Abstract Book

3rd Virtual International Conference on Carotenoids

VICC 2024

International Carotenoid Society

July 23rd, 24th, 25th

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*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Comments from the Organizers

We wish to thank each of the speakers and their co-authors, who through diligence and hard-work, have carried out the exceptional research that is represented by the abstracts that comprise VICC 2024. It is through their efforts and membership in the International Carotenoids Society that enables the maintenance of the Society's website, sponsorship of the Virtual Conferences, the Triennial International Symposium on Carotenoids, and support of research and education on Carotenoids.

VICC 2024 is pleased that outstanding original research abstracts from 18 different countries have been accepted for presentation in this three day conference.

The Organizers again wish to thank **Case Western Reserve University School of Medicine** for their gracious technical support of VICC 2024 and providing access of ICS through their Zoom account. Case Western has been supported each of the Societies previous virtual conferences

Special thanks go to Dr. Bruno Traber and ***CaroteNature*** for the generous sponsorship of this year's Awards for Outstanding Presentations by Students and Post-doctoral Fellows. This year, CaroteNature, as in the past conferences, has provided support for monetary awards to be given for outstanding Student and Post-doctoral Fellow presenters.

In addition, AstaReal, Inc. has provided funding to support the VICC awards and operations. We appreciate their support in making this conference a reality.

Following the past formats for VICC, each speaker has been assigned an 8-minute window to deliver a tightly focused presentation. Following each scheduled session's talks a Question and Discussion period will enable presenters to address specific Questions from our Conference Audience. We encourage all participants to submit Questions for the discussion periods via the **Zoom** '***chat box***' during talks. Moderators will select and read these to recognize questioners and to guide discussion. **Please keep microphones muted except when called on by a moderator**.

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Table of Contents

Tuesday, July 23rd

		Page
Organizing Committee Members		**2**
Comments of the Organizers		**4**
**********		
	Begin Conference	
**********		
Welcome		**5**
Session I	**Carotenoid Biosynthesis, Biotechnology, and Analysis - A**	**6**
Session II	**Carotenoid Biosynthesis, Biotechnology, and Analysis - B**	**7**
Session III	**Photophysics and Photoprotection and Emergent Topics in Carotenoid Science**	**8**
Session IV	**Carotenoids and the Environment**	**9**
Wednesday, July 24th		**10**
Session V	**Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - A**	**10**
Session VI	**Carotenoid Production by Microorganisms**	**11**
Session VII	**Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - B**	**12**
Session VIII	**Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - C**	**13**
Thursday, July 25th		**14**
Session IX	**Carotenoid and ApoCarotenoid Biology**	**14**
Session X	**Dietary Sources of Carotenoids and Nutritional Supplementation**	**15**
Session XI	**Carotenoid Synthesis and Carotenoids and Health II: Supplementation & Bioavailability – A**	**16**
Session VIII	**Carotenoids and Health II: Supplementation & Bioavailability – B**	**17**
VICC 2024 Concludes		**17**
ABSTRACTS		**18**
Presenter/Co-Author Index		**76**

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Tuesday July 23, 2024

7:30 am EDT **Pre-VICC Login Period**

8:00 am EDT **Welcome – Organizing Committee**

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8:04 am EDT

Session I
Carotenoid Biosynthesis, Biotechnology, and Analysis - A

Moderators: Associate Professor Chris Cazzonelli, Western Sydney University, Sydney, Australia
Professor Alexandrina Ligia Focsam, Valdosta State University, Georgia

8:07 am EDT
Abstract 1 Title: Alternative splicing of ZEAXANTHIN EPOXIDASE transcript harboring a premature termination codon and increased VIOLAXANTHIN DE-EPOXIDASE expression enhances zeaxanthin accumulation and triggers metabolic feedback regulation in capsicum fruits.

Presenter: *Cazzonelli, Christopher*

8:16 am EDT
Abstract 2 Title: Carotenoid and colour development during ripening of Mangoes

Presenter: Takagi, Tatsuyoshi

8:25 am EDT
Abstract 3 Title: Overexpression of the carotenoid biosynthetic gene LYCOPENE β-CYCLASE (LCYB) improves root growth and influences rhizospheric communication in tobacco and tomato

Presenter: Stra, Alice

8:34 am EDT
Abstract 4 Title: NUDIX23: A novel regulator of carotenoid biosynthesis and metabolon assembly

Presenter: Rao, Sombir

8:43 am EDT
Abstract 5 Title: Maximizing saffron apocarotenoid production in varied tomato fruit carotenoid contexts

Presenter: Lobato-Gómez, Maria

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8:52 am EDT **Questions & Discussion 15 min**

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9:07 am EDT **Session II**
Carotenoid Biosynthesis, Biotechnology, and Analysis - B

Moderators: Associate Professor Chris Cazzonelli, Western Sydney University, Sydney, Australia
Professor Alexandrina Ligia Focsam, Valdosta State University, Georgia

9:10 am EDT
Abstract 6 Title: Functional characterization of novel CYC-B non-functional alleles in tomato and their relation to earlier lycopene accumulation and cold stress tolerance

Presenter: Arruabarrena, Ana

9:19 am EDT
Abstract 7 Title: Carotenoid radicals and complexes analyzed using physiochemical techniques

Presenter: Focsan, Alexandrina

9:28 am EDT
Abstract 8 Title: METABOLIC ENGINEERING TO ATTAIN EFFICIENT PRODUCTION OF HIGH-VALUE XANTHOPHYLLS IN TOMATO

Presenter: Simanowitz, Marc

9:37 am EDT
Abstract 9 Title: Characterization and Genetic Mapping of a Novel Factor Affecting Carotenogenic Metabolic Flux in Melon Fruit

Presenter: Wrightstone, Emalee

9:46 am EDT
Abstract 10 Title: Genetic analysis provides new insights into the bixin biosynthesis of achiote (*Bixa orellana*)

Presenter: Rivera-Madrid, Renata

9:55 am EDT **Questions and Discussion** (15 min)

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10:10 am EDT **Break** (4 minutes)

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10:14 am EDT

Session III
Photophysics and Photoprotection
And
Emergent Topics in Carotenoid Science

Moderators: Assistant Professor Matthew Toomey, Univ. of Tulsa, Tulsa, Oklahoma
Ms. Sepalika Bandara, Research Associate, Case Western Univ., Cleveland, Ohio

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10:17 am EDT
Abstract 11 Title: Molecular events accompanying aggregation-induced energy quenching in Fucoxanthin-Chlorophyll Proteins

Presenter: Llansola-Portoles, Manuel

9:26 am EDT
Abstract 12 Title: How algae using the photoprotective diadinoxanthin cycle avoid the accumulation of violaxanthin cycle pigments

Presenter: Lohr, Martin

10:35 am EDT
Abstract 13 Title: Mixed Micelles of Water-soluble Carotenoid Crocin

Presenter: Polyakov, Nikolay

10:44 am EDT
Abstract 14 Title: Efficacy of the bio-solvents 2-methyltetrahydrofuran and ethyl lactate for ultrasound-assisted extraction of carotenoids from phytoene-enriched Dunaliella bardawil in comparison with other traditional solvents

Presenter: Morón-Ortiz, María de los Ángeles

10:53 am EDT
Abstract 15 Title: Evolution of carotenoid metabolism in the blind Mexican cavefish, *Astyanax mexicanus*

Presenter: Perez Guerra, David

11:02 am EDT **Questions and Discussion** (15 min)

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11:17 am EDT **Break** (4 minutes)

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11:21 am EDT

Session IV
Carotenoids and the Environment

Moderators: Assistant Professor Matthew Toomey, Univ. of Tulsa, Tulsa, Oklahoma
Ms. Sepalika Bandara, Research Associate, Case Western Univ., Cleveland, Ohio

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10:54 am EDT

Abstract 16 Title: Monitoring carotenoid content transitions by using RGB digital images as a tool for the early diagnosis of forest decline

Presenter: Ruiz de Larrinaga, Lorena

11:03 am EDT

Abstract 17 Title: β-cryptoxanthin loaded chitosome: A breakthrough in Raspberry preservation for enhanced shelf-life and quality

Presenter: Dutta, Debasmita

11:12 am EDT

Abstract 18 Title: Optimization of plant carotenoid production through manipulation of abiotic and biotic growth environment

Presenter: Demmig-Adams, Barbara

11:21 am EDT

Abstract 19 Title: The mediating effect of UVB on eustress and distress on the accumulation of carotenoids in Salicornia europaea

Presenter: Fitzner, Maria

11:30 am EDT

Abstract 20 Title: Mechanisms of carotenoid coloration in male house finches (*Haemorhous mexicanus*): the roles of fruit consumption, carotenoid intake during molt, and carotenoid status at molt onset

Presenter: Mc Graw, Kevin

11:40 am EDT **Session IV – Questions and Discussion** (15 min)

11:55 am EDT **Close, Day 1 VICC (Sessions I, II, III & VI)**

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Wednesday July 24, 2024

7:30 am EDT **Pre-VICC Login Period**

8:00 am EDT **Daily Updates – Organizing Committee**

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8:04 am EDT

Session V
Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - A

Moderators: Professor Joan Ribot, University of the Balearic Islands, Palma de Mallorca, Spain
Associate Professor Jaume Amengual, University of Illinois, Urbana-Champaign, IL

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8:07 am EDT

Abstract 21 Title: Unravelling the neuroprotective mechanisms of carotenes in differentiated human neural cells: Biochemical and proteomic approaches

Presenter: Magalingam, Kasthuri bai

8:16 am EDT

Abstract 22 Title: Sex-Specific Liver MicroRNA Profiles in Mouse Offspring of Western Diet-Fed Mothers: Impact of β-Carotene Supplementation during Lactation

Presenter: Abrego Guandique, Diana Marisol

8:25 am EDT

Abstract 23. Title: Dietary β-carotene promotes atherosclerosis resolution and reduces liver inflammation in mice

Presenter: Blanco Cirer, Amparo Heliodora

8:34 am EDT

Abstract 24 Title: Mechanisms involved in the protective effect of carotenoids in human skin cells under mitochondrial oxidative stress

Presenter: Darawsha, Aya

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8:43 am EDT

Abstract 25 Title: Haloarchaeal carotenoids as novel antiproliferative agents in human breast cancer: an *in vitro* study

Presenter: Giani Alonso, Micaela

8:52 am EDT **Questions and Discussion** (15 min)

****

9:07 am EDT

Session VI
Carotenoid Production by Microorganisms

Moderators: Professor Joan Ribot, University of the Balearic Islands, Palma de Mallorca, Spain
Associate Professor Jaume Amengual, University of Illinois, Urbana-Champaign, IL

9:10 am EDT

Abstract 26 Title: Exploring mutlitfunctional activities of pigment from Sporocarcina sp

Presenter: Thakker, Janki

9:19 am EDT

Abstract 27 Title: Metabolic engineering of cyanobacteria for phototrophic and secretory production of saffron pigments

Presenter: Song, Shanshan

9:28 am EDT

Abstract 28 Title: Improvement of neurosporaxanthin production in *Fusarium fujikuroi*

Presenter: Limón, M. Carmen

9:37 am EDT

Abstract 29 Title: *Cladosporium cladosporioides* converts crocin to crocetin

Presenter: Sliwka, Hans-Richard

9:46 am EDT **Questions and Discussion** (12 min)

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9:58 am EDT **Break** (**4** minutes)

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10:02 am EDT

Session VII
Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - B

Moderators: Professor Elizabeth Johnson, Tufts University, Boston U., Boston, Mass.
Assistant Professor Eroglu Abdulkerim North Carolina State University, Kannapolis, NC

10:05 am EDT
Abstract 30 Title: Does beta-carotene 9',10'-oxygenase (BCO2) contribute to the hypertrophy of the maternal heart during pregnancy?

Presenter: Kim, Youn-Kyung

10:14 am EDT
Abstract 31 Title: Dietary Tomato Powder Feeding Attenuated Non-Alcoholic Fatty Liver Disease Independent of Sirtuin 1 Activity in Mice

Presenter: Lee, Na Youn

10:23 am EDT
Abstract 32 Title: Relationship of Early Life Dietary Carotenoids to Macular Pigment Optical Density, Vision and Retinal Integrity in Middle-Aged Men and Women

Presenter: Liu, Zhe

10:32 am EDT
Abstract 33 Title: Understanding the molecular basis of postnatal heart growth defects in mice lacking β-carotene 9',10'-oxygenase (BCO2)

Presenter: Madonna, Elisabetta

10:41 am EDT
Abstract 34 Title: Characterizing the Colon Carotenoid Proteome in a Diet-Induced Obesity Animal Model

Presenter: Balbuena, Emilio

10:50 am EDT **Questions and Discussion** (15 min)

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11:05 am EDT

Session VIII
Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism - C

Moderators: Professor Elizabeth Johnson, Tufts University, Boston U., Boston, Mass.
Assistant Professor Eroglu Abdulkerim North Carolina State University, Kannapolis, NC

11:08 am EDT
Abstract 35 Title: Singlet oxygen scavenging activity of myxoxanthophylls from cyanobacteria *Synechocystis* sp. PCC6803 and *Anabaena* (Nostoc) sp. PCC7120

Presenter: Malec, Przemysław

11:17 am EDT
Abstract 36 Title: Circulating lycopene and lutein are associated with reduced adiposity and improved insulin sensitivity in healthy infants

Presenter: Senkus, Katelyn

11:26 am EDT
Abstract 37 Title: Assessing Seaweed-Derived Carotenoids for Mitigating Persistent Inflammation and Epigenetic-Driven Immune Deficiency and Aging Post-Tuberculosis

Presenter: Dube, Nondumiso

11:35 am EDT
Abstract 38 Title: Evaluating metabolic programming by β-carotene during the perinatal stage in mice lacking or expressing BCO1

Presenter: Rubí, Paula

11:44 am EDT
Abstract 39 Title: Prebiotic Effect of Lycopene on Gut Microbiome with Systemic Changes in Liver Metabolism, Skeletal Muscles and Skin in Moderately Obese Persons

Presenter: Petyaev, Ivan

11:53 am EDT **Questions and Discussion** (15 min)

12:08 am EDT **Organizer updates**

12:10 am EDT **Close, Day 2 VICC (Sessions V, VI, VII & VIII)**

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Thursday July 25, 2024

7:30 am EDT **Pre-VICC Login Period**

8:00 am EDT **Daily Updates – Organizing Committee**

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8:04 am EDT **Session IX**

Carotenoid and ApoCarotenoid Biology

Moderators: Associate Professor Jessica Cooperstone, The Ohio State University, Columbus, OH
Professor Salim Al-Babili, King Abdullah University of Science and Technology, Saudi Arabia

8:07 am EDT
Abstract 40 Title: Zaxinone Synthase overexpression modulates rice metabolism and physiology, improving growth and productivity under normal and low phosphate supply

Presenter: Ablazov, Abdugaffor

8:16 am EDT
Abstract 41 Title: The Receptors DWARF14 and KARRIKIN INSENSITIVE2 Bind and Mediate Arabidopsis Response to the Apocarotenoid Zaxinone

Presenter: Moreno Beltran, Juan Camilo

8:25 am EDT
Abstract 42 Title: The evidence of 9-cis-β-Apo-10'-carotenal and 9-cis-3-OH-β-Apo-10'-carotenal as the precursors of strigolactones in rice

Presenter: Wang, Jian You

8:34 am EDT
Abstract 43 Title: First confirmed detection of ketocarotenoids in *Phasianidae* plumage

Presenter: Hao, Lezhi

8:43 am EDT
Abstract 44 Title: Aster-B regulates mitochondrial carotenoid transport and homeostasis

Presenter: Bandara, Sepalika

9:52 am EDT
Abstract 45 Title: Mechanisms of ketocarotenoid metabolism in the house finch (*Haemorhous mexicanus*)

Presenter: Toomey, Matthew

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9:01 am EDT **Questions and Discussion** (18 min)

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9:10 am EDT

Session X
Dietary Sources of Carotenoids and Nutritional Supplementation

Moderators: Associate Professor Jessica Cooperstone, The Ohio State University, Columbus, OH
Professor Salim Al-Babili, King Abdullah University of Science and Technology, Saudi Arabia

9:22 am EDT

Abstract 46 Title: Structural models of beta-carotene ketolase (CrtW) and beta-carotene hydroxylase (CrtZ) -the active site puzzle

Presenter: Ghosh, Robin

9:31 am EDT

Abstract 47. Title: Carotenoid and Vitamin A Intake in Infants and Toddlers (0-24mo) – Sources, Contribution of Pro-vitamin A Carotenoids, Demographic Patterns (NHANES)

Presenter: Senawong, Taegen

9:40 am EDT

Abstract 48 Title: SAPONIFICATION TO DETERMINE THE CAROTENOID CONTENT IN UVAIA – A NATIVE FRUIT FROM THE BRAZILIAN ATLANTIC FOREST

Presenter: Taver, Isabela

9:49 am EDT

Abstract 49 Title: Intake of carotenoid rich foods, systemic concentrations, and relationship with cardiovascular health

Presenter: Marhuenda-Muñoz, María

9:58 am EDT **Questions and Discussion** (12 min)

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10:10 am EDT **Break** (4 minutes)

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10:14 am EDT

Session XI
Carotenoid Synthesis
And
Carotenoids and Health II: Supplementation & Bioavailability - A

Moderators: Assistant Professor Nancy Moran, Baylor College of Medicine, Houston, TX, USA
Assistant Michael P. Dzakovich, Baylor College of Medicine, Houston, TX, USA

10:17 am EDT
Abstract 50 Title: Preparation of Allylic Benzothiazolyl (BT) Sulfides for Carotene Synthesis via Julia-Kocienski Olefination

Presenter: Koo, Sangho

10:26 am EDT
Abstract 51 Title: Synthetic Approaches to Staphyloxanthin

Presenter: De Lera, Angel

10:35 am EDT
Abstract 52 Title: Cellular absorption characteristics and transport mechanisms of free and esterified xanthophylls in Caco-2 cell monolayers

Presenter: Wu, Fan

10:44 am EDT
Abstract 53 Title: Improved Stability and *in vivo* Bioavailability of Lutein in Emulsions Stabilized by Octenylsuccinylated Starch and Pea Protein Isolate

Presenter: Alam, Sayma

10:53 am EDT **Questions and Discussion** (12 min)

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11:11 am EDT

Session XII
Carotenoids and Health II:
Supplementation & Bioavailability - B

Moderators: Assistant Professor Nancy Moran, Baylor College of Medicine, Houston, TX, USA
Assistant Michael P. Dzakovich, Baylor College of Medicine, Houston, TX, USA

11:08 am EDT
Abstract 54 Title: Compelling clinical benefits of macular carotenoids, curcuminoids and vitamin D3 for dry eyes

Presenter: Rai, Deshanie

11:17 am EDT
Abstract 55. Title: Skin Carotenoids Measured by Reflection Spectroscopy Correlate with Vegetable Intake and Carotenoid Intake of Hispanic Children but not their Fathers in Houston, Texas

Presenter: Barajas, Karla

11:26 am EDT
Abstract 56 Title: Optimizing lutein emulsions stabilized by octenylsuccinylated starch-soy protein isolate for improved in vitro bioaccessibility and *in vivo* bioavailability of lutein

Presenter: Zhang, Yanqi

11:35 am EDT
Abstract 57 Title: Skin Carotenoid Scores Measured by Reflection Spectroscopy Correlate with Adolescent Vegetable Intake in Houston, Texas

Presenter: Onyekwere , Elizabeth

11:44 am EDT
Abstract 58. Title: Placental Carotenoid Association with Maternal and Infant Systemic and Ocular Carotenoid Status in the Lutein and Zeaxanthin in Pregnancy (L-ZIP) Trial

Presenter: Addo, Emmanuel Kofi

11:53 am EDT **Questions and Discussion** (15 min)

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12:08 pm EDT **Organizer's Wrap-Up and Announcements**
12:18 pm EDT **Conclusion VICC 2024**

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Abstracts

Day 1 – July 23, 2024

Session I, II, III, IV

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Abstract 1

Alternative splicing of ZEAXANTHIN EPOXIDASE transcript harboring a premature termination codon and increased VIOLAXANTHIN DE-EPOXIDASE expression enhances zeaxanthin accumulation and triggers metabolic feedback regulation in capsicum fruits.

