Book of Abstracts

2nd Virtual International Conference on Carotenoids

VICC 2022

International Carotenoid Society

April 12th, 13th, 14th

Conference Organizers

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Research Scientist, DSM Nutritional Products Ltd., Basel Switzerland
email: adrian.wyss@dsm.com

Comments from the Organizers

We wish to thank each of those who through diligence and hard-work have done the exceptional research that is represented by the abstracts that comprise VICC 2022.

The Organizers again wish to thank **Case Western Reserve University School of Medicine** for their gracious technical support of VICC 2022.

The outstanding success of VICC 2021 and the enthusiasm of those who attended were instrumental in our decision to organize this 2022 Virtual Conference on Carotenoids. VICC 2022 is pleased that outstanding original research abstracts from 23 different countries have been accepted for presentation in this three day conference.

Our special thanks go to ***CaroteNature*** for their generous sponsorship of the this year's awards for Outstanding Presentations by a Graduate Students and Post-doctoral Fellows.

Following the format of VICC 2021, each speaker has been assigned an 8-minute window to deliver a tightly focused presentation. Following each group of talks we have scheduled a 15 minute Question and Discussion period to enable participants to address specific Questions from our Conference Audience. We encourage all participants to submit Questions for the discussion periods via the '***chat box***' during talks. Moderators will select from among these to guide discussion.

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Tuesday, April 12

Tuesday April 12, 2022

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

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

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8:05 am EDT **Session I – Dietary Sources of Carotenoids and Nutritional Supplementation**

Moderators:

Assistant Professor Kristina Klajak
University of Zagreb
Zagreb Croatia
email: kkljak@agr.hr

Assistant Professor Rachel Kopec
The Ohio State University
Columbus, OH USA
email: kopec.4@osu.edu

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8:05 am EDT **Session I-A Dietary Sources of Carotenoids and Nutritional Supplementation**

Abstract 1 **CONSIDERATIONS FOR DEVELOPING AN AUSTRALIAN LUTEIN AND ZEAXANTHIN FOOD COMPOSITION DATABASE.**

8:06 am EDT

Presenter: **MS. NAOMI FITZPATRICK**
The University of Queensland, Australia
(emails: naomi.fitzpatrick@uq.net.au)

Abstract 2 **UNEXPECTED SIDE-EFFECTS OF ZEAXANTHIN BIOFORTIFICATION**

8:15 am EDT

Presenter: **MS. RIMJHIM AGARWAL**
The University of Queensland, Australia
(email: rimjhim.agarwal@uq.net.au)

Abstract 3 **BIOFORTIFYING MANGOES: FACTORS AFFECTING PRO-VITAMIN A CAROTENOIDS IN MANGO FLESH**

8:24 am EDT

Presenter: **MR. TATSUYOSHI TAKAGI**
The University of Queensland, Australia
(email: t.takagi@uq.edu.au).

Abstract 4 **RELATIONSHIP BETWEEN KERNEL PROPERTIES AND CAROTENOID DEGRADATION RATE IN MILLED MAIZE**

8:33 am EDT

Presenter: **DR. VERONIKA GUNJEVIĆ**
University of Zagreb, Zagreb, Croatia
(email: vgunjevic@agr.hr)

8:42 am EDT **Session I-A – Questions and Discussion** (15 min)
Moderators: **Profs. Kristina Kljak and Rachel Kopec**

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8:57 am EDT **Break** (4 minutes)

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9:01 am EDT **Session I-B – Dietary Sources of Carotenoids and Nutritional Supplementation**
Moderators: **Profs. Kristina Kljak and Rachel Kopec**

Abstract 5 **DID CAROTENOID CONSUMPTION AMONG BRAZILIANS CHANGE IN 10 YEARS?**

9:02 am EDT

Presenter: **ASSISTANT PROFESSOR LILIAN R. B. MARIUTTI**
University of Campinas, São Paulo, Brazil
(email: lilianma@unicamp.br)

Abstract 6 **LEMNA – RAPIDLY GROWING ZEAXANTHIN HYPERACCUMULATOR**

9:11 am EDT

Presenter: **PROFESSOR BARBARA DEMMIG-ADAMS**
University of Colorado Boulder, USA
(email: Barbara.Demmig-Adams@colorado.edu)

Abstract 7 **SKIN CAROTENOID ASSESSMENT AMONG SCHOOL CHILDREN IN THE HILLY REGION OF NEPAL**

9:20 am EDT

Presenter: **DR. RABA THAPA**
Tilganga Institute of Ophthalmology, Kathmandu, Nepal
(email: rabathapa@live.com)

