, which were then solubilized. The LH1-RC complex was subsequently isolated from the solubilized membranes via sucrose density gradient ultracentrifugation. To investigate the excitation energy transfer processes from Bchl *b* to carotenoids, sub-nanosecond time-resolved absorption spectroscopic measurements were performed on the isolated LH1-RC complex using Bchl *b* excitation in the Q_y absorption band (1010 nm).

3. Results and Discussion

Sub-nanosecond time-resolved absorption spectroscopy of the isolated LH1-RC complex did not afford the meaningful signals that are ascribable for the generation of the triplet carotenoids. This outstanding finding suggests that the photoprotective function of carotenoids that are bound to the LH1-RC complex from *Blc. viridis* may not be very effective.

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Decoding the molecular structures and functions of carotenoid radical species using resonance Raman spectroscopy and DFT calculations

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1. Introduction

Carotenoids, those precious pigments that safeguard light-harvesting (LH) and photoprotection in photosynthesis, play a vital role in our ecosystem [1]. When exposed to excessive light, the LH complexes increase the production of triplet chlorophylls that react with oxygen to create singlet oxygen. But scavenging of triplet chlorophylls by carotenoids represses the generation of singlet oxygen, thereby preserving the LH system. In the pigment-protein complexes, photo-excited state of carotenoids and their interplay with surrounding proteins are indispensable factors in expressing these critical functions. Diverse spectroscopic methods have been employed to investigate physical properties of carotenoids in the photoexcited states [2,3]. To decode these findings, quantum chemical calculations are instrumental. Moreover, it is known that cation radicals and dications of *trans*-carotenoids can produce *cis* isomers [4]. The carotenoid cation radicals play a vital role in photoprotection and serve as an antioxidant. The density functional theory (DFT) has enabled us to reproduce the Raman spectra of carotenoids with astounding precision. The solute-solvent interaction can be predicted through the Polarizable Continuum Model (PCM), utilizing the integral equation formalism variant (IEFPCM) [5-8].

2. Calculation method

Calculations were performed on spirilloxanthin, β -carotene, canthaxanthin, and astaxanthin using cc-pVTZ basis functions. Raman spectra of these carotenoids were calculated with the IEFPCM model, and the results were compared with experimental data.

3. Results and Discussion

Resonance Raman spectra of β -carotene, canthaxanthin, and astaxanthin and their radical species were recorded, and the results were compared with those of the DFT calculations. DFT calculation well reproduced the ground (S_0) state Raman spectra, while the resonance Raman spectra of the radical species can be regarded as the combination of the calculated Raman spectra of the radical cation and dication of the carotenoids. This is because that the resonance Raman spectra of the ground (S_0) species were recorded using the 532 nm laser light that is in resonance with the $S_0 \rightarrow S_2$ absorption of carotenoids, while the resonance Raman spectra of the radical species were recorded using the 808 nm laser light that is in resonance for both the radical cation and dication of the carotenoids. The agreement of the theoretically calculated Raman spectra with the experimentally observed resonance Raman spectra allows us to discuss the detailed molecular structures of the radical species of the carotenoids. As an example, the bond lengths of the ground (S_0) state, radical cation and dication of the carotenoids were compared. It is interesting to note that the bond alteration that can clearly be found in the ground (S_0) state species shows dramatic changes in the radical cation species; all the C=C double bond tends to elongate, while all the C-C single bond tends to shrink, which suggests that the bond orders of all the C=C and C-C bonds in the conjugated polyene approaches 1.5. In the case of the dication species, it is amazing that the bond alteration is reverted in the central part of the polyene chain; the C=C double becomes C-C single bond, and the C-C single bond becomes C=C bond. These drastic changes of the bond orders are well reflected to the vibrational modes, which were predicted based on the DFT calculations. The theoretically predicted molecular structures of the radical species of the carotenoids, which were supported experimentally by means of resonance Raman spectroscopy, can be a good tool to explore the functions of the radical species of the carotenoids in biological systems.

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Triplet-triplet-type photoprotective pathway in the spheroidene-reconstituted LH1 complex

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1. Introduction

Purple photosynthetic bacteria harness the power of sunlight using energy transfer from the light-harvesting (LH) complex to the reaction center (RC), where charge separation takes place [1]. Both the LH complex and RC rely on carotenoids to act as both light harvesters and photoprotectors. During light harvesting, the carotenoid (Car) is photoexcited to the S_2 singlet excited state and then relaxes to an optically forbidden S_1 singlet excited state, allowing for the transfer of excitation energy from the S_2 and S_1 states of Car to the S_2 (Q_x) and S_1 (Q_y) states of bacteriochlorophyll *a* (Bchl *a*), respectively. Alternatively, in the photoprotection process, Car can dissipate excess energy delivered to Bchl *a* through singlet to triplet (ST) and/or triplet to triplet (TT) energy transfer pathways. The ratio of ST/TT varies depending on the type of LH complexes. Notably, the LH2 complex from *Rhodobacter* (*Rba.*) *sphaeroides* strain 2.4.1, which contains spheroidene as Car, only undergoes TT-type energy dissipation [2], whereas the LH1 complex from *Rhodospirillum* (*Rsp.*) *rubrum* strain S1, which contains spirilloxanthin as Car, shows dominant ST-type energy dissipation [3].

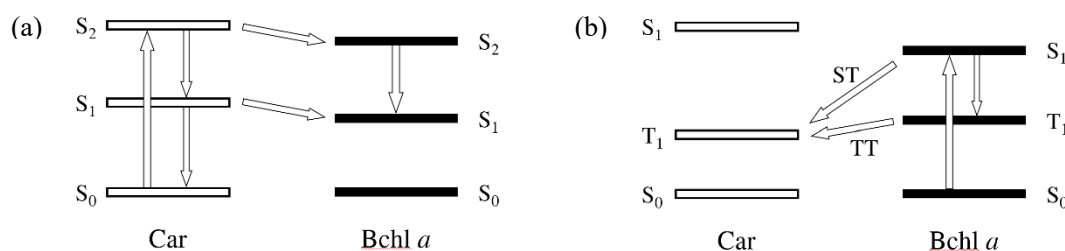


Figure 1. Schematic illustration of (a) light-harvesting and (b) photoprotection processes of Car.

2. Experimental

In this study, we extracted and purified spheroidene from *Rba. sphaeroides* strain 2.4.1 and reconstituted it into the carotenoidless LH1 complex from *Rsp. rubrum* strain G9+. By using femtosecond to sub-millisecond time-resolved absorption spectroscopy, we investigated how the species of Car bound to the LH1 complex affects the LH and photoprotection processes.

2. Results and discussion

Our findings indicate that the spheroidene-reconstituted LH1 complex exhibits the formation of the S^* state of spheroidene in the LH process and a dominant TT-type energy dissipation process in the photoprotection process. These mechanisms differ from those observed in the native spirilloxanthin-bound LH1 complex from *Rsp. rubrum* strain S1 but are similar to those in the native spheroidene-bound LH2 complex from *Rba. sphaeroides* 2.4.1 [2]. Therefore, we concluded that carotenoids, rather than the type of LH complexes, play a key role in altering energy flows in both the LH and photoprotection processes.

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P4-12

Exploring the Structure-Function Relationship of β -Apo-8'-Carotenal Isomers: Insights into Photosynthetic Pigments from Computational Chemistry Analysis

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1. Introduction

Photosynthesis is a remarkable process that harnesses the power of sunlight to generate biological energy from water and carbon dioxide. To accomplish this feat, nature has ingeniously selected conjugated polyene molecules, such as carotenoids and chlorophylls, as the key players. These pigment molecules possess unique structures that are intimately linked to their light-harvesting function, a critical component of the primary photosynthetic process [1]. In order to unravel the underlying mechanisms of photosynthetic light harvesting [2], it is imperative to gain a better understanding of the structure-function relationship of pigment molecules. This requires the determination of the most stable structure of the pigment molecule using computational chemistry, as well as the prediction of $\Delta\mu$, a crucial parameter that represents the direction and magnitude of charge transfer upon photoexcitation.

Our study focused on the *cis-trans* isomers of β -apo-8'-carotenal (refer to **Figure 1** for its chemical structure), whose $\Delta\mu$ has experimentally been determined [3]. We took on the challenge of comparing the $\Delta\mu$ of the all-*trans*- β -apo-8'-carotenal, which possesses a one-dimensional polyene backbone, with that of the *cis* isomers, where the polyene backbone extends in two dimensions, to shed light on the unique features of the latter. Utilizing the WinMOPAC 3.9 program (Fujitsu Ltd.), a semi-empirical molecular orbital calculation software, we determined the most stable structure of each isomer of β -apo-8'-carotenal and calculated their respective $\Delta\mu$ values. Our findings provide new insights into the fascinating world of photosynthetic pigments and pave the way for future investigations into their structure-function relationship.

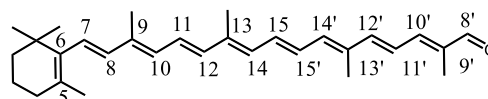


Figure 1. Chemical structure of all-*trans*- β -apo-8'-carotenal

2. Experimental

To identify the most stable structures of the all-*trans* isomers, we conducted minimum energy path calculations regarding the rotation around the C6-C7 bond, which represents the rotation of the β -ionone ring against the polyene backbone. The dihedral angle of the C6-C7 bond served as an initial parameter to determine the most stable structure of each *cis* isomer. Once we identified the most stable structures of each isomer, we calculated $\Delta\mu$ using the MOS-F program in WinMOPAC 3.9.

3. Results and Discussion

Our theoretical calculations yielded $\Delta\mu$ values that closely matched experimental observations, affirming their reliability. Intriguingly, a comparison of the $\Delta\mu$ directions between the isomers revealed a remarkable trend: while the all-*trans* isomer displayed a nearly parallel direction to its polyene backbone, the *cis* isomers exhibited progressively greater distances away from the all-*trans* fragment, in the order of all-*trans* < 7-*cis* < 9-*cis* < 13-*cis* < 15-*cis* < 13'-*cis*. This finding suggests that the *cis*-isomers' two-dimensional structure may facilitate intramolecular charge transfer along the arm of the carbonyl existing side of the molecule, offering fascinating insights into their unique functionality in photosynthesis.

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Replication of Absorption Spectrum of Carotenoid in Purple Photosynthetic Bacteria

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1. Introduction

In purple photosynthetic bacteria, the process of photosynthesis commences with the light-harvesting (LH) system capturing photons. The LH complex, which is responsible for this task, binds carotenoids (Car) that absorb sunlight in the visible wavelength range, as well as bacteriochlorophyll (Bchl), which strongly absorbs UV and near-infrared wavelengths. The efficiency of excitation energy transfer (EET) varies from 30% to 90% depending on the type of Car bound to the photosynthetic bacterium [1]. To determine the EET efficiency from Car to Bchl in kinetic terms, ultrafast relaxation processes of Car, which serve as a reference, have been observed in pure organic solvent systems. The lifetime of the excited state of Car is closely related to the dielectric environment surrounding it. Therefore, it is crucial to investigate the ultrafast relaxation processes of Car in solvent systems that accurately reflect the dielectric environment of the LH complex. In this study, we aim to replicate the absorption spectrum of Car bound to the LH complex using a mixed organic solvent system. By conducting transient absorption spectroscopy measurements using the mixed organic solvent system, we determine the rate constant for the internal conversion of Car from the singlet excited state.

2. Results and Discussion

We isolated chromatophores and spirilloxanthin from *Rhodospirillum (Rsp.) rubrum* strain S1. The cells of *Rsp. rubrum* strain S1 were disrupted using a French press, and the chromatophores were isolated by ultracentrifugation. Spirilloxanthin was extracted using organic solvents and purified through column chromatography. By using a mixed organic solvent system based on carbon disulfide and pyridine, we were able to replicate the wavelengths of the absorption spectra of the carotenoids in the chromatophores (refer to **Figure 1**). We investigate how the rate constant would be affected by the environment surrounding the carotenoids using femtosecond time-resolved absorption spectroscopy.

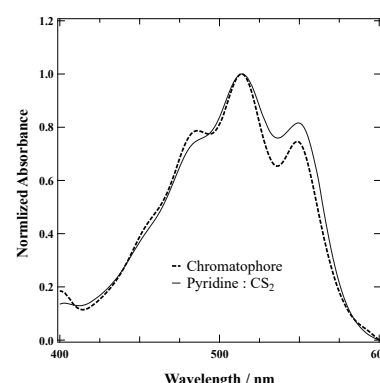


Figure 1. Comparison of the absorption spectra of spirilloxanthin in chromatophore and that in mixed solvent systems.

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Exploring the Excited State Properties of β -Apo-8'-Carotenal through Steady-State Fluorescence Spectroscopy

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1. Introduction

Photosynthesis sparks to life as chlorophyll seizes light energy, producing high-energy electrons. However, chlorophyll has limited absorption capabilities, leaving a gap in its wavelength region. This is where carotenoids step in - they are capable of absorbing light in this range, playing a critical role in transferring the captured energy to chlorophyll. An excited state known as intramolecular charge transfer (ICT) exists as an S_1 /ICT state through coupling with the S_1 state. It is present in carbonyl-containing carotenoids and enables highly efficient energy transfer to chlorophyll [1]. One such carotenoid is β -Apo-8'-carotenal (Apo-8'), which belongs to the aldehyde type. The ICT excited state arises from the presence of a carbonyl group at one end of the molecule. In this study, we measured the steady-state absorption, fluorescence, and fluorescence excitation spectra of Apo-8' and compared the photophysical properties of the ICT excited state with those of β -Carotene, shedding light on their intriguing characteristics.

2. Experimental

Steady-state absorption, fluorescence, and fluorescence excitation spectra were recorded using Horiba-Duetta fluorescence and absorbance spectrometer at room temperature. The carotenoid sample was dissolved in acetone, and the concentration was adjusted to an absorbance of approximately 0.25 at the maximum absorption wavelength. The fluorescence spectrum was measured by using this maximum absorption wavelength as the excitation wavelength. The fluorescence excitation spectrum was measured by using the wavelength of the peak of the fluorescence spectrum as the emission wavelength.

3. Results and Discussion

The fluorescence spectrum of the solution was measured using 457 nm excitation, and the results are presented in **Figure 1**. β -Carotene exhibited an $S_2 \rightarrow S_0$ emission spectrum, with a peak at 520 nm. Conversely, Apo-8' demonstrated an S_1 /ICT $\rightarrow S_0$ emission spectrum, with a peak at 850 nm. The fluorescence excitation spectrum detected at the peak wavelength of the emission spectrum exhibited a shape that correlated well with the absorption (1 - T) spectrum, albeit with an insufficient signal-to-noise ratio. In our future work, we aim to boost the signal-to-noise ratio and delve deeper into the solvent dependence.

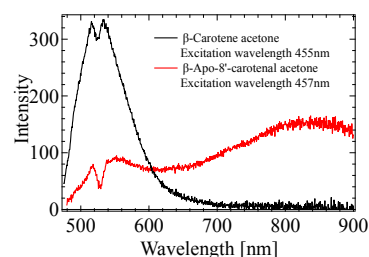


Figure 1. Fluorescence spectra of Apo-8' and β -Carotene in acetone

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Excited-State Dynamics of *Cis* Isomers of β -Apo-8'-Carotenal: Classification of Rate Constants and "Inverse Energy Gap Law"

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1. Introduction

Cis isomers of carotenoids play important roles in light-harvesting and photoprotection in photosynthetic bacteria, such as the reaction center (RC) in purple bacteria and the photosystem I (PS I) in cyanobacteria. Carotenoids bound to the RC of purple photosynthetic bacteria adopt a 15-*cis* configuration of the carotenoid [1]. In cyanobacterial PS I, there are 22 carotenoids, of which 17 are all-*trans*, 2 are 9-*cis*, and each one of the 9,9'-*cis*, 9,13'-*cis*, and 13-*cis* isomers [2]. However, the fundamental question of why the *cis* isomers rather than the all-*trans* isomers is selected still remains to be addressed. In this study, we systematically investigated the excited-state dynamics of the isomers of β -apo-8'-carotenal.

2. Experimental

We applied photoisomerization to the all-*trans* isomer of β -apo-8'-carotenal to obtain the 7-, 9-, 13-, 15-, 13'-, 9,13'-, 9,13-, and 13,13'-*cis* isomers. Each *cis* isomer was isolated and purified from the isomeric mixture using HPLC. We measured femtosecond transient absorption spectra of the highly pure *cis* isomers and applied Singular Value Decomposition and Global analysis to those datasets.

3. Results and Discussion

We investigated the excited-state dynamics of the *cis* isomers in terms of the relationship between the rate constant of the S_1 excited state (k_{cis}) and the energy gap between the S_1 and S_0 states (ΔE). We discovered that the k_{cis} can be further classified into three types: (13-, 15-) *cis*-bend group (solid circles), (13'-, 9-) *cis*-bend group (solid triangles), and 7-*cis* group (solid square). The defined $k_{cis} - k_{EGL}$ in **Figure 1** reflects the extent of the deviation of the experimentally determined rate of relaxation from the S_1 state of the *cis* isomer from that which can be estimated theoretically based on the energy gap law [3]. Interestingly, a good linear relationship was found between $k_{cis} - k_{EGL}$ and the $S_0 - S_1$ energy gaps for all the isomers. We call this trend an "inverse energy gap law" because it shows the opposite of the conventional energy gap law.

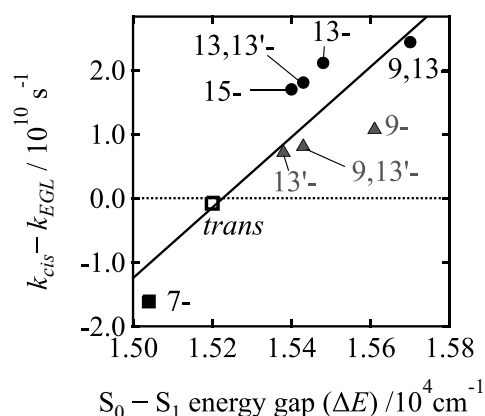


Figure 1. The relationship between $k_{cis} - k_{EGL}$ and the $S_0 - S_1$ energy gap of the isomers of β -apo-8'-carotenal in *n*-hexane.

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P4-16

Application of lipid-nanodisc to investigate the photoprotective function of carotenoids bound to photosynthetic light harvesting complex

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1. Introduction

In eukaryotic photosynthesis, carotenoids bound to light harvesting complex (LHC) play the role of efficient photon absorption and stabilization of LHC structure. Besides, quenching of harmful chlorophyll triplet ($^3\text{Chl } a^*$) generated within the complex is also crucial function to prevent the photodamage originating from ROS produced by $^3\text{Chl } a^*$. In spectroscopic studies about photosystem II-associated LHC (LHCII) isolated as detergent micelle from higher plants, it's revealed that $^3\text{Chl } a^*$ generated within LHC is quenched by two lutein molecules named Lut620, Lut621 (Fig.1A), however, detail and efficiency of $^3\text{Chl } a^*$ quenching in LHC in photosynthetic membrane hasn't been clarified yet. In this study, we aimed to reveal the $^3\text{Chl } a$ quenching for LHCII placed in lipid bilayer, by reconstituting isolated LHCII into lipid nanodisc. Lipid nanodisc is a discoidal lipid bilayer formed by self-assembly of lipid/protein/amphiphilic membrane scaffolding protein, and can disperse a single target protein in each disc (Fig.1B)[1]. Since this system can avoid the protein-protein interaction by randomly clustered proteins as often observed in liposome system, it's possible to obtain molecular photophysical properties of LHC in lipid interior.

2. Results and Discussion

LHCII isolated from spinach was reconstituted into a nanodisc with L- α -phosphatidylcholine and membrane scaffolding protein MSP1E3D1. The TEM image of prepared nanodisc (mnd-LHCII) showed a clear round shape with average diameter of 20 nm (Fig.1C). CD spectra revealed the structural difference between mnd-LHCII and conventional detergent micelle LHCII (DM-LHCII), indicating the interaction between LHCII and lipid bilayer in nanodisc. $^3\text{Chl } a^*$ quenching by two luteins was evaluated by ns-time resolved absorption spectroscopy, which revealed that contribution of lut620 for overall $^3\text{Chl } a^*$ quenching becomes higher in mnd-LHCII. Besides, conformational change induced by lipid-protein interaction enabled the further efficient $^3\text{Chl } a^*$ quenching by luteins, which resulted in 60% reduction of $^3\text{Chl } a^*$ left without being quenched (Fig.1D). Our result indicates that LHCII structure is well-designed to minimize harmful $^3\text{Chl } a^*$ and resulted photodamage when its located in the lipid membrane [2].

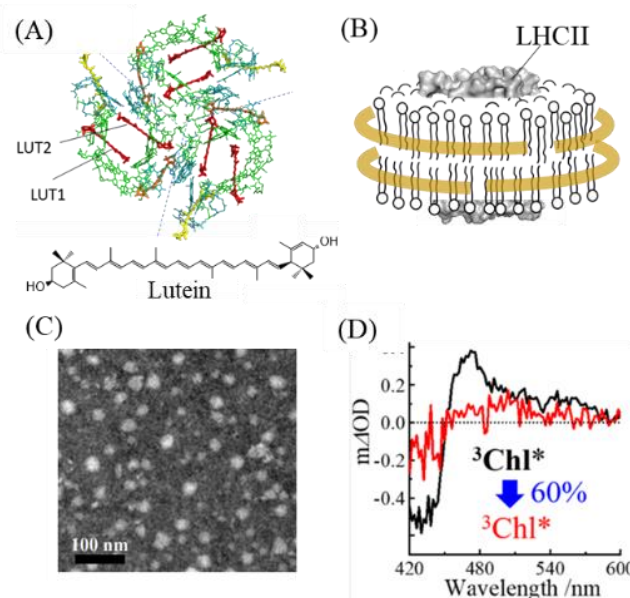


Figure 1. (A) X-ray crystal structure of LHCII (PDBID=2bhw) (B) Schematic picture of LHCII-nanodisc (C) TEM image of LHCII-nanodisc prepared with L- α -phosphatidylcholine and membrane scaffolding protein (D) Transient absorption spectra of $^3\text{Chl } a^*$ in LHCII micelle (black) and in nanodisc-LHCII (red), recorded by ns-time resolved absorption spectroscopy.

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Analysis of ICT state with fluorescence at low-temperature

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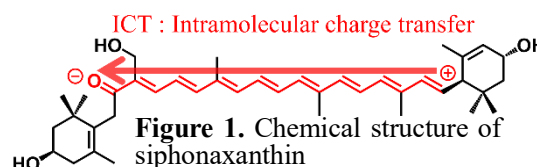
² Division of Molecular Materials Science, Graduate School of Science, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan.

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1. Introduction

The singlet energies of typical carotenoids can be expressed as S_0 ($1A_g^-$), S_1 ($2A_g^-$), and S_2 ($1B_u^+$). One-photon transition is symmetrically forbidden between the S_0 and S_1 states, and those carotenoids fluoresce little from the S_1 state. The S_1 state energy hardly depends on solvents due to the “covalent (-)” character of the state. Carbonyl carotenoids have conjugated carbonyl oxygen that induces intramolecular charge transfer (ICT) properties (Fig. 1) and are known to fluoresce from the S_1 state intense enough to be detected by a commercial fluorometer [1]. The ICT character was enhanced by solvent polarity and hydrogen bond formation. Enhanced ICT character result in stronger S_1 fluorescence and shorter S_1 lifetime. Here we measured low-temperature fluorescence spectra of a carbonyl carotenoid, siphonaxanthin (Sx), in a series of solvents to investigate the ICT properties of the S_1 state.



2. Experimental

Sx was totally synthesized as described [2]. Low temperature fluorescence spectra were measured in two non-polar and five polar solvents at 77 K using a commercial fluorometer. Excitation wavelength is 480 nm.

3. Results and Discussion

The 77K fluorescence spectrum of Sx in *n*-hexane shows clear vibrational structure of 0-0, 0-1, and 0-2 at 16,000, 14,600, and 13,600 cm^{-1} , respectively. Those energies are almost identical between in *n*-hexane and in methanol (Fig. 2). The matches in the vibrational energies were common to all solvent tested, including acetone, acetonitrile, toluene and decanol. This strongly suggested that the fluorescence 0-0 peak does not exhibit a solvent shift, which can be assigned to the S_1 to S_0 transition. Frank et al proposed that the solvent shift of the lifetime and spectral profiles can be explained as a vertical movement of the potential curve of the ICT state against that of the S_1 state [3]. However, our results suggest that the fluorescence is always from the S_1 state and not from the ICT state. A new model is needed to account for all investigations.

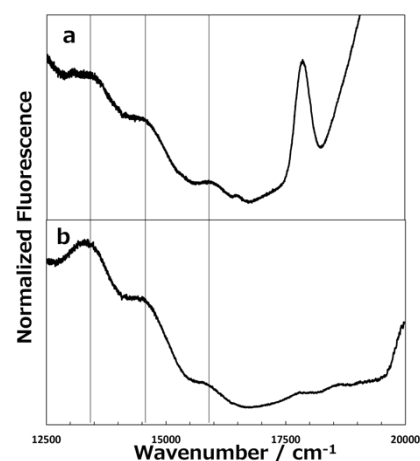


Figure 2. Fluorescence spectra of siphonaxanthin in hexane (a) and methanol (b) at 77 K.

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P5-1

Effects of Lycopene and β -Carotene on Cytokine Production and Secretion in Chronic Rhinosinusitis Without Nasal Polyps-Derived Nasal Fibroblasts: Differential Anti-Inflammatory Activities of Carotenoids in CRSsNP

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1. Introduction

Carotenoids such as carotene, lycopene and lutein are present in many foods and herbal medicines. Their structures are similar to vitamin A and retinoic acid. Humans-incapable of synthesizing carotenoids-obtain them by eating foods rich in these compounds. The predominant carotenoids found in human plasma are β -carotene, lycopene, and lutein, and their concentrations vary from 0 to 8 μ M, depending upon dietary intake [1]. Chronic rhinosinusitis (CRS), subdivided into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP), is a chronic disease affecting people of all ages and is one of the most prevalent chronic illnesses in the United States and countries worldwide. Given that dietary β -carotene prevents organ infection in vitamin A-deficient rats [2] and reduces ear infection in young children [3] and vitamin A deficiency compromises the mucosal barriers of several tissues [4], this study sought to determine the anti-inflammatory effects of two carotenoids, including lycopene and β -carotene, on CRSsNP-related stimuli-provoked CXC chemokine and cytokine production and secretion.