Christopher Cazzonelli, Western Sydney University, Sydney Australia
email: c.cazzonelli@westernsydney.edu.au

Capsicum fruit color is determined by carotenoid accumulation, an economically important trait selected for in horticultural breeding programs. We describe genetic mechanisms regulating carotenoid variation and metabolic feedback in immature green (35 days after pollination, DAP) and horticultural mature (65 DAP) ripe red 'Gina' and orange 'O06614' fruit cultivars. Immature fruits from the orange cultivar contained higher levels of lutein, β-carotene, antheraxanthin, violaxanthin and neoxanthin compared to the red cultivar. During ripening (45 and 55 DAP) total carotenoid levels rapidly increased in orange fruits, whereas at 65 DAP both varieties contained a similar total carotenoid content. Mature red fruits lacked lutein, violaxanthin and neoxanthin while capsanthin/capsorubin formed >50% of their total carotenoid composition. Orange fruits lacked capsanthin/capsorubin and zeaxanthin formed >40% of the total carotenoid pool. We demonstrate a 4-fold higher zeaxanthin content in orange relative to red fruits was due to homozygous and heterozygous single nucleotide polymorphisms respectively, in the 15th intron-exon boundary of *ZEAXANTHIN EPOXIDASE* (*ZEP*) that caused aberrant splicing leading to a premature termination codon in *ZEP*. The lack of capsanthin/capsorubin in fruits from the orange cultivar was due to a >2kb deletion in the upstream promoter region of *CAPSANTHIN-CAPSORUBIN SYNTHASE* (*CCS*) that caused a 960-fold reduction in *CCS* transcript levels. Transcriptomics and principal component analysis revealed variations between cultivars and during ripening. Gene ontological analysis revealed pathways enriched in phenylpropanoid biosynthesis, ascorbate biosynthesis, hormone-mediated signaling and polysaccharide metabolism during fruit ripening, while the mitotic cell cycle and apocarotenoid metabolism were enriched during immature green fruit development. A significant increase in *P450 β-RING CAROTENE 3-HYDROXYLASE* (CYP97A; 1.75-fold) and *VIOLAXANTHIN DE-EPOXIDASE* (*VDE;* 1.48-fold) expression correlated with zeaxanthin hyperaccumulation in mature orange fruit tissues. *VDE* was negatively correlated to *VITAMIN C2* involved in ascorbate biosynthesis. *CCS* expression was positively correlated with the expression of *PHYTOENE SYNTHASE*, *DWARF27* and *CAROTENE CIS-TRANS ISOMERASE*-like genes (spearman coefficient: >0.9, *p-value*: <0.0001) potentially indicating metabolic feedback regulation was linked to carotenoid signaling. We conclude that genetic perturbations impairing *ZEP* and *CCS* transcript levels enhance zeaxanthin without affecting total carotenoid composition in capsicum varieties thereby exerting beneficial antioxidant effects that can boost human health.

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Abstract 2

CAROTENOID AND COLOUR DEVELOPMENT DURING RIPENING OF MANGOES

[a]**TATSUYOSHI TAKAGI**, [b]Hung T. Hong, [c]Asjad Ali, [c]Natalie Dillon, [b]Peter Crisp and [a]Tim J. O'Hare

[a]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD, Australia (t.takagi@uq.edu.au; h.trieu@uq.edu.au; t.ohare@uq.edu.au) [b]School of Agriculture and Food Sciences, The University of Queensland, St Lucia, QLD, Australia (p.crisp@uq.edu.au). [c]Department of Agriculture and Fisheries, Mareeba, Queensland (natalie.dillon@daf.qld.gov.au; asjad.ali@daf.qld.gov.au)

Introduction: Mango (*Mangifera indica L.*) flesh can differ in colour depending on the cultivar, ranging from pale yellow to a dark orange when fully ripe. Prior to ripening, mango flesh is a pale yellowish cream colour, which during the ripening process can progressively develop darker and deeper colours due to the synthesis and accumulation of carotenoids. The total carotenoid concentration (TCC), individual carotenoid concentration and the proportions can vary strongly between cultivar as ripening progresses, which directly affects mango flesh colour. This study analysed differences in the carotenoid concentration and proportion, and the associated flesh colour as ripening progressed in six cultivars, ranging in colour from pale-yellow to dark-orange when fully ripe.

Materials and Methods: Fruit of six mango cultivars were harvested at mature hard-green from the germplasm collection at the Walkamin Research Facility (Queensland, Australia) and ripened at 23°C. The varieties were selected based on their known final flesh colour, ranging from dark orange (cvs. Creeper, Willard), orange-yellow (cvs. Phoenix, Thar) and pale-yellow (cvs. Himayat Pasand, Hong Sa). Carotenoid profiles and flesh colour were assessed at four ripening stages (3, 5, 7 and 10 days after harvest), with five fruit replicates assessed for each cultivar per ripening stage. Objective colour measurement was performed using a Konica chromameter (CR-400), and carotenoid extractions performed using the methodology of Hong et al. (2022), utilising ultra-high performance liquid chromatography coupled with diode array detection (UHPLC-DAD).

Results and Discussion: All six cultivars showed a decrease in hue angle (becoming more orange) and an increase in chroma (becoming more intensely coloured) as ripening progressed from 3 to 10 days. An overall increase in TCC was observed by 10 days in all cultivars.

As expected, the principal carotenoid in all six mango cultivars, all-trans β-carotene, followed a similar increase in concentration to that observed for TCC. Interestingly, the pattern of increase in all-trans β-carotene concentration during ripening was shown to be statistically significant between cultivars. However, the proportion was unique to each cultivar, and this proportion remained unchanged as ripening proceeded.

Each cultivar demonstrated different carotenoid concentrations and proportions, which resulted in their unique colour. However, while intensity of colour (chroma) was most strongly related to the total carotenoid concentration, flesh colour (hue) was most strongly related to the proportion of orange carotenoids, of which all-trans β-carotene was consistently the primary carotenoid in fully ripe fruit.

Acknowledgements
This project was funded by the Hort Frontiers Advanced Production Systems Fund (Genetics of Sensory Preference – AS19003) as part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from the Queensland Government and contributions from the Australian Government.

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Abstract 3

Overexpression of the carotenoid biosynthetic gene *LYCOPENE β-CYCLASE* (*LCYB*) improves root growth and influences rhizospheric communication in tobacco and tomato

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A main goal of plant biotechnology is the improvement of plant yield, resilience and nutritional value of crops. Carotenoids are essential pigments required for photosynthesis and the precursor of plant hormones and biologically active metabolites called apocarotenoids, which include vitamin A. Genetic engineering of carotenoid biosynthesis in crops has enabled the biofortification of grains, tubers and other edible organs with provitamin A carotenoids. However, recent studies showed that overexpressing the carotenogenic gene *LYCOPENE β-CYCLASE* (*LCYB*) also improved plant architecture, photosynthetic efficiency and stress tolerance. Here, we investigated the impact of overexpressing this gene on roots in tomato and tobacco. We show that *LCYB*-overexpressing plants exhibit enhanced root growth and biomass, due to the modulation of bioactive apocarotenoids and phytohormones. Moreover, the roots of these plants release fewer amounts of the phytohormone strigolactone, which affected their capability to establish and develop symbiosis with mycorrhizal fungi and reduced the activity of their root exudates in inducing seed germination in the root parasitic plant *Striga hermonthica.* Our findings highlight the importance of *LCYB* in regulating the growth and architecture of plants, as well as their rhizospheric interactions, and reveal the potential of this gene for increasing crop performance.

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Abstract 4

NUDIX23: A novel regulator of carotenoid biosynthesis and metabolon assembly

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Carotenoids are a group of pigments essential for plants. Carotenoid biosynthesis is highly regulated in response to diverse developmental and environmental signals. Carotenogenic enzymes are hypothesized to form metabolons to facilitate substrate channelling. However, the regulators conserved among plant species remain elusive and the facilitators required for metabolon formation are unknown. Phytoene synthase (PSY) is the major rate-limiting enzyme that catalyzes the highly regulated step of carotenogenesis. Geranylgeranyl diphosphate synthase (GGPPS) acts as a hub to interact with specific GGPP-utilizing enzymes for the synthesis of downstream isoprenoids. In this study, we discovered NUDX23, a Nudix domain-containing protein conserved among all plant species, as a novel posttranslational regulator of PSY and GGPPS, and an only known regulator required for carotenogenic enzyme metabolon assembly. Overexpression of *NUDX23* significantly increases PSY and GGPPS protein levels and carotenoid production, whereas knockout of *NUDX23* dramatically reduces their abundances and carotenoid accumulation in Arabidopsis. NUDX23 regulates carotenoid biosynthesis via direct interactions with PSY and GGPPS in chloroplasts, which enhances PSY and GGPPS protein stability in a large PSY-GGPPS enzyme complex. NUDX23 was found to co-migrate with PSY and GGPPS proteins in a high molecular weight complex and to be required for the complex assembly to facilitate metabolite channelling. The function of NUDX23 for the PSY-GGPPS enzyme complex assembly is conserved in bacteria and plants. Our findings uncover a new regulatory mechanism underlying carotenoid biosynthesis in plants and suggests a potential of *NUDX23* as an effective genetic tool for developing carotenoid-enriched food crops.

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Abstract 5

Maximizing saffron apocarotenoid production in varied tomato fruit carotenoid contexts

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Tomato ripe fruit is rich in liposoluble antioxidants, mainly lycopene, and low levels of β-carotene. Both carotenoids and vitamin C contribute to tomato antioxidant activity and benefit human health. Nevertheless, the water-soluble fraction of tomato is poor in antioxidant compounds. In our laboratory, we produced Tomaffron (TF), a transgenic tomato line expressing *Crocus sativus* CCD2 and accumulating high levels of saffron apocarotenoids, including crocins and picrocrocin, which are water-soluble apocarotenoids with high antioxidant activity. TF showed a higher overall antioxidant activity than its wild-type, MoneyMaker (MM). The precursors of *Cs*CCD2 are lutein and zeaxanthin; the second is not detected in MM ripe fruit, and the first is detected at low levels and disappears by the expression of *Cs*CCD2. Saffron apocarotenoids are also produced in *Gardenia jasminoides*. In addition to zeaxanthin and lutein, *Gj*CCD4a uses β-carotene and lycopene as substrates to produce saffron apocarotenoids.

We aimed to increase saffron apocarotenoid content in TF by crossing it with Xantomato, a quadruple mutant that accumulates high levels of zeaxanthin and β-carotene in the fruit. In addition, we expressed *Gj*CCD4a in MM and hp3/B^{Sh}, an intermediate mutant of Xantomato that accumulates high levels of β-carotene, to obtain saffron apocarotenoids from different carotenoid substrates.

From the cross of TF and Xantomato, we obtained different tomato plants accumulating higher levels of saffron apocarotenoids than TF and showing differences in carotenoid accumulation. In the T0 generation of the *Gj*CCD4a transformants, the accumulation of saffron apocarotenoids was 15 times higher in the hp3/B^{Sh} than in MM transgenic plants.

This work resulted in a wide diversity of tomato plants accumulating high levels of saffron apocarotenoids with different carotenoid profiles in ripe fruit, in addition to an accumulation of safranal, which is not present in wild-type fruit, and α and β-cyclocitral.

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Abstract 6

Functional characterization of novel CYC-B non-functional alleles in tomato and their relation to earlier lycopene accumulation and cold stress tolerance.

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Lycopene is a potent antioxidant carotenoid pigment present in tomatoes and other red fruits. This pigment is also important for the external quality of the fruit because it is responsible for the red color of tomatoes. Breeding plants with enhanced lycopene accumulation in fruits is the goal of numerous tomato breeding programs that aim to increase fruit quality. It has been reported that non-functional *CYC-B* mutants can accumulate more lycopene as it cannot be catabolized into β-carotene. Spontaneous *old gold* (*og*) and *old gold crimson* (*og^c*) mutants have been used in breeding programs to generate high-lycopene commercial cultivars. However, these mutations are linked to another mutation (*sp* allele) that confers determinate growth habits to tomato plants. Many fresh market crops require indeterminate growth cultivars, and consequently, the use of *og* and *og^c* mutations for breeding programs is limited. To overcome this, we used CRISPR/Cas9 to generate novel *CYC-B* non-functional alleles in indeterminate tomato breeding lines of INIA´s tomato breeding program. We previously characterized the LT16-*cycbΔ7* breeding line and found that lycopene accumulates earlier than in the LT16 wild-type isogenic line and that this is correlated with cold stress tolerance during postharvest storage. In the present study, we further characterized the novel *cyc-bΔ7* allele using a carotenogenic bacterial heterologous complementation system, explored the antioxidant properties of the LT16-*cycbΔ7* mutant line in response to cold stress during postharvest storage, and determined the phenotypic effects on another mutant line (LT46-*cyc-bΔ2*) that harbors a different *CYC-B* mutation. Increased cold stress tolerance was observed for the LT16-*cycbΔ7* genotype when breaker and mature green fruits were stored at 5°C for 13 days. In addition, the internal color index was higher in fruits from the LT16-*cycbΔ7* genotype after 20 days of storage at 5°C. However, no differences in the antioxidant properties were detected using the ABTS method for the lipophilic or hydrophilic fractions. Earlier internal color development was observed in both mutant lines when breaker and mature green fruit were stored at 25°C. Heterologous complementation assays in carotenogenic bacteria confirmed that the *cyc-bΔ7* allele was unable to transform lycopene into β-carotene. More studies are needed to explain the mechanism of lycopene involvement in cold stress tolerance in tomato fruits.

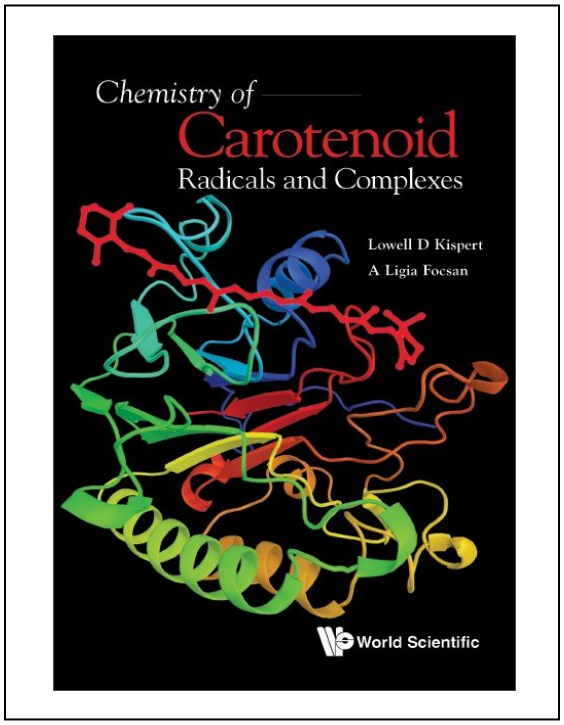
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Abstract 7

Carotenoid radicals and complexes analyzed using physicochemical techniques

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The continuous support from the U. S. Department of Energy for carotenoid research for almost 30 years enabled properties and structures of carotenoid radicals to be established. The most significant findings from this research have recently been gathered in a book titled "Chemistry of Carotenoid Radicals and Complexes." [1]. This book covers the chemistry of carotenoid radicals and special carotenoid complexes, as well as the physisorption, electron, and proton transfer processes that occur for carotenoids in different environments. Additionally, it details different techniques for the analysis of carotenoid radicals and complexes.

Among the numerous physicochemical techniques that have been employed in the studies of oxidation/reduction reactions of carotenoids and have been described in the book are: electrochemical measurements such as cyclic voltammetry (CV), 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 numerous other advanced EPR methods. Some CV results and EPR and ENDOR measurements performed in conjunction with DFT calculations, with possible implication in photoprotection, will be discussed in this session. These studies on carotenoid radicals are essential to understand some of the complex functions of the ubiquitous carotenoids.

[1] L. D. Kispert, A. L. Focsan, Chemistry of carotenoid radicals and complexes, December 2023, World Scientific Publishing Co. Pte. Ltd https://doi.org/10.1142/13471

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Abstract 8

METABOLIC ENGINEERING TO ATTAIN EFFICIENT PRODUCTION OF HIGH-VALUE XANTHOPHYLLS IN TOMATO

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Xanthophylls play pivotal roles in plant biology, contributing to the formation and function of photosynthetic complexes in leaves and imparting hues to reproductive organs, flowers, and fruits. Some of them have distinctive human health benefits, notably β-cryptoxanthin, lutein, and zeaxanthin. Consequently, significant efforts have been directed towards enhancing the content of these xanthophylls in crop plants, aiming to enrich the human diet. Our research endeavors have culminated in developing innovative strategies, including classical genetic approaches and genetic engineering techniques, to produce valuable xanthophylls in tomato fruits. Our focus lies in amplifying their biosynthesis and enhancing their accumulation within chromoplasts.

One notable achievement is the creation of a non-transgenic tomato line, dubbed 'Xantomato,' characterized by the accumulation of non-esterified zeaxanthin at concentrations surpassing 50 μg/g fresh weight, constituting approximately 50 percent of total carotenoids in the fruit. A possible limitation to the accumulation of zeaxanthin at higher concentrations lies in the manner of its sequestration in the cells. It has been shown that the esterification of carotenoids correlates with increased accumulation and stability within chromoplasts. The pivotal role of the acyltransferase enzyme PALE YELLOW PETAL 1 (PYP1) in fostering high concentrations of xanthophyll esters in flowers has been elucidated. To explore its impact in fruits, we have created transgenic tomato lines derived from the M82 variety, over-expressing the flower-specific SlPYP1 gene in the fruit. Although these transgenic fruits exhibited a marked increase in overall carotenoid content compared to the control variety M82, xanthophyll esters were absent, primarily due to the predominant accumulation of carotenes in the tomato fruit. Another possible bottleneck in β-xanthophylls biosynthesis is the rate of β-carotene hydroxylation. Therefore, a transgenic approach was applied by fruit-specific expressing of the CYP97A gene. Significant levels of zeaxanthin and β-cryptoxanthin were reached.

We are actively using this approach to investigate the effects of PYP1 expression with increased hydroxylation in zeaxanthin-accumulating and β-carotene-accumulating tomato lines, aiming to unravel its full potential in enhancing xanthophyll accumulation.