9:29 am EDT **Session I-B - Questions and Discussion** (15 min)
Moderators: **Profs. Kristina Kljak & Rachel Kopec**

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9:44 am EDT **Break** (4 minutes)

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9:48 am EDT **Session II - Carotenoid Production by Microorganisms**

Moderators:

Associate Professor Carmen Limon
University of Seville
Seville Spain
email: carmenlimon@us.es

Professor Robin Ghosh
University of Stuttgart
Stuttgart, Germany
email: robin.ghosh@bio.uni-stuttgart.de

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Abstract 8 **IMPACT OF CARBON SOURCES ON β-CRYPTOXANTHIN PRODUCTION AND STUDY OF ITS ANTIMICROBIAL EFFECT ON E. COLI**

9:49 am EDT ***Presenter:*** **MS. SAHA SANHITA**
National Institute of Technology Durgapur, West Bengal, India
(email: sahasanhita1@gmail.com)

Abstract 9 **ENHANCED PRODUCTION AND ISOLATION OF A NOVEL CAROTENOID LOROXANTHIN FROM SCENEDESMUS OBLIQUUS**

9:58 am EDT ***Presenter:*** **DR. AYŞEGÜL ERDOĞAN**
Ege University Application and Research Center For Testing and Analysis, İzmir-Turkey
(email: aysegul_erdogan@live.com)

Abstract 10 **REGULATION OF CAROTENOID BIOSYNTHESIS BY STRESS IN THE FUNGUS FUSARIUM FUJIKUROI**

10:07 am EDT ***Presenter:*** **ASSOCIATE PROFESSOR M. CARMEN LIMÓN**
University of Sevilla, Seville, Spain.
(e-mail: carmenlimon@us.es)

Abstract 11 **INVESTIGATING THE ROLE OF HMGBC, A HMG-BOX FAMILY PROTEIN, IN THE REGULATION OF CAROTENOGENESIS IN FUSARIUM**

10:16 am EDT ***Presenter:*** **MS. MARTA FRANCO-LOSILLA**
University of Seville, Sevilla, Spain
(email: martafranco@us.es)

Abstract 12. **PRELIMINARY STUDIES FOR THE OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF MICROALGAL CAROTENOIDS**

10:25 am EDT ***Presenter:*** **MS. ÁNGELES MORÓN-ORTIZ**
Universidad de Sevilla, Sevilla, Spain.
(email: amortiz@us.es *)*

10:34 am EDT **Session II - Questions and Discussion** (15 min)

Moderators: **Profs. Carmen Limon and Robin Ghosh**

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10:49 am EDT **Break** (4 minutes)

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10:53 am EDT **Session III - Carotenoids: Biotechnological and Analytical Methods**

Moderators:

Dr. Neal Craft
Craft Nutrition Consulting
Elm City, North Carolina USA
email: nealcraft@craftnutritionconsulting.com

Professor Susanne Baldermann
University of Bayreuth
Großbeeren Germany
email: baldermann@igzev.de

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Abstract 13 **CHARACTERIZATION STUDIES OF PROVITAMIN A CAROTENOID β CRYPTOXANTHIN ENCAPSULATED LIPOSOMES**

10:54 am EDT

Presenter: **MS. DUTTA DEBASMITA**
National Institute of Technology Durgapur, West Bengal, India.
(e-mail: mails.ddutta@gmail.com)

Abstract 14 **CAROTENOID STABILITY OF MARIGOLD FLOWERS (TAGETES ERECTA L.) DURING STORAGE IN DIFFERENT CONDITIONS**

11:03 am EDT

Presenter: **MS. SARA KOLAR**
University of Zagreb, Croatia
(emails: sara.kolar1993@gmail.com)

Abstract 15 **IMPACT OF HIGH PRESSURE PROCESSING ON EXTRACTABILITY AND DISTRIBUTION OF BIOACTIVE PLANT INGREDIENTS IN KALE**

11:12 am EDT

Presenter: **MR. MARIO SCHMIDT**
Friedrich Schiller University Jena, Jena, Germany
(email: mario.schmidt@uni-jena.de)

Abstract 16 **PERCEPTION OF ADEQUACY OF FRUIT AND VEGETABLE INTAKE AND ASSOCIATED SKIN CAROTENOID STATUS AMONGST ADOLESCENTS**