2. Experimental

Human CRSsNP nasal mucosa-derived fibroblasts (hNMDFs) were prepared from nasal mucosa tissues of CRSsNP patients. The ELISA was used to measure CXC chemokines, whereas the BDTM Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit was used to quantitatively and simultaneously determine interleukin-8 (IL-8), IL-1 β , IL-6, IL-10, tumor necrosis factor (TNF), and IL-12p70 protein levels in a single sample prepared from control and carotenoid-treated culture media of hNMDFs in challenge with various CRS-related proinflammatory mediators. The RT-PCR analysis was used to analyze mRNA expression level of the CXC chemokines in cell lysates.

3. Results and Discussion

In response to various CRS-related stimuli, including lipopolysaccharide (LPS), TNF- α , thrombin, U46619 (a thromboxane A2 analog), and bradykinin (BK), it was found that the CXC chemokine expression was induced but was differentially inhibited in the presence of lycopene and β -carotene in hNMDFs. The dexamethasone, a corticosteroid commonly used in the treatment of CRS, was used for comparison of their potencies. Among these, lycopene and β -carotene (both at 5 μ M) showed obvious and significant inhibitions on LPS-, thrombin-, and BK-induced CXC chemokine release. In parallel, the CBA assay also showed that a differential inducing effect on production of IL-8, IL-1 β , IL-6, IL-10, TNF, and IL-12p70 cytokines/chemokines in cultured medium of hNMDFs, which was also compromised in the presence of lycopene and β -carotene. However, it is surprising that lycopene and β -carotene did not affect CXC chemokine mRNA expression levels in an RT-PCR analysis. Taken together, we demonstrated here for the first time that lycopene and β -carotene are able to differentially attenuate production/secretion in CXC chemokines and some cytokines in human CRSsNP nasal mucosa-derived fibroblasts, possibly through a novel transcription-independent regulatory signaling pathway.

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P5-2

Anticancer effect of β -carotene in oxaliplatin-resistant colon cancer cell by regulating apoptosis and cancer stemness

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1. Introduction

β -Carotene (BC) is one of the carotenoids with antioxidant effect which can be found in vegetables and fruits. BC can act as a pro-vitamin A and has an ability to suppress tumorigenesis. Several epidemiological studies have shown that BC rich food consumption is related to a low risk of colorectal cancer (CRC) [1]. Oxaliplatin (OXA) is a diamine cyclohexane platinum, which commonly used in CRC chemotherapy. Platinum resistance is one of the main problems to delay cancer remedy [2]. Cancer stem cells (CSCs) are a sub-group of cancer cells with clonal tumor initiation features. Targeting CSCs to prevent cancer recurrence is important due to resistance against chemotherapy. In this study, the anticancer effect and mechanism of a combination of BC and OXA in various model systems, including CRC cell lines, OXA resistant colon cancer cells, and colon cancer organoid.

2. Experimental

To evaluate cell viability, we established OXA resistant HCT116 cells (see **Figure 1** for its IC₅₀ values). Levels of markers for apoptosis, CSC, and JAK/STAT signaling pathways in human CRC cells and OXA resistant cells were analyzed by western blot and RT-qPCR. FACS analysis was conducted to check cell cycle arrest in HCT116 cells and OXA resistant HCT116 cells. Human colon organoids were isolated and viability and differentiation markers were detected using MTT and immunofluorescence assay. Colony and sphere formation assays were performed to determine self-renewal capacity for CSCs.

3. Results and Discussion

A combination of BC and OXA induced cell cycle arrest in G₂/M phase and suppressed apoptosis in human CRC cells and OXA-resistant cells as shown in **Figure 2**. Although a combination of BC and OXA didn't affect normal organoids, it inhibited the growth of tumor organoids. Self-renewal ability and the expression of CSC markers were inhibited by a combination of BC and OXA in human CRC cells and OXA-resistant cells. In addition, BC can inhibit JAK/STAT signaling pathway-related markers. A combination of BC and OXA is more effective than individual treatment of BC or OXA in most experiments. In conclusion, BC improves the anticarcinogenic properties of OXA by regulating OXA resistance in CRC.

Table 1. IC₅₀ value of OXA and BC in three cell lines

Compounds	IC ₅₀		
	HCT116	HT29	HCT116/OXA
OXA (μ M)	1.57	2.62	4.15
BC (μ M)	32.51	32.70	31.52

BC, Beta carotene; OXA, Oxaliplatin; HCT116/OXA, Oxaliplatin resistant HCT116 cell line.

Figure 1. IC₅₀ values of OXA and BC in HCT116, HT29, and OXA resistant HCT116 cells.

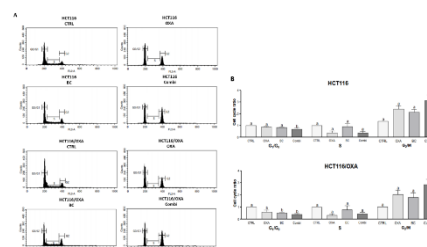


Figure 2. Cell cycle analysis of HCT116 and OXA resistant HCT116 cells after OXA and BC combination treatment.

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P5-3

An integrative transcriptomic analysis reveals the potential benefits of carotenoid for cancer therapy in female patients with colorectal cancer

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide. In 2020, it accounted for 10% of newly diagnosed cases, ranking third for men and second for women [1]. Comparing CRC incidence (2012-2016) and mortality (2013-2017) by gender in the U.S., a 31% higher incidence and 42% higher mortality rate was observed in males compared to females [2]. It has been reported that CRC prevalence and mortality are consistently higher in males than in females globally. Currently, numerous researches have been undertaken to distinguish differentially expressed genes, proteins, and metabolites as key factors contributing to sex differences in CRC. The present study integratively described a sex dimorphism in colon cancer patients using two separate GSE datasets, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

2. Experimental

Raw data of RNA sequencing (RNA-seq) of colon tissues was obtained from the open-source National Center for Biotechnology Information (NCBI) GEO database. RNA-seq data of normal human colon tissue was extracted from GSE66207 and GSE83687, whereas data of human colorectal tumor also including gender information was extracted from GSE80606 and GSE107422. To filter DEGs, an absolute value of log2 fold changes (FC) ≥ 1 and False Discovery Rate (FDR) ≤ 0.05 as the threshold identified both significantly upregulated and downregulated genes.

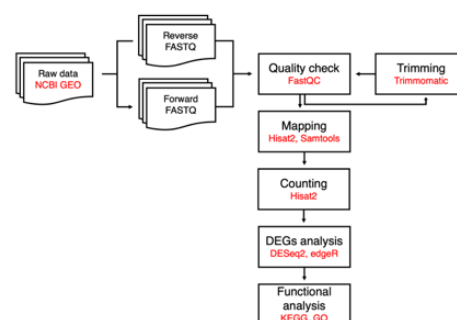


Figure 1 RNA-seq analysis pipeline for datasets extracted from NCBI database

3. Results and Discussion

We found that AKR1C3 aldo-keto reductase family 1 member C3 (AKR1C3) is downregulated in CRC tissue from female patients compared to male patients (GSE107422). RNA-seq demonstrated that female patients had enrichment of various fatty acids and hormone metabolic processes in the GO database. The AKR1C3 gene was incorporated in most enriched pathways which contain significantly downregulated genes in females. Above all, AKR1C3 is associated with the retinal/retinoid metabolic process and the doxorubicin metabolic process, suggesting its potential role in doxorubicin resistance in CRC. KEGG pathway analysis conjointly discovered that the PPAR signaling pathway (GSE107422) and the Wnt signaling pathway (GSE80606) are enriched respectively in downregulated and upregulated DEGs in female patients than male patients. Underlying molecular mechanisms of CRC need to be investigated and confirmed by further studies with carotenoid interventions, which provides the therapeutic potential of using various carotenoids, as retinal is a substrate of AKR1C3 [3]. The results suggest that the role of gender needs to be taken into account when treating patients with CRC.

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Effects of beta-carotene supplementation alone and in combination with metformin on tissue lipidomic profiling after high-fat diet feeding in mice

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1. Introduction

The combination of pharma with food bioactives is an attractive strategy in obesity management. We showed that mild treatments with beta-carotene (BC), metformin (MET), and their combination (BC+MET) can oppose the development of high-fat diet (HFD)-induced obesity (DIO) in mice [1]. The treatments including BC provided additional benefits regarding glucose control, insulin sensitivity, a more active BAT appearance, and a lower liver lipid content. The combined BC+MET treatment was the most effective counteracting DIO and hyperglycemia, and it uniquely potentiated hyperplastic over hypertrophic WAT expansion on the HFD, which may represent a healthier form of fat expansion. Lipid metabolites are involved in the pathology of obesity and tissue lipidomics change in response to an HFD. Using our previous study design, we here assessed the impact of BC supplementation alone and combined with MET on the lipidomic responses to HFD feeding in key homeostatic tissues - namely the liver, brown adipose tissue (BAT), and white adipose tissue (WAT).

2. Experimental

C57BL/6J male mice were challenged with an HFD (45% energy as fat) for 4 weeks while orally receiving a placebo (control HF) or BC (3 mg/kg/day), MET (100 mg/kg/day), or their combination (BC+MET); a fifth group received placebo and was fed a normal-fat diet (control NF). Metabolomics analyses were conducted in samples of 6-7 animals per group. Semi-polar metabolites were extracted from 10 mg of lyophilized tissue using 75% (v/v) methanol, 0.1% (v/v) formic acid, spiked with formononetin as internal standard, and analyzed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-HRMS). Non-polar metabolites were extracted from 5 mg of lyophilized tissue using methanol:chloroform, spiked with DL- α -tocopherol acetate as internal standard, and analyzed by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-HRMS). For each biological replicate, extractions were performed in triplicate. Full scan MS with data-dependent MS/MS was used for metabolite identification, prior to annotation pipeline (by using authentic standards, in house and public databases and mass fragmentation). Metabolites were quantified relatively by normalization on the internal standard amount.

3. Results and Discussion

A total of 118 lipid metabolites (LMs) were detected in the liver, 113 in BAT and WAT, and 98 in serum. Of them, 45 LMs (38%) were differentially accumulated between at least two of the experimental groups in the liver, 52 (46%) in BAT, 28 (25%) in WAT, and 7 (7%) in serum. The liver lipidome was similarly changed in the 4 groups on the HFD as compared with the NF group. In BAT, the HF group displayed increased relative levels of many LMs including triacylglycerol (TAG) species, phosphatidylcholine (PC) species, arachidonic acid and other PUFA metabolites, and a cholesterol metabolite (cholestanetriol), which could reflect BAT whitening and a pro-inflammatory environment compared with the NF group. For several of these LMs, the HFD-induced increase in BAT was attenuated by BC treatment and, especially, the combined BC+MET treatment. Strikingly, retinyl palmitate levels in BAT were increased in the animals receiving BC alone but decreased in the BC+MET mice, which also presented decreased levels of certain other carotenoid catabolites as compared with the BC animals. In WAT, the combined BC+MET treatment led to decreased levels of fourteen TAGs, three PCs, and six PUFA metabolites relative to levels in the HF or, especially, the MET group: thus, MET treatment appeared to enhance PUFA metabolism and incorporation into TAGs in WAT of mice on an HFD, and BC cotreatment opposed this effect. The profile of some BCO₂ metabolites was divergent between the MET group and the BC & BC+MET groups. Overall, the metabolomic analyses revealed changes that seem to be in good concordance with the biometric and histological data and may suggest interactions between MET and enzymes in carotenoid metabolism.

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Suppressive effects of fucoxanthin derived from a diatom, *Chaetoceros sp.*, on angiogenesis

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1.Objectives

Angiogenesis, formation of new blood vessels, is essential for growing solid cancer, which is one of angiogenic diseases, to get the nutrients. Angiogenesis is also crucial for other angiogenic diseases including diabetic retinopathy and macular degeneration. For treatment of these diseases, antiangiogenic agents are necessary, however, at present, only anti-vascular endothelial cell growth factor (VEGF) antibody and its related substances are clinically used. Since these agents sometimes cause specific side effects, other safer functional angiogenesis inhibitors have been sought for application in therapies for angiogenic diseases. Although it is reported that fucoxanthin derived from brown alga, *Undaria Pinnatifida*, suppresses angiogenesis in vitro and ex vivo, it is not clarified whether this compound inhibits angiogenesis in vivo. Biological functions of fucoxanthin produced by diatoms are also unknown. Therefore, we studied the effect of newly purified fucoxanthin derived from a diatom, *Chaetoceros sp.*, on angiogenesis of chicken embryo as an in vivo model.

2.Methods

We examined the effect of fucoxanthin purified from *Chaetoceros sp.* on angiogenesis, using chicken chorioallantoic membrane (CAM) presenting the in vivo vascular network. In addition, we evaluated the effect of this fucoxanthin on migration and tube formation, two important steps of angiogenic process, using bovine pulmonary microvascular endothelial cells.

3.Results

Fucoxanthin derived from *Chaetoceros sp.* exhibited the suppressive effect on angiogenesis of CAM and, also the inhibitory effect for both migration and tube formation by microvascular endothelial cells.

4.Conclusion

These results suggest that fucoxanthin derived from *Chaetoceros sp.* can be a source of agents for antiangiogenic therapy.

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P5-6

Anti-cancer effects of low-intensity exercise and genistein supplementation on high-fat diet stimulated breast cancer

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1. Introduction

An inactive lifestyle and high-fat diet (HFD) are well-known risk factors for breast cancer. Genistein, an isoflavone, is rich in soybeans which is structurally and functionally similar to the female hormone, estrogen (**Figure 1**). Genistein supplementation has been shown to exhibit anti-estrogenic effects and to lead to anti-cancer activity [1]. Regular physical activity causes muscles to contract and relax, secrete myokines which is known to have anti-cancer effects. The object of the present study is to investigate the effect of the combined treatment of physical activity and genistein on HFD-induced breast cancer growth and the possible mechanisms involved.

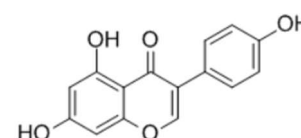


Figure 1. Chemical structure of genistein

2. Experimental

Five-week-old female Balb/c mice were randomized to five groups (n=10 per group) as follows: 1) control (CTRL), 2) HFD (H), 3) HFD + genistein (HG), 4) HFD + exercise (HE), and 5) HFD + genistein + exercise (HGE). The mice were fed AIN-93G diet (CTRL) or 60% high fat diet (HFD). Oil in the diets was changed from soybean oil to corn oil to prevent genistein contamination. In addition, genistein supplementation group received 200mg/kg of genistein 5 times a week through oral gavage. After 8 weeks, 4T1 mouse mammary tumor cells (1×10^4) were injected into the mammary fat pads. Low intensity exercising training was performed 5 times a week for the exercise group after 1 hr of genistein supplementation. The exercising training program consisted of warm-up exercise for 5m/min for 10 min, main exercise for 13m/min for 30 min, and cool-down exercise for 5m/min for 10 min. Tumor volume was measured twice a week by using a digital caliper. Tumor tissues were stained with hematoxylin and eosin (H&E). For cell division and apoptosis, levels of PCNA, bcl-2 and cleaved-caspase-3 were analyzed by RT-PCR and western blot assay.

3. Results and Discussion

Low-intensity exercise and genistein supplementation showed significantly effects on delaying breast cancer onset and reducing tumor volume. Tumor growth was inhibited in the HE and the HGE group compared with the H group or HG group (**Figure 2**). Compared to the rest of the groups, only the H group showed significantly lower adipose tissue weight and tended to exert lower muscle weight. However, steady low-intensity exercise and genistein supplementation showed preventive effects on the loss of adipose tissue associated with cancer cachexia. The results suggested that combined exercise and genistein supplementation delayed breast cancer growth and suppressed cancer cachexia-induced loss of adipose tissue. In conclusion, low-intensity exercise and genistein supplementation may benefit to prevent and treat the breast cancer.

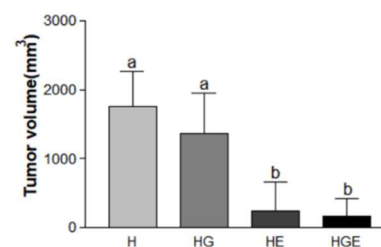


Figure 2. Effects of exercise and genistein on tumor volume in 4T1 breast cancer mice

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Evaluation of inhibitory effect of halocynthiaxanthin on differentiation into pro-inflammatory macrophages and its gastrointestinal absorption

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1. Introduction

Halocynthiaxanthin is one of marine carotenoids found in sea squirt (*Halocynthia roretzi*), and it has been known as a metabolite of fucoxanthin. In Japan, *H. roretzi* has been traditionally eaten, especially in the Northeast region. Halocynthiaxanthin have been reported to have a few bioactivities and could be expected to exert bioactivity due to its unique chemical structure, compared to other carotenoids (**Figure 1**). However, mechanisms of absorption and metabolism of halocynthiaxanthin in mammals have not been studied yet. In this study, we evaluated effects of halocynthiaxanthin on the macrophage differentiation to the pro-inflammatory phenotype, and its gastrointestinal absorption in mice to examine its biological processes.

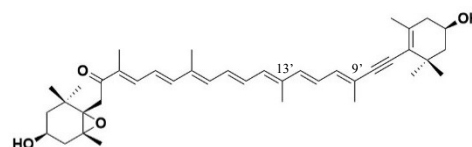


Figure 1. Chemical structure of all-*trans*-halocynthiaxanthin

2. Experiment

THP-1 cells (human monocytic leukemia cell line) were treated with phorbol-ester for 24 h and rested with fresh medium for another 24 h, for differentiation from monocyte to macrophage-like cells. After treatment with carotenoids at a concentration of 5 μ M for 24 h, differentiation into the pro-inflammatory phenotype was induced by IFN- γ and LPS for 6 h. The expression pro-inflammatory genes (i.e., CD14, TNF- α , MCP-1, IL-8, COX-2) was evaluated by qRT-PCR. A xanthophyll fraction rich in halocynthiaxanthin (88%) was extracted and purified from *H. roretzi*. Mice were orally administrated the xanthophyll fraction diluted with olive oil at the dose of 5 mg halocynthiaxanthin / kg body weight. The concentration of halocynthiaxanthin in plasma, liver and intestinal contents 1, 3, 6, 9 h after ingestion were quantified by HPLC-PDA.

3. Results and Discussion

Halocynthiaxanthin significantly suppressed the mRNA expression of CD14, TNF- α , MCP1, and COX-2 as well as other carotenoids with anti-inflammatory effects (i.e., fucoxanthin and siphonaxanthin) used for comparison. Therefore, halocynthiaxanthin may have a potential to prevent inflammation by suppressing the differentiation from macrophage-like cells into pro-inflammatory macrophages. The halocynthiaxanthin-rich xanthophyll fraction used in the animal study consisted mainly of the all-*trans* isomer ($\geq 94\%$), followed by the 13'-*cis* isomer and the 9'-*cis* isomer. After ingestion of the xanthophyll fraction, more *cis* isomers were found than the *trans*-isomer in mice plasma and liver. Plasma concentrations of all isomers increased until 6 h after administration. It is noteworthy that the 9'-*cis* halocynthiaxanthin became the major isomer accounting for more than 50% of total halocynthiaxanthin after 6 h, followed by the all-*trans* and the 13'-*cis* isomers. The isomer ratio in the liver also showed a similar tendency to plasma. Contrary to the trends in plasma and liver, all-*trans* halocynthiaxanthin was the primary isomer detected in small intestinal contents. Therefore, our results suggest that dietary all-*trans* halocynthiaxanthin might be transformed into *cis* isomers after intestinal absorption or that *cis* isomers might be absorbed rapidly from a small intestine.

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Buckwheat calli with different morphogenic potential as a unique system to study levels of both carotenoids and phenolic compounds

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1. Introduction

The morphogenic calli (MCs) of Tartary buckwheat consist of proembryogenic cell complexes (PECCs) and a 'soft' callus, the latter of which appears during the cyclical callus development and the disintegration of the PECCs. The 'soft' callus cells (SCCs) are formed during the loosening of the PECCs and primarily consist of elongated cells that are highly vacuolated. It has been shown that the calli of common buckwheat, which have a spherical structure, have the highest morphogenetic capacity [1]. According to the histological data, this type of callus consists of parenchymatous cells covered with several layers of meristematic cells [2]. The morphogenic calli (MCs) of common and Tartary buckwheat can be induced from immature embryos [2, 3]. Recent studies have shown that the hypocotyls can also induce the MCs of common and Tartary buckwheat. The data about the carotenoids and phenolics levels in calli with different morphogenic potential and their influence on somatic embryogenesis induction are absent in the literature.

2. Experimental

In this study, we used the calli with different morphogenic potential induced from the zygotic embryo of both common and Tartary buckwheat. We evaluated the levels and location of carotenoids and phenolics using the staining with dyes (DMCA, vanillin-HCL etc.) and spectrophotometry. The evaluation was performed during the PECCs production and somatic embryo induction.

3. Results and Discussion

Tartary buckwheat *in vitro* tissue culture of the morphogenic callus is characterised by low chromosome variability and the capacity for morphogenesis for up to ten years of culture, displaying an extraordinary level of stability. On the contrary, the non-morphogenic callus appears on the surface of the morphogenic callus after approximately two years of culture due to endoreduplication cycles. Non-morphogenic callus is characterised by aneuploidy, rapid growth rate and high hydrogen peroxide content, indicating that this tissue is in a constant state of oxidative stress. Common buckwheat embryogenic callus displays different morphological and histological features, remains stable for up to three years of culture, has a dense, globular structure, and can form embryoids but does not produce a non-embryogenic clone. We demonstrated that levels of both carotenoids and phenolics compounds fluctuated between the analysed calli. Tartary buckwheat non-morphogenic calli characterised the lowest levels of both components. The highest presence of phenolic compound was demonstrated for the surface of PECCs.

To summarise, understanding the morphological, physiological and molecular parameters that affect the levels of both carotenoids and phenolic compounds during the PECCs development and somatic embryos induction of the buckwheat plants will not only be relevant for revealing the complex mechanisms of these processes but also may have an impact on successfully modulating yield productivity and yield stability for future buckwheat breeding programmes.

The International Atomic Energy Agency supports this study under the framework of RER5024.

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Interaction of dietary lycopene with the ISX/SRB1 axis

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1. Introduction

It has been hypothesized that lycopene (or its metabolites) may have retinoid-like actions on retinoic acid receptors (RARs) and/or other nuclear receptors and influence gene expression of various downstream pathways. One of these pathways regulated through RARs is the intestinal specific homeobox (ISX) and scavenger receptor type 1 class B (SRB1) axis involved in the regulation of vitamin A levels in the body. ISX is a RAR-responsive gene that inhibits SRB1 and β -carotene oxygenase 1 (BCO1) expression. The objective of this study was to examine whether dietary lycopene influences intestinal gene expression of ISX, SRB1 and/or BCO1 and whether SRB1 expression is associated with the absorption and tissue accumulation of lycopene in mice.

2. Experimental

We utilized wild-type (WT) male and female mice (n=6-8/group). After weaning (3 weeks of age), mice were fed with chow diet for one week and switched to a vitamin A deficient AIN-93G diet supplemented with either 100 mg of lycopene/kg diet (VAD) or the same diet without lycopene (VAD vehicle). A third subset of mice received an AIN-93G vitamin A sufficient diet (4 IU/kg) supplemented with 100 mg of lycopene/kg diet (VAS). After four weeks on these diets, mice were fasted overnight and sacrificed for tissue and blood collection for HPLC and RT-PCR analyses to quantify lycopene levels and gene expression, respectively.

3. Results and Discussion

Hepatic lycopene level was ~2-fold higher in VAD mice compared to VAS mice ($p < 0.05$), while there were no differences in plasma levels between these groups. For gene expression in the duodenum, jejunum, and ileum, ISX was significantly upregulated in mice fed a VAD or VAS diet compared to mice fed a VAD vehicle diet. In regard to the target genes of ISX: SRB1 and BCO1, only mice fed a VAS diet presented with lower SRB1 and BCO1 expression compared to mice fed a VAD vehicle diet ($p = 0.02$ and $p < 0.001$, respectively). In conclusion, dietary lycopene (or its metabolites) appears to have retinoid-like activity and impact RAR receptor-mediated influence on the ISX/SRB1 axis.

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Dehydrogenation increases anti-inflammatory activity of siphonaxanthin

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1. Introduction

Siphonaxanthin is a carotenoid of specific green algae (e.g., *Codium fragile* and *Caulerpa lentillifera*). According to our previous studies, siphonaxanthin showed the most potent anti-inflammatory activities among carotenoids we tested [1, 2]. Anti-inflammatory activity of carotenoids is reported to be increased by dehydrogenation [3], and hence, we hypothesized that dehydrometabolites of siphonaxanthin would show more potent anti-inflammatory activities. In this study, we attempted to elucidate dehydrogenation of siphonaxanthin by liver S9 fraction and to evaluate anti-inflammatory activity of the dehydrometabolites of siphonaxanthin. Furthermore, we tried to reveal molecular mechanisms underlying their anti-inflammatory effect.

2. Experimental

We used rodent S9 fractions as an enzyme source to obtain dehydrometabolites of siphonaxanthin. Identification of the isolated metabolites was conducted by nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry analyses. Anti-inflammatory activities of the carotenoids were evaluated by measuring transcriptional activities of NF- κ B and interferon response factor (IRF).

3. Results and Discussion

Rat liver S9 fraction produced didehydro-siphonaxanthin (DDS), and mouse liver S9 fraction generated didehydro-metabolite (DDM) and tetrahydro-metabolite (TDM). These conversions would occur enzymatically, and NAD⁺ was the preferred cofactor over NADP⁺. Both microsome and cytosol fractions of rodent livers produced the dehydrometabolites, and the activities of microsome were higher than those of cytosol. NAD⁺-dependent microsomal enzymes would be responsible for the dehydrogenation of siphonaxanthin. All of the three dehydrometabolites exhibited inhibitory effects on triacyl lipopeptide-induced IRF activation. Their inhibitory activities were higher than that of siphonaxanthin and canceled in the presence of inhibitors of nuclear factor erythroid 2-related factor 2 (Nrf2). In conclusion, the NAD⁺-dependent dehydrogenation of siphonaxanthin increases its anti-inflammatory activity derived from Nrf2 activation.