Abstract 9

Characterization and Genetic Mapping of a Novel Factor Affecting Carotenogenic Metabolic Flux in Melon Fruit

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Carotenoids are critically important for human health and nutrition. Although the carotenoid biosynthetic pathway has been well-established, its regulatory controls remain to be elucidated. Melon (*Cucumis melo*) exhibits over a 1,000-fold difference in fruit flesh carotenoid level, making it a remarkable model crop species for novel gene discovery. β-carotene, the most prominent carotenoid in orange-fleshed varieties, accumulates due to the "golden SNP" allele of the *Orange* (*Or*) gene. This allele stimulates chromoplast biogenesis and posttranslationally regulates the stability of phytoene synthase (PSY), the key rate-limiting enzyme of carotenoid biosynthesis. We identified a unique melon genotype harboring the golden SNP allele but developed a carotenoid deficient mesocarp surrounding a β-carotene rich endocarp. In this study we characterized this unique melon in comparison to an orange-fleshed cultivar. PSY and Or sequence, expression, and protein abundances show no explanative differences. Additionally, white mesocarp PSY activity was confirmed by an *in vitro* bacterial assay. Metabolic flux analysis at two developmental stages revealed a significant white flesh tissue specific reduction of the PSY-catalyzed product phytoene and suggests a factor affecting PSY activity. We termed this novel component "*CmCAF*" (*CAROTENOID ACCUMULATION FACTOR*). Subsequent QTL-seq analysis of F_2 bulks created from these characterized melons identified two QTLs associated with the white flesh phenotype. Fine genetic mapping of *CmCAF1.1* using over 1,300 F_2 plants narrowed it to a candidate range spanning 206 Kb containing 42 genes. Among these genes, six are predicted to be localized to plastids, the site of carotenoid biosynthesis. Current investigation is underway to characterize these plastid-localized candidate genes. This research presents a thorough characterization of a unique melon genotype and the foundation to reveal a novel posttranslational regulator of carotenoid biosynthesis in melon fruit. Findings gained through this investigation are highly translational to crop carotenoid biofortification efforts.

Abstract 10

Genetic analysis provides new insights into bixin biosynthesis

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Achiote (*Bixa orellana*) is a plant that is highly valued worldwide for its economic importance in bixin pigment production. However, conclusive molecular or biochemical demonstrations of the bixin production route remain elusive. We have undertaken a research initiative to study the biosynthesis route of bixin, with a specific focus on seed tissues where the pigment primarily accumulates.

Carotene cleavage dioxygenases (CCDs) enzymes form a wide range of apocarotenoids, such as bixin. The first step of bixin biosynthesis involves a CCD oxidative cleavage of lycopene at the 5,6/5',6' double bonds, resulting in bixin aldehyde. A transcriptomic *B. orellana* analysis identified eight genes, BoCCD1s and BoCCD4s, potentially involved in bixin synthesis. The production of bixin aldehyde was confirmed by LC-ESI-QTOF-MS/MS analysis of extracts of *E. coli* cells that accumulate lycopene and express the BoCCD1 and BoCCD4 proteins. *In vitro* assays also revealed the presence of bixin aldehyde, suggesting that cleavage activity at the 5,6/5',6' bonds of lycopene is present in all BoCCD1 and BoCCD4 proteins analyzed. Interestingly, we found that the *in vivo* four BoCCDs convert bixin aldehyde to norbixin, the second product of the bixin pathway.

The present study analyzed RNAseq data derived from three developmental stages of achiote seeds, which corresponded to three distinct morphotypes. After enrichment analysis, our investigation revealed a crucial role of triterpenes, sesquiterpenes, and cuticular wax production pathways. We also found genes associated with these three pathways by WGCNA analysis, which strongly correlates with the amount of bixin.

TPM and correlation analysis probed the significance of these particular genes in further detail. The study results emphasized the complexity of our findings by demonstrating the intricate role of genes associated with the triterpene and carotenoid pathways in the growth glands of cells and the accumulation of bixin in the achiote seeds. The particular activity of orthologues of BoHMGR, BoFFP, BoDXS, and BoHDR was revealed by analysis of crucial genes of the mevalonate (MVA) and 2C-methyl-D-erythritol-4-phosphate (MEP) pathways. This suggests that isoprenoid formation is required for chemicals in growing seeds' reddish latex. The production of bixin was highly correlated with the carotenoid-related genes BoPSY2, BoPDS1, and BoZDS, which is consistent with the need for carotene precursors for apocarotenoid biosynthesis. In the last stage of seed development, there was a strong correlation between bixin and the BoCCD gene (BoCCD4–4), as well as certain BoALDH (ALDH2B7.2 and ALDH3I1) and BoMET (BoSABATH1 and BoSABATH8) gene members. This suggests that several genes may have a role in synthesizing apocarotenoids. The results of our study suggest that the synthesis of bixin involves the participation of several genes and metabolic pathways, making it a complex process.

Our findings in this regard may contribute to a better understanding of this pigment's biosynthetic pathway and enable us to explore its potential applications in various industries.

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Abstract 11

Molecular events accompanying aggregation-induced energy quenching in Fucoxanthin-Chlorophyll Proteins

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The light harvesting system in oxygenic photosynthetic organisms has the ability to switch into a photoprotective mode, dissipating the excess excitation energy as heat through a process known as non-photochemical quenching (NPQ). Diatoms, possessing an antenna system constituted of fucoxanthin chlorophyll-a/c proteins (FCP), exhibit very efficient NPQ which, as in plants, displays a fast, ΔpH-dependent component, and is accompanied by a xanthophyll cycle in which diadinoxanthin is de-epoxidized into diatoxanthin. In high-light environments, diatoms accumulate pigments from this cycle, and high amounts of diatoxanthin lead to a faster and more pronounced NPQ. The molecular mechanisms underlying NPQ in diatoms are still unclear, but it is possible to mimic some aspects of this process by inducing aggregation of isolated FCP proteins. We have assessed this model system by resonance Raman measurements of two peripheral antenna complexes purified from *Cyclotella meneghiniana*, the trimeric FCPa and nonameric FCPb, isolated from both high-light- and low-light-adapted cells. We show that quenching is associated with a reorganisation of these proteins, which affects the conformation of their bound carotenoids, and in a manner which is highly dependent on the protein considered.

Abstract 12

How algae using the photoprotective diadinoxanthin cycle avoid the accumulation of violaxanthin cycle pigments

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Carotenoids are essential in protecting photosynthetic organisms against damage caused by excessive light intensities. The plastids of land plants and many algae possess an inducible photoprotective mechanism known as the violaxanthin cycle. High light intensities activate the enzyme violaxanthin de-epoxidase (VDE) that catalyzes the conversion of violaxanthin to zeaxanthin, with the latter promoting the thermal dissipation of surplus excitation energy in the photosynthetic machinery. In subsequent low light, zeaxanthin is converted back to violaxanthin by zeaxanthin epoxidase (ZEP), thereby restoring efficient harvesting of sunlight for photosynthesis.

Algae such as diatoms and haptophytes that are major contributors to marine primary production and global carbon dioxide fixation use a different xanthophyll cycle for photoprotection. Here, VDE de-epoxidizes the carotenoid diadinoxanthin to diatoxanthin that promotes the dissipation of excessive energy in the photosystems similar to zeaxanthin. Again, the back reaction is catalyzed by a ZEP. In principle, diatoms and haptophytes could also use the violaxanthin cycle for photoprotection as violaxanthin is a biosynthetic precursor of diadinoxanthin and always present in small amounts. Under most conditions, however, the pigments of the violaxanthin cycle, and particularly zeaxanthin, do not accumulate to a significant extent in these algae.

We have identified mechanisms contributing to the preponderance of the diadinoxanthin cycle in diatoms and haptophytes. By comparing the substrate specificities of VDEs from land plants and various algae in vitro, we observed that VDE enzymes from organism with violaxanthin cycle de-epoxidize both violaxanthin and diadinoxanthin with similar efficiency, although the latter is no natural substrate. VDEs from algae with diadinoxanthin cycle, however, markedly favor diadinoxanthin over violaxanthin, thereby minimizing the formation of zeaxanthin from violaxanthin under temporal high light stress. In land plants and green algae, zeaxanthin in an obligatory intermediate in the de-novo-biosynthesis of violaxanthin. For diatoms and haptophytes, however, we also found evidence that they can synthesize violaxanthin by an alternative pathway bypassing zeaxanthin. The evolution of mechanisms to avoid the accumulation of zeaxanthin suggests that the diadinoxanthin cycle offers a selective advantage over the violaxanthin cycle, possibly because it enables faster acclimation to rapidly changing light conditions.

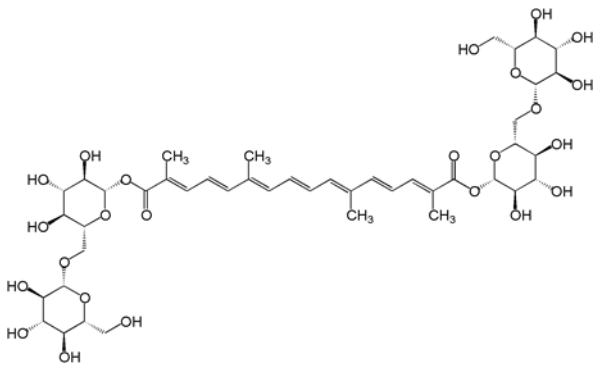
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Abstract 13

Mixed Micelles of Water-soluble Carotenoid Crocin

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Crocin (Figure 1) is a unique water-soluble carotenoid found in crocus and gardenia flowers.



Crocin has been shown to have a variety of pharmacological activities, such as antioxidant, anti-cancer, memory improvement, antidepressant, anti-ischemia, blood pressure lowering and aphrodisiac, gene protection and detoxification activities. However, similar to other carotenoids, practical application of crocin is restricted by its low stability in aqueous solution and high sensitivity to light, metal ions and oxygen species. The purpose of our study was to answer the questions, does crocin form mixed micelles or nanosized aggregates with water-soluble drug delivery systems glycyrrhizin and arabinogalactan, and how it effects on oxidation and photostability of this carotenoid. Earlier we have shown that these drug delivery systems form strong supramolecular complexes with various lipophilic carotenoids and can significantly improve their stability and solubility [see reviews 1-3]. It was demonstrated by a number of physical methods that due to amphiphilicity of crocin molecules, it forms concentration-dependent self-associate micelles in water solution, as well as mixed micelles with saponin glycyrrhizin, and nanosized aggregates with polysaccharide arabinogalactan. It was found that in mixed micelles with glycyrrhizin, crocin has much higher oxidation stability, but lower photo-stability in water solution. On the other hand, in the case of Fe(III) induced crocin photo-oxidation, both glycyrrhizin and arabinogalactan reduce the photo-oxidation rate. Also, antioxidant activity of crocin has been studied using model reaction of linoleic acid oxidation. The role of natural antioxidant ascorbate in metal induced lipid oxidation was also elucidated. In addition, the interaction of crocin with model lipid membrane has been investigated by nuclear magnetic resonance techniques and molecular dynamic simulation.

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Abstract 14

Efficacy of the bio-solvents 2-methyltetrahydrofuran and ethyl lactate for ultrasound-assisted extraction of carotenoids from phytoene-enriched *Dunaliella bardawil* in comparison with other traditional solvents

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Background: Phytoene is a colorless carotenoid that has not been extensively studied in comparison to colored carotenoids. Nevertheless, it is now clear that phytoene is a major carotenoid in our diet, is easily absorbed by the body, and may contribute to promoting health. The industry is increasingly focused on the adoption of more sustainable extraction technologies. Ultrasound-assisted extraction (UAE) offers higher efficiency in carotenoid extraction compared to traditional methods (e.g., maceration or Soxhlet extraction). There is also a trend to use greener solvents, recognized for their non-toxicity, recyclability, and biodegradability, as alternatives to the harmful solvents traditionally used in carotenoid extraction. Among these green solvents, ethyl lactate or 2-methyltetrahydrofuran (2-MeTHF) show promise and deserve further study. Additionally, research on microalgae continues expanding, not only as a source of carotenoids but also for their nutritional value and sustainability advantages.

Objective: This study focuses on evaluating the extraction efficiency of five different solvents in the UAE of microalgal carotenoids, especially phytoene. It also assesses the impact of ball mill pretreatment on the extraction process.

Methods: *Dunaliella bardawil* was used for this study, represented in fresh, freeze-dried, and encapsulated forms. The microalgae were incubated with an optimized amount of norflurazon herbicide for phytoene production. The UAE was carried out at 20 kHz, 30% amplitude, for 2 minutes. Five different solvents were tested: ethanol, methanol, ethyl lactate, 2-MeTHF, and dimethylsulfoxide (DMSO). The effect of pre-treatment of a ball mill (30 Hz, 5 min) was also studied.

Results: The carotenoids identified in the microalgae, were (15*Z*)-phytoene, (all-*E*)-lutein, (all-*E*)-β-carotene, (all-*E*)-phytoene, (all-*E*)-zeaxanthin, (9*Z*)-antheraxanthin, and (9*Z*)-β-carotene.

The ball milling pre-treatment did not significantly enhance carotenoid extraction across the tested solvents and matrices.

Regarding total carotenoid content (TCC), in the fresh matrix, methanol, ethanol, and 2-MeTHF demonstrated superior extraction efficiencies. Methanol was identified as the optimal solvent for the freeze-dried matrix and 2-MeTHF for the encapsulated matrix. Ethanol and 2-MeTHF were the best solvents for the extraction of (15*Z*)-phytoene. In the case of (all-*E*)-phytoene extraction, methanol was the optimal solvent in the fresh matrix. In the lyophilized matrix, all solvents tested except DMSO, were similarly effective. Finally, in the encapsulated matrix, ethanol and DMSO led to the highest extractions (Table 1).

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Matrix	Solvent	(15*Z*)-phytoene		(All-*E*)-phytoene		TCC	
Fresh	Ethanol	1674.7±117.4	A	238.3±45.2	B	3275±381.5	A
	Methanol	1763.7±32.6	A	442.6±6.4	A	3669.1±55.6	A
	Ethyl lactate	875.5±59.3	B	133.4±10	C	1524±126.2	B
	2-MeTHF	1720.9±20.4	A	274.5±7.1	B	3220.9±89.9	A
	DMSO	765±82.8	B	99.2±10.7	C	1374.6±124.3	B
Freeze-dried	Ethanol	2953.3±62.9	A	249.6±8.4	A	7936.3±118	B
	Methanol	3259.5±118.5	A	253.8±6.7	A	8464.1±273	A
	Ethyl lactate	2451.1±36.8	B	237.9±8.6	A	6430.7±171.3	C
	2-MeTHF	3145.3±216.3	A	236±21.8	A	7757.5±172.2	B
	DMSO	2307±31.2	B	195.8±5	B	5136.6±171.6	D
Encapsulated	Ethanol	81.1±2.7	A	6.4±0.4	A	166.4±4.1	AB
	Methanol	66.8±1	B	3.9±0.1	C	120.7±5.4	C
	Ethyl lactate	60.4±7.1	B	4.1±0.3	C	147.2±13	B
	2-MeTHF	82.1±2.8	A	5.3±0.5	B	186.9±6.2	A
	DMSO	83.3±3.4	A	6.9±0.4	A	161.5±7.1	B

Table 1. (15*Z*)-phytoene, (all-*E*)-phytoene, and TCC extracted with the different solvents tested from fresh, freeze-dried, and encapsulated phytoene-enriched *D. bardawil*. Different capital letters refer to differences between solvents in each matrix.

Conclusion: The application of the ball mill did not affect significantly carotenoid extraction in *D. bardawil*. 2-MeTHF emerged as a promising green solvent, especially for (15*Z*)-phytoene extraction, offering a possible new sustainable alternative for carotenoid extraction from microalgae. Life cycle assessment analysis is needed in the near future to better compare the sustainability of different extraction methodologies.

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Abstract 15

Evolution of carotenoid metabolism in the blind Mexican cavefish, *Astyanax mexicanus*

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How carotenoid metabolism evolves as animals adapt to diets of varying carotenoid content is not well understood. Obligate cave-dwelling species may experience severely limited carotenoid availability due to the absence of photosynthetic life. Such species may also have reduced carotenoid requirements as the need for vision and pigmentation is eliminated. We aim to understand how carotenoid metabolism evolves as animals adapt to carotenoid-limited diets using the Mexican tetra, *Astyanax mexicanus* as a model. *A. mexicanus* is a species of fish comprising a river-dwelling morphotype and an eyeless cave-dwelling morphotype that thrive in perpetually dark underground limestone caves in Northeastern Mexico. Surface fish consume plants and insects year-round while cavefish eat bat guano and seasonal flood debris. Both morphotypes are easy to maintain in the laboratory. Cavefish exhibit elevated fat accumulation and insulin resistance, traits that may provide them and advantage in an environment with infrequent access to food. We found that the visceral adipose tissue (VAT) of cavefish is strikingly yellow and has nearly 10 times the concentration of retinyl esters as surface fish fed the same laboratory diet, suggesting that cavefish have altered carotenoid metabolism. Some populations of field-collected cavefish also exhibit yellow VAT. We used surface/cave F2 hybrids to show that accumulation of yellow pigment in the VAT is not associated with eye loss but is associated with lower expression of *beta-carotene oxygenase 2a* (*bco2a*) in the gut. Cavefish also exhibit coding mutations in Bco2a that are predicted to form a truncated protein. We hypothesize that changes in *bco2a* expression or protein activity underlie altered carotenoid metabolism in cavefish. We will test this hypothesis using a cell-based assay to compare the enzymatic activity of surface fish and cavefish *bco2a*. We will also examine the impact of *bco2a* mutations on carotenoid accumulation and physiology *in vivo* using CRISPR-Cas9 gene editing to knock-out *bco2a* in surface fish and knock-in full length *bco2a* in cavefish. Our findings will lead to a better understanding of the genetic basis of natural variation in carotenoid bioavailability and the associated impacts on physiology and development.

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Abstract 16

Monitoring carotenoid content transitions by using RGB digital images as a tool for the early diagnosis of forest decline

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Anticipating drought-induced health transitions in trees has become crucial for reducing tree mortality, and therefore, minimizing forest decline. To address this challenge, ground-installing cameras have proven to be an effective and accessible tool. They monitor temporal changes in the vegetation structure and, thus, the leaves' phenology through the quantification of the red, green, and blue (RGB) colour channels. However, the association between digital imagery and the physiological status of the plant, based on biomarkers, such as carotenoid pools and photochemical efficiency, remains largely underexplored. This study aimed to evaluate the use of repeated imagery as a methodological approach for monitoring health transitions of *Quercus faginea* Lam. trees subjected to a drought experiment near Vitoria-Gasteiz (Basque Country, Spain). For this purpose, rain exclusion umbrellas (REUs) were installed on a total of 4 trees (simulating drought), while 4 other neighbouring trees were considered as control (no drought simulation). The REUs were specially designed to exclude precipitation and thus simulate drought events. The physiological status of each tree, including the carotenoid composition and the functioning of the photosynthetic apparatus of their leaves, was monitored alongside repeated imaging during the spring and summer of 2023. Our observations indicate that repeated imagery may accurately detect changes in the carotenoid content in the leaves of these trees, indicating thus the effectiveness of the near-remote techniques as monitoring systems and as possible indicators of tree health loss.

Keywords: Carotenoids, physiological status, RGB digital images, drought

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Abstract 17

β-Cryptoxanthin loaded chitosome: A breakthrough in Raspberry preservation for enhanced shelf-life and quality

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Fruit preservation is essential for extending shelf-life and maintaining optimal quality standards. However, there are some limitations, particularly with highly perishable fruits like raspberries. β-cryptoxanthin is a xanthophyll with provitamin A activity and has several benefits through its antioxidant characteristics. In this study, β-cryptoxanthin loaded chitosome, was used to coat raspberry, an exotic fruit with significant antioxidant activity. Raspberries coated with chitosomes experience reduced weight loss, sustained firmness and color retention, and exhibit lower levels of microbial growth, thereby significantly enhancing the shelf life. Furthermore, chitosomes show the potential in maintaining critical physicochemical features such as total soluble solids (TSS), titratable acidity, and antioxidant content during storage conditions. These findings highlight the potential of chitosome technology as a cost-effective and sustainable solution for improving raspberry preservation, significantly impacting the supply chain efficiency and overall market sustainability in the fruit industry.