11:21 am EDT

Presenter: **ASSOCIATE PROFESSOR ANNE MATHEWS**
University of Florida (UF), FL 32611, USA
(email: anne.mathews@ufl.edu)

Abstract 17 **SEPARATELY MAPPING ZEAXANTHIN AND MESO-ZEAXANTHIN IN THE HUMAN FOVEA WITH POLARIZED RESONANCE RAMAN MICROSCOPY**

11:30 am EDT

Presenter: **DR. BINXING LI**
University of Utah, School of Medicine, Salt Lake City, Utah USA
(e-mail: binxing.li@hsc.utah.edu)

11:40 am EDT **Session III – Questions and Discussion** (15 min)
Moderators: Dr. Neal Craft and Prof. Susanne Baldermann

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

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Wednesday April 13, 2022

7:30 am EDT Pre-VICC Login Period

8:00 am EDT Daily Updates – Organizing Committee

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8:05 am EDT Session IV – Carotenoid Biosynthesis & Biotechnology

Moderators:

Professor Giovanni Giuliano
Energy and Sustainable Development (ENEA) - Casaccia Research Center
Rome, Italy
email: giovanni.giuliano@enea.it

Assistant Professor Alexandra Jazz Dickinson
University of California, San Diego
San Diego, Ca USA
email: adickinson@ucsd.edu

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Abstract 18 **MICROTOM TANGERINE TOMATO CRTISO MUTANT ROOTS SHOW INCREASED ACCUMULATION OF ACYCLIC CAROTENOIDS, LESS ABSCISIC ACID, DROUGHT SENSITIVITY, AND IMPAIRED ENDOMYCORRHIZAL COLONISATION**

8:06 am EDT

Presenter: **MR. JWALIT J NAYAK**
Hawkesbury Institute for the Environment, Western Sydney University, Australia
*(*email: j.nayak@westernsydney.edu.au)

Abstract 19 **METABOLIC ENGINEERING TO ATTAIN EFFICIENT PRODUCTION OF HIGH-VALUE XANTHOPHYLLS IN TOMATO**

8:15 am EDT

Presenter: **MR. MARC C. SIMANOWITZ**
The Hebrew University of Jerusalem, Jerusalem, Israel
(email: marc.simanowitz@mail.huji.ac.il)

Abstract 20. **METABOLIC ENGINEERING FOR CROCIN PRODUCTION IN DIFFERENT PLANT, YEAST AND MICROALGAL PLATFORMS**

8:24 am EDT

Presenter: **PROFESSOR GIOVANNI GUILIANO**
Energy and Sustainable Development (ENEA), Casaccia Research Center, Rome Italy
(email: giovanni.giuliano@enea.it)

Abstract 21 **CONSTRUCTION OF A MODULAR EXPRESSION SYSTEM FOR HIGH-LEVEL PRODUCTION OF CAROTENOIDS IN THE PURPLE BACTERIUM, RHODOSPIRILLUM RUBRUM**

8:33 am EDT

Presenter: **Dr. CAROLINE AUTENRIETH**
University of Stuttgart, Stuttgart, Germany
(email: caroline.autenrieth@bio.uni-stuttgart.de)

Abstract 22 **ENGINEERING HIGH LEVELS OF SAFFRON APOCAROTENOIDS IN TOMATO**

8:42 am EDT

Presenter: **PROFESSOR ANTONIO GRANELL**
Consejo Superior de Investigaciones Científicas-Universidad Politécnica de València, Valencia, Spain
(email: A.granell@ibmcp.upv.es)

Abstract 23 **ESTABLISHMENT OF YEAST-BASED MICROBIAL CELL FACTORY FOR EFFICIENT BIOSYNTHESIS OF VIOLAXANTHIN**

8:51 am EDT

Presenter: **Ms. ANQI ZHOU**
University of California, Riverside California, USA
(email: aazhou036@ucr.edu)

9:01 am EDT **Session IV – Questions and Discussion** (20 min)
Moderators: **Profs. Giovanni Giuliano and Alexandra Jazz Dickinson**

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9:21 am EDT **Break** (4 minutes)

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9:25 am EDT **Session III – Photophysics and Chemistry**

Moderators:

Professor Bruno Robert
Saclay Institute of Biology and Technology
Gif Sur Yvette France
email: bruno.robert@cea.fr