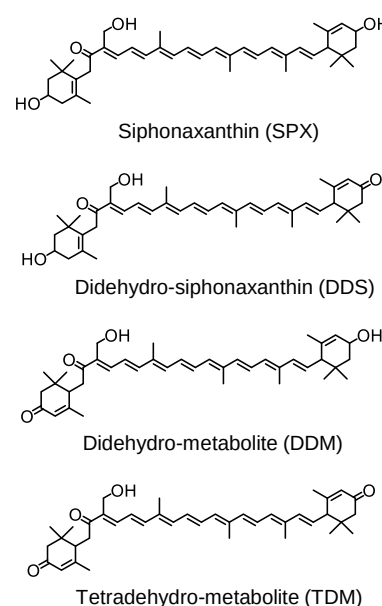


Figure 1. Chemical structures of siphonaxanthin and its metabolites

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Screening of structurally distinct lycopene β -cyclases for cyclic C40 carotenoid production of β -carotene and astaxanthin in *Corynebacterium glutamicum*

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1. Introduction

Lycopene β -cyclase (EC 5.5.1.19) is one of the key enzymes in the biosynthesis of β -carotene and derived carotenoids (**Figure 1**). It catalyzes an intracellular lyase reaction to form β -carotene from lycopene involving β -cyclization of both ends of lycopene. As carotenoids are hydrophobic molecules, they are usually located within the plasma membrane. Lycopene β -cyclases are widespread in nature. The soil bacterium *C. glutamicum* is a natural carotenoid producer, and thus has high potential in industrial strain development [1]. We systematically analyzed the phylogeny and transmembrane structures of lycopene β -cyclases from all kingdoms of life by bioinformatics approaches. Representative lycopene β -cyclases from four structurally different types (**Figure 2**) were screened in *C. glutamicum* for β -carotene and astaxanthin production.

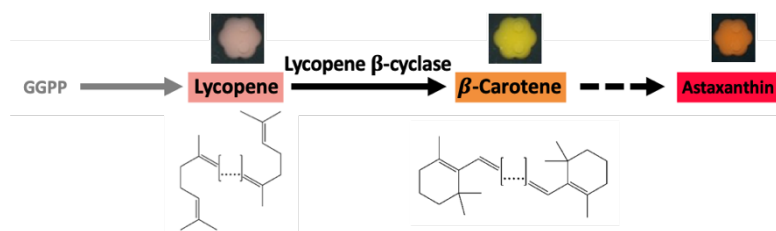


Figure 1. Reaction of lycopene β -cyclase from lycopene to β -carotene

2. Experimental

A collection of previously characterized lycopene β -cyclase polypeptide sequences was generated to identify the closest homologs of each of these bait sequences in a range of bacteria, archaea, fungi, algae and plant species with a previously developed Phyton script. Furthermore, a DeepTMHMM scan was applied to search for the presence of transmembrane domains. Nine enzymes from different phylogenetic lineages and with different transmembrane structure were chosen, expressed in *C. glutamicum* and carotenoids were quantified by HPLC.

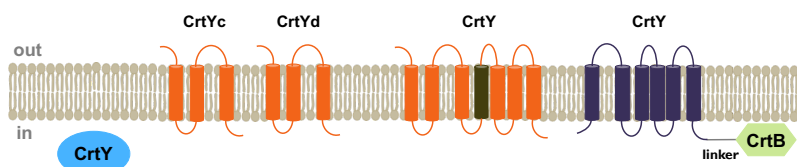


Figure 2. Four structurally different groups of lycopene β -cyclases

3. Results and Discussion

The resulting phylogenetic tree showed five distinct clusters and the DeepTMHMM scan revealed that lycopene β -cyclases can be organized in four structurally different groups distinguishing membrane-bound and cytosolic enzymes. Using the selected lycopene β -cyclases in our carotenoid producing strains [2] showed that this systematic screening facilitates the identification of new enzymes for carotenoid productions besides the commonly used ones. Higher astaxanthin production and less feedback inhibition on total carotenoid production was achieved with CrtL from *Synechococcus elongatus* and CrtYcd from *Brevibacterium linens* [3]. There were indications of different feedback-inhibition mechanisms between the candidates.

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Exploring the impact of dietary fibers on carotenoid bioaccessibility and bioavailability

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Introduction

Carotenoids form an important class of secondary plant metabolites in the human diet and their intake has been associated with positive health effects. In order to map their complete digestive fate in the body it is essential to take into account other dietary factors that form the bulk of human diet. It was shown that dietary lipids can improve bioavailability (the fraction of a nutrient that can be absorbed and used for physiological function). Additionally proteins could, depending on their solubility /digestibility, exert positive or negative effects on carotenoid absorption. Similarly, dietary fiber (DF) has also been linked to a potential reduction in carotenoid bioavailability, but further research is essential given the wide range of physiochemical characteristics, solubility, and fermentability of DF, factors that have to some degree been neglected thus far but which may impinge on carotenoid bioaccessibility and also on the fate of carotenoids in the colon.

Project Objectives

The primary research hypothesis is that DF would negatively modulate carotenoid bioaccessibility, cellular uptake and bioavailability, as determined by *in vitro*-digestion experiments/Caco-2 epithelial cell models and human bioavailability (triacylglycerol-rich lipoprotein fraction), but that prebiotic like fibers would exert less if any negative effects on the above parameters. The secondary hypothesis would be that carotenoids continue to be potentially available for absorption in the colon, but that this availability would likewise be a function of the type of DF, with soluble and highly fermentable DF (e.g. fructooligosaccharides) having a higher potential colonic bioaccessibility vs. poorly fermentable DF (e.g. cellulose), which may obstruct carotenoid release. Additionally through this research we also aim to elucidate carotenoid degradation, metabolites and whether they possess gut microbiota altering properties alone or in combination with dietary fibers.

Methodology

In order to systematically study interactions between carotenoids and dietary fiber, as well as additional factors such as co-consumed proteins, specific carotenoids (alone and in complex food matrices) will be gastro-intestinally (GI) digested together with various types of added individual DFs to study the impact on bioaccessibility and recovery of carotenoids, also investigating physico-chemical properties of the digesta such as viscosity. The next step involves an additional fermentation of samples GI- digested to study carotenoid degradation and also combined effects of carotenoids and DF on gut microbiota, employing batch fermentation and the SHIME model. LC-MS/MS will be employed to look for carotenoid breakdown products such as apocarotenoids. This will be followed by studying bioavailability of carotenoids with selected types of DF in a human randomized cross-over trial.

Expected Outcomes and Impact

With a particular focus on DF, the study aims to reveal differential aspects of DF on carotenoid bioavailability, as well as to bridge a knowledge gap existing for the colonic fate of carotenoids in relation to gut microbiota. Data on DF and carotenoid combinations will be of interest for the food/nutraceutical industry to formulate supplements and blends containing both DF and carotenoids, particularly prebiotics. Furthermore, since a significant portion of vegetarians and vegans, as well as in many under-developed areas of the world rely on carotenoids as their primary source of vitamin A, it could also be paramount to obtain an in-depth understanding of how different types of DF interact with carotenoids, thereby aiding to meet nutritional needs. The findings of this project will stimulate future projects in the area of carotenoids and health research.

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Peach palm fruit (*Bactris gasipaes*) peel as a source of Provitamin A carotenoids

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1. Introduction

Peach palm (*Bactris gasipaes*) mesocarp is traditionally cooked for consumption in South West regions of Colombia and its peel is usually discarded. However, this by-product is a source of carotenoid pigments that can be used as natural pigment or Provitamin A source. The flour obtained by drying fruit peels has been characterized as a raw material with high fiber and protein contents[1]. β -Ionone ring is required to provide provitamin A activity, thus, α - and β -carotene, and β -cryptoxanthin are considered carotenoids with high provitamin A activity. The aim of this work was to chemically characterize the provitamin A carotenoid content in the peel flours of two varieties of *B. gasipaes* from Colombia.

2. Experimental

Ripe peach palm fruits (*B. gasipaes*) were purchased in local markets of Palmira (Valle del Cauca, Colombia) and selected according to their ripeness characteristics. Two varieties were used, red (RV) and yellow variety (YV) which are widely consumed. The fruit peels were dehydrated in a convection oven (Binder ED 53 UL, GmbH) at $60 \pm 2^\circ\text{C}$ to obtain a moisture value close to 10-11%, and the dehydrated samples were crushed in an electric mill to a particle size ≤ 0.25 mm. Carotenoid extracts of RV and YV samples were obtained following the methodology of Anunciação *et al.* [2]. Then, the chemical composition was determined by HPLC-MS analysis, using a YMC C₃₀ column, an APCI interface operated in positive and negative modes. Provitamin A was expressed as Retinol Equivalent Activity (RAE).

3. Results and Discussion

The total carotenoid content (RV = 836.5 $\mu\text{g/g}$ DW; YV = 75.0 $\mu\text{g/g}$ DW; CV was below 9% in all measurements) and Provitamin A activity (RV = 33.1 $\mu\text{g RAE/g}$ DW; YV = 3.9 $\mu\text{g RAE/g}$ DW) were higher in red variety than in yellow variety. The main Provitamin A carotenoids in both varieties were: β -carotene, γ -carotene, Z - γ -carotene, 13-*cis*- β -carotene, δ -carotene, 9-*Z*- β -carotene, and α -carotene. Lycopene was also detected but it does not contribute to the provitamin A activity. The drying of *B. gasipaes* peel allows for a food additive with agro-industrial potential to reduce the environmental impact, which could be incorporate to fortify different foods.

Acknowledgment: The ANLA and Ministerio de Ambiente y Desarrollo Sostenible granted permission to collect samples (Contrato Marco de Acceso de Recursos Genéticos y sus Productos Derivados No. 357 del 17 de noviembre de 2022 suscrito entre el Ministerio de Ambiente y Desarrollo Sostenible y la Universidad Nacional de Colombia). Authors greatly acknowledge the financial support provided by Universidad Nacional de Colombia-Sede Palmira y Universidad del Valle-Sede Palmira, the Shimadzu Corporation and Merck Life Science.

[1] J. Martínez-Girón, C. Osorio, L. E. Ordoñez-Santos, *Food Sci. Technol Int.*, **2021**, 28, 535-544. [2] C. P. Anunciação, D. Giuffrida, D. C. Murador, G. X. de Paula Fihlho, G. Dugo, H. M. Pinheiro-Sant'Ana. *J. Food. Comp. Anal.* **2019**, 83, 103296.

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P7-2

Major Carotenoids of *Meiothermus ruber* Are Deinoxanthin Glucoside Esters, Not Meiothermoxanthin Glucoside Esters

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Meiothermus ruber DSMZ 1279^T was isolated from a hot spring in Kamchatka and was red in color. The major carotenoid present was reported to be 1'-(β-D-glucopyranosyloxy)-3,4,3',4'-tetrahydro-1',2'-dihydro-β,ψ-caroten-2-one after saponification [1]. In this study, we purified the major carotenoids in this species without saponification. We then reidentified the major carotenoids present using spectroscopic data, including CD, ¹H NMR, ROESY, ¹³C NMR, HSQC, HMBC and MS, and enzymatic hydrolysis of fatty acid moieties, and found deinoxanthin glucoside iso fatty acid esters (Fig. 1). The bound fatty acids present included four iso types, and their composition differed from cellular lipids. Moreover, the previously identified carotenoid glucoside was a saponification artifact of deinoxanthin glucoside esters. Ketomyxocoxanthin glucoside esters and 1'-hydroxytorulene glucoside esters were also present. Based on the identification of carotenoids and the whole genome sequence of *M. ruber*, we propose a carotenoid biosynthetic pathway and note the corresponding genes [2].

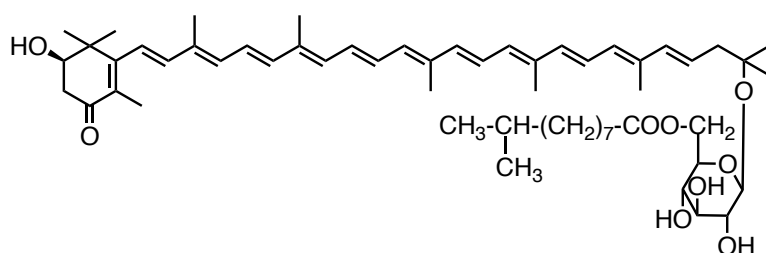


Figure 1. Chemical structure of deinoxanthin glucoside iso fatty acid esters

[1] M.L. Burgess, K.D. Barrow, C. Gao, G.M. Heard, D. Glenn, *J. Nat. Prod.* **1999**, 62, 859-863

[2] R. Asaka, K. Ohshima, S. Kawasaki, T. Maoka, C. Tode, Z.-Y. Wang-Otomo, S. Takaichi, *J. Nat. Prod.* **2022**, 85, 2266-2273.

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Identification Protocol of Carotenoids

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Eight criteria for identification of carotenoids have been recommended by Prof. S. Liaaen-Jensen (1995). I also summarize some revisions to general identification procedures as follows [1].

1. Identification of chromophore (conjugated C=C and C=O double bond system)
Wavelength of absorption maximum shows the number of conjugated C=C double bonds, and a broad absorption spectrum indicates the presence of conjugated C=O group using HPLC-PDA.
2. Estimation of polar group(s) from C₁₈-HPLC retention time
More polar carotenoids are eluted earlier, and non-polar carotenes are eluted later. Compare to known carotenoids, the polar groups (OH, C=O, epoxy, methoxy, etc.) can be estimated.
3. Molecular masses from mass spectra
Composition of C, H and O can be determined, that is, the number of polar groups. Some articles have missed to select molecular ion, and therefore miss identification due to have too much confidence in the database of MS.
4. Structure elucidation from ¹H-NMR and ¹³C-NMR spectra
NMR spectra finally elucidate the chemical structures of carotenoids.
5. Chirality elucidation from circular dichroism (CD) spectra
When the carotenoid has chiral structure, CD or chiral column analysis are essential.
6. “Carotenoids Handbook”
This book contains more than 700 carotenoids and is useful for checking the identity of any newly isolated carotenoids.
7. Compare with phylogenetic systematics of the species
In general, natural compounds and their metabolic pathways are strongly related to phylogenetic systematics of the organisms. Searching among closely related species.
8. Carotenogenic genes from genome DNA sequences
After a homologous search for the carotenogenic genes in the genome DNA sequences, the products of carotenoids can be predicted. This is a new approach.

Especially, absorption spectra, elution order on C₁₈-HPLC, and mass spectra are essential, and these are important data for identification of carotenoids. Some wrong identifications of carotenoids are found based on insufficient data and their interpretation, especially mass spectra.

[1] S. Takaichi (2020) Carotenoids in Phototrophic Microalgae: Distribution and Biosynthesis, In “Pigments from Microalgae Handbook (E. Jacob-Lopes et al. eds.)

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Hydrogen bonding and nonpolar environment applications to some rare carotenoids

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1. Introduction

Some rare carotenoids that have not been studied or have been less studied than the traditional carotenoids, contain more oxygen-containing functional groups such as hydroxyl, keto, ester and ether groups [1]. Besides the coordination bonds formed with a host molecule, and depending on the structure of the host molecule, these functional groups allow hydrogen bonding to occur on the surface of the host (**Figure 1**), or not occur. Depending on the functional groups present, carotenoids can act as either H-donor or H-acceptor, or as both. However, the surface of a host molecule can also be modified so that a carotenoid can act as only a H-bond donor, or as a H-bond acceptor.

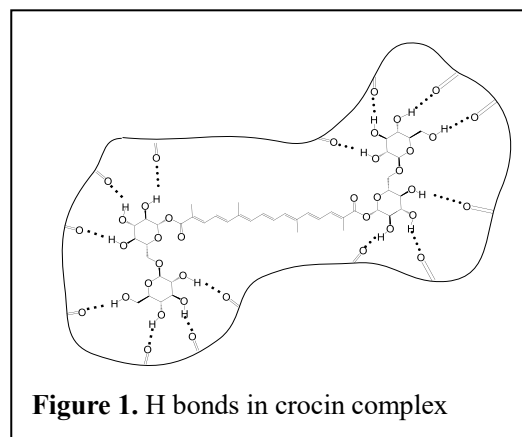


Figure 1. H bonds in crocin complex

2. Results and Discussion

Controlling H bonding is extremely important because we have found that H bonding affects the photostability of carotenoids. The photostability is related to the radical cation produced during photoirradiation. If the carotenoid is a H-bond donor, the formation of the H-bond increases the energy of the LUMO of the carotenoid which stabilizes the radical cation more than the neutral species, favoring photoinduced electron transfer and lowering photostability. The opposite is true if the carotenoid acts as H-bond acceptor.

The environment around the carotenoid also affects photostability. The photostability of a carotenoid is the highest if the environment of the carotenoid is nonpolar and no hydrogen bonds are formed. Our studies show that incorporation of carotenoids into nonpolar environment results not only in increasing of the oxidation potential but also in increased photostability, in the enhancement of their chemical stability. Thus, host molecules can be modified to decrease their polarity which in turn can increase the antioxidant activity of the carotenoid [1-3].

The new findings presented here give ways to improve the stability, oxidation potentials and antioxidant activity of the carotenoids, and to control the photooxidation efficiency of the carotenoids.

[1] N. E. Polyakov, A. L. Focsan, Y. Gao, L. D. Kispert, The endless world of carotenoids – insight from EPR and DFT studies, submitted to *Int. J. Mol. Sci.*

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[3] Y. Gao, A. L. Focsan, L. D., Kispert, Antioxidant activity in supramolecular carotenoid complexes favored by nonpolar environment and disfavored by hydrogen bonding. *Antioxidants* **2020**, 9, 625.

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Structural elucidation of β -carotene oxygen copolymers by complementary analytical methods

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1. Introduction

The degradation of carotenoids by oxidation is well described, however little is known about oxidatively formed polymers. Under ambient oxygen condition, β -carotene predominantly copolymerizes to an oxygen-rich mixture of compounds, termed OxBC, with an empirical formula of $C_{40}H_{60}O_{15}$ (compared to $C_{40}H_{56}$ for β -carotene). It mainly consists of oxygen-copolymers of different degrees as well as minor, low molecular weight norisoprenoids. Interestingly, these compounds show immunological activities, that are not related to vitamin A products or antioxidative behavior of large conjugated double bond systems such as in its precursor β -carotene [1,2]. The question arises whether the activity derives from polymeric or monomeric compounds as well as to which degree the latter are formed simultaneously during polymerization and/or are released from the polymer via further oxidation processes. Since this class of compounds has only recently been found, not many insights on its structural composition have been gained. Even though different spectroscopic approaches, LC- and GC-MS as well as elemental analysis have already given better understanding of the structural composition of OxBC, further research is necessary to fully understand the structure and relevance of this newly discovered class of compounds. Polymers have not only been synthesized but were also found in fresh and processed food [1,3,4]. We produced OxBC under atmospheric conditions to further elucidate the composition by investigating the polymeric compound as well as the released degradation products.

2. Experimental

In order to obtain OxBC, we incubated crystalline β -carotene at RT and atmospheric conditions with exposure to light for at least eight weeks. By measuring the absorbance from 190–700 nm in different solvents the completion of the polymerization was monitored. Since the copolymer gains a much higher polarity due to its high oxygen functionalization, its solubility in unpolar solvents strongly reduces. Therefore, the copolymer mixture was further purified by precipitation using THF as a solvent and *n*-hexane as the precipitating agent. The obtained fractions were analyzed by different methods, such as spectrophotometry, TLC, HPLC, UHPLC-QToF and GC-MS.

3. Results and Discussion

We were able to verify, that the polymerization of β -carotene at atmospheric condition is completed after 8 weeks by observing no significant changes in the absorption spectrum from 190–700 nm during additional 2 weeks. In addition, we could observe that compared to the more synthetic approach of producing OxBC by streaming oxygen through an ethyl acetate solution of β -carotene, less monomers were formed by air oxidation, as revealed by HPLC analysis [1]. Precipitation of OxBC in *n*-hexane gave much purer polymer, as indicated by a reduction of monomeric peaks in the HPLC chromatogram. Presently our work focuses on gaining more insights into the structure by the application of complementary analytical methods such as NMR, Raman-Spectroscopy and GC-MS.

[1] G.W. Burton, J. Daroszewski, J.G. Nickerson, J.B. Johnston, *et al.*, *Can. J. Chem.* **92** (2014) 305–316.

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[4] T.J. Mogg, G.W. Burton, *Can. J. Chem.* **99** (2021) 751–762.

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Synthesis of phenolic acids esters of carotenoids

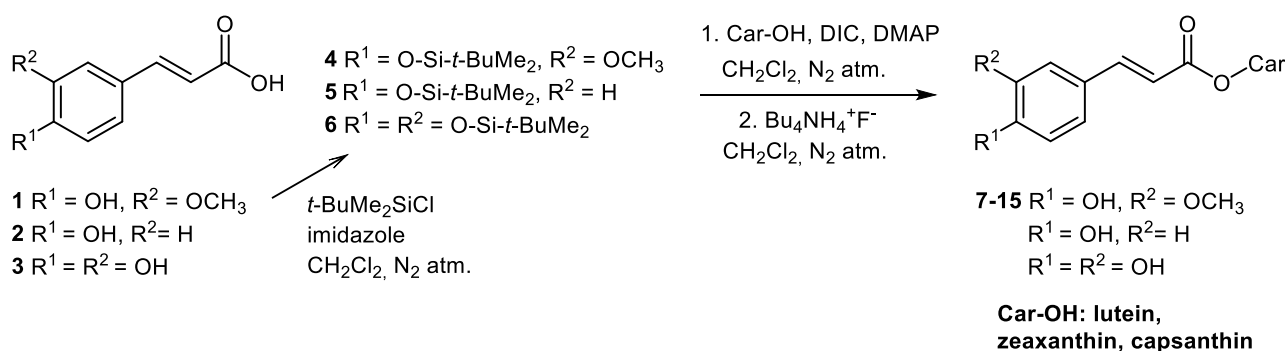
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1. Introduction

Naturally occurring phenolic acids due to their structure possess advantageous physiological effects, such as antioxidant, neuroprotective and anti-inflammatory properties. In the present research, different hydrophobic carotenoids were covalently coupled with hydrophilic phenolic acids in order to increase their stability, bioavailability and antioxidant effect, even synergistically.



2. Experimental

Phenolic hydroxyl groups of natural ferulic (**1**), o-coumaric (**2**) and caffeic acids (**3**) were protected by *tert*-butyldimethylsilyl group. The produced silylated compounds (**4-6**) were used to esterify three carotenoids (zeaxanthin, capsanthin and lutein) in the presence of *N,N*-diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP). The silyl protective groups were removed by tetrabutylammoniumfluoride giving the corresponding bisphenolic acid-carotinoid derivatives (**7-15**). Desilylation could also be performed without the isolation of the silylated esters.

3. Results and Discussion

We applied Steglich esterification-like reaction for coupling of silylated phenolic acids to carotenoids, which was proven to be a mild and appropriate method for carotenoids [1]. The following deprotection does not require the isolation of the silylated intermediate, the coupling and desilylation can be performed as a one-pot reaction. The novel carotenoid-phenolic acid conjugates were obtained in reasonable yields. The synthesized bis(phenolic acid)-carotinoid esters may have increased antioxidant effect, as well as, having amphipathic structure, they showed self-assembly in aqueous medium.

[1] I. Línzembold, D. Czett, K. Böddi, T. Kurtán, S. B. Király, G. Gulyás-Fekete, A. Takátsy, T. Lóránd, J. Deli, A. Agócs, V. Nagy, *Molecules*, **2020**, 25, 636.

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Cactaceae as new carotenoid sources: Carotenoid characterization in *Opuntia* fruits and cladodes (*O. ficus-indica*, *O. dillenii* and *O. joconostle*)

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1. Introduction

The Cactacea family includes more than 200 species which grow abundantly in arid regions of the world in countries such as in Mexico, United States, Spain and Italy, among others [1]. Edible species such as *Opuntia ficus-indica*, *Opuntia dillenii* and *Opuntia joconostle* are of special interest (Figure 1) because of their potential to use as (i) raw material and (ii) to revalorize their subproducts from manufacturing industries (jams, juices). Previously in our laboratory, we reported for the first time the carotenoid composition of *Opuntia ficus-indica* fruits [2] and showed that the most abundant carotenoids in these fruits were (all-*E*)-lutein and (all-*E*)- β -carotene. The objective this study was the characterization and quantification of carotenoids in peels and pulps of 10 prickly pear fruits (*O. ficus-indica* and *O. dillenii*) and 4 prickly pear cladodes (*O. ficus-indica* and *O. joconostle*) to assess their potential as healthy foods and ingredients.

2. Experimental

Carotenoids were determined by HPLC-PDA-MS (APCI+), using a reverse phase C30 column. Carotenoid extraction and chromatographic conditions were previously published [2]. The individual carotenoid identification was carried out by comparing retention times, UV-Vis absorption and mass spectra to those authentic standards.

3. Results and Discussion

The carotenoid profiles of 11 different varieties of *Opuntia* fruits and 4 samples of *Opuntia ficus-indica* cladodes were characterized and quantified for the first time. A total of 11 xanthophylls and 3 hydrocarbon carotenes were identified in *Opuntia* extracts. All carotenoids in Cactaceae tissues were present in their free form (no esters were found). For example, Figure 2 shows the carotenoid content ($\mu\text{g/g}$ dry weight) in *Opuntia ficus-indica* var. Naranja cladodes and fruit skin, being (all-*E*)-lutein (196.9 $\mu\text{g/g}$ dry weight) the most abundant xanthophyll, followed by (all-*E*)-lycopene (59.8 $\mu\text{g/g}$ dry weight) and (all-*E*)-carotene (88.4 $\mu\text{g/g}$ dry weight) in cladodes. The results obtained show the interest of the skin fruit tissue of the fruits and cladodes as a source of carotenoids of interest for human health.

Acknowledgements

This work was supported by Spanish Ministry of Science and Innovation (project PID2020-118300RB-C21).

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[2] M. P. Cano, A. Gómez-Maqueo, T. García-Cayuela, J. Welti-Chanes Food Chem. 237 (2017) 612-622

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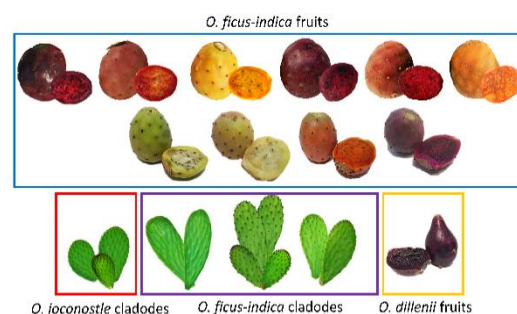


Figure 1. *Opuntia* prickly pear (*O. ficus-indica*, *O. joconostle* and *O. dillenii*) fruits and cladodes.