Abstract 18

Optimization of plant carotenoid production through manipulation of abiotic and biotic growth environment

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We briefly review underlying theory of how leaf carotenoid content and composition is expected to change in response to environmental conditions, including biotic and abiotic factors, that impact the size of the photosynthetic apparatus via source-sink regulation. We illustrate how this framework can guide design of plant growth conditions that maximize production of multiple carotenoids of interest to the human consumer, while simultaneously minimizing input of light energy. We demonstrate such effects of growth conditions for members (genus *Lemna*) of the fast-growing *Lemnaceae* that are particularly suitable for a pre-harvest protocol designed to simultaneously maximize plant content of lutein, β-carotene, stable zeaxanthin, and high-quality protein. As aquatic floating plants, *Lemnaceae* can withstand sudden transfer of low-light-grown plants (that exhibit maximal levels of lutein and β-carotene as well as a uniquely high protein content) to very high light intensity (for additional accumulation of ample amounts of stable zeaxanthin) in a short pre-harvesting finishing procedure. This protocol minimizes the requirement for light-energy input while allowing production of a highly nutritious plant. In addition, we show that leaf carotenoid content declines under increasing atmospheric CO_2 in the common circumstances when elevated CO_2 triggers downsizing of the photosynthetic apparatus and its chlorophyll and carotenoid constituents. We illustrate how this negative effect in carotenoid content (as seen from the perspective of the human consumer) can be counteracted or prevented by the presence of microbiome and its interactions with *Lemna*.

Data presented will include unpublished data placed into the context of information from some of the sources below.

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Abstract 19

The mediating effect of UVB on eustress and distress on the accumulation of carotenoids in *Salicornia europaea*

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Halophytes (salt-tolerant plants) have the potential to be considered as alternative vegetables for future food production (Fitzner *et al.*, 2023b). Indoor cultivation allows the targeted modulation of cultivation conditions in favor of plant secondary metabolites (PSMs), e.g. carotenoids. A higher content of PSMs is desirable because of their health-promoting properties. One way of inducing PSMs is by UV radiation. Low or medium doses of UVB radiation can induce eustress leading to the accumulation of carotenoids, as has been shown for example in pepper and broccoli sprouts (León-Chan *et al.*, 2017). Higher doses of UVB radiation, on the other hand, can cause distress leading to the accumulation of reactive oxygen species (ROS) and a decrease in carotenoids. In particular, the response of halophytes to UVB radiation and the changes in carotenoid content and profile are poorly understood (Fitzner *et al.*, 2023a). The study summarizes the carotenoid accumulation in response to UVB eustress and distress in the halophyte *Salicornia europaea*. Furthermore, UVB signaling was investigated by analyzing the crosstalk between phytohormones, reactive oxygen species and carotenoids, and a correlation between oxidative stress and carotenoid accumulation was observed. The oxidative stress-induced accumulation of carotenoids was supported by model experiments inducing menadione stress. Taken together, we were able to provide insight into the UVB response and carotenoid accumulation of *S. europaea* and demonstrate the application potential of UVB radiation in indoor farming.

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Abstract 20

MECHANISMS OF CAROTENOID COLORATION IN MALE HOUSE FINCHES (*HAEMORHOUS MEXICANUS*): THE ROLES OF FRUIT CONSUMPTION, CAROTENOID INTAKE DURING MOLT, AND CAROTENOID STATUS AT MOLT ONSET

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The brilliant colors of birds have attracted special attention by researchers studying sexual selection and communication in recent years. The condition-dependent, sexually selected, carotenoid-based coloration of male house finches (*Carpodacus mexicanus*) is among the best-studied in this context. Numerous field and experimental studies indicate that elaborate expression of plumage redness in house finches depends upon adequate carotenoid intake, general nutrition, and health state during feather growth. Yet surprisingly the actual food sources that house finches obtain to acquire the carotenoids for becoming red are unknown; artificial colorants (e.g., purified astaxanthin supplements, tangerine juice) can produce red coloration in ornamental feathers, but otherwise house finches held in captivity during the molt period on a normal grain diet grow yellow feathers without exception. To further investigate dietary mechanisms underlying red color development in house finch plumage, we conducted a series of lab experiments using wild-caught house finches from Arizona, in which we fed birds different natural and colorful fruits (e.g., papaya, *Carica papaya*; prickly pear cactus, *Opuntia* sp.) or housed them in captivity for different durations of time prior to and during molt. Finches fed the orange and red fruit never developed red plumage; papaya-fed birds grew orange feathers despite circulating high concentrations of the carotenoid (β-cryptoxanthin) previously argued to be the main determinant (metabolic precursor) of red plumage pigmentation. In contrast, for the first time in this species, we found that house finches will grow red feathers in captivity when they have already started molting and circulating red plumage ketocarotenoids (e.g., 3-hydroxy-echinenone) through plasma at capture. Our studies fail to identify what house finches eat in nature to become red, but instead advance our understanding by showing that (a) diet at the precise time of feather growth does not have to contain suitable carotenoid pigments (i.e. non-xanthophyll precursors) for house finches to develop red plumage, and (b) ketocarotenoid status of males during early stages of molt is a key driver of red plumage color expression.

Key words: carotenoid pigmentation, condition dependence, plumage color, sexual signaling

Day 2 – July 24, 2024

Sessions V, VI, VII, & VIII

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Abstract 21

Unravelling the neuroprotective mechanisms of carotenes in differentiated human neural cells: Biochemical and proteomic approaches

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Carotenoids, which are fat-soluble pigments commonly found in plants and fruits, are known for their ability to protect against damage from free radicals. However, the potential neuroprotective effects of a complex of palm mixed carotenes (PMC) derived from virgin crude palm oil have not been thoroughly investigated. Hence, this study aimed to explore the neuroprotective properties of PMC on specialized human neural cells in the face of cytotoxicity induced by 6-hydroxydopamine (6-OHDA). Human neural cells were differentiated using retinoic acid over six days. Subsequently, these differentiated cells were pre-treated with PMC for 24 hours before exposure to 6-OHDA. The results showed that pre-treatment with PMC significantly reduced 6-OHDA-induced cytotoxicity, as evidenced by increased activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Additionally, PMC boosted the levels of dopamine and tyrosine hydroxylase (TH) within the neural cells. Notably, PMC pre-treatment effectively mitigated the overexpression of α-synuclein induced by 6-OHDA. Proteomic analysis revealed that PMC altered the expression of various proteins, including ribosomal proteins, α/β isotypes of tubulins, protein disulphide isomerases (PDI), and heat shock proteins (HSP), in the differentiated human neural cells. Overall, these findings highlight the potent antioxidant properties of PMC, derived from natural palm phytonutrients, and its significant neuroprotective effects against oxidative stress induced by free radicals.

Abstract 22

Sex-Specific Liver MicroRNA Profiles in Mouse Offspring of Western Diet-Fed Mothers: Impact of β-Carotene Supplementation during Lactation

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Introduction: Maternal unbalanced diets cause adverse metabolic programming and affect the offspring's liver microRNA profile. The liver is a site of β-carotene (BC) metabolism and a target of BC action. We studied the interaction of maternal Western diet (WD) and early-life BC supplementation on the epigenetic remodeling of offspring's liver microRNAs.

Methods: Mouse offspring of mothers fed a WD prior to and during gestation and lactation were orally given daily a placebo (controls) or BC (~3-fold the vitamin A ingested through milk) from day 2 to day 21 of life. The liver miRNAome was analyzed in recently weaned animals (26 days old) by microarray hybridization. Differentially expressed (DE) miRNAs were identified based on sex. Bioinformatics approaches were used for miRNA profiling. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of predicted DE miRNA target genes was conducted.

Results: BC impacted the liver miRNAome differently in male and female offspring, with no overlap in DE miRNAs between sexes and more impact in females. Bioinformatic analysis of DE miRNA predicted target genes revealed enrichment in biological processes/pathways related to metabolic processes, regulation of developmental growth and circadian rhythm, liver homeostasis and metabolism, insulin resistance, and neurodegeneration, among others, with differences between sexes. Fifty five percent of the overlapping target genes in both sexes identified were targeted by DE miRNAs changed in opposite directions in males and females.

Conclusions/Discussion: The results identify sex-dependent responses of the liver miRNA expression profile to BC supplementation during suckling and may sustain further investigations regarding the long-term impact of early postnatal life BC supplementation on top of an unbalanced maternal diet.

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Abstract 23

Dietary β-carotene promotes atherosclerosis resolution and reduces liver inflammation in mice

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Introduction: Atherosclerosis is the underlying cause of cardiovascular disease, and therefore, contributes to millions of deaths worldwide. Our recent findings show that β-carotene promotes atherosclerosis resolution (=healing) by decreasing the number of macrophages in the atherosclerotic lesion and reducing local inflammation. Hypothesis: Based on these findings, we hypothesize that β-carotene supplementation during atherosclerosis resolution could also contribute to mitigating liver steatosis and inflammation. Methods: We performed a secondary analysis in which we examined liver samples in two experimental models for atherosclerosis resolution. In both models, we fed our mice a vitamin A-deficient Western diet (41% fat + 0.3% cholesterol). After 12 or 16 weeks on diet, we harvested a subset of mice (Baseline), while the remaining mice continued on a diet (Control group) or were switched to a diet containing 50 mg/kg of β-carotene (BC group). Results: We observed that the BC group showed reduced fat and macrophage contents in the liver in comparison to Baseline and Control groups. These effects were abrogated in mice lacking β-carotene oxygenase 1 (BCO1), suggesting these effects depend on vitamin A formation. We did not observe changes in adipose tissue lipid or macrophage contents. This data suggests that dietary β-carotene during atherosclerosis resolution strategies could have a dual effect on ameliorating plaque and liver inflammation in mice.

Abstract 24

Mechanisms involved in the protective effect of carotenoids 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 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. Mitochondrial respiration was measured using the Seahorse and transcriptional activities were measured by reporter gene assays.

3. Results and Discussion

Rotenone almost completely reduced mitochondrial respiration and increased mitochondrial and cytosolic ROS. This was followed by increased transcriptional activities of the antioxidant response element/Nrf2 (ARE/Nrf2), NFkB and AP-1 transcription systems, and by apoptotic cell death, and MMP1 secretion and decreased collagen secretion. Pretreatment with carotenoid rich tomato extracts, restored mitochondrial respiration, 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 NFkB 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. To determine if activation of NFkB is a mechanism for cell damage, this transcription system was inhibited using the inhibitors, IKK-16 and JSH-23. This study indicates that carotenoids protect skin cells from damage caused by mitochondria generated ROS and thus, may delay skin aging and improve skin health.

Abstract 25

Haloarchaeal carotenoids as novel antiproliferative agents in human breast cancer: an *in vitro* study

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Breast cancer (BC) is still the leading cause of death (7%) in women worldwide. Carotenoids are well-known for their health-beneficial properties, including antioxidant, antiproliferative, immunomodulatory and antitumoral activities. Nevertheless, the properties of C_{50} carotenoids, such as the haloarchaeal carotenoid bacterioruberin, have not been addressed lately. Haloarchaeal carotenoids have excellent antioxidant activity, but there is still scarce information concerning their potential effects on human health.

Here, the aim was to evaluate the in vitro cytotoxic effect of *Haloferax mediterranei* bacterioruberin-rich carotenoid extracts (BRCE) (0–100 µg/ml) in six BC cell lines, representative of the intrinsic phenotypes and a healthy mammary epithelium cell line.

Cell index values were obtained using xCELLigence RTCA System. Furthermore, cell diameter, viability, and concentration were measured at 12 h, 24 h, and 30 h. We found that BC cells were selectively affected by BRCE (SI>1, p<0.005). After 30 h, the population of BC cells exposed to 100 µg/ml was 11.7–64.6% of the control ($p = 0.0001–0.0009$). Triple-negative cells were significantly affected (MDA-MB-231 (IC_{50} 51.8 µg/ml, $p < 0.0001$) and MDA-MB-468 (IC_{50} 63.9 µg/ml, $p < 0.0001$)). Cell size was also reduced after 30 h treatment in 3.8 (± 0.1) µm and 3.3 (± 0.02) µm for SK-BR-3 ($p < 0.0001$) and MDA-MB-468 ($p < 0.0001$), respectively.

In conclusion, *Hfx. mediterranei* BRCE exerts a cytotoxic effect on BC cell lines representative of all studied intrinsic subtypes. Furthermore, results obtained for MDA-MB-231 and MDA-MB-468 are very promising, considering the aggressive behaviour of the triple-negative BC subtype.

Abstract 26

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Exploring mutlitfunctional activities of pigment from Sporocarcina sp.

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Microbial pigments have received a lot of attention in recent years for its unique bioactive attributes and prospective applications in a variety of sectors. In this study, we extracted and studied crude pigment derived from Sporocarcina sp., which exhibited positive results for its antioxidant, antidiabetic, DNA-protective, and antibacterial characteristics. Crude pigment was extracted in methanol and its various applications were studied. Antioxidant activity was found to be 69.4% and 78.1% for maximum concentration of 500µg/ml by ABTS and DPPH methods. It had antibacterial activity against Gram negative strains (*Serratia marcescens*) and Gram-positive strains *(Staphylococcus aureus, Bacillus megaterium*), making it a potential candidate for antimicrobial applications. Pigment also showed anti-*Malassezia* activity, *Malassezia* is a dandruff causing fungus found in human scalp. Anti-amylase and antiglucosidic activity was found to be 94.7% and 40.05% respectively for highest concentration of 500µg/ml showing its application in diabetes. Its SPF was found to be 16.9. As it showed antimicrobial activity, further the dyeing efficacy was checked and antimicrobial activity of the dyed cloth was also checked which showed positive results for antimicrobial activity against *Serratia marcescens* and *Staphylococcus aureus*. It showed DNA protection activity against Fenton's reagent. Toxicity assay was done on *C. elegans* and it was found to be nontoxic, which may open its way to be used for humans after further confirmation.

Overall, our findings show that *Sporocarcina* sp., pigment are multifunctional bioactive compounds with numerous uses, including textiles, medicine, and biotechnology.

Keywords: antioxidant, antibacterial, textile

Abstract 27

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Metabolic engineering of cyanobacteria for phototrophic and secretory production of saffron pigments

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The large application potential of the saffron pigments crocetin and crocins in food, human healthcare and cosmetics has spurred increasing research into metabolically engineered production of these valued compounds in various microorganisms and plants. Present strategies have focused on enhancing accumulation of these saffron pigments and their isolation from engineered cells, which requires extensive biomass harvesting, extraction, and recovery. Here, we report the generation of the first crocetin-secreting *Synechocystis* sp. PCC 6803 strain, notable for utilizing sunlight and carbon dioxide to produce crocetin and facilitating its continual extraction from the growth medium. The co-expression of *carotenoid cleavage dioxygenase 2* (*CCD2*) from *Crocus sativus* and an endogenous *aldehyde dehydrogenase* (*SynALDH*) led to the extracellular secretion of crocetin, achieving a yield of 0.3 mg/L in three-day cultivation, which was further increased to 1.5 mg/L by adding glucose, without hindering cell growth. By sequentially linking cyanobacteria and *Escherichia coli* via a spatial separation approach, the final products crocins were successfully synthesized, reaching approximately 0.25 mg/L in 6 hours in the engineered *Escherichia coli* strain, equipped with *Gardenia glycosyltransferase GjUGT74F8*. Remarkably, over 95% of the total crocetin and crocins were secreted from cells into extracellular medium, laying the groundwork for a straightforward and effective purification method directly from growth medium. Taken together, this study provides an efficient biotechnological strategy superior to the common “cells-biomass-pigments” route and opens up the possibility for a truly continuous and carbon-negative manufacturing of saffron pigments.

Abstract 28

Improvement of neurosporaxanthin production in *Fusarium fujikuroi*

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Fusarium fujikuroi is a filamentous fungus that synthesizes neurosporaxanthin when it grows under illumination or under stressful conditions. Extracts rich in neurosporaxanthin has been demonstrated to have higher antioxidant properties than β-carotene *in vitro* (Parra-Rivero et al, 2020) but also to be a precursor of vitamin A. Experiments with mice have also shown that neurosporaxanthin is more soluble and more available than beta-carotenoid and astaxanthin (Miller et al, 2023). In our group we are studying the regulation of the synthesis of carotenoids in *Fusarium* to improve the production of neurosporaxanthin. The pathway for carotenoid synthesis has two branches, one that gives neurosporaxanthin and another one that gives β-carotene which is further converted in retinal, the precursor of vitamin A. This last step is carried out by the enzyme CarX. And the pathway is downregulated by CarS protein.

Carotenoid overproducer mutants have been isolated from a Δ*carX* strain with UV-irradiation. Optimization of culture conditions has been done in these mutants to improve the production of neurosporaxanthin. These super-producers accumulate up to 73% β-carotene when grown on optimized medium in flasks. The intense orange pigmentation in *F. fujikuroi* is associated with mutation of the *carS* gene, the main negative regulator of carotenogenesis in Fusarium. Although no mutations in this gene were found in any of the super-producing mutants in the Δ*carX* background, RT-PCR analysis showed a significant decrease in *carS* gene mRNA levels in them. For this reason, the genomes of two mutants were sequenced, revealing polymorphisms and structural variations in genes that could regulate carotenoid synthesis in *F. fujikuroi.*

Carotenoid super-producing mutants have been obtained by UV-irradiation mutagenesis from a ΔcarX mutant. These super-producers accumulate up to 73% β-carotene when grown on optimized medium in flasks. The intense orange pigmentation in F. fujikuroi is associated with mutation of the carS gene, the main negative regulator of carotenogenesis in Fusarium. Although no mutations in this gene were found in any of the super-producing mutants in the ΔcarX background, RT-PCR analysis showed a significant decrease in carS gene mRNA levels in these mutants. For this reason, the genome of mutants SG292 and SG313 was sequenced, revealing polymorphisms and structural variations in genes that could regulate carotenoid synthesis in F. fujikuroi. However, targeted mutations of the most promising candidates will be necessary to find out which of the variations found are responsible for the super-producing phenotype.

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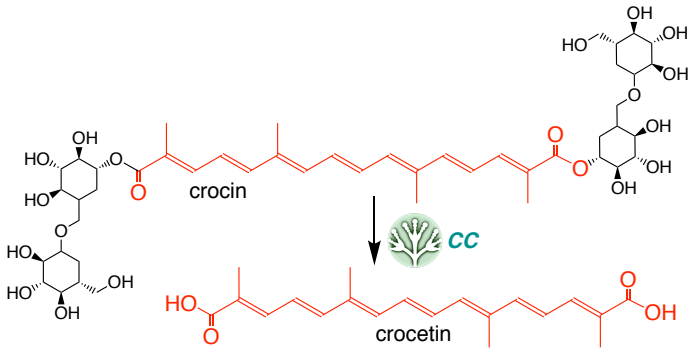
Abstract 29

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Cladosporium cladosporioides converts crocin to crocetin

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A commercial sample of crocin, purchased for determining the surface properties and solubility of the compound,[1] was found to be highly contaminated. During the purification process, diluted crocin solutions remained, which were collected in an open Erlenmeyer flask. After some weeks an adventitious spot was observed in the Erlenmeyer flask. Over time, the spot grew into a filamentous structure, suggesting the presence of an organism that thrives in a crocin rich environment, and raising questions as to its identity and mode of interaction with crocin. The organism was grown on three different culture media at 22°C, and subsequently identified, on the basis of its morphological characteristics and nuclear ribosomal DNA internal transcribed spacer sequence analysis, as *Cladosporium cladosporioides* (*CC*), a common airborne fungus.[2] *CC* from a different stock of the three cultural media was added to each of the three crocin solutions below the aggregation concentration c_{agg} = 0.8 mg/ml. Daily VIS spectroscopic measurements of the solutions for one week showed the gradual decrease until disappearance of crocin (λ_{max} = 442 nm) and the simultaneous formation of crocetin (λ_{max} = 420 nm). Unexpectedly, we have found, that crocetin can easily be synthesized by an airborne fungus. The synthesis with *CC* is straightforward compared to recent microbiological pathways for crocetin.[3]
Expectedly, *CC* did not hydrolyze bixin to norbixin.