Professor Hideki Hashimoto (Emeritus Professor of Osaka City University)
Kwansei Gakuin University
Sanda, Hyogo Japan
email: hideki-hassy@kwansei.ac.jp

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Abstract 24 **EFFECT OF NON-CONJUGATED FUNCTIONAL GROUP TO THE OPTICAL PROPERTIES OF CARBONYL CAROTENOID.**

9:26 am EDT

Presenter: **MR. SOICHIRO SEKI**
Osaka City Univ., Osaka, Japan
(e-mail: d21sb001@vx.osaka-cu.ac.jp)

Abstract 25 **MODELING EXCITED STATES AND RAMAN SPECTRA FOR CAROTENOID AND THEIR COMPLEXES**

9:35 am EDT ***Presenter:*** **ASSOCIATE PROFESSOR MINDAUGAS MACERNIS**
Vilnius University, Lithuania
(email: mindaugas.macernis@ff.vu.lt)

Abstract 26 **CORONA AND CAROTENOIDS – MORE IN COMMON THAN INITIAL LETTER C?**

9:44 am EDT ***Presenter:*** **PROFESSOR HANS-RICHARD SLIWKA**
Norwegian University of Science and Technology, Trondheim, Norway
(email: richard.sliwka@ntnu.no)

Abstract 27 **STEREOCONTROLLED SYNTHESIS OF LIPOFUSCIN PIGMENT A2E, A PYRIDINIUM BISAPOCAROTENOID**

9:53 am EDT ***Presenter:*** **DR. CLAUDIO MARTÍNEZ**
University of Vigo, Vigo, Spain.
(email: claudiom@uvigo.es)

10:02 am EDT **Session V – Questions and Discussion** (15 min)
Moderators: **Profs. Bruno Robert and Hideki Hashimoto**

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10:17 am EDT **Break** (**4** minutes)

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10:21 am EDT **Session VI - Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism**

Moderators:

Professor Loredana Quadro
Rutgers University
New Brunswick, NJ USA
email: lquadro@sebs.rutgers.edu

Professor Yoav Sharoni
Ben-Gurion University
BEER-SHEVA Israel
email: yoav@bgu.ac.il

10:21 am EDT **Session VI-A - Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism**

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Abstract 28. **THE PROTECTIVE EFFECT OF CAROTENOIDS, POLYPHENOLS AND SEX HORMONES ON SKIN CELLS UNDER OXIDATIVE STRESS**

10:22 am EDT ***Presenter:*** **Mrs. AYA DARAWSHE**
Ben-Gurion University of the Negev, Beer-sheva, Israel
(email: ayadar@post.bgu.ac.il)

Abstract 29 **DIETARY BETA-CAROTENE SUPPLEMENTATION TO ISX-/- DAMS DURING LATE GESTATION AND LACTATION IMPACTS OFFSPRING RESPONSES TO A HIGH-FAT DIET IN ADULTHOOD**

10:31 am EDT

Presenter: **PROFESSOR M. LUISA BONET**
Universitat de les Illes, Balears-CIBER, Palma, Spain.
(email: luisabonet@uib.es)

Abstract 30 **ANTIOXIDANT, ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF THE HALOARCHAEAL CAROTENOID BACTERIORUBERIN**

10:40 am EDT

Presenter: **MS. MICAELA GIANI**
University of Alicante, Alicante, Spain.
(e-mail: micaela.giani@ua.es)

Abstract 31 **TISSUE LYCOPENE ACCUMULATION IN TRANSGENIC MICE LACKING ONE OR BOTH CAROTENOID CLEAVING ENZYMES**

10:49 am EDT

Presenter: **MS. MADELYN BRADLEY**
University of Illinois Urbana Champaign, Urbana, IL
(email: madelynbradley@gmail.com)

10:58 am EDT **Session VI-A – Questions and Discussion** (15 min)
Moderators: Profs. Loredana Quadro and Yoav Sharoni

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11:13 am EDT **Break** (4 minutes)

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11:17 am EDT **Session VI-B- Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism**
Moderators: Profs. Loredana Quadro and Yoav Sharoni

Abstract 32 **β-CAROTENE MITIGATES LIVER INFLAMMATION DURING ATHEROSCLEROSIS REGRESSION**

11:18 am EDT

Presenter: **DR. PAULA MAPELLI-BRAHM**
University of Illinois at Urbana-Champaign, Urbana, IL
(email: pmapelli@illinois.edu)