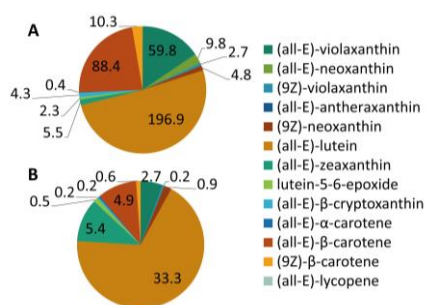


Figure 2. Carotenoid content ($\mu\text{g/g}$ dry weight) in *O. ficus-indica* var. Naranja: A, cladodes and B, fruit skin

P8-2

Carotenoid and Carotenoid ester deposition in plastids of *Pouteria lucuma*, *Persea americana* and *Diospiros kaki* and its effect on bioaccessibility

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1. Introduction

Carotenoids play critical roles in human nutrition and health as essential components of the human diet. However, their bioaccessibilities are affected by other components present in fruits such as fiber, starch and lipids as well as their physical deposition state. Lucuma (*Pouteria lucuma*, var. Beltran), avocado (*Persea Americana* Mill, var. Hass) and persimmon (*Dyospiros kaki*, var. Rojo Brillante) are rich sources of carotenoids and carotenoid esters with diverse profiles and macromolecular composition. The aim of this study was the assessment of the relationship among carotenoid plastid deposition on the stability and bioaccessibility of carotenoid and carotenoid esters in lucuma, avocado and persimmon fruits, following the adapted INFOGEST recommendations assay the gastro-intestinal digestion and evaluate the individual carotenoid and carotenoid esters stabilities and bioaccessibilities.

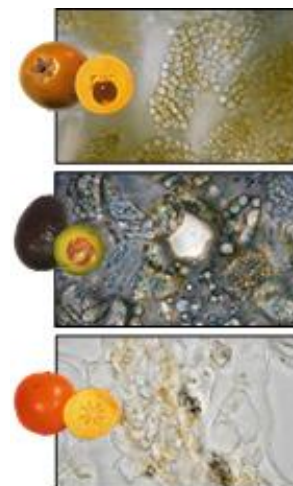


Figure 1. Carotenoid deposition in plastids of lucuma, avocado and persimmon.

2. Experimental

Fresh fruit pulp sections were analyzed by confocal and optical microscopies to examine their carotenoid deposition and also in each digestive phase [1]. Individual carotenoid identification and quantification was analyzed by HPLC-DAD-MS using a reversed-phase C30 column [2].

3. Results and Discussion

In lucuma pulp carotenoids were deposited in amyloplasts in the membrane, enclosing the starch granules (**Figure 1**). During digestion, carotenoids were concentrated in the amyloplasts due to the dissolving of starch in the digestive medium. Carotenoids in avocado were found in elaioplasts along with a large content of lipid vesicles. In avocado, the high Fatty acid content (637 mg FAME/ g dry weight) and their profile contributed to high carotenoid bioaccessibility (**Figure 2**). Carotenoids in persimmon were found in globular chromoplasts in the cells surrounding vascular bundles. They were the least bioaccessible ones because of (i) low fatty acid content and (ii) because they were trapped in the pectin polysaccharide chains during digestion.

Acknowledgements

This work was supported by Spanish Ministry of Science and Innovation (project PID2020-118300RB-C21).

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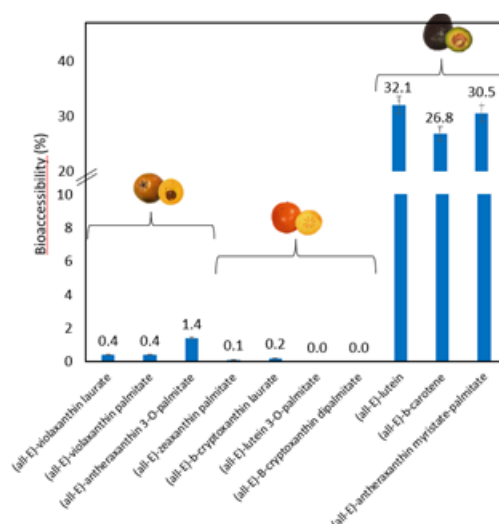


Figure 2. Carotenoid bioaccessibility (%).

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P8-3

Comparative assessment of Cas9/sgRNA ribonucleoprotein complexes and herbicide treatment for PDS knock-out in chlorophyte microalgae

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1. Introduction

Phytoene (7,8,11,12,7',8',11',12'-octahydro- ψ,ψ -carotene) is the first uncoloured carotenoid, precursor of the rest of carotenoids, produced by condensation of two molecules of geranylgeranyl pyrophosphate (GGPP) in a reaction catalysed by phytoene synthase (PSY). Phytoene is appreciated in cosmetic for its ability to absorb UV radiation and has been shown to be involved in several health-promoting actions, being particularly remarkable its antioxidant, anticarcinogenic, and anti-inflammatory activities [1]. In this study, phytoene accumulation has been induced in the chlorophyte microalga *C. reinhardtii*, by, either generating targeted PDS knock-out mutants with the CRISPR-Cas9 editing technology; or by treatment with optimized concentrations of the bleaching herbicide norflurazon. The phytoene containing strains generated by these treatments have been characterized at phenotypic and transcriptomic level.

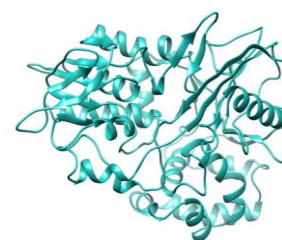


Figure 1. 3D structure of crPDS

2. Experimental

Chlamydomonas reinhardtii ARG defective strain CC-3403 (RU-387 *nit1 arg7 cw15 mt-*) was grown in liquid or agar solidified standard Tris-acetate phosphate (TAP). For knock out of the *PDS* encoding gene, two Cas9-gRNA Ribonucleoprotein complexes (RNP-Cas9) were prepared as previously reported [2]. Carotenoid content in the selected PDS knock out mutants and in norflurazon-treated strains was determined by Hitachi Chromaster HPLC-DAD equipped RP-18 column [3]. Expression levels of carotenogenic genes were studied by RT-PCR in a Mx3000P Multiplex Quantitative PCR System (Stratagene).

3. Results and Discussion

The doses of norflurazon able to induce the accumulation of the colourless carotenoid phytoene in *Chlamydomonas reinhardtii*, was optimized. Two preassembled *Cas9/sgRNA* ribonucleoprotein complexes (RNPs), containing PDS specific guide RNAs, delivered into the nucleus of *Chlamydomonas reinhardtii* by electroporation. We found that both, herbicide treatment and RNPs knock-out, induce the accumulation of phytoene in *Chlamydomonas*. The expression of PDS, PSY and ZEP is strongly down-regulated in norflurazon-treated cells.

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Stereoselective orobanchol biosynthesis catalyzed by dirigent domain containing protein in tomato

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1. Introduction

Strigolactones (SLs) are plant apocarotenoids that function as both endogenous plant hormones and rhizosphere signaling molecules. In our previous study on tomato (*Solanum lycopersicum*), we identified the cytochrome P450 SICYP722C as the enzyme responsible for converting carlactonoic acid (CLA), a biosynthetic precursor of SLs, to orobanchol. However, recombinant SICYP722C protein converted CLA to a mixture of orobanchol and *ent*-2'-*epi*-orobanchol, the C-ring configuration of which is opposite to orobanchol, suggesting the presence of additional determinants regulating the C-ring configuration [1]. We here report the identification of the stereoselective ring-formation factor (SRF) which regulates C-ring configuration of orobanchol, functioning downstream of SICYP722C in the process of BC-ring formation.

2. Experimental

For biochemical functional analysis, recombinant SICYP722C and SRF proteins were expressed in *E. coli* and purified. A SRF candidate SRF was selected from the list of genes co-expressed genes with SL biosynthesis genes, based on RNA-seq datasets in orobanchol-producing plants. To verify the function of SRF in plants, we examined alteration of the composition of SLs in *srf*-knockout plants generated through CRISPR-Cas9 mediated genome editing. The subcellular localization of SRF was analyzed by transiently expressing the signal peptide of SRF fused with a fluorescent protein in *Nicotiana benthamiana* leaves. Furthermore, we investigated the stereoselective mechanism of SRF using model structure predicted by AlphaFold2 and molecular dynamics simulations, followed by experimental site-directed mutagenesis to verify the importance of predicted catalytic residues in SRF.

3. Results and Discussion

Detailed analysis of the enzyme reaction revealed that SICYP722C catalyzed conversion of CLA to 18-oxo-carlactonoic acid (18-oxo-CLA), which spontaneously cyclized to form the BC ring without stereoselectivity. We targeted a gene encoding dirigent domain-containing protein as SRF candidate. The recombinant candidate protein stereoselectively converted 18-oxo-CLA to orobanchol. The *srf*-knockout tomato plants produced both orobanchol and *ent*-2'-*epi*-orobanchol, confirming its function as SRF. Subcellular localization analysis revealed co-localization of SICYP722C and SRF at the endoplasmic reticulum membrane, which may facilitate the transfer of 18-oxo-CLA. Through computational analysis, we predicted that residues of Y90, D124, and R131 form a hydrogen bond network to fix the carboxyl group of 18-oxo-CLA, controlling its conformation. Additionally, residue of D37 plays a critical role in protonation at the 18-aldehyde group of 18-oxo-CLA, which is necessary to initiate cyclization. Mutation of these residues significantly decreased the enzyme activity of SRF, thus validating the proposed stereoselective BC-ring formation mechanism. Our proposed novel mechanism of stereoselective BC-ring formation provides a molecular basis for understanding the SL structural diversity.

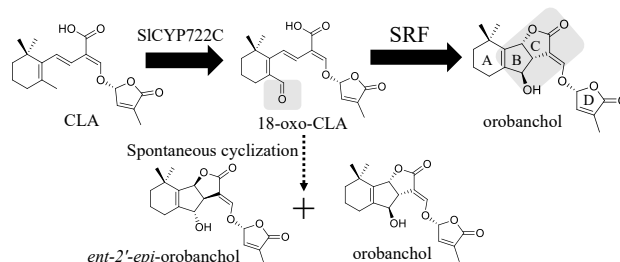


Fig. Biosynthetic pathway of orobanchol in tomato elucidated by this study

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Buckwheat - a unique plant system to improve carotene production by using mutagenesis

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1. Introduction

Buckwheat, a grain crop belonging to the Polygonaceae family, is essential for food production and medicine. Buckwheat seeds are also valuable because they do not contain gluten. Among buckwheat species, *Fagopyrum esculentum* (common buckwheat) and *Fagopyrum tataricum* (L.) Gaertn. (Tartary buckwheat) are the most cultivated ones. Especially Tartary buckwheat has been popular internationally because of its excellent dietary sources of nutrients, amino acids, minerals, phenolic compounds, and carotenoids. Because of the importance of carotenoids for plant and human health, the carotenoid biosynthesis pathway has been well-studied in higher plants for several decades. These are examples of the effects of internal and environmental factors on carotenoid biosynthesis in buckwheats [1, 2]. Because of the importance of buckwheat species, improving some nutritional status is desirable by using mutagenesis. Until now, common buckwheat cultivars with high antioxidative activity or high rutin levels were developed by physical mutagenesis. The knowledge in the field of buckwheat mutagenesis is not comprehensive at present, and there needs to be data on carotenoid level improvements in this way.

2. Experimental

In this study, we are using chemical and physical mutagenesis as an effective strategy for improving the carotenoid production in the buckwheat species. Our study aimed to enhance carotenoid level by using chemical and biological mutagenesis. For this purpose, we have used gamma rays and two chemical mutagens: N-nitroso-N-methylurea (MNU) and maleic acid hydrazide (MH), to treat seeds of *Fagopyrum tataricum* and *F. esculentum*, cv. Panda and Kora.

3. Results and Discussion

The preliminary results showed significant differences in the cytological response of genomes of *F. esculentum* and *F. tataricum* in the M1 generation to mutagenic treatment. All cytogenetic parameters describing the genotoxic effect of mutagens showed that the *F. tataricum* genome is less sensitive to MH than the *F. esculentum* one. We did not notice the difference in the sensitivities of *F. esculentum* cv. Panda and Kora. The harsh conditions in which the Tartary buckwheat grows may impact its sensitivity to mutagens. This knowledge will contribute significantly to advancing breeding programmes of *Fagopyrum* species as practical biological tools towards the development of functional foods with the increased level of carotenoids. The analysis of the carotenoid content in the obtained M3 generation followed by mutagenesis is planned.

The International Atomic Energy Agency supports this study under the framework of RER5024.

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P8-6

Relations among carotenoids and vitamin E compounds in a diverse inbred line collection of temperate maize

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1. Introduction

The consumption of maize products that are high in carotenoids and vitamin E compounds (tocols) has been linked to a range of health benefits [1]. They are the two most abundant groups of lipid-soluble antioxidants in maize kernels, with majority of carotenoids present in endosperm and majority of tocopherols in germ. Previous work showed that selection for these two groups could be performed simultaneously in sweet corn and broccoli [2]. In this study, we focused on relations among lutein, zeaxanthin, α - and β -cryptoxanthin, α - and β -carotene, α -, γ - and δ - tocopherol, α - and γ -tocotrienol content in grain of 88 accessions of temperate maize from Croatian maize genebank.

2. Experimental

A field trial with 88 maize inbred lines in two replicates was conducted at two different locations in Croatia in 2019. The inbreds have different proportions of native germplasm (maize landraces from Southeastern Europe) in their pedigrees [3]. Extraction and quantification of carotenoids and tocopherols from whole maize grain were performed as previously described [4].

3. Results and Discussion

The ranges of grain content of the analyzed compounds in $\mu\text{g g}^{-1}$ on a dry weight basis were as follows: lutein (0.8 – 32.7), zeaxanthin (1.5 – 21.6), α -cryptoxanthin (0.2 – 8.6), β -cryptoxanthin, (0.2 – 5.9) α -carotene (0.1 – 2.2), β -carotene (0.1 – 4.2), α -tocopherol (0.8 – 30.4), γ -tocopherol (2.9 – 80.0), δ -tocopherol (0.3 – 4.5), α -tocotrienol (1.2 – 7.9) and γ -tocotrienol (0.9 – 19.3). Correlations among carotenoids and tocopherols were shown in tocopherol and α -tocotrienol, but not other α -branch carotenoids. As expected, the tightest positive correlations were between zeaxanthin and β -cryptoxanthin, as

well as between γ - and δ -tocopherol. Generally, it suggests that selection for carotenoids and vitamin E compounds could be achieved simultaneously.

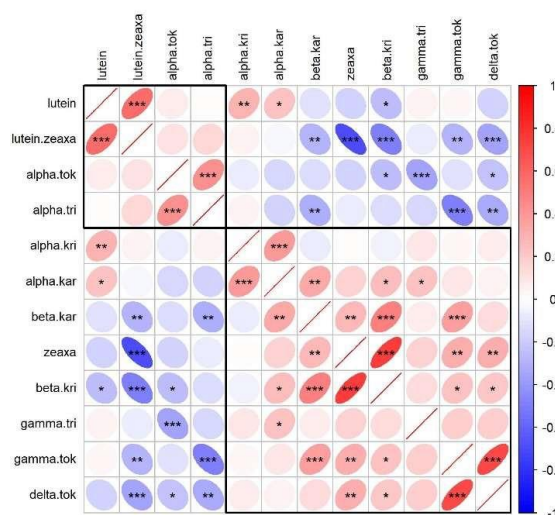


Figure 1. Two squares represent major clusters of the hierarchical clustering using a complete linkage method. Interestingly, the first cluster includes lutein, α -**Figure 1.** Correlogram of the accession mean values between carotenoids and tocopherols. *, **, **** represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

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Identification of candidate genes affecting mango flesh colour and validation through GWAS

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1. Introduction

Mango flesh is a rich source of vitamins like carotenoids, which have antioxidant properties and are important for healthy vision, and immune functions. Variation in flesh colour is one of the key determinants of the concentration and composition of carotenoids [1]. Mango varieties can vary in their ripe flesh colour from dark orange to pale/green-yellow (**Figure 1**). Identification of molecular markers (SNPs and candidate genes) associated with flesh colour and carotenoid contents can assist in exploring the carotenoid biosynthesis pathway in mango and employing marker assisted selection in rapid genetic improvement for these traits. Over the years, genome wide association studies (GWAS) have been used in trait specific marker discovery in several crop species. In this study, candidate genes which affect the flesh colour of mangoes will be predicted using 26 varieties of mangoes and will be validated by conducting a GWAS using 223 varieties of mangoes with corresponding flesh colours (using genome sequence-based SNP markers).

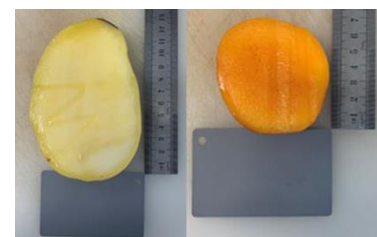


Figure 1. Ripe flesh colour of a pale/green-yellow mango (left) and a dark orange mango (right)

1. Experimental

Candidate genes for mango flesh colour were predicted based on HPLC carotenoid analysis of a fully-ripe subset of 26 varieties of mangoes, ranging in flesh colour (hue) and colour intensity (chroma), as determined by a Minolta CR400 chromameter. The relationship between carotenoid profile, colour (hue) and colour intensity (chroma) were determined by multiple linear regressions to understand the causal relationships between carotenoid profile and mango flesh colour. Concurrently, a larger data set of 223 mango varieties were whole genome sequenced (DAF) to identify single nucleotide polymorphisms (SNP) within the mango genome. The 223 varieties were subsequently phenotyped for flesh colour using a subjective 1-5 scale (1 = dark-orange, 3 = orange-yellow, 5 = greenish-yellow), based on Royal Horticultural Society Colour chart cards. The SNP markers were subsequently sorted for quality, based on % call rate and polymorphic information content. Using the flesh colour phenotypic data and SNP markers, a GWAS will be conducted using TASSEL software [2]. Significant markers will be aligned with the mango reference genome sequence to confirm or disprove the association of the candidate genes predicted from the smaller subset, and also to identify genes influencing flesh colour that are not directly associated with the carotenoid biosynthesis pathway.

2. Results and Discussion

Carotenoid profile analysis of the 26-mango variety subset (~10% of the mango variety population) revealed a clear relationship between total carotenoid content and decrease in hue, or 'orangeness' of the flesh. This suggests the involvement of PSY (Phytoene synthase) which is situated towards the beginning of the carotenoid biosynthesis pathway and is one of the candidate genes predicted to influence flesh colour. Other candidates include lycopene β -cyclase and β -hydroxylase which influence the inflow into the β -branch and levels of all-trans β -carotene, an orange carotenoid, respectively. This along with other candidate genes will be validated using 223 mango accessions using 13K SNP markers selected from the 900K for this study.

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Changes in Carotenoid and Tocopherol Contents of Cherry Tomato Lines Improved by Radiation Induced Mutation Breeding

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1. Introduction

The tomato, which has an important place among vegetable crops in terms of human health and nutrition, varies according to year, but its production in 2022 was 187 million tons [1]. In the selection of varieties used in production, besides yield and tolerance to abiotic and biotic stress conditions, nutrient content values are one of the important parameters, especially in recent years. Therefore, considering the decreasing production areas, it is seen that studies on obtaining new varieties with high nutrient content through breeding studies gain more importance in today's world. Classical breeding methods and biotechnological approaches are the methods used in breeding studies. The technique of mutation breeding, which is one of these methods, appears as an alternative method for creating new variations in the limited gene pool by using physical or chemical mutagens. To date, there are 26 registered cultivars registered in the International Atomic Energy Agency mutant variety database (MVD), which have been developed by improving different characteristics through mutation breeding [2]. In this study, it was aimed to enrich the genetic pool with radiation-induced mutation breeding in the cherry tomato cultivar, which has started to receive more demand in greenhouse cultivation in recent years, in order to develop new cultivar candidates with higher nutrient content.

2. Material and Method

Seeds of a commercial cherry tomato cultivar were used in this study. First, the effective mutation dose (EMD) of the genotype was determined. Then, seeds were irradiated in a cobalt 60 gamma source with the EMD of 80 Gy. Following the irradiation, classical mutation breeding steps were performed. In order to determine the nutritional content of the fruits of 8 mutant lines, which were found superior in terms of fruit structure, plant growth, and yield, samples were conducted to HPLC analysis. Carotenoid and tocopherol contents of mutant lines, which growth under controlled conditions were followed for 3 years from the M4 generation. The method described by Abushita and his co-workers ([3], [4]) was used for the extraction of carotenoids and tocopherols for tomato samples. Carotenoids and Vitamine E (tocopherols) extractions were made with the liquid-liquid partition technique. All extractions were made just before the injection. Sample preparation was done from each sample in duplicate, and each extract was injected in triplicate. Two separate HPLC systems (A Varian ProStar HPLC, Waters HPLC system with a Waters 2695 Separations Module and a Waters 2475 Multi-L Fluorescence Detector) were used in the tocopherol and carotenoid analyses.

3. Results

According the analysis report, the content of nutritional parameters followed was found to be for beta-carotene 2.061-0.775 mg/100 g of mutant samples whereas 1.382 mg/100 g of control sample; lutein 0.079-0.033 mg/100 g of mutant samples whereas 0.046 mg/100 g of control sample; lycopene 6.992-2.761 mg/100 g of mutant samples whereas 5.351 mg/100 g of control sample; gamma tocopherol 0.220-0.519 mg/100 g of mutant samples whereas 0.186 mg/100 g of control sample; deltha tocopherol 0.036-0.044 mg/100 g of mutant samples whereas 0.036 mg/100 g of control sample and gamma tocopherol 0.116-0.263 mg/100 g of mutant samples whereas 0.169 mg/100 g of control sample. By these findings, the mutant line 114/15 has the highest content of beta-carotene and lycopene in all samples. The results showed that the dose of 80 Gy achieved by using a ⁶⁰Co gamma irradiator resulted in some differences in the phytonutrient capacity of mutant cherry lines. The average nutrient rate is rather above the control.

4. References

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Conversion of carlactonoic acid to various strigolactones in plants

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1. Introduction

Strigolactones (SLs) are plant apocarotenoids which act as germination stimulants for root parasitic weeds and as rhizosphere signaling molecules for hyphal branching of arbuscular mycorrhizal fungi. SLs also act as endogenous plant hormones that regulate root and shoot architecture. Carlactonoic acid (CLA) is the biosynthetic intermediate of a series of SLs, which is derived from β -carotene by the sequential reactions catalyzed by D27, CCD7, CCD8, and MAX1. We selected high SL-producing plants and established biosynthetic pathways in each plant leading to various SLs at chemical, biochemical and molecular levels.

2. Experimental

High SL-producing plants were selected, cowpea (*Vigna unguiculata*) for orobanchol, cotton (*Gossypium arboreum*) for 5-deoxystrigol (5DS) and sorgomol, cotton (*G. hirsutum*) for 5DS and strigol, and sorghum (*Sorghum bicolor*) for 5DS and sorgomol. Biosynthetic precursors, CLA, 5DS and 4-deoxyorobanchol (4DO), were administered independently to each plant grown hydroponically and treated with fluridone, a phytoene desaturase inhibitor, to confirm the biosynthetic sequence of SLs. Genes exhibiting expression patterns similar to known SL biosynthetic genes were selected from RNA-seq data of each plant and the functions of heterologously expressed proteins were characterized.

3. Results and Discussion

CLA was converted to orobanchol without intermediacy of 4DO in cowpea and to 5DS in sorghum and cotton. 5DS was converted to sorgomol in sorghum and *G. arboreum* and to strigol in *G. hirsutum* (**Figure**). Inhibition of the conversions by uniconazole-P suggested the involvement of P450 in each step. Selection of candidate genes from RNA-seq data and the following functional analyses elucidated involvement of VuCYP722C in orobanchol biosynthesis in cowpea [1]. However, in vitro enzyme reaction of VuCYP722C with CLA as a substrate resulted in both orobanchol and its diastereomer *ent*-2'-*epi*-orobanchol, implying involvement of a mechanism which regulates stereoselective cyclization of an intermediate, most probably 18-oxo-orobanchol. On the other hand, GaCYP722C and GhCYP722C converted CLA to 5DS in a stereoselective manner in cotton [2]. Another enzyme of cotton introduced a hydroxy group at C-5 of 5DS to generate strigol. SbCYP728B introduced a hydroxy group at C-9 of 5DS to generate sorgomol in sorghum [3]. Identification of the genes responsible for biosynthesis will enable qualitative and quantitative manipulation of SL production in plants for further study on their biological significance.

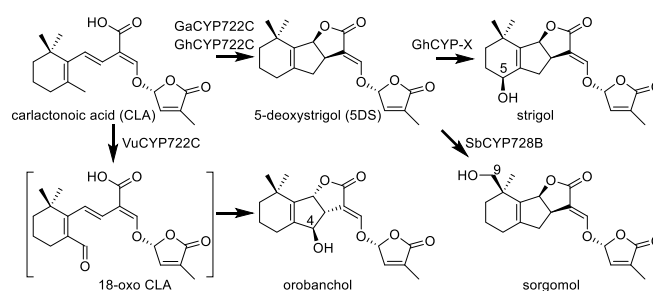


Figure Conversion of CLA to orobanchol, strigol and sorgomol, in cowpea, cotton and sorghum, respectively. Each enzyme involved in the synthesis of these SLs was identified.

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P8-10

Proteome profiling of sweet pepper genotypes (*Capsicum annuum* L.) with beneficial mutations

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1. Introduction

Since the dawn of the 21st century, the main problem has been the exploitation of the earth's resources to feed the growing population. One of the ways is to increase the quality of food, the main deficiency of which is the lack of substances with antioxidant properties. Among them, carotenoids play a major role, and their increase provides a healthy food. Mutation breeding, accelerated by advanced molecular technology, can lead to genetic diversity and thus greatly limit the problem of malnutrition. Pepper is a major natural source of β -carotene, which determines the quality of plant food. In pepper, the approach of integrated application of nuclear techniques and proteomics approaches provides new opportunities for its increase in fruit. The proposed characterization of *de novo* developed mutant pepper genotypes is a prerequisite for the selection of improved mutant varieties to provide a reliable basis for breeding programs oriented towards sustainable agricultural systems in Bulgaria.