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Abstract 30

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Does beta-carotene 9',10'-oxygenase (BCO2) contribute to the hypertrophy of the maternal heart during pregnancy?

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Study background: Pregnancy imposes dynamic alterations in the physiology of the mother that is usually reversed postpartum. Specifically, the maternal heart undergoes metabolic and morphological adaptations to maintain the health of the mother and the developing fetus. Maternal hypertrophy is a physiological condition that the heart transiently enlarges to adapt to persistent increased blood flow[1-2]. Maternal cardiac hypertrophy begins with a mechano-stress or hormonal stimulus followed by activation of various signaling pathway cascades including the renin-angiotensin system (RAS)[3]. Intake of carotenoids such as β-carotene - the most abundant dietary precursor of vitamin A - have been linked to the prevention of cardiovascular diseases[4]. β-carotene 9',10'-dioxygenase (BCO2), an enzyme that metabolizes β-carotene into apo-carotenoids, is the only β-carotene cleavage enzyme expressed in the adult heart. We found that *Bco2*$^{-/-}$ female mice have significantly smaller hearts than wild-type (WT) controls, both pre- and post-puberty. We also found that cardiac *Bco2* mRNA expression is upregulated during pregnancy in WT dams. Therefore, we wondered whether BCO2 may play a role in regulating maternal cardiac hypertrophy.

Materials and Methods: Age matched WT and *Bco2*$^{-/-}$ pregnant female mice, maintained on standard chow diets, were sacrificed at mid- (14.5 dpc) and late-gestation (17.5 dpc) to perform morphometric assessment of heart size, measure cardiac retinoid levels by HPLC and LC/MS and to measure cardiac mRNA expression of retinoid and hypertrophy regulatory genes. Age-, genotype- and diet-matched non pregnant females were used as controls.

Results and Discussion: Morphometric assessment (heart weight/tibia length) showed that the magnitude of the maternal cardiac growth was reduced during pregnancy in *Bco2*$^{-/-}$ dams. Interestingly, HPLC and LC-MS/MS analyses showed that, unlike the WT strain, cardiac retinyl ester levels in *Bco2*$^{-/-}$ dams were decreased while the retinoic acid levels were increased during gestation. Furthermore, cardiac mRNA expression analysis indicated that, retinoid biosynthesis and storage were attenuated and key components of the RAS pathway remained unchanged in the heart of *Bco2*$^{-/-}$ dams compared to WT controls. Overall, these and other findings provide novel critical insights into a potential role of BCO2 in regulating maternal heart hypertrophy.

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Abstract 31

Dietary Tomato Powder Feeding Attenuated Non-Alcoholic Fatty Liver Disease Independent of Sirtuin 1 Activity in Mice

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Background: Sirtuin 1 (SIRT1) is a highly conserved NAD^+-dependent protein deacetylase that modulates lipid metabolism, oxidative stress, and inflammation. We have previously shown that whole tomato (tomato powder, TP) and lycopene inhibited high-fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC) by modulating SIRT1 activity in animal models. Whether dietary TP inhibits NAFLD and HCC dependent on SIRT1 activity remains unknown.

Methods: Mice with homozygous ablation in catalytic activity of SIRT1 (MT) and their corresponding wild type littermates (WT) were injected with the carcinogen diethylnitrosamine at two weeks of age. At six weeks of age, mice were fed with a HFD (60% total calories from fat) with or without TP (containing lycopene 100 mg/kg diet or equivalent to dietary lycopene intake of 8.1 mg or 2-3 medium size of raw tomatoes (~300 g wet weight) per day in humans) for 34 weeks. Pathological and biochemical variables were assessed in the liver, mesenteric adipose tissue, and plasma.

Results: TP feeding significantly reduced plasma levels of triglycerides, hepatic steatosis score, and inflammatory foci in both HFD-fed WT and MT mice at comparable hepatic lycopene concentrations. However, the beneficial effects of TP feeding were achieved through different molecular mechanisms: In WT mice, the protective effect of TP was associated with increased hepatic SIRT1 protein expression and activity, along with decreased mRNA expressions of caspase-1 and fatty acid-binding protein 1 and lowered the concentration of hepatic IL-1β protein; In MT mice, TP feeding increased the mRNA expressions of glutathione peroxidase-1 and AMP-kinase phosphorylation, and decreased levels of malondialdehyde, acetylated-p53, and acetyl-CoA carboxylase in the liver. Furthermore, TP feeding significantly lowered the mRNA expression of IL-6 in mesenteric adipose tissue and the protein concentration of IL-6 in both plasma and the liver. Neither the lack of SIRT1 activity nor TP feeding altered the incidence, multiplicity, and volume of HCC in both WT and MT mice.

Conclusions: The present study demonstrates that dietary TP, independent of SIRT1 activity, ameliorates HFD-induced NAFLD, oxidative stress and inflammatory responses in the liver and mesenteric adipose tissue.

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Abstract 32

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Relationship of Early Life Dietary Carotenoids to Macular Pigment Optical Density, Vision and Retinal Integrity in Middle-Aged Men and Women

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Purpose: We previously reported that older women who were breastfed as infants in the 1920's-1940's had greater area of the carotenoids lutein (L) and zeaxanthin (Z) in their retina (macular pigment) than those who were fed infant formulas.† It has been established that having higher macular pigment is associated with better visual acuity and contrast sensitivity. We contribute to mounting evidence for the importance of L and Z in infancy to lifelong eye health, results of a pilot study in middle-aged men and women born in years when commercial formula lacking L and Z was common.

Methods: We conducted a retrospective cohort study to ascertain L and Z exposure during infancy (as determined by having been breast or formula fed), as well as mid-life L and Z intake, were related to their optical density in the macula, and measures of vision function and retinal structure. Seventy-seven participants ages 40 to 65 years were recruited by mass email to the University of Wisconsin community and by flyers in Ophthalmology offices. Before enrollment, telephone interviews were conducted to determine whether they met inclusion criteria: Could recall whether they were primarily breast or formula fed in their first six months of life, were not aware of any retinal disease, and did not consume >1 mg/day of LZ in supplements. Macular pigment optical density (MPOD) was measured by heterochromatic flicker photometry. Spectral-domain optical coherence tomography scans of each eye were acquired to measure retinal thickness. Vision functions were assessed (best corrected visual acuity (BCVA) and contrast sensitivity (CS). Means in ocular outcomes were assessed using linear regression.

Results: Participants who were formula fed as infants tended to have a lower MPOD at 0.5^{o} than those breastfed if their current dietary intake of L and Z was <2 mg/day (mean (SE) = 0.38 (0.06) vs. 0.54 (0.07), P= 0.09).

Conclusions: Maintaining adequate intake of lutein and zeaxanthin may be more important to retinal integrity and vision function in mid-life in individuals who were not exposed to retinal carotenoids through breastmilk or infant formulas as infants.

†Liu Z, et al. Exposure to lutein in infancy via breast milk and later life macular pigment optical density. IOVS, 2015; 56(7):192.

Abstract 33

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Understanding the molecular basis of postnatal heart growth defects in mice lacking β-carotene 9',10'-oxygenase (BCO2)

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Vitamin A is an essential micronutrient that supports many critical biological functions in mammals throughout the life cycle [1]. The most abundant dietary source of vitamin A is β-carotene [2]. Our lab recently demonstrated that three-month-old female mice lacking β-carotene 9',10'-oxygenase (*Bco2*$^{-/-}$), the only enzyme of the adult heart that cleaves β-carotene into vitamin A and its derivatives, i.e., retinoids, display cardiac retinoic acid insufficiency [3]. We also found that the heart of both male and female *Bco2*$^{-/-}$ mice maintained on the regular chow diet is smaller (ratio heart weight/tibia length) compared to the wild-type counterpart already at postnatal day 4 (P4), implying that the absence of the enzyme impairs the postnatal growth of this organ. The heart grows by proliferation in the first 7 days after birth, after which a shift from glucose to fatty acids as the main energy source, results in cardiomyocyte cell cycle arrest and a switch from proliferative to hypertrophic growth [4, 5]. This study aims to understand the molecular mechanism underlying the heart phenotype of the *Bco2*$^{-/-}$ mice. Preliminary analysis showed that *Bco2*$^{-/-}$ females' hearts have a reduced number of nuclei as well as a reduced number of mitotic events at P4 compared to their WT counterpart. However, by three months of age, *Bco2*$^{-/-}$ and WT control females display the same number of cells in the heart, even though the cardiomyocytes are significantly smaller in the heart of the mutant female mice. Based on these and other data, we hypothesized that the cardiac phenotype of the *Bco2*$^{-/-}$ may arise from impaired proliferation in the first few days of life, lack of cell cycle arrest at P7, as well as defective hypertrophy from P7 onwards. Experiments are ongoing to test this hypothesis by analyzing mRNA and protein levels of known markers of heart proliferation and hypertrophy, and cardiac mitochondrial functions at different postnatal stages in the mutant mice.

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Abstract 34

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Characterizing the Colon Carotenoid Proteome in a Diet-Induced Obesity Animal Model

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Carotenoids are natural pigments in carrots that display antioxidant and anti-inflammatory potential in multiple tissues of mammalian organisms, including along the intestinal tract. A high-fat diet can cause gut impairment by inducing barrier permeability, leading to low-grade chronic inflammation via metabolic endotoxemia. Our hypothesis for this study is that the dietary intake of carotenoid-rich foods will alleviate obesity-associated gut inflammation due to decreased intestinal barrier function. We used a diet-induced obesity animal model in which male C57BL/6J mice consumed one of four experimental diets for 20 weeks: Low-fat diet (LFD, 10% kcals from fat), high-fat diet (HFD, 45% kcals), HFD with 20% white carrot powder (HFD + WC), or HFD with 20% orange carrot powder (HFD + OC). High-performance liquid chromatography (HPLC) analysis revealed that carotenoids were not detected in the HFD+WC pellets, while HFD+OC pellets possessed high concentrations of α-carotene and β-carotene. Colon tissues, specifically distal sections, were harvested and subjected to a bottom-up proteomics workflow in which tryptic peptides were labeled with isobaric tandem mass tags. These peptides were separated by nano liquid chromatography coupled with mass spectrometry (nano LC-MS/MS) using a state-of-the-art mass spectrometer, an Orbitrap Eclipse Tribrid. Quantitative proteomics revealed a total of 4410 differentially expressed proteins. We then used bioinformatic tools for functional enrichment analysis and found that intestinal barrier-associated proteins, particularly those involved in mucin production and secretion, were highly upregulated in mice fed with orange carrots. Our study provided the first proteomic evidence that carotenoid-rich foods can promote barrier function.

Abstract 35

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Singlet oxygen scavenging activity of myxoxanthophylls from cyanobacteria *Synechocystis* sp. PCC6803 and *Anabaena* (*Nostoc*) sp. PCC7120.

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Myxoxanthophylls (myxol 20-glycosides) are carotenoid glycosides that occur almost exclusively in cyanobacteria. Recently, based on a new, liquid adsorption chromatography method, high-purity myxoxanthophyll fractions were isolated from two model species of cyanobacteria: filamentous *Anabaena* (*Nostoc*) sp. PCC7120 (accumulating myxoxanthophyll (3R,2‘S)-myxol 2’-fucoside) and 4-ketomyxoxanthophyll ((3S,2’S)-4-ketomyxol 2’-fucoside), and unicellular *Synechocystis* sp. PCC 6803 (accumulating myxoxanthophyll ((3R,2‘S)-myxol 2’-dimethyl-fucoside))[1,2]. The absorption maxima as observed in solutions of isolated myxoxanthophylls were amenable to solvent properties, displaying solvent-dependent shifts in the visible region (solvatochromism). In organic solvents, myxoxanthophylls show the absence of any specific solvent–solute interactions, as the energy of their electronic transitions increases only with solvent polarizability. In contrast, an aqueous environment promotes intermolecular interactions (aggregation), which are directly manifested as changes in the absorption and circular dichroism spectra of myxoxanthophylls[2].

The singlet oxygen scavenging activity of myxoxanthophylls has been analyzed in non-cellular experimental system, containing different ethanol:D_2O ratios. $^1O_2$ was generated by the excitation of rose Bengal dye (2mM) by an integrated nanosecond DSSNd:YAG laser (550 nm) and a time-resolved phosphorescence at 1270 nm was recorded using photon-counting mode. The ability of myxoxanthophylls to quench singlet oxygen was determined directly by measuring the effect of increasing concentrations of a myxoxanthophyll on lifetime of singlet oxygen photogenerated by rose Bengal in ethanol:D_2O mixtures.We found that both myxoxanthophyll and 4-ketomyxoxanthophyll were effective quenchers of singlet oxygen. Similar to other carotenoids, the observed rate constants for myxoxanthophylls were $>10^9$ [$M^{-1}*s^{-1}$], irrespective of their monomolecular or aggregated forms. Our results show that in carotenoid glycosides, the presence of sugar moiety has a little or no effect on the global singlet oxygen scavenging activity. In contrast, the presence of additional functional groups may affect the rate of $^1O_2$ quenching by myxoxanthophylls *in vitro*.

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Abstract 36

Circulating lycopene and lutein are associated with reduced adiposity and improved insulin sensitivity in healthy infants

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Objectives: Excess adiposity and metabolic abnormalities in infancy have been linked to future risk of obesity and cardiometabolic disease. Carotenoids exert metabolic benefits in adults, but their role in infancy is unknown. The purpose of this ancillary study was to determine if blood carotenoid concentrations are inversely associated with adiposity and positively associated with insulin sensitivity in healthy infants. **Methods:** Plasma carotenoid concentrations (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) were measured by HPLC in 12 healthy infants at 4, 6, and 8 months old (n=22 observations). Adiposity was estimated using skin fold thickness measurements and expressed as % body fat (%BF). Glucose and insulin were assessed, and the glucose-insulin ratio was calculated. Bivariate correlations and linear regression were employed. **Results:** Plasma lycopene was moderately and significantly inversely associated with %BF (r=-0.492, p=0.020). This remained significant after adjusting for breastfeeding status. Plasma lutein was moderately and inversely associated with %BF (r=-0.414, p=0.055). Plasma lycopene and lutein were moderately and significantly inversely associated with insulin (r=-0.571, p=0.041; r=-0.584, p=0.036, respectively). Further, plasma lutein was strongly correlated with glucose-insulin ratio (r=0.659, p=0.014), a measure of insulin sensitivity, and plasma lycopene was moderately associated with glucose-insulin ratio, albeit non-significant (r=0.538, p=0.058). Remaining carotenoids were weakly and non-significantly associated with outcomes. **Conclusions**: Results from this pilot study suggest that plasma carotenoid concentrations, namely lycopene and lutein, are associated with reduced adiposity and greater insulin sensitivity. Additional research investigating the molecular mechanisms by which carotenoids influence adipose tissue and cardiometabolic health in infants is warranted.

Abstract 37

Assessing Seaweed-Derived Carotenoids for Mitigating Persistent Inflammation and Epigenetic-Driven Immune Deficiency and Aging Post-Tuberculosis

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BACKGROUND: Post-treatment of tuberculosis (TB) can leave behind damaging epigenetic marks that lead to continued immune suppression and accelerated aging. Similar patterns are noticeable in individuals recovering from HIV and schistosomiasis, indicating a potential shared epigenetic disruption. Rich in antioxidants and anti-inflammatory agents, seaweed-derived carotenoids present a unique avenue for therapeutic intervention. They could potentially counteract these post-infectious epigenetic changes. Our research delves into how carotenoids specifically target and may reverse epigenetic-driven immune suppression and the premature aging seen in survivors of TB, HIV, and Schistosomiasis.

METHODS: Our investigation enlisted participants from a TB-recovered cohort in ESwatini, alongside healthy individuals residing in the same households, over a period from 2013 to 2020. The study tracked 167 post-TB patients and 275 control subjects, conducting assessments over 12 months. Biomarker analyses were performed at baseline and at the end of TB therapy, evaluating inflammation, oxidative stress, DNA methylation, and immune function. Approximately 60% of TB survivors continued to demonstrate high inflammation levels, as evidenced by C-reactive protein (CRP) exceeding 3 mg/dL, irrespective of HIV status, gender, or age. We employed high-performance liquid chromatography (HPLC) to quantify carotenoid concentrations in plasma from TB, HIV, and Schistosomiasis patients, pre- and post-treatment.

RESULTS: Our Results indicate enduring inflammation and heightened lipid peroxidation, alongside diminished reduction capacity and Carotenoid levels, in participants newly diagnoses with TB. Notably, theses parameters did not return to baseline levels even after successful therapy. Lipid peroxidation (oxidized LDL), induces atherosclerosis, and remains 2-times above the upper limit of normal in 61% of “cured” TB patient participants (HHC 1.7, TB 16, TB EOT 9.6; $p<0.0001$). Similarly, carotenoids, a lipophilic dietary reducer of lipid peroxidation, normalized in only 30% of cured TB survivors.

CONCLUSIONS: These findings suggest a potential link between carotenoid deficiency and premature aging in post-infectious individuals. Addressing inflammation and restoring carotenoid levels may mitigate premature aging in TB survivors, with potential implications for HIV and Schistosomiasis as well. Further investigations will elucidate the therapeutic potential of seaweed-derived carotenoids in this context.

Abstract 38

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Evaluating metabolic programming by β-carotene during the perinatal stage in mice lacking or expressing BCO1

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The perinatal stage is an essential window of time in which nutrition influences metabolic health in adulthood. Maternal diet can modify milk composition and, therefore, affect the nutritional status of the offspring during lactation and adulthood. β-carotene, which is present in human breast milk, is cleaved to vitamin A by the β-carotene oxygenase 1 (BCO1). Retinoic acid, the transcriptionally active form of vitamin A, is essential during embryonic development and is involved in the regulation of energy metabolism in adulthood. Whether intact β-carotene has additional biological roles besides serving as a vitamin A precursor remains unclear. To explore the bioactive role(s) of β-carotene in milk as (1) an intact molecule and (2) a vitamin A precursor, we provided β-carotene to pregnant Bco1-/- dams carrying Bco1-/- and Bco1+/- pups, and Bco1+/+ dams carrying Bco1+/+ pups. A subset of pups was weaned onto a normal-fat (NFD) or high-fat diet (HFD) for three months, while another subgroup was harvested at weaning time. **Results**: We detected β-carotene in the milk of Bco1-/- dams fed β-carotene, and in plasma and tissues in the Bco1-/- offspring at weaning time. Preliminary data suggest that Bco1-/- female mice fed HFD displayed a reduction in adiposity and liver fat accompanied by a tendency towards a higher expression of insulin sensitivity markers and a downregulation of adipogenesis markers compared to Bco1+/? females. These effects were not observed in Bco1-/- males. **Conclusion**: A β-carotene-enriched maternal diet might offer unique protection to female offspring against diet-induced obesity. This effect might be independent of β-carotene conversion to vitamin A.

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Abstract 39

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Prebiotic Effect of Lycopene on Gut Microbiome with Systemic Changes in Liver Metabolism, Skeletal Muscles and Skin in Moderately Obese Persons

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Lycopene rich food and dark chocolate are among the best-documented products with broad health benefit. This study explored the systemic effect of lycopene and dark chocolate (DC) on gut microbiota, blood, liver metabolism, skeletal muscle tissue oxygenation and skin.