Abstract 33 **β-CAROTENE ENHANCES ATHEROSCLEROSIS RESOLUTION BY REDUCING INFLAMMATION AND INCREASING PLAQUE STABILITY**

11:27 am EDT

Presenter: **MS. JOHANA CORONEL**
University of Illinois at Urbana-Champaign, Urbana, IL
(email: acoronel@illinois.edu)

Abstract 34 **ROLE OF LECITHIN-RETINOL ACYLTRANSFERASE IN TRIGLYCERIDE SECRETION**

11:35 am EDT

Presenter: **MR. DONALD MOLINA CHAVES**
University of Illinois at Urbana-Champaign, Urbana, IL
(e-mail: dfm4@illinois.edu)

11:44 am EDT **Session VI-B– Questions and Discussion** (15 min)
Moderators: Profs. Loredana Quadro and Yoav Sharoni

11:59 am EDT **Close, Day 2 VICC (Sessions IV, V, & VI)**

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Thursday April 13, 2022

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

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

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8:05 am EDT **Session VII - Carotenoids and Health II: Supplementation & Bioavailability**

Moderators:

Assistant Professor Lilian Mariutti
University of Campinas
São Paulo *Brazil*
email: lilianma@unicamp.br

Associate Professor Tim O'Hare
The University of Queensland
Brisbane, Queensland Australia
email: t.ohare@uq.edu.au

8:05 am EDT **Session VII-A - Carotenoids and Health II: Supplementation & Bioavailability**

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Abstract 35 **CAROTENOID DIGESTIBILITY IN COMMERCIAL MAIZE HYBRIDS IS AFFECTED BY GRAIN PROPERTIES**

8:06 am EDT

Presenter: **Ms. DORA ZURAK**
University of Zagreb, Zagreb, Croatia
(email: dzurak@agr.hr)

Abstract 36 **BIOACCESSIBILITY OF CAROTENOIDS IN BABY FOOD**

8:15 am EDT

Presenter: **MS. ADRIELE HACKE**
University of Campinas, Department of Food Science and Nutrition, São Paulo, Brazil
(email: h_adriele@hotmail.com)

Abstract 37 **TRAIT STACKING SIMULTANEOUSLY ENHANCES MINERAL AND PROVITAMIN A CAROTENOID BIOACCESSIBILITY IN BIOFORTIFIED SORGHUM BICOLOR**

8:24 am EDT

Presenter: **DR. MICHAEL P. DZAKOVICH**
USDA-ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
(email: michael.dzakovich@bcm.edu)

Abstract 38 **ASSOCIATION BETWEEN SKIN CAROTENOID LEVELS AND BMI WITH FAMILY FUNCTIONING AND PEDIATRIC QUALITY OF LIFE IN CHILDREN FROM LOW-INCOME HOUSEHOLDS**

8:33 am EDT

Presenter: **MS. SURANJANA DEY**
Nationwide Children's Hospital, Columbus, OH, USA
(email: Suranjana.dey@nationwidechildrens.org)

Abstract 39 **ROLE OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR IN THE UPTAKE AND EXCRETION OF CAROTENOIDS**

8:42 am EDT

Presenter: **Mr. WALTER CATALAN**
University of Illinois Urbana, Champaign, Urbana, IL, USA
(email: walter24@illinois.edu)

8:51 am EDT **Session VI-A – Questions and Discussion** (15 min)
Moderators: Profs. Lilian Mariutti and Tim O'Hare

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9:06 am EDT **Break** (4 minutes)

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9:10 am EDT **Session VII-B - Carotenoids and Health II: Supplementation & Bioavailability (cont'd)**
Moderators: Profs. Lilian Mariutti and Tim O'Hare

Abstract 40 **TISSUE DISTRIBUTION OF LUTEIN IN NEONATAL SPRAGUE-DAWLEY RATS REARED BY MOTHERS CONSUMING A NORMAL- OR A HIGH FAT DIET**

9:11 am EDT

Presenter: **MS. YANQI ZHANG**
The University of Alabama, Tuscaloosa, Alabama, USA;
(email: yzhang309@crimson.ua.edu)

Abstract 41. **BIOPOLYMER STABILIZED EMULSIONS IMPROVED STORAGE STABILITY AND IN VITRO BIOACCESSIBILITY OF LUTEIN**

9:20 am EDT

Presenter: **MS. YANQI ZHANG**
The University of Alabama, Tuscaloosa, Alabama, USA;
(email: yzhang309@crimson.ua.edu)