The success of breeding programs can be enhanced by the efficient and integral application of various molecular methods to characterize mutants. On the other hand, identifying new cost-effective methods is important in a resource-constrained environment. To this end, we compared the proteomic profiles of pepper fruit intended for fresh consumption of a mutant variety “Oranzheva kapia” with high β -carotene in fruits, “Albena” (an anthocyanin-free variety), and a mutant line “OKal” (possessing high β -carotene concentration and anthocyanin-free) with the corresponding initial variety “Pazardzhishka kapia 794” (initial), which are shown in **Figure 1**.

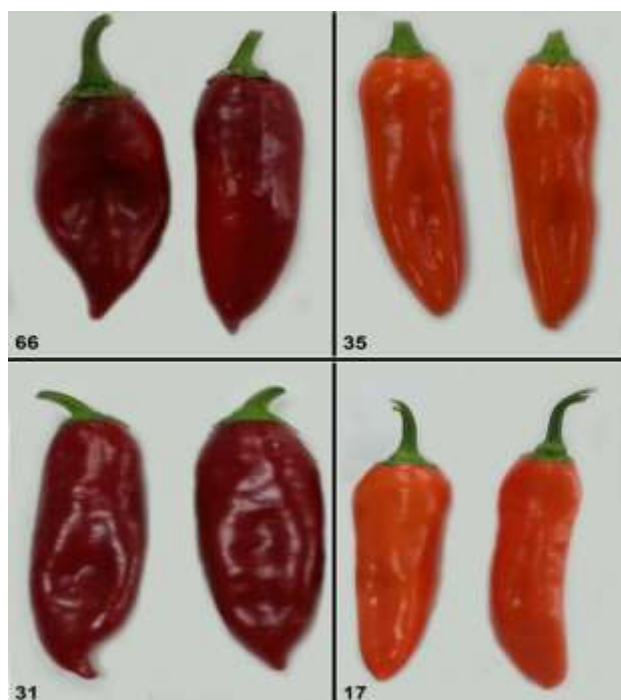


Figure 1. Pepper mutant genotypes

2. Experimental

The proteomics characterization of the mutant genotypes was performed by 2D-DIGE and shotgun LC-MS approaches. For better sensitivity, we used also protein enrichment with combinatorial hexapeptide ligand library (CPLL) to enrich low abundant proteins from mutants.

3. Results and Discussion

P8-10

More than thousand proteins were identified in mutant lines differing in their abundance, as shown in **Figure 2**, involved in different cellular processes: primary and secondary metabolism, signaling, growing and maturation, stress and transport (**Figure 3**).

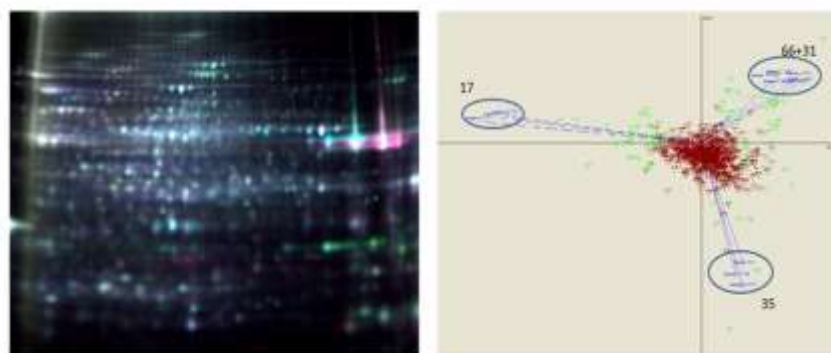


Figure 2. 2D DIGE (left). On left is represented multiplexed image of gel with internal standard and mutant lines. Next is the distribution of mutants according to normalized volumes of detected spots in PCA space (right). Spots with highest contribution in PC1 and PC2 (green) are targeted for spot picking and MALDI TOF/TOF analyses

Our results showed that the down-regulation of capsanthin/capsorubin synthase (CCS) in the mutant variety “Oranzheva kapia” is related with the color fruit altogether with increased β -carotene accumulation in the mutant variety.

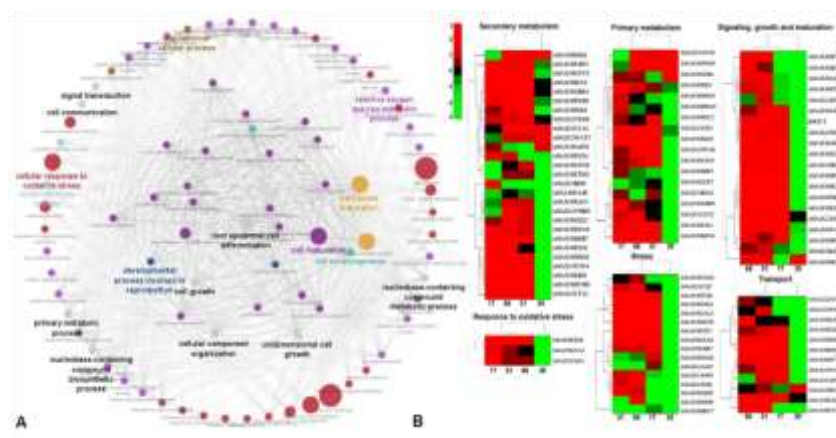


Figure 3. Shotgun LC/MS. A) GO enrichment analysis. B) Heat map of HCA clustering of identified proteins

Characterization of chromoplasts as natural food colorants and their stability under different storage conditions

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1. Introduction

Carotenoids are natural hydrophobic pigments, and accumulated in a particular plastid in nature, so called chromoplasts. Chromoplasts from different plants contain various substructures within them, e.g., plastoglobules, tubules, membranes, and crystalloids (see **Figure 1** for their structural model) [1], where the aggregation of carotenoids in the form of lipid-dissolved, liquid-crystalline, or solid-crystalline occurs. Diverse aggregation states of carotenoid molecules arouse a shift of absorption band, resulting in changes in visual color compared to free monomers. Recent study found that the degradation rates of crystal lycopene existed in tomato chromoplasts were lower than those of free carotenoids [2]. Thus, the carotenoid-containing chromoplasts might be considered as a potential natural food colorant with higher stability. ZDP (zeaxanthin dipalmitate) are the major carotenoids in goji berries and primarily deposited in tubular chromoplasts. In this study, we isolated chromoplasts from fresh goji berries (*Lycium barbarum* L.) and investigated their aqueous stability under different environmental conditions as natural food colorants.

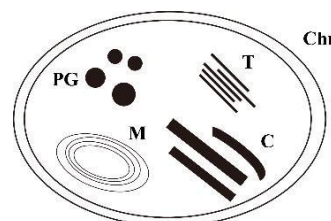


Figure 1. Schematic diagram of chromoplast. Chr chromoplasts, PG plastoglobules, T tubules, M membranes, C crystalloids

2. Experimental

We firstly isolated chromoplasts from fresh goji berries using a protocol of sucrose density gradient centrifugation and conducted morphological observations and particle size analysis. The storability was evaluated by pigment color attributes (*CIELab** parameters), kinetic degradation model, and antioxidant capacity under different temperatures (4, 25 and 40 °C) and light exposure conditions (dark and UV-light).

3. Results and Discussion

The tubular chromoplasts of goji berries had intact bilayer membranes observed through transmission electron microscope, and their mean diameter was $3.53 \pm 0.11 \mu\text{m}$. Colorimetric parameters, especially ΔE was lower both at 4 °C and in the dark compared with other conditions during storage as shown in **Figure 2**. The degradation rate (k) of ZDP accelerated with increasing temperature, and the lowest k value of ZDP was found at 0.02 in the dark for a 15-day storage. The antioxidant capacity of chromoplasts showed a similar downward trend among different conditions and was more affected by high temperature. Through initial exploration, we found that tubular chromoplasts of goji berries are more stable at low temperature and in dark conditions during storage, and more efforts may be needed to study their protective effect on carotenoids as natural colorants in the future.

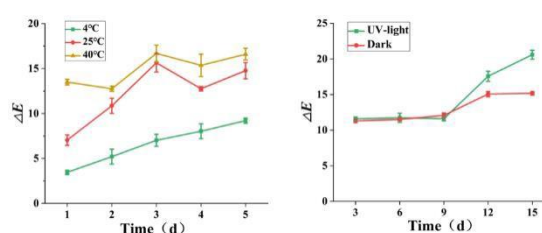


Figure 2. Effect of storage temperature (4, 25 and 40 °C) and lightness (dark or UV-light) on total color difference (ΔE) of chromoplasts.

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Role of apocarotenoids in the management of fusariosis in flax

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1. Introduction

Diseases caused by soil-borne pathogens result worldwide in significant yield losses in economically important crops. In addition to causing heavy yield losses fungi are producing mycotoxins, so even if the crops survive, the products are contaminated and not suitable for food and animal feed. Flax (*Linum usitatissimum*) is a valuable crop plant that give two types of products – fibre and oil. Additionally, flax seeds contain numerous other valuable components including vitamins, phytosterols and lignans. Also the materials considered as waste-products are the source of valuable components which make flax perfectly suitable to the “zero-waste” slogan. The cultivation of flax is restricted by environmental stress factors. In Poland, the main cause of losses in the flax crops are fusarioses making fusarium wilt of flax economically important disease. Due to the pathogen’s ability to survive in soil for long periods, it is essential to identify fusarium wilt resistance mechanisms and identify molecules which may serve as protectants. Over the years we investigated several pathways, in particular of secondary metabolite synthesis in order to improve flax resistance to pathogens and in the same time plant properties in terms of fibre, oil quality and bioactive metabolites content useful for human nutrition and medicine. Recently we concentrated our efforts on terpenoid synthesis pathway when we noticed that its mevalonate branch is strongly activated during pathogen infection.,

2. Experimental

The expression of key genes of carotenoid and apocarotenoid pathway was studied by RT-PCR. The carotenoids and apocarotenoids levels were measured by UPLC and GC-MS method. The transgenic plants with lower levels of ABA were obtained by silencing NCED expression. The transgenic plants were generated via *Agrobacterium* method. The mycotoxin production by *Fusarium oxysporum* was measured using HPLC

3. Results and Discussion

The increased inexpression of genes leading to carotenoid synthesis was observed but without accompanying increase in carotenoid content. While some of the carotenoid pool is expected to serve as protectants against ROS produced during infection they are also substrates for many other molecules, some of them with still unidentified function. During flax infection significant changes in expression of carotenoid cleavage dioxygenases were observed. In particular, increase in expression of NCED genes leading to abscisic acid was noticed. This was accompanied by increased levels of ABA in flax tissue [1]. While the ABA role in plant infection is still somehow controversial there are more reports published recently supporting our theory that ABA is one of key players in flax response to *Fusarium* infection. The importance of ABA for plant survival during infection was confirmed by analysis of transgenic flax plants with changed ABA levels. Plants with lower levels of ABA were shown to be much more susceptible to fungal infection. Our more recent research shows that ABA is probably only one of the many molecules from apocarotenoid pathway involved in signalling and plant response during *Fusarium* infection. Some of the molecules produced on this pathway like ionones can also directly influence fungal growth and mycotoxin production and induce PR gene expression.

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Possible reasons for high zeaxanthin in orange capsicums

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1. Introduction

Zeaxanthin, an orange-coloured carotenoid, has been associated with slowing the progression of macular degeneration [1], the leading cause of blindness in developed countries. Zeaxanthin is obtained from dietary sources but compared to other carotenoids such as beta-carotene or lutein, it is a relatively uncommon carotenoid in the western diet. Orange capsicums (*Capsicum annuum*), also known as bell peppers, have been specifically reported to be an excellent source of zeaxanthin, with a greater concentration of zeaxanthin than any other dietary vegetable. Past studies have indicated the role of key genes such as *Capsanthin/Capsorubin Synthase (CCS)* and *Zeaxanthin Epoxidase (ZEP)* in determining the zeaxanthin concentration. However, little comprehensive information is available on the factors responsible for this high accumulation of zeaxanthin. This study, therefore, aims to explore the factors such as the effect of total carotenoid concentration, zeaxanthin percentage, mutation and gene expression of *CCS* and *ZEP* genes in dictating zeaxanthin concentration in orange varieties.

2. Experimental

Carotenoid profiles of 11 orange capsicum varieties were determined and their zeaxanthin concentration was quantified using UHPLC-DAD-MS. Furthermore, the presence/absence of a functional *CCS* was determined using PCR primers while mutation (SNP) in *ZEP* was identified with CAPS primers. Lastly, q-PCR studies were conducted to understand the variation in gene expression in these varieties.

3. Results and Discussion

A comparison of the carotenoid profile of orange bell pepper (*C. annuum*) accessions indicated that both total carotenoid concentration (TCC) and the percentage of zeaxanthin present played an important role in the overall concentration of zeaxanthin, which ranged from 6.2 to 27.0 mg/100g FW. It was observed that varieties with a high percentage of zeaxanthin could still have a relatively low zeaxanthin concentration within the orange bell pepper accessions. A 'dark orange' accession, '179-8' had the lowest zeaxanthin concentration (0.22 mg/100g FW) of all the bell peppers assessed. Despite this accession having a dark orange colour, it had both a very low percentage of zeaxanthin (6%) and a low TCC (3.7 mg/100g FW). Rather than its orange colour being due to the presence of zeaxanthin, it was shown to be due to a combination of red capsanthin and yellow violaxanthin carotenoids.

Molecular studies further suggest that in orange bell pepper accessions, two of three primer sets targeting *CCS* failed to amplify sections within the first two-thirds of the gene while quantitative PCR indicated no transcription of the *CCS* gene at any stages of fruit maturity. The PCR analysis showed that a 250 bp region at the 3' end of the *CCS* gene was still present in all red, orange, and dark orange accessions. Apart from the inactivation of the *CCS* gene, it was also observed that orange accessions had a modified *ZEP* gene while red and dark-orange varieties had a wild type 'A' *ZEP* allele. An SNP substitution (G for A) within the *ZEP* gene, appears to prevent the *ZEP* enzyme from being fully functional, thereby hindering (but not disabling) the downstream carotenoid pathway, leading to an accumulation of zeaxanthin in orange varieties. This study suggests that TCC and zeaxanthin percentage play an important role in determining the zeaxanthin concentration in orange capsicums. The presence of the mutant 'G' *ZEP* allele and the absence of the *CCS* gene are other factors resulting in a high accumulation of zeaxanthin in orange capsicums.

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In Silico Methodologies to Improve Antioxidant Evaluation of Carotenoids in Soybean and Protein Hydrolysates from Soybean Dregs (Okara)

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1. Introduction

Approximately almost 10% of the world's soybean production is used directly for human food. Soybean has several bioactive compounds including carotenoids that will be beneficial for human health. After, the production of soymilk and tofu results in an insoluble by-product soybean dregs, or called okara with limited market value and is usually used as animal feed[1]. Okara has a high nutritive value due to its high-quality protein, fat, carbohydrates, fiber, vitamin, and minerals that are still can be utilized further. The antioxidant properties of carotenoids and also soy protein hydrolysates have been ascribed to the cooperative effect of several properties including their ability to scavenge free radicals, act as a metal-ion chelator, oxygen quencher, or hydrogen donor, and the possibility of preventing the penetration of lipid oxidation initiators by forming a membrane around oil droplets. *In silico* analysis is one approach of the common types of bioinformatics that is widely used in analyzing the amino acid sequence, protein domain, and protein structure, through a computational approach [2]. The outcome of this study, although preliminary, could add to a better understanding of the potential of carotenoids in soybean and also soybean dregs as protein-rich food wastes, as a source of health-promoting peptides.

2. Experimental

1. Docking of carotenoids in soybean with antioxidative enzyme

A molecular docking study was performed using AutoDock Vina-4.2.6. The Crystal Structure of Bovine XO (PDB ID: 3NVY) was retrieved from the RCSB Protein Data Bank 1 (<https://www.rcsb.org/>), and carotenoids in soybean such as lutein, beta carotene, and zeaxanthin were retrieved from PubChem. Both protein and carotenoids as ligands were prepared using PyRix. The activity was generated through the global docking method.

2. In silico proteolysis of soybean dregs (okara) and molecular docking of peptides with antioxidative enzyme

The assessment of the biological potential of glycinin and the β and α subunits of β -conglycinin as the main protein in soybean dreg was carried out using the BIOPEP analysis [<http://www.uwm.edu.pl/biochemia/index.php/en/biopep>]. Protein parents were also subjected to in silico proteolysis to predict the theoretical peptide sequences cleaved by papain. The antioxidant peptides were predicted using the Peptide Ranker [<http://bioware.ucd.ie/compass/biowareweb/>] tool and the sensory of peptides was also determined. A molecular docking study was performed using AutoDock Vina-4.2.6. The Crystal Structure of Bovine XO (PDB ID: 3NVY) was retrieved from the RCSB Protein Data Bank 1 (<https://www.rcsb.org/>), and peptides derived were retrieved from PubChem. Both protein and peptides were prepared using PyRix. The activity was generated through the global docking method.

3. Results and Discussion

We found that several carotenoids in soybean can dock the antioxidative enzyme. However, as shown in **Figure 1**, several interactions of ligands (carotenoids) to the protein can be seen in 2D and 3D. The protein hydrolysates of soybean dregs (okara) also have antioxidative activity based on the in silico approach. Several antioxidant bioactive peptides in the hydrolysates such as Proline-Histidine-Phenylalanine (PHF), Alanine-Aspartic Acid-Phenylalanine (ADF), Tyrosine-Tyrosine-Leucine (YYL), Proline-Histidine-Histidine (PHH), Isoleucine-Arginine (IR), Serine-Valine-Leucine (SVL) that predicted using papain *in silico*. The sensory activities such as umami, bitterness, and sourness of the antioxidant peptides will be important for further application in foods and nutraceuticals. Further evaluation such as in vitro and in vivo assessment will be needed to see several functional properties of carotenoids from soybean and the protein hydrolysates from soybean dregs (okara).

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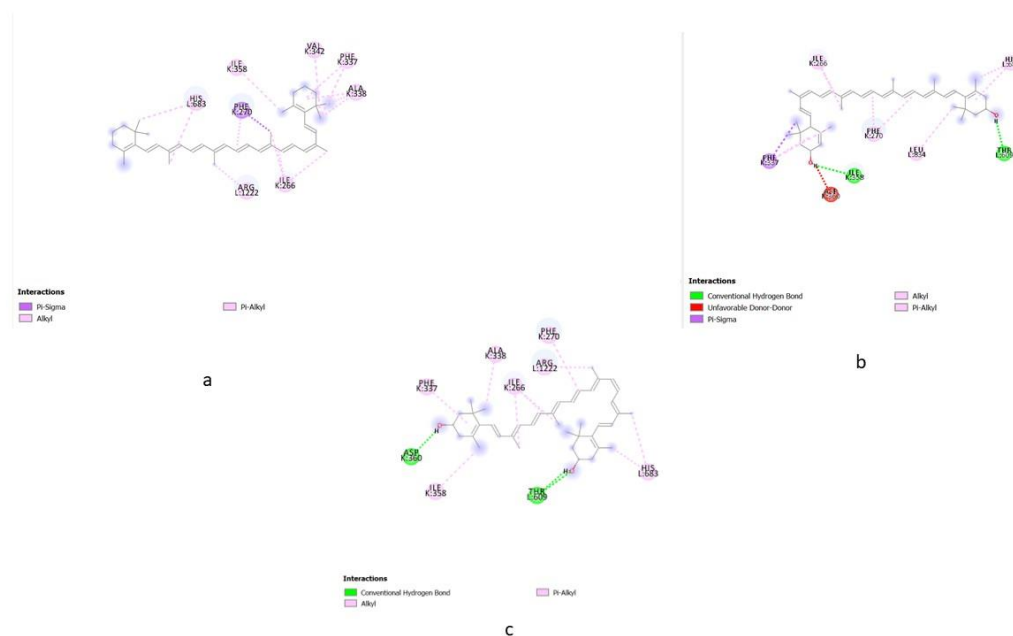


Figure 1. Ligand- Protein interaction 2D (a) β -carotene ;(b)lutein; (c) zeaxanthin

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Modification of chloroplast pigment composition by overexpression of carotenoid biosynthesis enzyme genes

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1. Introduction

In green leaves of plants, carotenoids have antioxidant properties and are important in alleviating excessive light stress. Lutein, the most abundant carotenoid in green leaves, has been reported to prevent cataracts and age-related macular degeneration in humans. Therefore, lutein has attracted attention as a functional ingredient and has recently been widely marketed as an eye supplement. In this study, we attempted to modify the carotenoid composition of leaves by overexpression of carotenoid synthase genes in order to grow crops that accumulate high levels of lutein.

2. Experimental

In *Arabidopsis thaliana*, lycopene β -cyclase (*LcyB*), lycopene ϵ -cyclase (*LcyE*), CYP97A3 (*Lut5*), and CYP97C1 (*Lut1*) have been identified as enzyme genes involved in lutein biosynthesis from lycopene. To identify the most important enzymes for increasing lutein accumulation, we inserted genes for *LcyB*, *LcyE*, *Lut5*, *Lut*, phytoene synthase (*PSY*), which regulates total carotenoid levels, and *ORANGE* protein, which interacts with *PSY*, into the *pBYR2HS* plasmid, a transient high expression vector, respectively. Each vector was introduced into *Agrobacterium*, and the *Agrobacterium* was inoculated into *Nicotiana benthamiana* green leaves to overexpress the enzyme genes, and chloroplast pigment composition of the inoculated leaves were analyzed.

3. Results and Discussion

While we found no significant increase in lutein content in leaves overexpressing each enzyme gene alone, we found that leaves overexpressing *LcyE* and *Lut5* simultaneously increased lutein content per chlorophyll by more than 20% over the control. This indicates that expression of an appropriate combination of multiple genes is effective in increasing lutein content. In addition, the changes in lutein content of the inoculated leaves over time showed that the amount of lutein per chlorophyll and the percentage of lutein in total carotenoids tended to increase up to 5~6 days after inoculation. However, leaf browning was observed after day 5~6. Therefore, we investigated the effects of foliar application of the antioxidant ascorbic acid or changes in light intensity during incubation with the aim of suppressing leaf browning. As a result, no effect of ascorbic acid spraying was observed. On the other hand, browning was suppressed by irradiating the plants with intense light for 3 days prior to *Agrobacterium* inoculation. These results could be due to the increased stress tolerance of the plants after high-intensity irradiation, suggesting the possibility of application during overexpression experiments of genes other than the carotenoid biosynthetic enzyme genes.

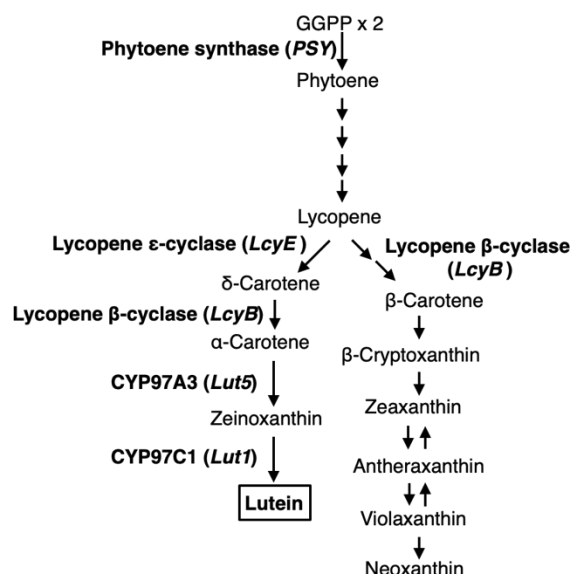


Figure1. Carotenoid biosynthesis pathway in *Arabidopsis*

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P8-16

Exploring mass spectrometry technologies to decipher metabolomic shifts during floral transition in *Arabidopsis thaliana*

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As sessile organisms, plants have evolved a multitude of mechanisms to acclimate to their environment. This enables the plant to optimise development and reproduction and fight off or resist both biotic and abiotic stresses they may encounter through their life cycles. One such mechanism is a communication system involving the release of volatile organic compounds (VOCs). VOCs are produced from different biosynthetic pathways, by nearly all plant tissues, and under diverse physiological processes, thus providing a rich complexity. VOCs are extensively studied but little is known about their biosynthesis and biological functions.

With the aim to discover new VOCs, different methods using mass spectrometry (MS) were explored to select compounds of interest involved during plant development and more precisely during the floral meristem transition. VOC signatures were suggested for seven main tissue types in *Arabidopsis*, and for the four floral organs. A temporal analysis was also performed to determine metabolomic shifts during floral development, and therefore new VOC putative roles during floral transition, morphogenesis, or anthesis. Finally, mutants in the carotenogenesis pathway were investigated to decipher the floral transition in more depth. The *clb5* mutant was shown to display important remodelling of the gene regulatory system, accumulating a signal that trigger a very early floral meristem transition. Putative signalling molecules and signalling pathways suggested carotenoids are involved in floral development initiation. The carotenoids act as signals to activate the resources needed by the plants to enter into transition (e.g., sugars, tricarboxylic acid cycle, methyl salicylate, L-proline) and were shown for the first time to be linked to floral development.

Overall, the results presented in this thesis extend our global understanding of VOC roles and their importance in the plant development. Given the chemical fingerprint analysis and deciphering floral transition, MS technologies provide new opportunities for VOC discovery and crop application to increase plant growth, yield, and protection against diverse stresses.

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Zeaxanthin loaded nanoemulsion systems – preparation, characterization and stability

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1. Introduction

Zea is an important contributor to eye and brain health and function but its bioaccessibility is low and is influenced by many host or dietary factors. The lipid components in food are essential for the absorption of carotenoids and can modulate their bioaccessibility, depending on the concentration, unsaturation degree and chain length [1, 2]. The aim of our work was to develop Zea and zeaxanthin dipalmitate (ZeaDP) loaded oil-in water nanoemulsions with oils having different composition of fatty acids (ratio between saturated and unsaturated fatty acids, medium or long chain fatty acids) and to examine their properties and stability in various conditions.