30 volunteers were recruited for this trial, 15 women and 15 men with a mean age of 55 $\pm$ 5.7 years and with moderate obesity, 30 < BMI < 35 kg/m^2. They were randomized and divided into five equal interventional groups: three received different formulations of lycopene, one of them with 7 mg of daily dose and two with 30 mg, another group was given 10 g of DC with 7 mg lycopene embedded into its matrix, and the last group received 10 g DC. The trial was double-blinded for three lycopene groups and separately for 2 DC groups; the trial lasted for 1 month. By the end of the trial there were dose-dependent changes in the gut microbiota profile in all three lycopene groups with an increase of relative abundance of e.g. *Bifidobacterium adolescentis* and *Bifidobacterium longum*. This was also accompanied by a dose-dependent changes in the blood, liver metabolism, skeletal muscle and skin parameters. Consumption of DC resulted in increased relative abundance of e.g. *Lactobacillus* and a reduction of corneocyte exfoliation. This is the first study, which reports the prebiotic potential of lycopene and DC.

Day 3 – July 25, 2024

Sessions IX, X, XI, & XII

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Abstract 40

***Zaxinone Synthase* overexpression modulates rice metabolism and physiology, improving growth and productivity under normal and low phosphate supply**

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The rice *Zaxinone Synthase (ZAS)* gene encodes a carotenoid cleavage dioxygenase (CCD) that forms the apocarotenoid growth regulator zaxinone *in vitro*. Here, we generated and characterized constitutive *ZAS*-overexpressing rice lines, to better understand *ZAS* role in determining zaxinone content and regulating growth and architecture. *ZAS* overexpression enhanced endogenous zaxinone level, promoted root growth and meristem size, and increased the number of productive tillers, leading to an up to 30% higher grain yield per plant. Hormone analysis revealed a decrease in strigolactone (SL) content, which we confirmed by rescuing the high-tillering phenotype through application of a SL analog. Metabolomics analysis revealed that *ZAS* overexpressing plants accumulate higher amounts of monosaccharide sugars, in line with transcriptome analysis. Moreover, transgenic plants showed higher carbon (C) assimilation rate and elevated root phosphate, nitrate and sulfate level, enhancing the tolerance towards low phosphate (Pi) and indicating a generally better nutrient uptake. Our study confirms *ZAS* as an important determinant of rice growth and architecture and shows that *ZAS* regulates hormone homeostasis and a combination of physiological processes to promote growth and grain yield, which makes this gene an excellent candidate for sustainable crop improvement.

Key words: Zaxinone, Zaxinone Synthase, Apocarotenoids, Rice, Strigolactone, Tiller number

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Abstract 41

The Receptors DWARF14 and KARRIKIN INSENSITIVE2 Bind and Mediate Arabidopsis Response to the Apocarotenoid Zaxinone

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Strigolactones (SLs) are plant hormones involved in plant growth, stress response and plant-organism interactions. DWARF14 (D14) and KARRIKIN INSENSITIVE2 (KAI2) are α/β hydrolases involved in the perception of SLs and Karrikins (KAR), a smoked-derived growth regulator. Zaxinone is an endogenous plant apocarotenoid growth regulator that enhances SL and ABA contents in Arabidopsis *via* a yet unknown perception machinery. Here, we show that zaxinone affects the transcription of 96 genes including the up-regulation of the SL biosynthetic genes *CAROTENOID CLEAVAGE DEOXYGENASE7* (*CCD7*) and *CCD8* and resulting in increased SL content. Moreover, this transcriptional regulation depends on *At*D14 and the F-Box protein MORE AXILLARY BRANCHING2 (*At*MAX2) but not *At*KAI2. *In vitro* binding and competition assays demonstrate that zaxinone is a novel ligand of *At*D14. Structural analysis and pull-down assays revealed that zaxinone occupies the SL-binding pocket in *At*D14 acting as a SL antagonist and interrupting the SL-triggered interaction between *At*D14 and *At*MAX2. In addition, *in vitro* and *in silico* assays show that zaxinone binds to KAI2 and that this receptor is required for a significant part of the Arabidopsis transcriptional response to zaxinone. These findings uncover a perception mechanism for the hormone candidate zaxinone in Arabidopsis and demonstrate the capability of *At*D14 and *At*KAI2 to bind different types of endogenous signalling molecules and mediate their transduction, revealing one more unique feature of these proteins with a dual receptor/enzyme function.

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Abstract 42

Evidence of 9-*cis*-β-Apo-10'-carotenal and 9-*cis*-3-OH-β-Apo-10'-carotenal as the precursors of strigolactones in rice

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Strigolactones (SLs) are apocarotenoid-derived plant hormones that regulate plant architecture and mediate rhizospheric communications. Several *in vitro* assays using heterologously produced enzymes unraveled the conversion of all-*trans*-β-carotene *via* the intermediate 9-*cis*-β-apo-10'-carotenal into the SL precursor carlactone (CL). Besides, recent studies indicate that plants can also synthesize 3-OH-CL from all-trans-β-zeaxanthin via the intermediate 9-*cis*-3-OH-β-apo-10'-carotenal. However, direct evidence for the formation of SLs from 9-*cis*-β-apo-10'-carotenal and 9-*cis*-3-OH-β-apo-10'-carotenal remains elusive. Herein, we supplied rice seedlings with both ^{13}C-labeled 9-*cis*-β-apo-10'-carotenal and ^{13}C-labeled 9-*cis*-3-OH-β-apo-10'-carotenal, and monitored the synthesis of SLs using liquid chromatography-mass spectrometry (LC-MS) and Striga bioassay. We further validated the biological activity of the two apocarotenoid-derived SLs using the *ccd7* SL-deficient rice mutant, which cannot catabolize 9-*cis*-configuration carotenoids into 9-*cis*-apo-10'-carotenals. Our results demonstrated that both 9-*cis*-β-apo-10'-carotenal 9-*cis*-3-OH-β-apo-10'-carotenal are the SL biosynthetic intermediate *in planta*. Moreover, we proved that the application of labeled long-chain apocarotenoids as a promising approach to investigate apocarotenoid metabolism, paving the way for investigating apocarotenoid-derived growth regulators.

Abstract 43

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

First confirmed detection of ketocarotenoids in *Phasianidae* plumage

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Phasianidae (peafowls, pheasants, quails, etc.) males exhibit some of the most extreme plumage ornaments and colors in birds, presumably driven mainly by sexual selection, yet to date the underlying plumage pigmentation pathways have been poorly characterized in this lineage. More ancient bird groups such as the gamebirds (*Galliformes*) predominantly use melanin pigments in their black, brown, gray, etc. feathers, but several *Phasianidae* species display potential carotenoid-based red, orange, and yellow plumage colors. We used high-performance liquid chromatography (HPLC) to detect ketocarotenoids within orange nape feathers of male golden pheasants (*Chrysolophus pictus*), representing the first confirmed detection of ketocarotenoids in *Phasianidae* plumage. We identified 9 major carotenoid peaks on the HPLC chromatogram, 5 of which had absorbance properties consistent with ketocarotenoids (including echinenone and canthaxanthin) and 4 of which had absorbance profiles typical of xanthophylls (including lutein). Our findings suggest that ketocarotenoid-based plumage colors already evolved in this old family of birds, and open up interesting future research questions regarding the evolution and mechanisms of ketocarotenoid-based plumage coloration as a potential rare and novel trait in ancient avian lineages.

Abstract 44

Aster-B regulates mitochondrial carotenoid transport and homeostasis

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The scavenger receptor class B type 1 facilitates uptake of cholesterol and carotenoids into the plasma membrane of mammalian cells. Downstream of SR-B1, Aster-B protein mediates the non-vesicular transport of cholesterol to mitochondria for steroidogenesis. Mitochondria also are the place for the processing of carotenoids into diapocarotenoids by β-carotene oxygenase-2. Therefore, our purpose is to understand the role of these lipid transport proteins in carotenoid metabolism. We generated expression plamids to express the StART-like domain binding protein in carotenoid synthesizing *E. coli*. We established protocols to purify the carotenoprotein complexes for spectroscopic analysis. We established a novel carotenoid uptake assay to determine carotenoid uptake and distribution in human cell lines. We used HPLC and other analytical methods to determine carotenoid concentrations in different subcellular compartments. We showed that the recombinant StART-like lipid-binding domain of Aster-A and B preferentially binds oxygenated carotenoids such as zeaxanthin using recombinant protein co-expression with carotenoids. We demonstrated that Aster-B expressing A549 cells transport zeaxanthin and lutein to mitochondria. The pure hydrocarbon β-carotene is not transported to the organelles, consistent with its metabolic processing to vitamin A in the cytosol by β-carotene oxygenase-1. Depletion of the plasma membrane from cholesterol by methyl-β-cyclodextrin treatment enhanced zeaxanthin but not β-carotene transport to mitochondria. Loss-of function assays by siRNA in A549 cells and the absence of zeaxanthin accumulation in mitochondria of ARPE19 cells, confirmed the pivotal role of Aster-B in this process. Our study in human cell lines established Aster-B protein as key player non-vesicular transport of zeaxanthin to mitochondria and elucidated the molecular basis of compartmentalization of the metabolism of non-provitamin A and provitamin A carotenoids in mammalian cells. Expression of these components in the human retina suggests that these mechanisms contribute to macular pigment distribution in the eyes.

Abstract 45

Mechanisms of ketocarotenoid metabolism in the house finch (*Haemorhous mexicanus*)

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Abstract: The carotenoid-based colors of birds are a celebrated example of biological diversity and an important system for the study of evolution. The house finch (*Haemorhous mexicanus*) has become a textbook example color signal evolution where the ketocarotenoid-based plumage coloration of males appears to be a quality-dependent signal used in mate choice. However, the molecular and biochemical mechanisms of house finch color expression are not well understood. Recently a two-step mechanism, with the enzymes Cytochrome P450 2J19 (CYP2J19) and D-β-hydroxybutyrate-like (BDH1L), was described for the biosynthesis of ketocarotenoids in the avian retina and plumage of some bird species. We hypothesized that house finch color expression relied on this same mechanism and tested this by examining the catalytic function of the house finch homologs of these enzymes and tracking their expression in house finch tissues. Contrary to our hypothesis, we found that CYP2J19 and BDH1L did not catalyze the production of the primary house finch ornamental pigment, 3-hydroxy-echinenone, though they do produce many other ketocarotenoid types. Gene expression analyses (RNA-seq) revealed essentially no expression of *CYP2J19* in the putative sites of pigment metabolism (liver and growing feather follicles). We propose that house finches use an alternative enzymatic pathway to produce their characteristic red ketocarotenoid-based coloration, which may explain the exceptionally large color variation observed in male house finches and its sensitivity to physiological condition.

Abstract 46

STRUCTURAL MODELS OF β-CAROTENE KETOLASE (CrtW) AND β-CAROTENE HYDROXYLASE (CrtZ) - THE ACTIVE SITE PUZZLE

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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In bacteria and many plants, the pathway leading to the ketocarotenoid astaxanthin involves the consecutive, and combinatorial action of two small enzymes, β-carotene ketolase (CrtW) and β-carotene hydroxylase (CrtZ) [1]:
Despite the fact that many CrtWs and CrtZs have been employed for the production of keto- and hydroxycarotenoids in recombinant hosts [2], little is known about the enzymatic mechanisms of the enzymes, and no atomic stuctures are yet available. We are currently using the purple bacterium, *Rhodosprillum rubrum*, as both a production and overexpression host for CrtW and CrtZ from *Paracoccus sp*. N81106 (formerly *Agrobacterium aurantiacum*) (Fig. 1):

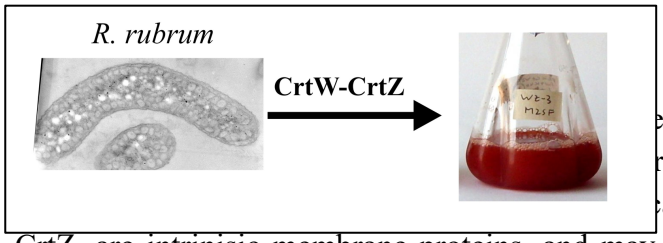


Fig. 1. Production of plant keto- and hydroxycarotenoids introducing a codon-optimized synthetic *crtW-crtZ* cistron into *R. rubrum*, and producing dark

have performed preliminary modelling of the
prediction algorithm [4], together with additional
cal analysis. We predict that CrtW, and possibly
microaerophilic conditions (right panel)
(manuscript in preparation).

CrtZ, are intrinisic membrane proteins, and may use Fe or di-Fe as a cofactors. However, the prediction of the putative active site shows a puzzling aspect. We will report on this here.

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Abstract 47

Carotenoid and Vitamin A Intake in Infants and Toddlers (0-24mo) – Sources, Contribution of Pro-vitamin A Carotenoids, Demographic Patterns (NHANES)

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Carotenoids contribute to infant vitamin A requirements and are associated with pediatric neurodevelopment. For children 2–19 years, the average vitamin A intake was 497 to 680 mcg retinol activity equivalents. However, typical amounts and patterns of carotenoid, preformed vitamin A, and total vitamin A intake in very young children are poorly understood. Cross-sectional data from 1682 infants and toddlers aged 0 to 24 months from the 2015-2020 National Health and Nutrition Examination Surveys (NHANES) were analyzed. Two models were used: multivariate logistic regression and gamma GLM with a log link function to define the significant age, poverty status, race, and ethnic correlates with of the outcomes of interest (total vitamin A intake, vitamin A equivalents from carotenoids [VAC], provitamin A carotenoid intake [PVA], and total carotenoid intake [TCI]). Carotenoid and vitamin A intake from human milk could not be evaluated due to missing data in the NHANES database. Mean total carotenoid intake was 1333.8 ± 3020.7 mcg, 3710.2 ± 3970.7 mcg, and 4444.9 ± 4620.3 mcg in 0-6,6.1-12-, and 12.1-24-month-olds, respectively. Age was associated with significant intakes of total vitamin A ($p = 0.000$), preformed vitamin A (retinol, $p = 0.000$), TCI ($p = 0.000$), VAC ($p = 0.000$), and PVA ($p = 0.000$). There was also a significant association between male sex and increased retinol intake ($p = 0.039$) and between "Not in poverty" status and decreased intake of retinol ($p = 0.001$) and increased VAC ($p = 0.013$). Pro-vitamin A carotenoids provided 21% of the RDA for the 0–6-month-olds, 36% for the 6.1–12-months, and 35% for the 12.1–24-month-olds. Compared to other food categories, pureed baby food vegetables provided the highest amounts of PVA, TCI, and VAC. Milk and dairy accounted for the greatest amount of retinol intake per day (38%). Baby food vegetables are the major source of carotenoids during this life stage. Young children's carotenoid intake increases significantly with age and feeding stage, and provitamin A carotenoid intake contributes substantially to their vitamin A requirements. Future research on the biological roles of carotenoids and contribution of human milk to carotenoid intake in early life are warranted.

Abstract 48

SAPONIFICATION TO DETERMINE THE CAROTENOID CONTENT IN UVAIA – A NATIVE FRUIT FROM THE BRAZILIAN ATLANTIC FOREST

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Introduction: Uvaia (*Eugenia pyriformis* Cambess) is a native fruit of the Atlantic Forest belonging to the Myrtaceae family. It is primarily found in the southeastern region of Brazil and is known for its pale-yellow to bright orange colour and oval shape. The fruit has a delicate skin, with a juicy flesh containing one to three seeds. Uvaia has great potential for consumption due to its flavour, aroma, and functional properties [1]. Despite its lack of commercial cultivation, research on the uvaia fruit is increasing in various areas such as genetics, fruit quality and storage. However, there is still conflicting information regarding to the main carotenoid present [1,2]. The current study focuses on analysing the carotenoid profile of uvaia fruit samples before and after saponification, highlighting the importance of the saponification process in profile analysis of uvaia. **Materials and Methods:** Ripe uvaia fruit from the 2022 season was harvested from the Laboratory of Postharvest (ESALQ – USP, Brazil) germplasm collection. Fruit samples were freeze-dried and carotenoids extracted using a 70:30 hexane:DCM solution. An unsaponified aliquot was subsampled, and the remaining portion was saponified. The extracts were analysed using LC-MS and carotenoids were quantified using standard curves derived from carotenoids obtained from Sigma-Aldrich. All processes were conducted according to the methodology outlined by Hong et al. [3]. **Results and Discussion:** Twenty-nine carotenoid compounds were identified in unsaponified uvaia samples. Among these, seven were free carotenoids, with trans-β-carotene being the most abundant. The remaining 22 compounds were identified as carotenoid esters, such as lutein-palmitate, zeaxanthin-oleate, and β-cryptoxanthin myristate. The esterified peaks hindered identification and quantification of carotenoids, necessitating saponification. Following saponification, 16 compounds were identified, with a total carotenoid concentration of 84.3 ± 2.6 mg 100 g^{-1} DW, comparable to that of carrot (approximately 78.0 mg 100 g^{-1} DW), a well-known carotenoid source [4,5]. The major carotenoid identified in uvaia was β-cryptoxanthin (18.42 mg 100 g^{-1} DW), which plays a vital role in antioxidant defence and serves as a precursor of vitamin A [6]. Including all isomers of cryptoxanthin, the total cryptoxanthin concentration accounted for 87% of the total carotenoid present in uvaia.

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Abstract 49

Intake of carotenoid rich foods, systemic concentrations, and relationship with cardiovascular health.

Marhuenda-Muñoz, M., Rinaldi de Alvarenga, J.F., Domínguez-López, I., Langohr, K., Hernáez, Á., Tresserra-Rimbau, A., Martínez-González, M.Á., Salas-Salvadó, J., Corella, D., Fitó, M., Estruch, R., Tinahones, F., Ros, E., Gómez Melis, G., Martínez-Huélamo, M., Lamuela-Raventós, R.M.

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Carotenoids intake has been reported to be associated with improved cardiovascular health. Nevertheless, there is little information on actual plasma concentrations of these compounds as biomarkers of cardiometabolic risk. Due to their lipophilic nature, co-ingestion of fat appears to increase their bioavailability via facilitating transfer to the aqueous micellar phase during digestion. However, the extent to which high fat intake may contribute to increased carotenoid plasma concentrations is still unclear.

Our objective was to cross-sectionally examine the degree to which the consumption of different amounts of both carotenoid-rich foods and fats is associated with plasma carotenoid concentrations within the context of a Mediterranean lifestyle. We also investigated the association between circulating plasma carotenoids and different cardiometabolic risk factors and the plasma fatty acid profile.
These analyses were performed in a subcohort (106 women and 124 men) of an ongoing multi-factorial lifestyle trial for primary cardiovascular prevention. The study population was categorized into four groups according to their self-reported intake of F&V and fat. Carotenoids were extracted from plasma samples and analysed by HPLC-UV-VIS-QqQ-MS/MS. Fatty acids were measured by LC-GC. Determination of glucose, glycated haemoglobin, triglycerides, total cholesterol, and HDL-cholesterol was performed by standard laboratory enzymatic methods.

Carotenoid plasma concentrations were greater in high consumers of F&V than in low consumers of these foods, circulating concentrations showed a trend towards being decreased when total fat intake was very high, although this did not reach statistical significance. These results were driven by the provitamin A carotenoid fraction, for which we observed the same findings, unlike nonprovitamin A carotenoids, that were not different between fat consumers.

Regarding the cardiovascular risk factors, carotenoid concentrations were cross-sectionally inversely associated with serum triglyceride concentrations, lower levels of plasma saturated fatty acids and higher levels of plasma polyunsaturated fatty acids in the whole population. Plasma carotenoid concentrations were also associated with higher plasma HDL-cholesterol in women, and lower fasting plasma glucose in men.

In summary, high consumption of F&V is associated with greater systemic levels of total carotenoids, in particular when fat intake is low-to-moderate rather than very high. Plasma carotenoids correlate with a better circulating lipid and fatty acid profile. In females they also correlate with higher levels of circulating HDL-cholesterol and in males with lower levels of glucose.