2. Experimental

Zea and ZeaDP were obtained at a purity of >95% from plant material by solvent extraction and purification on column and thin layer chromatography. Four vegetable oils (coconut MCT, olive, sunflower, linseed and palm oils) were characterized in terms of fatty acids and carotenoid composition by GC-MS and C30-HPLC-PDA. Zea and ZeaDP oil in water (O/W; 1:9 v/v) nanoemulsions doped with carotenoids (0.05 mg/ml in the final emulsion) were obtained by probe ultrasonic emulsification, in the presence of Tween 20 (at 1 %) as emulsifier. All emulsions were exposed to various pH values (3,4,5,6,7,8), temperatures (4, 20, 37, 50, 60°C) and NaCl concentrations (0, 25, 50, 100 and 250 mM), over a time interval of up to 30 days. Nanoemulsions were characterized by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). The stability of the emulsions was evaluated by measuring the mean diameter and distribution of the particles, zeta potential and residual carotenoid content.

3. Results and Discussion

Very good incorporation yields of Zea and ZeaDP in nanoemulsions were found for all vegetable oils (up to 98%). The average hydrodynamic diameter was variable (100-200 nm), depending on the type of liquid oil (chain length, unsaturation degree). The polydispersity index was less than 0.3 and the surface was negatively charged (zeta potential). The emulsions obtained with solid fats (palm oil) had higher diameters, up to 250 nm and higher polydispersity indices compared with those obtained with liquid oils. Nanoemulsions with ZeaDP had a larger diameter than those with free Zea, regardless of the type of fat used. All nanoemulsions had a good visual stability after 30 days, at room temperature and in the dark, with a retention of carotenoids higher than 75 % (30 days). They were stable at temperatures suitable for possible industrial applications (refrigerated foods, nutraceuticals), namely at 4, 20°C, at salt concentrations below 50 mM and at moderate pH values (5-8), even at longer storage period (15-30 days).

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P9-2

Bioaccessibility and cellular uptake of zeaxanthin from oil-in-water nanoemulsions

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1. Introduction

Zeaxanthin (Zea), together with lutein, are widely known as the macular carotenoids, due to their accumulation in the human retina. Although Zea is present in the human diet, its accumulation in human tissues is limited by its reduced bioavailability. It is known that the lipid fraction of food is essential for the absorption of carotenoids and it can increase their bioaccessibility [1]. The aim of our work was to develop Zea and zeaxanthin dipalmitate (ZeaDP) loaded oil-in water nanoemulsions with oils having different composition of fatty acids (ratio between saturated and unsaturated fatty acids, medium or long chain fatty acids) and to examine their bioaccessibility and cellular uptake.

2. Experimental

Zea and ZeaDP were separated and purified from vegetal sources by chromatography. Four vegetable oils (coconut MCT, olive, sunflower, linseed and palm oils) were characterized in terms of fatty acids by GC-MS. Zea and ZeaDP oil in water (O/W; 1:9 v/v) nanoemulsions doped with carotenoids (0.05 mg/ml, 1% Tween 20) were obtained by probe ultrasonic emulsification. Bioaccessibility of carotenoids was determined by the INFOGEST standardized *in vitro* digestion protocol [2]. Cellular uptake in Caco-2 and RPE cells was determined by C30-HPLC-PDA and visualized using a Raman microspectrometer (InVia, Renishaw). Raman spectra were recorded with a 532 nm laser at a spectral resolution of $\sim 4\text{ cm}^{-1}$. All data were analyzed using the QUASAR software and principal component analysis (PCA) was used to identify multiple cellular components.

3. Results and Discussion

The bioaccessibility of free zeaxanthin was higher than that of ZeaDP, regardless of the oil used. The bioaccessibility of both carotenoids was higher for nanoemulsions compared to non-emulsified oils doped with carotenoids. Nanoemulsions with Zea obtained with coconut oil and those with ZeaDP and olive oil showed the highest bioaccessibility, with 60% and 42%, respectively, which is far superior to the bioaccessibility of Zea from known food matrices. Cellular uptake and secretion of Zea in intestinal and retinal cells was superior from nanoemulsions compared to solvent delivery (DMSO), and dependent on the type of emulsions. The highest values were obtained for nanoemulsions with unsaturated oils. The presence of Zea in the cytosol of RPE cells, near the nucleus, was qualitatively confirmed by Raman mapping, based on specific and intense signals at wavenumbers 1152 cm^{-1} (C-CH₃ stretching) and 1520 cm^{-1} (C=C stretching) (see Figure 1).

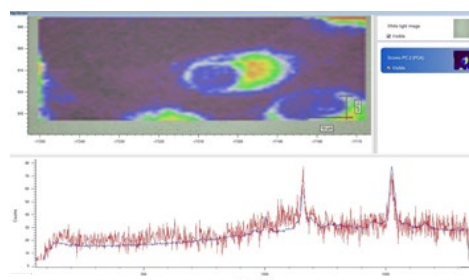


Figure 1. Raman mapping of Zea in RPE

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-1172, number 243/2021 within PNCDI III.

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Low-tech production of astaxanthin-rich copepods: Towards a self-sustainable and decentralized food production system

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1. Introduction

Human beings are currently facing the greatest challenge of our species. Immersed in a migration from fossil fuels to renewable energy, we need to increase food production to feed the ever-growing population. Unfortunately, current food production methods (agriculture, livestock, fishing and aquaculture) are severely impacted by climate change, and in turn, contribute to its exacerbation. To adapt to this scenario we must transform existing food production systems and establish new ones that are climate-neutral, are resistant to global warming and employ low-tech approaches, to contribute to the food security of all, including low-income communities.

2. Experimental

We developed and tested a proof-of-concept prototype bioreactor to continuously co-produce astaxanthin-rich copepods (*Tigriopus californicus*) in a non-sterile continuous culture with a single species of microalgae (*Tetraselmis chuii*) as the feed source. The prototype operated using solar light and atmospheric air (Fig. 1).

3. Results and Discussion

The rich microbial ecosystem established in the reactor regulated the water's pH and consumed dissolved nitrogen and phosphorus, thus maintaining water quality. This allowed for zero effluent discharges during a 3-month running period [1]. The projected annual zooplankton biomass productivity of the prototype was 1.56 Kg m² year⁻¹ (ww), which is triple the average biomass productivity of soybean, at 0.45 Kg m² year⁻¹ (ww). The zooplankton was composed (dw) of 66% protein containing all essential amino acids, 1.6% of the omega-3 fatty acids EPA and DHA, and 1.9% of the carotenoid all-trans 3S,3'S-astaxanthin [2].

Figure 1. Zooplankton harvested from the prototype bioreactor.



We are currently applying for funding to upscale the bioreactor to a commercial scale. Challenges include reducing energy use as the volume/area ratio in the bioreactor increases, minimizing land use, testing automatic collection devices, and evaluating the composition and safety of the zooplankton. We will use the astaxanthin-rich copepods produced as aquafeeds, in applications that need of this carotenoid to achieve appropriate coloration, and as direct human food, starting with premium applications such as a sushi ingredient, leveraging our research center's experience conducting human trials.

The protein production methods currently being developed, such as single-cell protein production, rely on technologies such as hydrogen production, natural gas flare use, or atmospheric CO₂ capture that may limit their implementation in low-income communities, which in turn are the most greatly affected by the climate change. Our low-tech copepod production method aims to provide these communities with a resilient method of food production that can be used to produce aquafeeds and for direct human consumption.

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Preparation, Evaluation and Bioavailability of a Novel Lutein-fortified Yoghurt: Fortixan

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1. Introduction

The health benefits of carotenoids have been extensively demonstrated in animal and clinical studies. Lutein is a prominent carotenoid in human health, as it protects the retina from photo-oxidative and inflammatory damage. The main dietary source of lutein is green-leafy vegetables, but due to the generally low intake of these foods, this carotenoid is not sufficiently consumed. These facts have attracted market interest, and lutein is currently commercialized as a dietary supplement worldwide. Although these supplements provide a means of increasing lutein intake, their use is limited in certain populations, such as children and the elderly. Thus, providing more convenient methods to supplement lutein intake in these population groups is desirable. In this study (Fortixan), we developed a lutein-fortified yoghurt and conducted a bioavailability study in human subjects to evaluate its effectiveness to increase lutein levels in blood.

2. Experimental

We developed a yoghurt with 14.6 mg of lutein per 100 g of product. For this, we used a commercial lutein emulsion compliant with the European Food Safety Authority (EFSA) regulations. The entire preparation process rigorously adhered to a Hazard Analysis and Critical Control Points (HACCP) plan. The safety of the yoghurt were confirmed through component analysis and microbiological testing. The sensory attributes of the yoghurt were assessed by independent experts. The content and stability of lutein in the yoghurt was determined using high-performance liquid chromatography (HPLC). The bioavailability of lutein in the yoghurt was evaluated over a 72-hour period.

3. Results and Discussion

Lutein remained stable in the yoghurt throughout the 35-day shelf life (14.60 ± 0.41 at day 1 and 14.60 ± 0.19 mg/100 g at day 35). Also, an expert third-party sensory analysis of the yoghurt yielded satisfactory parameters in terms of appearance and flavor. In the Fortixan bioavailability study, a single-dose of lutein-fortified yoghurt (100 g) significantly increased blood lutein levels by 45.2 ± 19.1 µg/L and followed a typical pharmacokinetic absorption and decay curve. These findings confirm that our novel lutein-fortified yoghurt is suitable for commercial use.

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β -carotene Degradation in a Sport Isotonic Beverage

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1. Purpose

β -carotene is a precursor for vitamin A, which is an essential vitamin required for vision. This carotenoid is used as a natural food additive for food colourant purposes. Research shows that when exposed to light and/or high temperatures, carotenoids will degrade. The purpose of this study was to evaluate β -carotene degradation in a sport isotonic beverage.

2. Experimental Materials & Methods

β -Carotene content of a sport isotonic beverage was quantified with spectrophotometry and high-performance liquid chromatography (HPLC) under three accelerated storage conditions, including high temperature (50°C), high light exposure (fluorescent light), and low light exposure refrigeration (3.4°C). β -carotene analysis was conducted weekly over a four-week period. The sport isotonic beverage assessed for this experiment was within shelf life of expiry date.

3. Results

β -Carotene concentration at baseline was 1.25 mg/500 mL. After a period of one-month, β -carotene showed degradation in all three storage conditions: 92.1% in high temperature, 74.5% in high light exposure, and 28.8% in low light exposure refrigeration. The storage condition that degraded β -carotene the most was high temperature, followed by high light exposure, and finally in low light exposure refrigeration.

4. Conclusion

This study confirms that carotenoids used in commercial food products such as isotonic beverages are subject to degradation, and the conditions in which they are stored has a significant impact on the rate of degradation. This work has implications for functional foods using carotenoids and should inform regulatory guidelines.

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P9-6

Simultaneous Determination of Fat-Soluble Vitamins in Beverages Using a Triple Quadrupole Mass Spectrometer

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1. Introduction

Vitamins are responsible for maintaining normal body functions. Because they cannot be synthesized in the body, they need to be obtained from food. Vitamins A, D, E, and K, which are not soluble in water, are called fat-soluble vitamins. Vitamin A mainly refers to retinol, which has the function of keeping eyes and skin healthy and enhancing immunity. On the other hand, α -carotene and β -carotene are called provitamin A because they are converted to retinol in a body. The efficiency of the conversion to retinol differs between them, and the bioavailability of α -carotene and β -carotene is estimated to be 1/24 and 1/12, respectively [1]. To evaluate the activity of vitamin A, individual quantification of α -carotene and β -carotene is necessary. Since these are isomers, it had made separation difficult with LC-MS/MS in the past. In this study, we introduce simultaneous determination of fat-soluble vitamins, which achieved individual quantification of α/β -carotene by examining a LC condition. We performed individual quantification of α/β -carotene and recovery tests on vegetable juice using this method. Since α/β -carotene does not have ester compounds, the alkaline hydrolysis treatment normally required for sample preparation can be omitted. Good recovery rates were achieved with a simple extraction operation.

2. Experimental

Adding 5 g of anhydrous sodium sulfate to 1 g of commercially available vegetable juice, ethanol was added and the mixture was shaken for 5 minutes. After centrifugation at 4,000xg for 5 minutes, the supernatant was collected. The above extraction process was repeated twice with the remaining sediment. The collected supernatants were combined and made up to 40 mL with ethanol. After diluting the extraction solution 100 times with ethanol, the supernatant was collected as the extraction sample after centrifuging at 15,000 rpm for 10 minutes. The analysis was carried out using a system that combined a triple quadrupole mass spectrometer LCMS-8050 and an ultra-high-speed liquid chromatograph Nexera X3.

3. Results and Discussion

We achieved separation of α -carotene and β -carotene on MS chromatogram by optimized a LC condition. Accurate and wide-range quantification was successful. Calibration curves were in the range of 0.5 to 1000 ng/mL for alpha-carotene and 0.5 to 500 ng/mL for beta-carotene. Using the developed method, individual determination of alpha-carotene and beta-carotene in commercial vegetable juices was attempted. Two types of vegetable juices were used, both with good recoveries. It was confirmed that a simple pretreatment without saponification could be used for accurate quantification.

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Impact of High Pressure Processing on Lipophilic Bioactive Plant Ingredients in Kale and a Microemulsion System

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1. Introduction

High pressure processing (HPP), a gentle food preservation technique, is characterized by a minimal impact on sensory, nutritional and functional food characteristics, considering pressure regimes up to 600 MPa [1]. In contrast to hydrophilic compounds (e.g. vitamin C), effects on lipophilic ingredients such as carotenoids and vitamin E are still less investigated. Low pressure regimes (<200 MPa) may contribute to an enrichment of carotenoids via *de-novo* biosynthesis prior to preservation at high pressure [2]. Thermodynamically stable microemulsions containing lipophilic ingredients of increasing complexity may help to unravel pressure-driven interactions in food matrices such as lipoxygenase (LOX)-mediated carotenoid co-oxidation processes.

2. Experimental

Crushed kale leaves (Retsch Grindomix GM200) were pressurized at room temperature at 10 - 600 MPa for 5 - 40 min and steam-sterilized at 120 °C for 20 min. Carotenoids and chlorophylls as well as vitamin E were analysed via RP-HPLC-DAD and NP-HPLC-FLD, respectively. Antioxidant capacities were determined via lipophilic TEAC-/ORAC-assays. Bioaccessibilities in pressurized kale were investigated by an *in-vitro* digestion model [3]. An oil-in-water microemulsion containing Tween 80, Transcutol®HP and Capryol™90 [4] was applied to study pressure stabilities and interactions of compounds in combination with aqueous enzyme extracts from soybeans in presence of linoleic acid. (Figure 1)

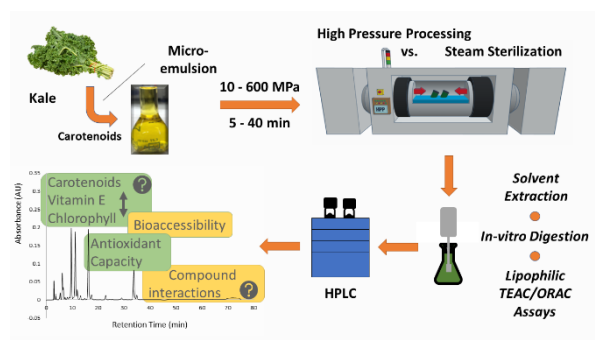


Figure 1: Investigation of effects of high pressure processing on carotenoids in kale and a microemulsion model system.

3. Results and Discussion

A gentle character of HPP was determined by significantly increased ($p < 0.05$) antioxidant capacities compared to steam-sterilized kale considering heat-labile carotenoids and chlorophylls. Operations at 100 MPa reduced the total carotenoid content and bioaccessibilities of (*all-E*)- β -carotene and (*all-E*)-lutein in kale significantly, potentially caused by activated LOX. Pressure-labile properties were observed for (*all-E*)- β -carotene and chlorophylls in a microemulsion. The addition of vitamin E to a microemulsion containing a raw enzyme extract with LOX and linoleic acid resulted in increased contents of (*all-E*)- β -carotene during storage studies. Further pressure-driven experiments on LOX-activation and carotenoid stability in microemulsions of more complex composition will be presented to increase knowledge about observed trends of carotenoid retention in kale pressurized at low and high pressure (10 - 600 MPa).

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P9-8

Bioefficacy of β -cryptoxanthin in comparison to β -carotene using stable isotopes

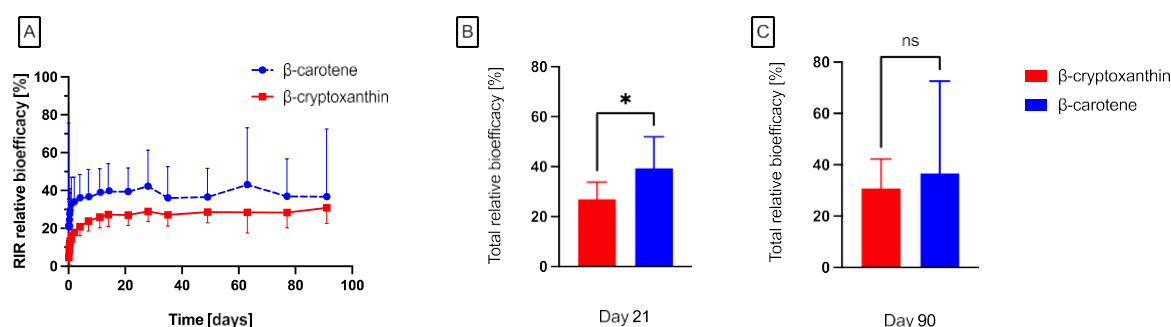
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Efficacy studies have demonstrated that increasing provitamin A carotenoid (pVAC) intake through consuming vitamin A- biofortified crops results in increased circulating β -carotene (β C) and vitamin A (VA) body stores (1,2). However, despite the fact that β -carotene is the primary focus of breeding programs for provitamin A biofortified maize, considerable degradation of β C has been observed in maize under typical storage conditions, processing and cooking (3). Since β - cryptoxanthin (β CX) has been shown to be more stable during storage compared to β C (4) and has an apparent bioavailability of 1.8 times higher than that of β C (5), we explored if β CX could be used in biofortified maize. However, since β CX has a 4 times lower affinity to the carotenoid cleavage enzyme β -carotene 15,15'-oxygenase (BCO1) than β C (6), we explored the bioefficacy of both pVACs over a 90-day period in healthy human volunteers through the application of ^{13}C -labeled pVACs and ^2H -labeled retinol.

Stable isotopes were administered in a β CX: β C ratio of 2:1, with 2mg of [$^{13}\text{C}_{14}$]- β CX and 1mg of [$^{13}\text{C}_{10}$]- β C applied alongside 1mg of [$^2\text{H}_6$]-retinyl acetate as reference dose. Blood samples were drawn at 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h on day 0, followed by single blood draws on days 1, 2, 4, 7, 11, 14, 21, 28, 35, 49, 63, 77, and 91 in 8 volunteers. Relative bioefficacy of both carotenoids were calculated based on the plasma retinol isotope ratio (RIR) as outlined previously (7).

Intestinal relative bioefficacy of [$^{13}\text{C}_{14}$] β CX and [$^{13}\text{C}_{10}$] β C were $17.1\% \pm 6.3\%$ (mean $\pm$ SD) and $35.3\% \pm 14.3\%$, respectively. Thus, intestinal relative bioefficacy was 2.1fold higher for [$^{13}\text{C}_{10}$] β C compared to [$^{13}\text{C}_{14}$] β CX. This difference in relative bioefficacy using the RIR method between both carotenoids declined as time went on (**Figure 1A**). Total relative bioefficacy increased for both carotenoids due to post-intestinal bioefficacy to $31.9\% \pm 8.8\%$ (mean $\pm$ SD) for [$^{13}\text{C}_{14}$] β CX and to $41.3\% \pm 22.4\%$ for [$^{13}\text{C}_{10}$] β C. Therefore, the significant difference in total relative bioefficacy between both carotenoids at day 1 and day 21 was no longer significant at day 90 ($p > 0.05$, paired t-test) (**Figure 1B, C**).



Relative and post-intestinal bioefficacy of [$^{13}\text{C}_{10}$] β -carotene and [$^{13}\text{C}_{14}$] β -cryptoxanthin. Panel A) relative bioefficacy of [$^{13}\text{C}_{10}$] β -carotene and [$^{13}\text{C}_{14}$] β -cryptoxanthin from day 0 to day 90 calculated using the plasma RIR (19); Panel B & C) Total relative bioefficacy of [$^{13}\text{C}_{10}$] β -carotene and [$^{13}\text{C}_{14}$] β -cryptoxanthin using the plasma RIR at day 21 and 90; Data are represented as geometric means $\pm$ 95% CI. * significant different at $p < 0.05$ (paired t-test).

In summary, due to a 2.8fold higher post-intestinal bioconversion of β CX compared to β C, the observed lower relative bioefficacy of β CX is reduced over time. This makes β CX an attractive alternative source for biofortification compared to the traditional breeding of β C enriched genotypes.

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P10-1

Assessment of dietary supplements containing lutein, zeaxanthin and *meso*-zeaxanthin and/or omega-3 fatty acids for compliance to label claim

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1. Introduction

The food supplement industry has seen substantial growth in recent years, particularly in the area of carotenoids and omega-3 fatty acid supplements. While specific food supplements can have a significant positive impact on human health, as demonstrated by our laboratory, consumer knowledge and information about the active supplement ingredients and their stability over shelf life is limited. Carotenoids are permitted under Directive 2002/46/EC, however there are no statutory regulations for these supplements. Our pilot data on products tested so far suggests major disparities between label claim and active ingredients measured, and issues with degeneration of products during their shelf life.

2. Experimental

For our initial work, we have analyzed 94 products containing either carotenoids and/or the omega-3 fatty acids EPA and DHA. Products analyzed were formulated as either soft gels, powder capsules or hard capsules. Carotenoids were analyzed by chiral HPLC and fatty acids were analyzed by GC-FID at our campus company Supplement Certified Ltd. All products were analyzed in triplicate and quantified using externally prepared calibration lines as previously described [1, 2].

3. Results and Discussion

Our analysis reveals that out of the 94 products analysed to date, 46 complied with their label claim, whereas 48 did not. As part of our research and development work, we also present here a comprehensive summary of the various regulatory frameworks applicable to dietary supplements in Europe, the US, and Canada. Our research shows that there are gaps in the regulatory requirements in these territories that allow companies to sell and distribute supplements containing carotenoids and/or fatty acids without appropriate testing to confirm product label claim. In order to address these issues in terms of product quality and regulatory challenges, we strongly recommend that changes in policy and regulatory guidelines, to include third-party assessment of product active ingredients are needed.

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P10-2

Imaging Macular Carotenoids and Their Related Proteins in the Human Retina with Confocal Resonance Raman and Fluorescence Microscopy

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1. Introduction

The retinal content of carotenoids is inversely associated with the risk of human retinal disease such as age-related macular degeneration (AMD), a common cause of blindness in the elderly. Carotenoid supplementation can reduce the risk of AMD. The xanthophyll carotenoids lutein and zeaxanthin are concentrated specifically at the center of the human retina with extremely high concentration, forming a visible yellow spot called the *macula lutea*; therefore, these two carotenoids are also called the macular carotenoids. Genetic and biochemical studies have revealed many proteins involved in the specific tissue distribution, transport, and metabolism of macular carotenoids; however, the mechanisms underlying the formation of the *macula lutea* are still not fully elucidated. In this work, we systematically investigated the retinal distribution of the currently known macular carotenoid-related proteins and that of the carotenoids using confocal resonance Raman microscopy and fluorescence microscopy.

2. Experimental

Human donor eyes were fixed in 4% PFA for one hour, then an 8-mm macular punch was taken from each eye. One punch was used to generate a flat-mounted retina, while the other was embedded for retinal sections as before. We then checked the distribution of carotenoid-binding proteins (GSTP1 and StARD3), carotenoid cleavage enzymes (BCO2 and BCO1), carotenoid transporters (SR-BI, ABCA1, LIPC, CD36, and LDLR), and two newly reported carotenoid-related proteins (Aster and TTC39B) using our established immunohistochemistry (IHC) procedure. All primary and secondary antibodies were purchased from vendors, and the secondary antibody was conjugated with Rhodamine-Red fluorophore. Fluorescence imaging measurement was conducted using the fluorescence spectrometer mode of the Horiba XploRA Plus confocal resonance Raman microscope with the excitation of a 532-nm green laser, while the carotenoids were detected by the Raman spectrometer mode with a 473-nm blue laser. All the data were analyzed using Horiba LabSpec 6 software.

3. Results and Discussion

On flat-mounted human retina, more GSTP1 was detected in the fovea than the peripheral retina, showing a similar distribution pattern to the macular carotenoids. Stronger signals of BCO2, BCO1, Aster, and CD36 were detected in the fovea as well. TTC39B is strongly expressed in the fovea and optic nerve regions (**Figure 1**). Interestingly, stronger expression of LIPC was found in sub-foveal relative to the peripheral RPE. No specific retinal distribution was found for the other proteins. Similar distribution patterns were seen in the human retinal sections. Our data offer insights into the formation mechanism of *macula lutea*.

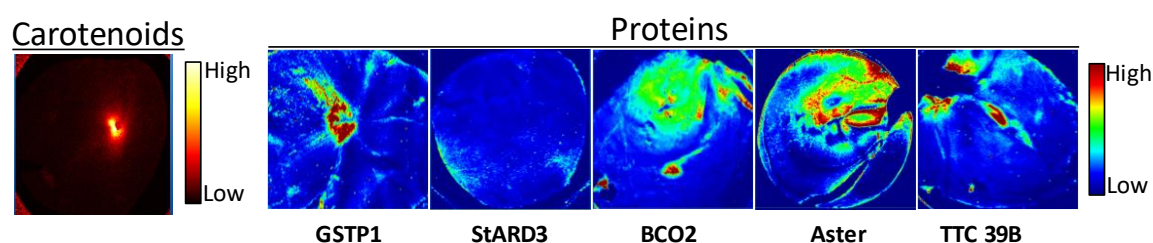


Figure 1. Retinal distribution of macular carotenoids and their related proteins (selected).

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P10-3

Is electronic device use related to macular pigment optical density, dietary and plasma lutein and zeaxanthin in healthy Australian adults?

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1. Introduction

Lutein and zeaxanthin (L/Z) are dietary carotenoids highly concentrated in the macula in the eye which can be measured by macular pigment optical density (MPOD). Low L/Z concentration has been associated with increased risk and severity of age-related macular degeneration. [1] L/Z may protect the macula through direct and indirect antioxidant activity, and absorption of blue light. [1] Blue light exposure from electronic device screen exposure has been hypothesised as an environmental factor impacting MPOD status due to its potential to cause photochemical damage. [2] This cross-sectional study aimed to investigate the associations between electronic device use, dietary L/Z intake, plasma L/Z and MPOD status in healthy Australian adults.

2. Methods

MPOD was measured via heterochromatic flicker photometry, plasma L/Z was analysed via high performance liquid chromatography. Dietary L/Z intake was assessed via a 91-item screener with a month recall timeframe developed for the study. Daily electronic device use was assessed via frequency questionnaire purposely developed to recall usual electronic device use as an indication of screen-emitted blue light exposure. Body fat percentage was measured via bioelectrical impedance scales. The relationship between variables was assessed by multiple linear regression analysis, and a p value of <0.05 was considered statistically significant.

3. Results

Participants included 65 females and 31 males with a median age of 27 years (Table 1). Plasma L/Z was the only variable individually correlated with MPOD, $r = 0.28$, $R^2 = 0.35$, ($p = 0.008$). Dietary L/Z intake was significantly correlated with plasma L/Z, $r = 0.28$, $R^2 = 0.35$, $p = 0.008$. There were no significant associations in the models to predict MPOD from age, sex, electronic device use, and dietary L/Z ($F(4, 87) = 1.396$, $p = 0.24$, adjusted $R^2 = 0.06$) or plasma L/Z ($F(4, 77) = 2.927$, $p = 0.026$, adjusted $R^2 = 0.087$ respectively).

4. Discussion and conclusions

The study outcomes suggested blue light exposure from habitual electronic device use is not related to MPOD. The memory recall bias previously reported with recall of dietary intake and activities such as electronic device use [3, 4], in addition to the homogeneity of participant characteristics (predominantly female, tertiary educated and high device users), may have contributed to the observed lack of relationship between MPOD, age, sex, electronic device use, dietary L/Z intake, and plasma L/Z in this study. Future studies may benefit from a more diverse study population, in particular with diversity in electronic device use.

Table 1. Participant characteristics^a, n = 96

Age (years)	27 (24 – 40)
Body Fat (%)	27 (8.4)
MPOD (ODU)	0.42 (0.16)
Plasma L/Z concentration ($\mu\text{g/mL}$) [*]	0.24 (0.15 – 0.31)
Dietary L/Z intake (mg/day)	4.6 (2.7 – 7.4)
Mean of all devices (hours/day) [^]	9.1 $\pm$ 3.1
Television (hours/day)	3.0 (1.0 – 5.0)
Computer (hours/day)	8.5 $\pm$ 4.1
Handheld (hours/day)	4.8 (3.0 – 7.0)

^aData as median (25th – 75th percentile) or mean $\pm$ standard deviation. ^{*}n = 86. [^]n = 91. ODU, optical density units; L/Z, lutein and zeaxanthin; n = number of participants

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P10-4

An Evaluation of Xanthophyll Carotenoids, Visual Function and Sport Performance in an Athlete Population

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1. Introduction

A shooter's visual system is always under intense pressure to achieve stability of hold, timing of triggering, aiming accuracy and cleanliness of trigger, which influences shooting performance and are partly dependent on visual stimuli. There is currently limited data on dietary or supplemented lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) and visual performance for athletes. Here we report descriptive data for shooting athletes who are members of the Sport Singapore Institute (SSI) in an attempt to establish relationships, if any, between carotenoid status, visual function (VF) and sports performance.

2. Experimental

VF was assessed by measuring contrast sensitivity (CS); sporting performance was analysed using a shooting score at the end of 60 shots obtained at the shooting practice range; and carotenoid status was measured by dietary intake of L and Z and skin carotenoid concentrations.

3. Results and Discussion

A total of 24 athletes were assessed in the SSI. The mean (range) age of the participants was 28 (18 to 50) years, and 50% (n = 12) were women. Mean $\pm$ SD dietary intake of L and Z was $3,214 \pm 3513$ μ g/day and mean $\pm$ SD skin carotenoid concentration was $32,083 \pm 15736$. Mean $\pm$ SD CS photopic and mesopic was 2.88 ± 1.13 % and 4.9 ± 2.61 %, respectively, and mean $\pm$ SD shooting performance score was 601.76 ± 49.85 . There was no significant difference between men and women for any of the variables assessed ($p > 0.05$, for all). In addition, we were unable to identify any relationship between carotenoid status, VF, and sport performance. This null finding may be explained by the small sample size to date. Continuation of this study to recruit 100 athletes is likely to provide valuable data relating to the proposed link between carotenoid nutrition, VF, and sport performance.

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P10-5

Lutein and its metabolites modulate inflammation, oxidative stress, and lipid peroxidation at glutamate-induced neurotoxicity

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1. Introduction

The relevance of the xanthophyll lutein in visual health, mainly improving age-related macular degeneration and light-induced oxidative stress, is well-known [1]. Lutein has been published with benefits to cognitive function together with its antioxidant and anti-inflammatory features in neuron and microglia as well [2,3]. Lutein can be metabolised to 3'-epilutein and 3'-oxolutein among others. The 3'-epilutein is a natural metabolite of lutein in humans also can be extracted from *Caltha palustris* petals. The 3'-oxolutein is the main oxidation product of lutein. The bioavailability of lutein derivatives might be varying; thus their effects can also alter.

Glutamate, the pivotal excitatory neurotransmitter in central nervous system (CNS), plays a key role in regulating brain function. Excessive accumulation of glutamate accelerates an increase in the concentration of reactive oxygen species (ROS) in neurons [4]. Glutamate induces toxicity and ROS production in neurons in concentration dependent way, suggesting that the toxic effect of glutamate is related to oxidative stress [5]. ROS in CNS plays a key role in regulating inflammatory responses, iron homeostasis, apoptosis and lipid peroxidation inter alia [6]. We focused on the effect of lutein, 3'-epilutein and 3'-oxolutein on glutamate-induced neurotoxicity.

2. Experimental

SH-SY5Y human neuroblastoma cells were treated with glutamate to induce oxidative stress. The cells were treated with lutein, 3'-epilutein or 3'-oxolutein in a concentration and time-dependent manner. ROS measurements were carried out and cytokine ELISA measurements were obtained. Total antioxidant capacity was determined, Real-time PCR experiments were performed and thiol concentration was measured in the cells.

3. Results and Discussion

Glutamate treatment increased intracellular ROS in SH-SY5Y cells, while lutein, 3'-epilutein and 3'-oxolutein downregulated ROS. Lutein and its metabolites altered the secretion of inflammatory cytokines upregulated by glutamate. The total antioxidant capacity of cells was decreased upon glutamate while lutein, 3'-epilutein and 3'-oxolutein significantly increased it alone and in the presence of glutamate as well. Glutamate upregulated the expression of lipid-peroxidation related genes, which was modified upon carotenoids. Thiol content of the cells showed elevation after glutamate treatment, while lutein, 3'-epilutein and 3'-oxolutein decreased it.

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[3] R. Pap, E. Pandur, G. Jánosa, K. Sipos, A. Agócs, J. Deli, *Antioxidants*, **2021**; 10, 3–363.

[4] H. Xin, Y. Cui, Z. An, Q. Yang, X. Zou, N. Yu. *RSC Adv.* **2019**, 9, 34735–34743

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[6] J.-F. Ying, Z.-B. Lu, L.-Q. Fu, Y. Tong; Z. Wang, W.-F. Li, X.-Z. Mou. *Am. J. Cancer Res.* **2021**, 11, 1895–1912.

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P10-6

Xanthophylls as moderators of cognitive functional improvement during a 6-month cognitive training intervention in older persons at risk for Alzheimer's disease or a related dementia

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1. Introduction

Macular pigment (MP) is composed of xanthophyll carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin. Of these three carotenoids, L+Z are the dominant carotenoids in diet and human neocortex. Retinal and cortical L+Z levels are strongly correlated, and past studies have shown that supplementing L+Z improves cognitive function and changes brain activation and brain function in older people at risk for Alzheimer's disease or a related dementia (ADRD). In this study, we investigate whether these xanthophylls can moderate cognitive functional improvement from a cognitive training program designed for older persons at risk for ADRD.

2. Experimental

Older participants ($M=72.7\pm4.9$ years; 83% White/Caucasian; 65.1% Female) with early-stage cognitive impairment and less than 3-years of past musical experience were invited to participate in a clinical trial on the effects of a technology-delivered music (piano) learning cognitive training intervention. Participants completed a baseline diagnostic interview and neuropsychological assessment that included a 45-minute cognitive battery using the CNS Vital Signs test platform. Cognitive assessments were repeated after 6-months of participation in the cognitive training intervention. During the intervention, participants attended weekly piano lessons at the University of Georgia Institute of Gerontology and completed daily practice at home. The primary outcome was change in raw scores in the composite memory domain; the secondary outcome was change in global cognitive function (measured as the computed neurocognitive index [NCI] score). Macular pigment was measured at baseline using customized heterochromatic flicker photometry at 30-minutes of eccentricity, compared to a 7-degree parafoveal reference.

3. Results and Discussion

Here, we present data from the sub-sample of participants who have already completed the ongoing intervention ($n = 29$). To determine the relation between baseline MPOD and magnitude of change in memory and global cognition endpoints, difference scores were computed between baseline and 6-month raw composite memory scores and computed NCI scores. Given the fact difference scores in memory and global cognition were not normally distributed, Spearman ρ was computed to determine the relation between baseline MPOD and magnitude of cognitive change over the 6-month intervention. At baseline, MPOD ranged from 0.13-0.77 log units ($M=0.47\pm0.18$). Participants with higher baseline MPOD had larger difference scores in both the NCI ($r_s=0.47$, $p=0.018$) and composite memory ($r_s=0.50$, $p = 0.010$) measures. Past studies have shown increasing dietary xanthophylls can improve cognitive function in older people at risk for ADRD. The mechanisms for how L+Z impact brain and cognitive function are not entirely known, but our laboratory has shown that MPOD is related to white matter integrity in older persons. White matter integrity is also improved by cognitive training, particularly working memory training. These results suggest that CNS L+Z may moderate the effects of cognitive training on memory function.

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P10-7

Infant Ocular and Systemic Effects of Prenatal Carotenoid Supplementation in the Lutein and Zeaxanthin in Pregnancy (L-ZIP) Trial

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1. Purpose

Lutein (L) and zeaxanthin (Z) are carotenoid pigments that accumulate in developing infants' retinas and brains *in utero* and are detectable even at birth, suggesting a possible maternal transfer. Our understanding of how prenatal carotenoid supplementation impacts infants' ocular and systemic carotenoid status is limited. Therefore, for our secondary aim in this randomized clinical trial (NCT03750968), we investigated the effects of prenatal L and Z supplementation on newborn infants' ocular and systemic carotenoid status.

2. Methods

We randomly assigned 47 pregnant women to receive standard-of-care daily prenatal vitamins with added 10 mg L and 2 mg Z (Carotenoid Group) or standard-of-care daily prenatal vitamins with a placebo (Control Group) for 6 to 8 months. Infants' skin, serum, and macular pigment were assessed using resonance Raman spectroscopy, high-performance liquid chromatography, and RetCam, respectively. Student's t-test compared infants' carotenoid status in the study groups, and a p-value of 0.05 was considered statistically significant.

3. Results

Infants whose mothers were in the Carotenoid Group ($n = 19$) had a significant 5-fold increase in cord blood L + Z concentrations, over a 3-fold increase in cord blood total carotenoids, and a 38% increase in skin carotenoid status compared to the Control Group ($n = 18$) ($p < 0.0001$ for all). Although not statistically significant, infants whose mothers were in the Carotenoid Group had a 20% increase in macular pigment and more matured foveal parameters relative to those in the Control Group ($p = 0.242$).

4. Conclusion

Prenatal carotenoid supplementation remarkably enhanced infants' systemic and ocular carotenoid status during pregnancy and could provide health benefits to babies. Our findings will facilitate the design of future large-scale studies on prenatal lutein and zeaxanthin supplementation in nutritionally compromised women and high-risk pregnancies.

P10-8

 β , β -carotene-9',10'-oxygenase 2 (*Bco2*^{-/-}) Mice Are Protected against Oxygen-Induced Retinopathy (OIR) Relative to Wild-type (WT) Mice

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*Department of Ophthalmology and Visual Sciences, John A. Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT***1. Introduction**

Retinopathy of prematurity (ROP) is one of the leading causes of childhood blindness in the United States and throughout the globe. The mouse oxygen-induced retinopathy (OIR) model mimics human ROP. Premature infants at risk of ROP are carotenoid-deficient because they miss placental transfer of lutein (L), and zeaxanthin (Z), during the third trimester of pregnancy. Oxidative stress is one of the major causative factors of ROP, and we recently demonstrated that prenatal supplementation with L or Z ameliorates OIR in *Bco2*^{-/-} mice. We utilized β , β -carotene-9',10'-oxygenase 2 (*Bco2*^{-/-}) mice because unlike wild-type (WT) mice, they are genetically engineered to take up L and Z into the retina, and we noted that knockout of *Bco2* without carotenoid supplementation partially protected against OIR. In this study, we explore the inherent possible protective effects of *Bco2*^{-/-} knockout on OIR.

2. Methods

Male and female *Bco2*^{-/-} or WT mice were set up on a normal breeder diet (3980X, Envigo). P7 mouse litters of *Bco2*^{-/-} or WT mice along with their nursing mothers were exposed to 75% oxygen for 5 days (P7-12, vasoobliteration phase) in a ProOx model P360 hyperoxia chamber (BioSpherix, Ltd.) and returned to room air (21% oxygen) for 5 days (P12-P17, neovascularization phase). Pups were sacrificed, and the whole eyes were collected at P12 and P17, (n=4) mice/time point. The isolated retinal tissue was stained with Isolectin GS-IB4 (Alexa Fluor 568) and was analyzed for the central avascular area (P12) and intravitreal neovascularization (INV) (P17). The central avascular areas, the total retinal areas, and INV area were measured manually using Image J and Image pro-plus software (v.2018).

3. Results

The high levels of VO and INV confirm that we are modeling advanced stage ROP. WT mouse pups exhibit higher VO (31.6 ± 3.0 %) at P12 compared to *Bco2*^{-/-} pups (27.8 ± 1.9 %). The WT mice at P17 show VO (4.9 ± 1.6), but surprisingly we did not observe any VO in *Bco2*^{-/-} at P17. WT mice pups exhibit higher INV (9.2 ± 2.4 %) at P17 compared to *Bco2*^{-/-} (7.4 ± 1.5 %). Overall, *Bco2*^{-/-} mice exhibited better protection against OIR relative to WT mice.

4. Conclusion

β , β -carotene-9',10'-oxygenase 2 (*Bco2*) act as a carotenoid cleavage enzyme, but additional functions of *Bco2* are less characterized. In the current study, *Bco2* appears to be a modulator for OIR-induced oxidative stress. The molecular mechanisms underlying this effect deserve further exploration.

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Carotenoid-rich microalgae extracts as antimicrobial agents against marine pathogens

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1. Introduction

The alarming spread of antibiotic-resistant pathogens is a global threat that makes evident the need to find new antimicrobial compounds. Aquaculture is the fastest growing activity in the food production sector, however, infectious diseases compromise its viability [1]. Microalgae are versatile fast-growing unicellular photosynthetic microorganisms able to produce great variety of active agents including unexplored antimicrobial compounds [2]. Carotenoids have been traditionally exploited for their colour, provitaminic, antioxidant and UV-light protective properties beside their health-promoting abilities. In the present study, we will investigate the antimicrobial ability of carotenoid-enriched microalgal extracts against some of the most threatening pathogens species in aquaculture.

2. Experimental

The chlorophyte microalga *Chlorella sorokiniana* was cultured in Tris-acetate phosphate (TAP) liquid medium under continuous agitation and illumination ($200 \mu\text{E m}^{-2} \text{s}^{-1}$) at 27°C . Methanol microalgal extracts were obtained by agitation with glass beads and incubation at 70°C in methanol for 15 min. The typical fish pathogens and the control Gram negative and Gram positive strains *Escherichia coli* and *Micrococcus luteus* were grown at their corresponding optimal conditions. The carotenoid profile of the extract was assayed by Hitachi Chromaster HPLC equipped with a DAD detector HPLC.

3. Results and Discussion

The carotenoid extract obtained contained 937 and $67 \mu\text{g mL}^{-1}$ of chlorophylls and carotenoids, respectively, being lutein the majority carotenoid. The typical fish pathogens *Pseudomonas anguilliseptica*, *Pseudomonas baetica*, *Pseudomonas plecoglossicida*, *Pseudomonas moraviensis*, *Aeromonas salmonicida*, *Vibrio harveyi*, *Vibrio anguillarum*, *Vibrio tapetis*, *Vibrio aestuarianus* and *Photobacterium damsela*, were used to test the antimicrobial activity of the extracts. Lysis halos, measured as previously described [1], revealed that the most susceptible species were the *Vibrio* species, *V. anguillarum* and *V. tapeti*. These data demonstrate that methanol extracts with a high content of carotenoids and chlorophylls, obtained from the chlorophyte microalga *Chlorella sorokiniana*, have a considerable antibacterial activity *in vitro* against some of the most important marine pathogens. Further studies are needed to determine the compound responsible for this activity and include them in new formulations for their use as therapeutic agents in aquaculture.

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[2] Schüller et al. Trends and strategies to enhance carotenoid, TAG and LC-PUFA production in microalgae. *Algal Research.* 25, 263–273, 2017.

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P11-2

***In situ* feeding of non-cognate apocarotenoids to Bacterial rhodopsins.**

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1. Introduction

Proton pump rhodopsins are stand-alone molecular machineries that can convert light energy into proton motive force. Based to the photochemical property of retinal, the light-absorbing apocarotenoid pigment tethered to the core of rhodopsins, each rhodopsin can absorb only a limited range of wavelength. Attempts have been made to shift the wavelength range utilized by rhodopsins, mostly by amino acid substitutions (mutations) [1], while *in vitro* feeding artificial retinal with altered photochemical properties could be effective in modulating action spectrum of rhodopsins [2]. In this study, we constructed a biosynthetic pathway for retinal analogs with different conjugation numbers for *in situ* feeding to pump rhodopsin to change their action spectrum.

2. Experimental

Retinal was biosynthesized by additionally expressing β -carotene oxygenase (Blh) to the β -carotene biosynthetic pathway established in *Escherichia coli* (1). To this system, (2) β -carotene ketotransferase (CrtW) from a marine bacterium, or (3) retinal dehydrogenase (CYP27C1 from *Danio rerio* and FDX1/FDXR from *Bos taurus*) were additionally expressed. The action spectrum (wavelength dependence in performance) of rhodopsin RxR from *Rubrobacter xylanophilus* was taken by monitoring the pH change in the suspension of *E. coli* co-expressing rhodopsin (RxR) and the biosynthetic pathways established above.

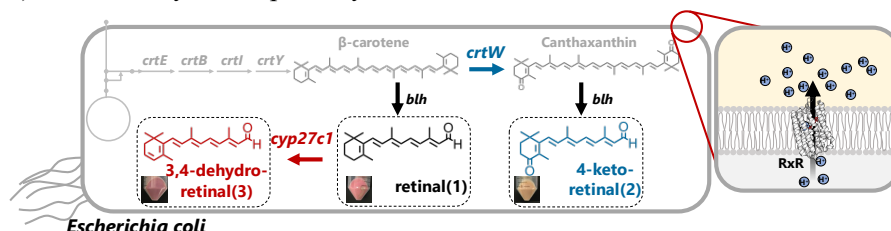


Figure 1. *In situ* feeding of 4-keto- and 3,4-dehydro- retinals to pump rhodopsin.

3. Results and Discussion

Additional expression of CrtW in retinal producing *E. coli* strain resulted in accumulation of retinal and 4-ketoretinal in nearly equal amounts (ca. 10.8 $\mu\text{g/g-DCW}$). RxR co-expressed in this strain enveloped blue shifted appearance and action spectrum. Co-expression of CYP27C1 in retinal producing *E. coli* strain resulted in the conversion of all retinal to 3,4-dehydroretinal (17.4 $\mu\text{g/g-DCW}$) with the aid of a mitochondrial reductase (FDX1/FDXR). Co-expression of RxR with the biosynthetic genes for 3,4-dehydroretinal resulted in the deep-red cellular hue, and RxR expressed therein exhibited red-shifted action spectrum (by 33 nm) compared with that charged with the cognate retinal.

[1] Engqvist, M. et al, J.Mol.Biol., **427** (2015) 205-220.

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P11-3

Resource recovery using mixed purple phototrophic bacteria for carotenoid production

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1. Introduction

The agri-industry, specifically animal feeds, is a prime market for immune-nutritional additives such as carotenoids. Due to their antioxidant and disease-preventing properties and their coloring capacity the world demand is increasing, particularly for naturally sourced products. In this context, purple phototrophic bacteria (PPB) have been proposed as mediator for resource recovery [1] and as an alternative protein source e.g., to substitute fishmeal [2]. The PPB biomass is generally characterized by high protein contents as well as an arsenal of carotenoids (crt) and bacteriochlorophylls (BChls), which are synthesized to harvest photons to perform anoxygenic photosynthesis. PPB have the potential to produce large amounts of carotenoids and can be grown on low-cost substrates, which promises low overall production costs. The aim of this study was to investigate the potential of PPB mixed cultures for carotenoid production by assessing different illumination conditions while using advanced analytical methods for their detection and quantification.

2. Experimental

PPB mixed cultures were subjected to varying light intensities and wavelengths. A method for PPB cultivation in microplates was developed to ensure optimal illumination and to obtain live-cell spectrophotometric information. A UPLC-UV-HRMS method using a C₃₀ column, was applied to quantify neurosporene, lycopene, other crt and BChl *a* content. Metaproteomic and 16S analyses were conducted.

3. Results and Discussion

Our results showed that the highest lycopene content (5.13 and 3.47 mg gVS⁻¹) and other crt were obtained at lower light intensities (1.7 and 5.7 Wm⁻²). PPB treated with only IR light produced more crt than those treated with other wavelength ranges. The major crt in all experimentss were lycopene, 3,4-dihydrorhodopin, and rhodopin. Metaproteomic analysis of mixed culture identified ~26 proteins associated with photosynthetic metabolism. The relative expression of individual proteins varied with different light intensities, with photosynthetic protein expression maximized at low light intensities and suppressed in the absence of light. In addition, two lycopene isomers differing from the analytical standard (*all-trans*) were detected. Our study highlights the potential of PPB for crt production and presents a robust method for their detection and quantification while providing insights into the pigment metabolism of photoheterotrophic purple bacteria, which can aid in optimizing carotenoid content and profile for industrial application.

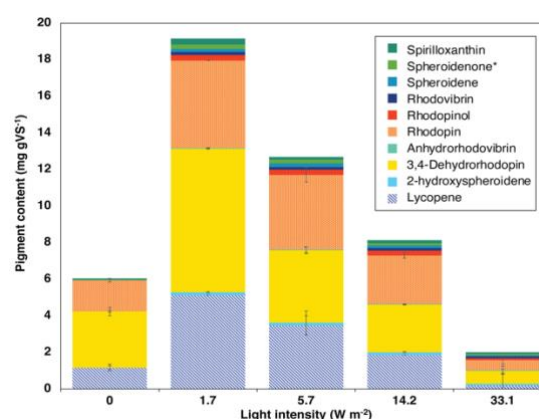


Figure 1. Carotenoid contents in mixed PPB treated with varying light intensities (0, 1.7, 5.7, 14.2 and 33.1 W m⁻²).

[1] T. Hülsen, D. J. Batstone and J. Keller, *Water Res.*, 2014, **50**, 18–26.

[2] J. Delamare-Deboutville, D.J. Batstone, M. Kawasaki, S. Stegman, M. Salini, S. Tabrett, R. Smullen, A. C. Barnes and T. Hülsen, *Water Res. X*, 2019, **4**, 100031.

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P11-4

Formulation of a potential Healthy Ingredient using Papaya Carotenoid green extracts to Improve Carotenoids Stability and Bioaccessibility

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1. Introduction

Papaya fruits are rich sources of bioactive compounds, such as carotenoids [1]. Recently, Lara-Abia et al [2] reported the green ultrasound-assisted extraction (UEA) of papaya peel carotenoids using vegetable oils. The carotenoid enriched-oil extracts could be directly used to formulate food carotenoid ingredients. In the present study, peel papaya carotenoid vegetable oil UEA green extracts were used to formulate O/W emulsions. Stability and bioaccessibility of the main individual encapsulated carotenoids were studied, also conducting the microstructural analysis of O/W microemulsions.

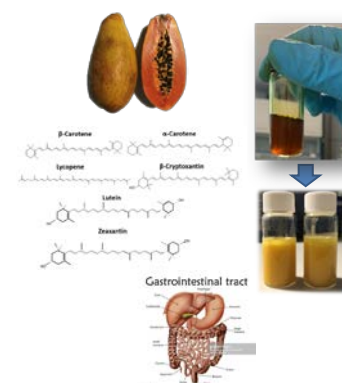


Figure 1. Overview of the study

2. Experimental

UAE sunflower and soybean oil green extracts obtained from peels of papaya fruits var. Sweet Mary [2] were used to obtain a O/W emulsions. Pectin, Tween 20 and papaya carotenoid enriched-oil extracts was used to obtain O/W emulsions. INFOGEST methodology was used to evaluate the bioaccessibilities of papaya individual carotenoids in O/W emulsions. Microstructural analysis (confocal and optical microscopy) of the O/W emulsions and the *in vitro* digestion phases were also carried out.

3. Results and Discussion

Sunflower O/W microemulsions showed smaller mean particle size, higher negative ζ -potential and higher viscosity, than soybean O/W microemulsions. Particle size reduction in sunflower microemulsions favoured the bioaccessibility of papaya carotenoids, being (all-*E*)-lycopene, the carotenoid with the higher bioaccessibility (71.4%), followed by (all-*E*)- β -carotene (18.4%), (all-*E*)- β -cryptoxanthin (15.0%) and (all-*E*)- β -cryptoxanthin laurate (7.2%). Slightly lower bioaccessibilities were obtained for these carotenoids in soybean O/W emulsions. These results highlight the potential of using direct UAE carotenoid-enriched vegetable oils to formulate O/W emulsions to enhance carotenoids bioactivities, avowing their degradation and increasing their *in vitro* bioaccessibilities.