Abstract 50

Preparation of Allylic Benzothiazolyl (BT) Sulfides for Carotene Synthesis via Julia-Kocienski Olefination[1]

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The Julia-Kocienski (J-K) olefination is an improved version of the original Julia-olefination, condensing its two-step process of sulfone coupling and elimination into a single reaction. In the original version, the sulfone coupling necessitates very low temperatures to prevent retro-reaction, especially in sterically-hindered cases, and sulfone elimination typically demands high temperatures. Remarkably, the J-K reaction permits operation at very low to room temperatures, facilitating both coupling and elimination in one pot. Despite its appeal, this convenient one-pot olefination is infrequently employed in carotenoid synthesis, primarily due to challenges in preparing allylic benzothiazolyl sulfides from vinyl carbiol derived from ionone.

We found that palladium ($L_2Pd_2Cl_2$) efficiently catalyzed the *S*-allylation of vinyl carbinols **1a** or **1b** (derived from α- or β-ionone) with 2-mercaptobenzothiazole, yielding the corresponding allylic benzothiazolyl (BT) sulfide **2a** or **2b** in high yields. This reaction involves the activation of allylic alcohols by Pd(II) catalyst in [A], followed by the addition of thiol and Pd to the terminal alkene, forming the intermediate [B]. β-Elimination of $L_2Pd(OH)Cl$ from [B] yields allylic BT-sulfide **2**. The $L_2Pd_2Cl_2$ catalyst can be regenerated by reacting with in situ generated HCl, concurrently removing H_2O (**Figure A**). The BT-sulfides **2** can be oxidized to the corresponding BT-sulfones, which undergo Julia-Kocienski olefination with 2,7-dimethyl-2,4,6-octatrienedial to produce α-carotene and β-carotene, respectively.

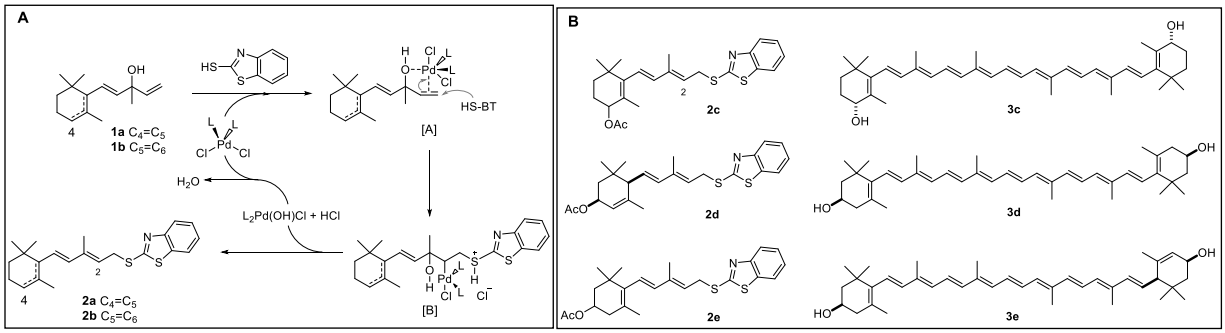


Figure A: Mechanism of Pd-catalyzed *S*-allylation of vinyl carbinol **1**. **Figure B**: Xanthophylls **3** which were synthesized from the resulting allylic benzothiazolyl (BT) sulfides **2c-2e**.

This Pd-catalyzed *S*-allylation specifically targets free allylic alcohol, showing preference over allylic acetate and BT-sulfides **2c**, **2d**, and **2e** containing an acetate substituent in the ring were easily prepared. Subsequently, the resulting BT-sulfones reacted with 2,7-dimethyl-2,4,6-octatrienedial to provides diverse xanthophylls, including isozeaxanthin **3c**, zeaxanthin **3d**, and lutein **3e** (**Figure B**).

[1] A. Golikov, C. Seo, S. Koo,* *Adv. Synth. Catal.* **2024**, *366*, 82-90.

Abstract 51

Synthetic Approaches to Staphyloxanthin

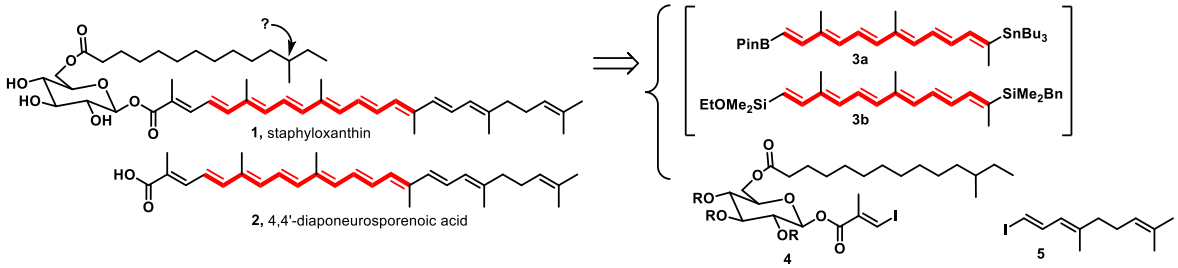
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Human infections by *Staphylococcus aureus* are a concern in present times, since these bacteria have been included within the Priority 2 (HIGH) group in the WHO pathogen list. The ability of *S. aureus* to quench reactive oxygen species (ROS), which are produced by the white cells of the organism to combat infections, acts as a virulence factor for the microorganism. The polyene chain of the C_{30} carotenoid staphyloxanthin or α-D-glucopyranosyl 1-*O*-(4,4'-diaponeurosporen-4-oate)-6-*O*-(12-methyltetradecanoate) (**1**), an unphosphorylated saccharolipid identified in *S. aureus*, is partly responsible for ROS quenching (and for the golden appearance of the microorganism).[1] Interestingly, *in vivo* anticancer activity of staphyloxanthin produced by *S. gallinarum* KX912244, isolated at the gut microflora bacterium of the insect *Bombyx mori,* has also been reported.[2] Photolysis of staphyloxanthin in methicillin-resistant *S. aureus* has been shown to potentiate killing by ROS, thus pointing out to a potential therapy for the diseases caused by this microorganism.[3]

Although this apocarotenoid was first isolated in 1981,[1a] the configuration of the stereocenter of the fatty acid has not yet been determined. We have addressed the stereocontrolled synthesis of staphyloxanthin (**1**) by a bidirectional palladium-catalyzed cross-coupling approach, which required the preparation of the non-symmetrical boronate/stannane (**3a**) and a hexaenyl-bissilane counterpart (**3b**), to connect any of the diastereomers of the protected glucose (**4**) functionalized with 12-methyltetradecanoic acid at the primary alcohol and the iodoalkenoic acid at the anomeric position. The same approach could be applied to simpler apocarotenoids isolated from the same source, such as 4,4'-diaponeurosporenoic acid (**2**).[1]



Scheme 1. Synthetic approach to staphyloxanthin (**1**) and 4,4'-diaponeurosporenoic acid (**2**).

References: [1] (a) Marshall, J. H.; Wilmoth, G. J. *J. Bacteriol.* **1981**, *2147*, 900-913; (b) Götz, F. *et al. J. Biol. Chem.*, **2005**, *280*, 32493-32498. [2] Barretto, D. A.; Vootla, S. K. *Indian J. Microbiol.*, **2018**, *58*, 146-158. [3] Seleem, M. N.; J.- Cheng, X. *et al. Adv. Sci.*, **2019**, *6*, 1900030.

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Abstract 52

Cellular absorption characteristics and transport mechanisms of free and esterified xanthophylls in Caco-2 cell monolayers

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Zeaxanthin and lutein are two important xanthophylls, also well known as macular pigments, which play a crucial role on human visual and neural function. They are abundant in various foods, existing in both free and esterified forms. However, limited knowledge on intestinal absorption and transport of xanthophyll esters is available. Therefore, this research was designed to explore the difference on absorption characteristics and transport mechanisms of free and esterified xanthophylls using Caco-2 cell monolayer, with lutein, zeaxanthin and their dipalmitate as representatives. The result showed that esterified xanthophylls were mainly absorbed in the form of free xanthophylls by Caco-2 cells, while the absorbed free xanthophylls were not found to be re-esterified intracellularly. Transport of free zeaxanthin and lutein was mediated by SR-B1, NPC1L1, and ABCG5 transporters, while that of their esters were mediated by SR-B1, CD36, and PPARγ transporters. However, what is similar is that all of their micelles could be absorbed directly through endocytosis pathways. This study provided a fundamental understanding on the transmembrane transport of free and esterified xanthophylls, which would facilitate future investigation on improvement of their uptake efficiency and subsequent function of their bioactivity.

Abstract 53

Improved Stability and *in vivo* Bioavailability of Lutein in Emulsions Stabilized by Octenylsuccinylated Starch and Pea Protein Isolate

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Objective: Lutein is an important carotenoid for infant eye and brain health. However, the potential utilization of lutein as a food fortification is limited due to its susceptibility to chemical deterioration under high temperature, oxygen, and light exposure, and its low bioavailability. The aim of the study is to develop a lutein emulsion stabilized by octenylsuccinylated starch (OSS) and pea protein isolate (PPI) to improve lutein's chemical stability, bioaccessibility and bioavailability.

Method: A simplex-centroid mixture design was used to evaluate the proportions of OSS, PPI, and water in the aqueous phase on the properties of the emulsions. Lutein was dissolved into olive oil to obtain a 0.1% (w/v) of lutein ester in oil solution, which was then combined with the aqueous phase at a fixed 70% oil volume fraction and homogenized at 20,000 rpm to prepare the emulsions. Visual appearance and emulsification index were used to indicate the successful formation of emulsion. The emulsion samples were tested for droplet size and distribution, *in vitro* infant bioaccessibility, thermal stability, and photostability after seven days of storage at room temperature. For all the tests, free lutein dissolved in olive oil was used as the control. To assess the bioavailability of lutein, a daily dose of 2mg/kg body weight of an optimal lutein emulsion and free lutein was fed to neonatal Sprague-Dawley rats from postnatal day 3 (P3) to P18. On P18, pups were euthanized and blood from the vena cava and all the key organs, including liver, spleen, brain, eye, kidney, lung, small intestine, white adipose tissue (WAT) and brown adipose tissue (BAT) were collected. Serum and tissues were analyzed using ultra-performance liquid chromatography (UPLC) to measure the concentration of lutein.

Result: Homogenous lutein emulsions were formed by three compositions. The mean droplet size of the three samples was 0.98 ± 0.84, 1.33 ± 0.99, and 1.08 ± 0.92μm, respectively. The in vitro bioaccessibility for the lutein emulsions was 69%, 52%, and 63%, respectively; in the control group, it was 56%. After 7 hours of thermal treatment, lutein retention in the three emulsions was 90%, 95%, and 100%, respectively, whereas that in the control group was merely 69%. Following a 7-hour UV treatment, the three emulsions displayed retention rates of lutein of 82%, 94%, and 84%, respectively, while only 69% were retained in the control group. After administering lutein daily for two weeks, lutein concentrations significantly increased by 2.53-fold in the serum, 2.99-fold in the liver, 2.29-fold in the spleen and 6.86-fold in the brain in the lutein emulsion group as compared to the free lutein group.

Conclusion: In conclusion, OSS and PPI stabilized lutein emulsion provided enhanced physical and chemical stability and bioavailability of lutein.

Abstract 54

Compelling clinical benefits of macular carotenoids, curcuminoids and vitamin D3 for dry eyes.

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Background: Dry eye syndrome is multifactorial typically characterized by compromised eye hydration along with inflammation and ocular surface discomfort. It is widely prevalent and cited as one of the most common reasons for visiting the eye doctor, but treatment options are unsatisfactory. According to the Tear Film and Ocular Surface Dry Eye Workshop Reports, there is growing evidence that dietary factors and nutritional supplementation can help as a first line therapy for dry eye management. Given the role of oxidative stress in mediating dry eye issues, we conducted a clinical study to reconfirm the efficacy of a proprietary, antioxidant formula including 24 mg macular carotenoids from marigold flower extract, 200 mg curcuminoids from turmeric extract, and supplemented with 600 IU vitamin D3 (Nutritears™).

Methods: This study was a randomized, double-blind, placebo-controlled design conducted at four sites in the USA. It included one hundred and sixteen healthy adult subjects who met the relevant inclusion and exclusion criteria as recommended by clinical guidelines for evaluating dry eye syndrome. Subjects received Nutritears™ or placebo (soybean oil) over a 56-day study period. The study outcomes were focused on the widely established clinical markers of dry eye syndrome using validated methodologies and included tear volume, tear film stability and tear osmolarity. Severity and progression of dry eye symptoms along with ocular surface health using corneal and conjunctival staining and levels of matrix-metalloproteinase-9 (MMP-9) in the tears was also measured.

Results: With respect to indices of eye hydration and tear quality, Nutritears™ supplementation significantly increased tear volume and tear film stability and reduced tear osmolarity over the course of the study with significant differences measured by day 14 for tear break-up time. Corneal and conjunctival staining along with MMP- 9 levels in the tears were significantly reduced supporting the role of Nutritears™ in favorably influencing aspects of ocular surface integrity and inflammation. Finally, dry eye discomfort severity as measured by Ocular Surface Disease Index revealed that Nutritears™ was able to reduce discomfort within two weeks of supplementation.

Conclusion: The combination of bioactive components, lutein, zeaxanthin isomers, curcuminoids, and vitamin D3, well-known for their role in eye health, demonstrated clinically meaningful benefits for the management of dry eye symptoms vs. placebo. The benefits for eye hydration and reduced dry eye discomfort were observed within two weeks of supplementation and sustained through the study.

Abstract 55

Skin Carotenoids Measured by Reflection Spectroscopy Correlate with Vegetable Intake and Carotenoid Intake of Hispanic Children but not their Fathers in Houston, Texas.

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Fruit and vegetable (FV) intake are components of a healthy dietary pattern for children and adults. Objective assessment methods of FV intake in Hispanic children and their parents are crucial for evaluating interventions directed to increase FV intake in this population. Portable pressure-mediated reflection spectroscopy (RS) provides a rapid, non-invasive method to measure skin carotenoid concentrations, a non-invasive biomarker of carotenoid, fruit, and vegetable intakes. The aim of this study is to evaluate the feasibility of skin carotenoid scores (SCS) as a biomarker of FV intake in Hispanic children and fathers participating in a 10-week nutrition and physical activity behavior intervention called *Papás saludables, Niños Saludables* (PSNS).

This study is a secondary analysis of data obtained from a study in Houston, TX evaluating the feasibility of PSNS for Hispanic fathers with overweight or obesity (n = 36) and their 5–11-year-old children (n = 36). This study randomized families to intervention or control and measured skin carotenoid scores (SCS) pre- and post-intervention using a pressure-mediated reflection spectroscopy device (Veggie Meter) as a secondary outcome. Dietary intake of fruits, vegetables, total carotenoids, and individual carotenoid species (β-carotene, α-carotene, lutein & zeaxanthin, β-cryptoxanthin, and lycopene) was assessed with Block food frequency questionnaires (FFQ), as well as demographics and anthropometric measurements. The data was tested using fixed effect models and included BMI, sex, and age as variables. SCS in children pre- and post-intervention were positively associated with intake of total carotenoids (mg/d) ($\beta = 0.025$, $P = 0.0232$), β-carotene ($\beta = 0.092$, $P = 0.0113$), α-carotene ($\beta = 0.294$, $P = 0.0181$), total vegetable servings ($\beta = 0.21$, $P = 0.0126$) and total FV servings ($\beta = 0.06$, $P = 0.0467$). Distinctly, children's total fruit serving intake was not associated with skin carotenoids (P = 0.1423). Conversely, no positive significant associations were noted between fruit, vegetable, or carotenoid intake with SCS within the fathers, which may have been related to the attenuated strength of the biomarker in adults with overweight or obesity.

SCS measured by RS provides a potential method of an accessible, non-invasive biomarker of carotenoid, and vegetable intake in Hispanic children. Additional research is needed before skin carotenoid biomarkers can be utilized in Hispanic fathers with overweight and obesity.

Abstract 56

Improved *in vitro* bioaccessibility and *in vivo* bioavailability of lutein

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Objective: Lutein is a hydrophobic carotenoid that acts as the macular pigment in the retina and is the dominant carotenoid in the brain of infants. It plays a crucial role in neonatal visual and cognitive development. However, the application of lutein as a nutritional intervention is limited due to its poor stability and water solubility. Oil-in-water emulsions are widely used delivery systems of lipophilic nutrients that can protect carotenoids from chemical degradation and improve their bioavailability. Our previous research showed that lutein emulsion stabilized by octenylsuccinylated starch (OSS), a biopolymer emulsifier approved for use in infant foods, significantly increased the stability and bioavailability of lutein. As the initial step of developing a lutein emulsion-fortified infant formula, the purpose of this study was to add soy protein isolate (SPI) in the emulsion system and determine the optimal concentrations of OSS and SPI to improve the stability, *in vitro* bioaccessibility, and *in vivo* bioavailability of lutein.

Methods: A total of 17 biopolymer solutions containing various proportions of deionized water, OSS, and SPI were made according to a simplex-centroid mixture design. Lutein emulsions were prepared by mixing biopolymer dispersions and lutein in olive oil at 70% oil volume fraction using a homogenizer. The microstructure, photo-stability, thermal stability, and simulated *in vitro* infant gastrointestinal release of lutein emulsions were assessed. An optimal lutein emulsion was selected for a feeding study in neonatal Sprague-Dawley rats. Rat pups were fed either free lutein or lutein emulsion from postnatal day 3 (P3) to P18. At P19, blood from vena cave, liver, spleen, brain, eye, kidney, and lung were collected. Lutein concentration in the serum and tissues were analyzed utilizing ultra-performance liquid chromatography (UPLC).

Results: The OSS-SPI based lutein emulsions at biopolymer dispersions of 74%-87% water, 9%-22% CTA, and 0-8% SPI successfully formed emulsions without phase separation. The mean droplet size of lutein emulsions fell into the range from 2.09 ± 1.69 μm to 6.23 ± 8.46 μm and decreased with increasing SPI concentration. After 7-hour UV light exposure, OSS-SPI based lutein emulsions improved lutein stability than the solo OSS-based emulsion, and all emulsions had higher lutein retention than free lutein. After a 7-hour water bath at 80 °C, lutein retention was significantly higher in all emulsions than in free lutein. Using *in vitro* infant digestion model, the lutein release from the emulsions were higher than those of free lutein. Notably, while the emulsion with 78% water, 14% OSS, and 8% SPI (W78-O14-S8) was 2.16-fold higher, the other emulsions showed 1.11 to 1.92-fold higher release. Based on *in vitro* testing, the W78-O14-S8 lutein exhibit the best overall performance and was selected for the *in vivo* study. After two weeks of daily dosing, rat pups in the lutein emulsion group exhibited a significant 2.18-fold higher serum lutein concentration compared to those receiving free lutein. Lutein concentration in the liver, spleen, brain, eye, kidney, and lung was significantly higher by 3.84-, 1.80-, 6.73-, 3.60-, 2.22-, and 1.95-fold, respectively, in the lutein emulsion group as compared to the free lutein group.

Conclusion: The W78-O14-S8 lutein emulsion showed the best stability, highest *in vitro* lutein bioaccessibility, and improved lutein *in vivo* bioavailability, offering promising potential for enhancing lutein's nutritional benefits in infant foods, such as infant formula.