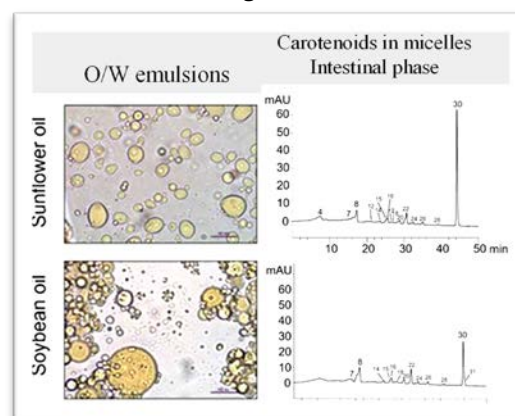


Figure 2. Images of optical microscopy of Papaya carotenoids O/W emulsions and chromatograms of carotenoids micellarized in the intestinal phase.

Acknowledgements

This work was supported by Spanish Ministry of Science and Innovation (project PID2020-118300RB-C21).

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[2] S. Lara-Abia, J. Welte-Chanes, M. P. Cano, *Molecules*, **27** (2022) 638-656

Luncheon Seminars
and
Sunset Seminar

Measuring Health: Future Considerations for Nutrition Research

Abstract

Nutrition science requires a unique approach to clinical design that measures health instead of disease. Clinical studies designed for pharmaceutical applications examine specific drug targets and measure disease markers. A fundamentally different approach is needed to look at dietary interventions that have multi-system effects throughout the body, and which are intended to improve healthspan. This panel discussion will explore emerging tools and research design schemes that help us make evidence-based recommendations for maintaining and optimizing health through nutrition.

Speakers



Jennifer Lovejoy, PhD, is the Director of Translational Science at the Institute for Systems Biology (ISB) in Seattle, WA, USA. Prior to ISB, she was Chief Translational Science Officer at Arivale, and Senior VP of Clinical and Quality Support at Alere Wellbeing (formerly Free & Clear). Her areas of expertise include clinical trials, behavioral science, and clinical application of multi-omic data.



Suzan Wopereis, PhD, is the Principal Scientist at TNO where she works within the systems biology team on ‘quantification of health and effects of lifestyle on health’. Her focus has been on phenotypic flexibility as a measure of health, where she uses standardized challenge tests to study the response of a multitude of biological processes to quantify resilience in health optimization and chronic lifestyle related diseases.



Professor John Nolan, PhD, is a principal investigator and founder of the Nutrition Research Centre Ireland (NRCI) where he studies the role of nutrition and lifestyle for human well-being. His current research interests include the study of key nutrients for cognitive function and brain health with a major goal of identifying ways to reduce risk of Alzheimer’s disease.

Moderators

Vincent Wood, President AstaReal Pte. Ltd. (Singapore)

Karen Hecht, PhD, Scientific Affairs Manager, AstaReal Inc. (USA)

Monday 10th July

Agenda

Monday July 10th, 2023

12:15pm – 1:45pm Japan time.

12:15 pm Vincent and Karen, welcome and introduction.

12:30 pm Dr. Jennifer Lovejoy (joining by teleconference), on systems biology and P4 medicine.

12:55 pm Dr. Suzan Wopereis (joining by teleconference), on phenotypic plasticity and challenge test.

1:20 pm Dr. John Nolan (joining in person), on carotenoids and health optimization.

1:40 pm Vincent and Karen, concluding remarks.

Macular Carotenoids and the Eye-Brain Connection: A Panel Discussion on the Evolving Basic Science and Clinical Translation Landscapes

Rudrani Banik, M.D.¹, Deshanie Rai, PhD^{2,3*}

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³*Friedman School of Nutrition Science and Policy, Tufts*

1. Introduction

The macular carotenoids, lutein, RR-zeaxanthin and RS-*meso*-zeaxanthin are widely recognized by the academic community in supporting several aspects of vision performance. Accumulating scientific and clinical research over the past 200 plus years have lent credence to their biological plausibility for retinal health. These include: i] levels of accumulation in the macula correlates with dietary intakes and thereby influence macular pigment optical density; ii] of all the dietary carotenoids consumed and that can be measured in human serum, only lutein and the zeaxanthin isomers are preferentially accumulated in the macula; iii] presence of lutein and zeaxanthin in uveal structures: iris, ciliary body, and RPE/choroid; iv] unique biophysical, anatomical and biochemical capacity to influence processes supporting normal retinal health as well as affect the pathogenesis and progression of retinal diseases. These include age-related macular degeneration, dry eye syndrome, cataract, and other blinding disorders.

The role of macular carotenoids in overall health and wellness can be further appreciated thanks to research over the past two decades demonstrating their preferential accumulation in the brain. Similar to the deposition in the macula, this accumulation is relevant through all lifestyles. Epidemiological and clinical supplementation studies have shown functional and nutritional relevance to brain health and aspects of cognitive performance. These data both at the basic and clinical levels, builds a rationale for the pivotal role of these macular carotenoids in mediating the cross-talk between the visual and cognitive centres of the human body.

2. Goal and Outcomes:

Given the overall theme of the International Carotenoid Symposium 2023 (Toyama, Japan), this panel discussion will address how the mechanistic science of macular carotenoids has evolved over the past 200 plus years as well as research gaps and opportunities for the next 200 years to come. Additionally, we will address how this mechanistic science has shaped/is shaping the clinical landscape, both in terms of healthy individuals and those with retinal disease conditions. The panel will focus on how these scientific and clinical insights are influencing patient-doctor interactions, not just in terms of supplementation, but building awareness and educating both the health care professional community as well as patients and consumers. These insights are particularly relevant given that the digital revolution has changed the way we use our eyes to interact with the world – it is therefore not surprising that digital eye strain/computer vision syndrome is a common phenomenon both among children and adults. This digital era is likely not going away soon and along with rising health care costs around the world, scientists and clinicians are compelled to come together to further the mechanistic understanding of macular carotenoids and the clinical applications thereof.

The panel will include subject matter experts representing the basic sciences and clinical ophthalmology fields to share their perspectives on these topics and their thoughts on where we go from here. In other words, what does the future trajectory of the science on macular carotenoids in vision and cognitive performance look like.

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Tuesday 11th July

Why and how to fortify with carotenoids ?

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Carotenoids are widely found in plants but natural intake with diet is often below recommended levels. In spite of a consumer trend towards more healthy eating and plant-based diets, carotenoid intake from fruits and vegetables varies broadly, as will be shown based on latest intake data from several countries. Therefore, fortification of foods and beverages with carotenoids is a common way to improve carotenoid intake.

Looking at animal nutrition, modern livestock production depends on the provision of carotenoid-supplemented feeds for pigmentation of fish, crustaceans, broiler skin and egg yolks to meet consumer demands.

However, fortification of foods and feeds is not simple. Carotenoids are highly sensitive, fat-soluble substances. For use as health ingredients or colorants in foods and feeds, key challenges to be managed are:

- 1) Providing miscibility with the target matrix. For example, achieving water-miscibility of the carotenoid to allow for use in beverages and – on the feed side – in post-pelleting liquid applications.
- 2) Protecting the carotenoid against oxygen, light and heat to allow high stability during food and feed processing; e.g., tableting, pelletting and extrusion.
- 3) Using carotenoids as natural-source or nature-like colors enabling yellow to red color shades in foods; e.g., margarine, lemonades; and in animal nutrition; e.g., egg yolk or salmonid pigmentation.

To address these challenges, microencapsulation technologies are used in the production of carotenoids. The carotenoid is embedded in a specific protective matrix to stabilize the carotenoid, to manage its product properties, and to facilitate its use in various target applications. The most prominent examples are carotenoid “beadlets,” which provide high carotenoid stability, water-dispersibility, and ease of use. Moreover, consumer lifestyle preferences can be taken into account, for example, by producing vegetarian, kosher, halal, allergen-free, gluten-free, or plant-based carotenoid formulations. In the luncheon seminar, a better understanding of carotenoid microencapsulation will be gained based on practical examples: 1) natural mixed carotenoids for tableting; 2) beta-carotene for coloring of beverages; 3) astaxanthin for aquaculture; and, 4) C-30 ester and canthaxanthin for egg yolk pigmentation.

Based on these modern technologies, the health benefits and coloring properties of carotenoids can be made available in foods and feeds for optimum nutrition of both humans and animals.

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Lutein as a modulator of oxidative stress: implications to health and disease.

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Lutein is a xanthophyll carotenoid found in a variety of food such as dark green leafy vegetables, corn, egg yolk, and avocados. Lutein has been shown to have antioxidant activity *in vitro* and *in vivo* to mitigate possible oxidative injury by deactivating free radicals. Reducing oxidative stress may also reduce inflammation. These activities may aid in the understanding of how lutein functions in health and diseases. Of note is the research that has been performed in ocular, cognitive and skin health. Lutein has been shown to be positively related to neural health, and oxidative stress related to the risk of ocular diseases and cognitive decline. Mechanistic studies have been performed to demonstrate lutein's neuroprotective effect during oxidative stress and inflammation. As with the eye, the skin is sensitive to oxidative damage due to light exposure. As sunlight exposure produces free radicals, oxidation arises so that an antioxidant response can provide photoprotection. Indeed, supplementation with lutein has been shown to enhance the skin's innate resistance against UV-induced erythema (sunburn). *In vivo* studies have reported lutein supplementation to be effective against UV light damage to affect the skin's immune function. Due to its antioxidant and anti-inflammatory properties, lutein has been considered promising in several areas of health, such as ocular, cognitive, and skin health, but is likely to function in other areas as well due to these functions.

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Importance of Lutein throughout life stages

The retina is an important and sensitive part of the eye. Especially in children, when the lens is not fully developed, protection is essential. During all life stages the retina is exposed to light stress and oxidative stress. Therefore, a high MPOD (macular pigment optical density) ensures protection of the central retina and may prevent retinal degenerative diseases later in life. In addition, a high MPOD also improves visual performance, i.e. contrast sensitivity and vision in bright light.

A high MPOD is a good marker for brain lutein concentration. It was demonstrated in many studies that higher lutein brain concentrations improve verbal memory, neuronal processing, just to mention two brain health benefits. Across the lifespan lutein has an impact on brain health and cognitive function.

To reach the two main target organs bioaccessibility and bioavailability need to be optimized through product form design. Particle size, matrix and formulation technology will influence plasma and tissue lutein levels and also ensure product stability.

All these aspects will be explained and discussed during the DSM luncheon seminar.

Prof Paul Bernstein (University of Utah, USA)

“Lutein for Eye Health Throughout the Lifespan”

Dr Lisa Renzi (University of Georgia, USA)

“Brain Health: towards healthy aging”

Dr Christian Schaefer (DSM, Switzerland)

“Designing product forms with bioavailability in mind”

Index (sort with family name)

A		Böddi Katalin	68, 118
Acevedo Leslie	75	Böhm Volker	82, 181
Addo Emmanuel Kofi	93, 190	Böhn Torsten	81, 86, 150
Agarwal Rimjhim	170	Bombarely Aureliano	107
Agreda-Laguna Kenny Alejandra	75	Bonet M. Luisa	45, 94, 142
Al-Babili Salim	72	Bowtell Joanna	84, 185
Albreht Alen	77	Bradley Madelyn	147
Allah Nawaz	25	Brenya Eric	120
Allman Susan	189	Briskey David	84, 185
Amengual Jaume	43, 45, 59, 147	Buhiniček Ivica	162
Ando Hiroto	108, 109, 111	Bunea Andrea	175
Andreu Palou	45, 94, 142	Bwar Wai	20
Aoi Wataru	26	C	
Araki Yui	108, 109	Cafarella Cinzia	151
Ariumi Masashi	173	Calame Julie	97
Asaka Ryo	152	Cano M. Pilar	157, 158, 194
Asaoka Ryo	89	Capra Sandra	84, 185
Ashino Hiromi	143	Cazzonelli Christopher I.	116, 120
Attila Agócs	68, 118, 156, 187	Chachay Veronique	84, 185
Autenrieth Caroline	29	Chang FU-Yen	184, 190
B		Charalambos Andreou	37
Baldermann Susanne	11, 155	Charalambos Tselios	37, 126
Bandino Elisa	158	Chattopadhyay Atrayee	54
BANDO-UOTANI Miki	122, 123	Chen Nan	168
Banh Anh Thi-Mai	9	Chino Tomoki	192
Banik Rudarani	199	Cogdell Richard J.	3, 130, 132, 136
Barja Victoria	97	Coman Cristina	176
Bassi Roberto	101	Cooperstone Jessica	57
Batstone Damien	193	Cordoba Elizabeth	75
Beer Jenay	188	Czett Dalma	68, 118
Benítez-González Ana M.	80	D	
Beppu Fumiaki	58	Dall'Osto Luca	101
Bernstein Paul S.	89, 93, 184, 189,	Dandurand Jade	188
	190, 202	Dang Kelly	56
Betekhtin Alexander	146, 161	Dan-Hong Li	36
Black Molly	147	Darawshe Aya	46
Boba Aleksandra	169	Daròs José-Antonio	8, 101

Deguchi Shigeru	67	Gherasim Cristina	175, 176
Deli József	68, 118, 156, 187	Ghosh Robin	29
Demurtas Olivia Costantina	74, 101	Giordano Elena	54
Dergunov Sergey	55	Giuffrida Daniele	64, 149
Dillon Natalie	163	Giuliano Giovanni	74, 101
Diretto Gianfranco	8, 74, 142	Giuseppe Aprea	101
Drapal Margit	96	Gloria Lobo	157, 158, 194
Dufossé Laurent	18	Goddard Alice	182
Dugo Paola	64	Gohto Yuko	89
Dulf Francisc V.	175	Golczak Marcin	52
E		Gómez-Gómez Lourdes	8
Echigo Mayu	133	Gomez-Green Marina	186
Ekafitri Riyanti	171	Gomez-Maqueo Andrea	157, 158
Emmanuel K. Addo	93, 189, 190	González Jesus	191
Erdman John	17, 147	Gonzalez-Calquin Christian	73
Eugenia Enfissi	96	Gorka Joanna E.	189
Everett Michael	56	Göttl Vanessa L.	102, 149
F		Grassino Maria	193
Faber Marius	82	Green Marina	47, 49, 178, 179
Fehn Simon	181	Gu Lina	66
Ferrante Paola	74, 101	Guignard Cedric	86
Fitzner Maria	11	H	
Fitzpatrick Naomi	84, 185	Hama Yujiro	122, 124
Flynn Róisín	179	Hamaguchi Tasuku	127
Focsan A. Ligia	69, 119, 154	Hammond Billy	90, 188
Focsan Monica	175	Han Hyejin	144
Fraser Paul D.	7, 96	Hanna Nazeeh	54
Frede Katja	11	Harbart Vanessa	11
Frusciante Sarah	8, 74	Harper Steve	170
Fujii Ritsuko	39, 114, 138	Harriet Berry	96
Fujisaka Shiho	25	Harth Jacob	90
Furubayashi Maiko	103	Hashida Megumi	147
G		Hashimoto Hideki	3, 34, 129, 130, 131, 132, 133, 134, 135, 136
Galic Vlatko	162	Hasunuma Tomohisa	100
Gannon Bryan	20	Hayward Alice	170
Gantenbein Manon	86	Hecht Karen	197
Gao Yunlong	69, 119, 154	Hegemann Peter	159
Gardiner Alastair	132	Henke Nadja A.	102, 121, 149
Gentleman Susan	55		

Heo Seung Chul	140	Kamiya Nobuo	123, 125
Hilke Alberts-Hubatsch	121	Kantoğlu K. Yaprak	164
Hirschberg Cosima	200	Kantoğlu Ömer	164
Hoffmann Julian	155	Kashino Yasuhiro	122, 127
Hogan Cassidy	188	Kawai-Noma Shigeko	110, 192
Hong Hung	163, 170	Kawakami Keisuke	122, 123, 125, 127
Honma Masato	160, 165	Kawasaki Shinji	152
Hopfhauer Sofia	181	Kelterborn Simon	159
Horiuchi Kota	129, 133, 135, 136	Kida Masatoshi	125, 128
Horváth Adrienn	187	Kim Dahye	22, 66
Hosokawa Masashi	58	Kim Hyein	66
Hulsen Tim	193	Kim Junghyun	144
Hussain Mahmood	54	Kim Minsoo	22
Hyeonju Yu	141	Kim Youn-Kyun	54
I		Kim Yunji	66
Iç Erhan	164	Kim Yuri	140, 141, 144
Ichikawa Satomi	83	Kiser Philip	56
Iddir Mohammed	81, 86	Kishida Ikuya	130
Ikedo Chiaki	145	Kispert Lowell D.	69, 119, 154
Inoue-Kashino Natsuko	122, 127	Kljak Kristina	162
Ioannou Aristos	37	Ko Kwang	141
Ishihara Daichi	108, 110	Kobayashi Manami	180
Ishikawa Marumi	113	Kochneva Yelyzaveta	169
Isobe Kazuki	160	Koepsel Christian	200
Iwasaki Miki	110	Kojima Risa	128
Iwase Katsuro	143	Koo Sangho	22, 66
J		Kosumi Daisuke	34, 122, 123, 124, 125, 128, 136
Jackman Sarah	84, 185	Kousoulaki Katherina	121
Jambrovic Antun	162	Koutsoupakis Constantinos	37
Jánosa Gergely	187	Kranjc Krištof	77
Johnson Elizabeth	88, 201	Kristanti Dita	171
Johnson Scott	120	Kulma Anna	169
Jude Abueg	56	Kumalasari Rima	171
Juliana Almeida	96	Kuwahara Daichi	98
Jung Hyunuk	22	Kuznetsova Valentyna	40
K		Kwasniewska Jolanta	146, 161
Kado Tomonobu	25	Kwon Hyeji	144
Kakuta Yumeka	131		
Kamei Yasuhiro	114		

L			
Lánzembold Ildikó	156	Miura Kenji	173
Lara-Abia Sara	194	Miyayama Masami	83
Larondelle Yvan	86	Mizuno Khoki	173
Leclercq Céline	166	Mizutani Masaharu	160, 165
Ledenčan Tatjana	162	Mladenov Petko	166
Lee Jung Hyeun	140	Mobashwer Alam	163
Leners Bernadette	86	Mochida Keiichi	113
León Patricia	75	Mohsen Sharifzadeh	189
León Rosa	159, 191	Molina-Márquez Ana	159, 191
Leroux Julie	174	Mondello Luigi	64, 151
Li Binxing	184, 190	Morelli Luca	107
Li Li	12	Morifuji Masashi	83
Lietz Georg	182	Morillo David	191
Lim Boram	22	Morita Yuya	134
Lin Xinhua	54	Moriwaki Yoshitaka	160
Llorente Briardo	97	Morote Lucía	8
Lovejoy Jennifer	197, 198	Muhammad Bilal	25
Lozano-Omeñaca Aurora	112	Mukai Katsuyuki	143
Lu Shan	117	Munizaga Fabiana	73
M		Muntean Patricia	175, 176
Maeshima Nozomi	180	Murakami Akio	125
Manabe Yuki	44, 83, 145, 148	Muto Shigeki	89
Maoka Takashi	28, 58, 63, 145, 148, 152	N	
Martí Maricarmen	101	Nagashima Aya	108
Martín-Chamorro Rocío A.	94	Nagy Veronika	68, 118, 156
Matsubara Shizue	9	Naito Yuji	24
Mcquinn Ryan	75, 174	Nakagawa Takashi	25
Meléndez-Martínez Antonio J.	80	Nakajima Hiroshi	143
Mertinez-Giron Jader	151	Nakamura Mieko	89
Metličar Valentina	77	Nakaniwa Tetsuko	39
Meyer Florian	121	Nayak Jwalit J.	116
Miller Anthony	59	Nicholls Joshua	97
Miller Mark Js	27	Nicolia Alessandro	101
Minakata Katsuya	110	Ningrum Andriati	171
Mini Paola	74, 101	Nishida Yasuhiro	25
Misawa Norihiko	6	Nishino Hoyoku	48
Mitani Koki	132	Nistor Mădălina	176
Miura Aya	89	Nogueira Marilise	96
		Nohara Taiki	123

Nolan John M.	47, 49, 92, 177, 178, 179, 183, 186, 197	Pintea Adela	175, 176
Nomura Toshihisa	113	Pittois Denis	86
Nozue Miho	89	Planchon Sébastien	166
O		Pogson Barry J.	75, 76, 174
Oakley Donna	183	Poliakov Eugenia	55
Obana Akira	89	Polívka Tomas	33, 38
Obermueller-Jevic Ute	200	Polyakov Nikolay E.	69, 119, 154
Oh Yeonsoo	141, 144	Pooja Acharya	54
O'hare Tim	163, 170	Poudel Sujata	97
Oh-Oka Hirozo	128	Power Tommy	178, 183
Ohshima Kazuki	152	Prado-Cabrero Alfonso	177, 178, 179, 183
Ohta Daisaku	160	Pryor Colette	178
Ojima Takumi	21, 108, 109, 111	Pucker Boas	149
Oka K.	143	Q	
Oka Naohiro	114	Quadro Loredana	54
Okazawa Atsushi	160	Quian-Ulloa Rocío	73
Onozato Yumi	110	R	
Ordonez-Santos Luis Eduardo	151	Rai Deshanie	199
Osorio Coralia	151	Ramkumar Srinivasagan	52
Oussama Ahrazem	8	Ranganathan Arunkumar	189, 190
Oxley Anthony	182	Ranganathan Arunkumar	189, 190
Özmen Dilan	164	Redmond T. Michael	55
P		Renaut Jenny	166
Palacios-Rojas Natalia	20	Rengel R.	159, 191
Palou Andreu	45, 94, 142	Renzi-Hammond Lisa	90, 188, 202
Pandur Edina	187	Rey Florencia	112
Pap Ramóna	187	Ribot Joan	45, 94, 142
Papageorgiou Marios	126	Rigano Francesca	151
Parralejo-Sanz Sara	157, 158, 194	Robert Bruno	16
Pelletier Wolf	200	Roche Warren	47, 186
Peng Yu	168	Rocheford Torbert	10
Perez-Fons Laura	96	Rodrigo Maria J.	13, 112
Perez-Perez Renee	146	Rodríguez Ana M.	94
Pervin Mahfuza	120	Rodriguez-Concepcion Manuel	19, 107, 117
Peters-Wendisch Petra	102, 121	Rogozin Igor	55
Petrillo Maria Grazia	74	Rubio-Moraga Ángela	8
Pinakoulaki Eftychia	126	Rugina Dumitrita	175, 176
Pinhassik Eugene	55	Ruit Sanduk	93
		Ryan P. Mcquinn	75, 174

S

Sadamatsu Hisato	143	Stahl Wilhelm	82
Sago Yuma	135	Stange Claudia	73
Saito Kyoichi	192	Stein Dan	199
Saito Yohei	192	Stinco Carla M.	80
Sakimura Kana	58	Stojnic Bojan	142
Sala Katarzyna	146	Stringham James M.	186
Sandmann Gerhard	7, 99	Stringham Nicole	47, 49
Sarcevic Hrvoje	162	Sugawara Tatsuya	44, 83, 145, 148
Sarifudin Achmat	171	Sugimoto Yukihiro	160, 165
Schaefer Christian	202	Sugiyama Kenjiro	115
Schmidt Mario	181	Sulli Maria	74, 101
Schmitt Ina	121	Suzuki Kengo	113
Schwarzenbolz Uwe	181	Suzuki Ryuju	67
Sebelik Vaclav	38	T	
Sei Ryuya	129		
Seki Soichiro	39, 114, 138	Tadeo Francisco R.	13
Seki Takahiro	192	Takagi Tatsuyoshi	163
Sepalika Bandara	52	Takaichi Shinichi	115, 152, 153
Serikawa Hiroki	123	Takatani Naoki	58
Setiaboma Raima	171	Takátsy Anikó	118
Sharoni Yoav	46	Takayanagi Yuji	89
Shen Jian-Ren	32	Takeda Satomi	173
Shi Gaosheng	66	Takemoto Kayo	192
Shi Hao	178	Takikawa Hirosato	160, 165
Shigeta Takumi	160	Talon Manuel	13
Shimizu Kentaro	160	Tamaki Shun	113
Shindo Kazutoshi	65	Tanumihardjo Sherry	20
Shinzawa-Itoh Kyoko	122, 127	Tao Ruohan	131, 136
Shiotani Nanami	160	Tauber Svenja	181
Shore Angela	84, 185	Terada Tohru	160
Shukla Anushka	150	Terol Javier	13
Sierra Julio	75	Thapa Raba	93
Simic Domagoj	162	Thiele Bjoern	9
Simova Ivana	38	Thomas Linda	52
Sipos Katalin	187	Tianhu Sun	12
Sivaperuman Parimala	186	Tobe Kazuyuki	25
Sliwka Richard	62	Tode Chisato	152
Solano Yasmeen	56	Tomlekova Nasya	166
		Tomonaga Nami	145
		Toomey Matthew	91

Torres-Montilla Salvador	107	Yao S.	143
Trachtenberg Aviram	46	Yasuhara Kazuma	67
Truong Thuy T.	174	Yonekura Koji	122, 123, 127
Tsai Yih-Jeng	139	Yoo Hyebin	22
U		Yoshida Kazuhiro	138
Uchida Hiroko	125	Yukihira Nao	132
Umeno Daisuke	21, 108, 109, 110, 111, 192	Z	
Uppal Sheetal	55	Zacarias Lorenzo	13, 112
Uragami Chiasa	129, 130, 131, 132, 133, 134, 135, 136	Zacarias-Garcia Jaime	13
V		Zerres Sarah	82
Varner Michael W.	189	Zeytin-Schüning Sinem	121
Varotsis Constantinos	37, 126	Zhang Jian-Ping	36, 137
Verónica Aragonès	8, 101	Zheng Hui	117
Vigara Javier	159, 191	Zheng-Yu Wang-Otomo	152
Von Lintig Johannes	45, 52	Zoccali Mariosimone	64
W			
Wada Tatsuya	145		
Wakabayashi Takatoshi	160, 165		
Wan Zihe	184		
Wang Peng	137		
Wang Xiang-Dong	42		
Weber Bernard	86		
Wen Xin	168		
Wendisch Volker F.	102, 121, 149		
Werner Gellermann	89		
Wood Vincent	197		
Wopereis Suzan	197, 198		
Wu Fan	168		
Wu Pi-Hui	139		
Wu Wen-Bin	139		
Wysocky Cameron	188		
Y			
Yamada Koji	113		
Yamamoto Hayata	125		
Yamano Nami	36, 137		
Yamano Yumiko	58, 114, 138		
Yanai Yu	143		
Yao Hai-Dan	36		