Abstract 57

Skin Carotenoid Scores Measured by Reflection Spectroscopy Correlate with Adolescent Vegetable Intake in Houston, Texas

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Objective methods are needed to assess adolescent fruit and vegetable (FV) intake to better evaluate interventions aimed at improving FV intake. Skin carotenoid concentration measures provide a potential objective biomarker of FV intake, but the plausibility and robustness must be established across adolescent populations. The objective of this study is to evaluate the relationship between pressure-mediated reflection spectroscopy-measured skin carotenoid scores (SCSs) and self-reported FV intake among US racial and ethnic minority adolescents in Houston, TX. This is a secondary analysis of data collected on adolescents participating in the randomized, controlled study "Teens Committed to Health Through Activity, Relationships, and Good Eating (Take CHARGE!)." This was approved by the University of Houston IRB, and registered at ClinicalTrials.gov (#NCT04362280). Participants were adolescents (13.6 (SD 1.5) years of age; 86.2% Hispanic, 12.6% African American, 1.2% Asian) (N=167) who participated in a year-long healthy lifestyles program as their physical education class in Houston, TX from August 2018 to 2019. Over the course of a year, participants' SCSs were measured by pressure-mediated reflection spectroscopy and self-reported FV intake frequency was assessed using questions from the School Physical Activity and Nutrition questionnaire at four separate time points. The relationship between adolescent SCSs and FV intake was tested using generalized linear mixed models, controlling for BMI z-score, gender, time-point, group assignment, and age. SCSs were positively predicted by self-reported intake frequencies for FV (p=0.028), vegetables (p=0.010), and orange and dark green vegetables (p=0.019), with no significant relationship observed with fruit intake frequency (p=0.613). Self-reported total FV (combined), vegetable, and orange and green vegetable (combined) intake is a predictor of SCSs among racial and ethnic minority adolescents in Houston, TX. Skin carotenoid measurement merits further investigation as a biomarker of vegetable intake in US adolescents.

Abstract 58

Placental Carotenoid Association with Maternal and Infant Systemic and Ocular Carotenoid Status in the Lutein and Zeaxanthin in Pregnancy (L-ZIP) Trial

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

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Introduction: Macular pigment carotenoids transfer from the maternal systemic circulation via the placenta in the third trimester of pregnancy to support crucial retinal and brain development in the growing fetus. Until now, the effect of prenatal carotenoid supplementation on the placenta is limited. Therefore, we explored the associations between placental carotenoids with postpartum maternal and infant systemic and ocular carotenoid status in the L-ZIP trial (NCT03750968).

Methods: 47 pregnant women were randomized 1:1 to receive standard-of-care daily prenatal vitamins with or without 10 mg of lutein (L) and 2 mg of zeaxanthin (Z) for 6 to 8 months. The primary outcome assessed maternal carotenoid status in the serum, skin, and eye at the end of each trimester and postpartum with HPLC, resonance Raman spectroscopy, and dual-wavelength autofluorescence, respectively. The secondary outcome measured infants' carotenoid concentrations in the umbilical cord blood, skin, and eyes using techniques similar to those above but optimized for infants. As an exploratory outcome, we collected and analyzed placentas for their carotenoid status with HPLC at delivery.

Results: Placental L+Z in mothers in the Carotenoid Group were significantly higher than those in the Control Group ($t(36) = 5.70$, $p<0.0001$; Mean difference = 1886). There was a positive statistically significant association between placental L+Z with postpartum maternal and infant serum and skin carotenoid status ($p<0.0001$, for all). Although placental L+Z correlated positively with maternal and infant macular pigment, these associations were not statistically significant.

Conclusion: Increased placental carotenoid concentration following prenatal carotenoid supplementation could offer insight into carotenoids' mechanistic and potential role in mitigating oxidative stress that characterizes some disorders of pregnancy, such as preeclampsia. Collaboration with experts is underway to delineate prenatal carotenoid effects on the placenta.

Presenter-Author Index*

Ablazov, Abdugaffor	Abstract 40	Page 61
	Abstract 41	Page 63

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Abrego-Guandique, Diana Marisol	Abstract 22	Page 43
Acharya, Pooja	Abstract 38	Page 59
Adams, William W. III	Abstract 18	Page 38
Addo, Emmanuel K.	Abstract 58	Page 80
Aguilar-Espinosa, Margarita.	Abstract 10	Page 29
Ahlén, Catrine	Abstract 29	Page 50
ALAGOZ, Y.	Abstract 3	Page 22
Alam, Sayma	Abstract 53	Page 75
	Abstract 56	Page 78
AL-BABILI, S.	Abstract 3	Page 22
	Abstract 40	Page 62
	Abstract 41	Page 63
	Abstract 42	Page 64
Albakri, Asma'a	Abstract 23	Page 44
Alberton, Bruna	Abstract 16	Page 36
Alexandre, Maxime T. A.	Abstract 11	Page 30
Ali, Asjad	Abstract 2	Page 21
Allman, Susan J.	Abstract 58	Page 80
Álvarez, Rosana	Abstract 51	Page 73
Amengual, Jaume	Abstract 23	Page 44
	Abstract 38	Page 59
Arlinghaus, Katherine R.	Abstract 57	Page 79
Arold, Stefan	Abstract 41	Page 63
Arruabarrena, Ana	Abstract 6	Page 25
Artetxe, Unai	Abstract 16	Page 36
Autenrieth, Caroline	Abstract 46	Page 68
Avalos, Javier	Abstract 28	Page 49
Balakrishna, Aparna	Abstract 41	Page 63
Balbuena, Emilio	Abstract 34	Page 55
Baldermann, S.	Abstract 19	Page 39
Bandaletova, Tatyana	Abstract 39	Page 60
Bandara, Sepalika	Abstract 44	Page 66
Barajas, Karla	Abstract 55	Page 77
Barbour, Krista	Abstract 54	Page 76
Bashmakov, Yuriy	Abstract 39	Page 60
Bekker, Marlize	Abstract 48	Page 70
Bernstein, Paul S.	Abstract 58	Page 80
Berqdar, Lamis	Abstract 40	Page 61
Bjørk, Tina Beate	Abstract 29	Page 50
Blanco, Amparo	Abstract 23	Page 44
Blilou, Ikram	Abstract 40	Page 62
Blodi, Barbara A.;	Abstract 32	Page 53
Bonet, M. Luisa	Abstract 22	Page 43
	Abstract 38	Page 59
Borrego-Serrano, Ismael	Abstract 28	Page 49

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Büchel, Claudia	Abstract 11	Page 30
Buschbeck, Paul	Abstract 12	Page 31
Cannataro, Roberto	Abstract 22	Page 43
Cao, Hongbo	Abstract 4	Page 23
Cárdenas-Conejo, Yair	Abstract 10	Page 29
Caroleo , Maria Cristina	Abstract 22	Page 43
Cazzonelli, Christopher	Abstract 1	Page 20
Cepni, Aliye B.	Abstract 57	Page 79
Chaly, Natalia	Abstract 39	Page 60
CHEN, G.-T. E.	Abstract 3	Page 22
Chen, Guan-Ting	Abstract 40	Page 61
	Abstract 42	Page 64
Cheng, Junrui	Abstract 31	Page 52
Chernyshova, Marina	Abstract 39	Page 60
Cione, Erika	Abstract 22	Page 43
Collins, Leonard	Abstract 34	Page 55
Corella, D.	Abstract 49	Page 71
Crisp, Peter	Abstract 2	Page 21
Curiel Yuste, Jorge	Abstract 16	Page 36
Dai, Nai-Chiang	Abstract 40	Page 61
Darawshe, Aya	Abstract 24	Page 45
Dautermann, Oliver	Abstract 12	Page 31
de Lera, Ángel R.	Abstract 51	Page 73
Demmig-Adams, Barbara	Abstract 18	Page 38
Dhandhukia, Pinakin	Abstract 26	Page 47
Dillon, Natalie	Abstract 2	Page 21
DiNardo, Andrew	Abstract 37	Page 58
Domalpally, Amitha;	Abstract 32	Page 53
Domínguez-López, I.	Abstract 49	Page 71
Dube, Nondumiso	Abstract 37	Page 58
Dushkin, A.V.	Abstract 13	Page 32
Dutta, Debasmita	Abstract 17	Page 37
Dutta, Debjani	Abstract 17	Page 37
Duvós, Valentina	Abstract 6	Page 25
Eroglu, Abdulkerim	Abstract 34	Page 55
Esteban, Raquel	Abstract 16	Page 36
Estruch, R.	Abstract 49	Page 71
Fei, Zhangjun	Abstract 9	Page 28
Fiorilli, Valentina	Abstract 40	Page 61
Fitó, M.	Abstract 49	Page 71
Fitzner, M.	Abstract 19	Page 39
Focsan, A. Ligia	Abstract 7	Page 26
	Abstract 13	Page 32
Galmès, Sebastià	Abstract 22	Page 43
	Abstract 38	Page 59

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Gascua, Clement	Abstract 37	Page 58
Germain, Alexandre de Saint	Abstract 41	Page 63
Gerson, Jeffry	Abstract 54	Page 76
Ghosh, Robin	Abstract 46	Page 68
Giani, Micaela	Abstract 25	Page 46
Gioia, Neda	Abstract 54	Page 76
Golikov, Aleksei	Abstract 50	Page 72
Gómez Melis, G.	Abstract 49	Page 71
González-Arcos, Matías	Abstract 6	Page 25
Goss, Reimund	Abstract 12	Page 31
Granell, Antonio	Abstract 5	Page 24
Gundermann, Kathi	Abstract 11	Page 30
Haider, Imran	Abstract 40	Page 61
Haleagrahara, Nagaraja	Abstract 21	Page 42
Hameed, Umar Shahul	Abstract 41	Page 63
Hao, Lezhi	Abstract 43	Page 65
Hereş, Ana-Maria	Abstract 16	Page 36
Hernáez, Á.	Abstract 49	Page 71
Hill, Geoffrey E.	Abstract 45	Page 67
Hirschberg, Joseph	Abstract 8	Page 27
Holloway, Chelsee	Abstract 30	Page 51
	Abstract 33	Page 54
Hong, Hung T.	Abstract 2	Page 21
	Abstract 48	Page 70
Hsing, Yue-Ie Caroline	Abstract 40	Page 61
Iruiñ, Xabat	Abstract 16	Page 36
Isoherranen, Nina	Abstract 30	Page 51
Jacomino, Angelo P.	Abstract 48	Page 70
JAMIL, M.	Abstract 3	Page 22
Jamil, Muhammad	Abstract 40	Page 61
	Abstract 41	Page 63
	Abstract 42	Page 64
Jennings, Brooke	Abstract 54	Page 76
Julia Marente, Gabriel	Abstract 28	Page 49
Kim, Hanjoe	Abstract 57	Page 79
Kim, Youn-Kyung	Abstract 30	Page 51
	Abstract 33	Page 54
Kiremitci, Buse Zeren	Abstract 34	Page 55
Kispert, Lowell. D.	Abstract 7	Page 26
	Abstract 13	Page 32
Klochkov, Victor	Abstract 39	Page 60
Kłodawska, Kinga	Abstract 35	Page 56
Koch, Rebecca E.	Abstract 45	Page 67
Kone, Ngalulawa	Abstract 37	Page 58
Kong, Lingyan	Abstract 53	Page 75

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

	Abstract 56	Page 78
Koo, Sangho	Abstract 50	Page 72
Kot, Witold	Abstract 39	Page 60
Krüger, Tjaart P.J.	Abstract 11	Page 30
Krych, Łukasz	Abstract 39	Page 60
Kuijer, Hendrik NJ	Abstract 40	Page 61
Kyle, Nigel	Abstract 39	Page 60
Lado. Joanna	Abstract 6	Page 25
Lai, Ming-Hsin	Abstract 40	Page 61
Lamuela-Raventós, R.M.	Abstract 49	Page 71
LANFRANCO, L.	Abstract 3	Page 22
Lanfranco, Luisa	Abstract 40	Page 61
Langohr, K.	Abstract 49	Page 71
Lawler, Thomas;	Abstract 32	Page 53
Ledoux, Tracey A.	Abstract 57	Page 79
Lee, Na Youn	Abstract 31	Page 52
León, Rosa	Abstract 14	Page 33
León-Vaz, Antonio	Abstract 14	Page 33
Li Li	Abstract 4	Page 23
	Abstract 9	Page 28
LIEW, K. X.	Abstract 3	Page 22
Liew, Kit Xi	Abstract 40	Page 61
	Abstract 41	Page 63
Limón, M. Carmen	Abstract 28	Page 49
Lin, Pei-Yu	Abstract 40	Page 61
Liu, Zhe	Abstract 32	Page 53
Llansola-Portoles, Manuel J.	Abstract 11	Page 30
Lobato-Gómez, Maria	Abstract 5	Page 24
Lohr, Martin	Abstract 12	Page 31
Longlax, Santiago Carrero	Abstract 37	Page 58
López-Pozo, Marina	Abstract 18	Page 38
Lorenzo, Paula	Abstract 51	Page 73
Lowery, Ryan	Abstract 54	Page 76
Madonna, Elisabetta	Abstract 33	Page 54
Magalingam, Kasthuri Bai	Abstract 21	Page 42
Maghrebi, Moez	Abstract 40	Page 61
Malec, Przemysław	Abstract 35	Page 56
Mapelli-Brahm, Paula	Abstract 14	Page 33
	Abstract 23	Page 44
Mares, Julie A.	Abstract 32	Page 53
Marhuenda-Muñoz, M.	Abstract 49	Page 71
Martínez-Espinosa, Rosa María	Abstract 25	Page 46
Martínez-González, M.Á.	Abstract 49	Page 71
Martínez-Huélamo, M.	Abstract 49	Page 71
Martín-Gómez, Andrés Noel	Abstract 14	Page 33

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Mazzarella, Teresa	Abstract 40	Page 61
McGraw, Kevin J.	Abstract 20	Page 40
	Abstract 43	Page 65
Meléndez Martínez, Antonio Jesús	Abstract 14	Page 33
Melino, Vanessa	Abstract 40	Page 61
Mi, Jianing	Abstract 41	Page 63
Milhem, Fadia	Abstract 34	Page 55
Moltini, Ana Inés	Abstract 6	Page 25
Montoyo-Pujol,	Abstract 25	Page 46
Moon, Jean	Abstract 44	Page 66
Moran Nancy E.	Abstract 36	Page 58
	Abstract 47	Page 69
	Abstract 55	Page 77
	Abstract 57	Page 79
Morde, Abhijeet	Abstract 54	Page 76
Moreno, Juan C.	Abstract 3	Page 22
	Abstract 41	Page 63
Morón-Ortiz, María de los Ángeles	Abstract 14	Page 33
Musaad, Salma	Abstract 47	Page 69
	Abstract 55	Page 77
Narváez-Zapata, Jose A,	Abstract 10	Page 29
Nguyen, Thuy	Abstract 47	Page 69
Nielsen, Dennis Sandris	Abstract 39	Page 60
Noor Hason,	Abstract 55	Page 77
NOVAK, O.	Abstract 3	Page 22
O'Connor, Teresia	Abstract 55	Page 77
O'Hare, Tim J.	Abstract 2	Page 21
	Abstract 48	Page 70
Onyekwere, Elizabeth	Abstract 57	Page 79
Padhye, Isha	Abstract 33	Page 54
Padigaru, Muralidhara,	Abstract 54	Page 76
Padilla, Rita	Abstract 28	Page 49
Partali, Vassilia	Abstract 29	Page 50
Pascal, Andrew A.	Abstract 11	Page 30
Peiró, Gloria	Abstract 25	Page 46
Perez-Guerr, David	Abstract 15	Page 35
Pérez-Revenga, Víctor	Abstract 51	Page 73
Periman, Laura M.	Abstract 54	Page 76
Perry-Raymond, Jennie;	Abstract 32	Page 53
PETRIK, I.	Abstract 3	Page 22
Petyaev, Ivan	Abstract 39	Page 60
Pinos, Ivan	Abstract 23	Page 44
Polyakov, N.E.	Abstract 13	Page 32
Poteet, Julie	Abstract 54	Page 76
Pristensky, Dmitry	Abstract 39	Page 60

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Quadro, Loredana	Abstract 30	Page 51
	Abstract 33	Page 54
Radhakrishnan Ammu Kutty	Abstract 21	Page 42
Rai, Deshanie	Abstract 54	Page 76
Rao, Sombir	Abstract 4	Page 23
	Abstract 9	Page 28
Ribot, Joan	Abstract 22	Page 43
	Abstract 38	Page 59
Riddle, Misty R.	Abstract 15	Page 35
Rinaldi de Alvarenga, J.F.	Abstract 49	Page 71
Rivas, Aurea	Abstract 51	Page 73
Rivera-Madrid, Renata	Abstract 10	Page 29
Robert, Bruno	Abstract 11	Page 30
Ros, E.	Abstract 49	Page 71
Roy, Danyalaxmi	Abstract 26	Page 47
Rubí, Paula	Abstract 38	Page 59
Ruiz de Larrinaga, Lorena	Abstract 16	Page 36
Ryan, Robert	Abstract 54	Page 76
Salas-Salvadó, J.	Abstract 49	Page 71
San Miguel Oti, Francisco	Abstract 16	Page 36
Sarna, Tadeusz	Abstract 35	Page 56
Schreiner, M.	Abstract 19	Page 39
Selvaduray, Kanga Rani	Abstract 21	Page 42
Senawong, Taegen	Abstract 47	Page 69
Senkus, Katelyn E.	Abstract 36	Page 57
	Abstract 47	Page 69
Seo, Chibeom	Abstract 50	Page 72
Sharoni,Yoav	Abstract 24	Page 45
Sharp, Matthew	Abstract 54	Page 76
Shu, Qingbo	Abstract 34	Page 55
Simanowitz, Marc C.	Abstract 8	Page 27
Sliwka, Hans-Richard	Abstract 29	Page 50
Smith, Christopher;	Abstract 32	Page 53
Somanath, Sushela Devi	Abstract 21	Page 42
Song, Shanshan	Abstract 27	Page 48
Soni, Nidhi	Abstract 26	Page 47
Srinivasagan, Ramkumar	Abstract 44	Page 66
STRA, ALICE	Abstract 3	Page 22
Szewczyk, Grzegorz	Abstract 35	Page 56
TAKAGI, TATSUYOSHI	Abstract 2	Page 21
Tan, Libo	Abstract 53	Page 75
	Abstract 56	Page 78
Taver, Isabela B.	Abstract 48	Page 70
Tester, Mark	Abstract 40	Page 61
Thakker, Janki N.	Abstract 26	Page 47

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.

Tinahones, F.	Abstract 49	Page 71
Toomey, Matthew B.	Abstract 45	Page 67
Tresserra-Rimbau, A.	Abstract 49	Page 71
van Grondelle, Rienk	Abstract 11	Page 30
Varner, Michael W.	Abstract 58	Page 80
Veremeienko, Vasyl	Abstract 11	Page 30
Vidal, Sabina	Abstract 6	Page 25
Vigani, Gianpiero	Abstract 40	Page 61
von Lintig, Johannes	Abstract 15	Page 35
	Abstract 38	Page 59
	Abstract 44	Page 66
VOTTA, C.	Abstract 3	Page 22
WANG, J. Y.	Abstract 3	Page 22
Wang, Jian You	Abstract 40	Page 61
	Abstract 42	Page 64
Wang, Xiang-Dong	Abstract 31	Page 52
Wiese, Maria	Abstract 39	Page 60
Williams, Taufika Islam	Abstract 34	Page 55
Wilson, Jacob	Abstract 54	Page 76
Wrightstone, Emalee	Abstract 9	Page 28
Wu, Fan	Abstract 52	Page 74
Yoel Genaro	Abstract 25	Page 46
Żbik, Paweł	Abstract 35	Page 56
Zhang, Yanqi	Abstract 53	Page 75
	Abstract 56	Page 78
Zhang, Yufeng	Abstract 45	Page 67
Zheng, Xiongjie	Abstract 40	Page 61

*All Presenters &Co-authors are ICS members and emails that are not found on the abstract page can be found by a query on the ICS website.