Abstract 42 **SYSTEMATIC REVIEW OF WORLDWIDE INFANT BLOOD AND HUMAN MILK CAROTENOID CONCENTRATIONS**

9:29 am EDT

Presenter: **ASSISTANT PROFESSOR NANCY E. MORAN**
USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
(email: Nancy.Moran@bcm.edu)

Abstract 43. **THE RELATIONSHIPS BETWEEN POSTPARTUM MATERNAL AND INFANT OCULAR AND SYSTEMIC CAROTENOID STATUS IN THE LUTEIN AND ZEAXANTHIN IN PREGNANCY (L-ZIP) TRIAL**

9:38 am EDT

Presenter: **Dr. EMMANUEL KOFI ADDO**
University of Utah, School of Medicine, Salt Lake City, UT
(email: emmanuel.k.addo@utah.edu)

9:47 am EDT **Session VI-B – Questions and Discussion** (15 min)
Moderators: Profs. Lilian Mariutti and Tim O'Hare

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10:02 am EDT **Break** (4 minutes)

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10:06 am EDT **Session VIII – Carotenoid and Apocarotenoid Biology**

Moderators:

Assistant Professor Matt Toomey
University Of Tulsa
Tulsa, Oklahoma USA
email: mbtoomey@gmail.com

Assistant Professor Kerim Eroglu
North Carolina State University
North Carolina USA
email: aeroglu@ncsu.edu

10:06 am EDT **Session VIII-A – Carotenoid and Apocarotenoid Biology**

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Abstract 44 **STUDYING THE ROLES OF APOCAROTENOIDS IN TOMATO BY GENOME EDITING OF THE CAROTENOID CLEAVAGE DIOXYGENASE (CCD) GENES**

10:07 am EDT

Presenter: **MRS. TAL MAKOV BOUANICHE**
The Hebrew University of Jerusalem, Jerusalem, Israel
(email: tal.makov@mail.huji.ac.il)

Abstract 45 **RETINOL AS A REGULATOR OF ENERGY HOMEOSTASIS DURING EMBRYOGENESIS**

10:16 am EDT

Presenter: **DR. YOUN-KYUNG KIM**
Rutgers University, New Brunswick, NJ, USA
(emails: ykkim5@sebs.rutgers.edu)

Abstract 46 **THE SYNTHETIC RETINOID FENRETINIDE INHIBITS THE CONVERSION OF B-CAROTENE TO VITAMIN A IN MICE**

10:25 am EDT

Presenter: **Mr. ANTHONY MILLER**
University of Illinois at Urbana-Champaign, Urbana, IL USA
(email: apmille2@illinois.edu)

Abstract 47 **CHARACTERIZATION OF MAMMALIAN CAROTENOID CLEAVAGE DIOXYGENASES: HETEROLOGOUS EXPRESSION, PURIFICATION, ENZYME ASSAYS, AND SUBSTRATE SELECTIVITY.**

10:34 am EDT

Presenter: **Dr. SEPALIKA BANDARA**
Case Western Reserve University, School of Medicine, *Cleveland, Ohio USA*
(email: sxb1081@case.edu *)*

Abstract 48 **CAROTENOID ACCUMULATION IN ISX/BCO2 DKO SERVES AS A MACULA PIGMENT MOUSE MODEL**

10:43 am EDT

Presenter: **MS. LINDA D. THOMAS**
Case Western Reserve University, School of Medicine, Cleveland, OH
(email: ldt29@case.edu)

10:52 am EDT **Session VIII-A – Questions and Discussion** (15 min)
Moderators: Profs. Matt Toomey and Kerim Eroglu

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11:07 am EDT **Break** (4 minutes)

****

11:11 am EDT **Session VIII-B – Carotenoid and Apocarotenoid Biology**
Moderators: Profs. Matt Toomey and Kerim Eroglu

Abstract 49. **GENETIC DISSECTION OF THE VITAMIN A DELIVERY PATHWAYS TO OCULAR TISSUES IN MICE**

11:12 am EDT

Presenter: **Ms. JEAN MOON**
Case Western Reserve University, School of Medicine, Cleveland, OH
(email: jxm1019@case.edu)

Abstract 50 **MECHANISMS OF CAROTENOID-BASED SPECTRAL FILTERING IN THE AVIAN VISUAL SYSTEM**

11:21 am EDT

Presenter: **ASSISTANT PROFESSOR MATTHEW B. TOOMEY**
University of Tulsa, Tulsa, OK, USA
(email: mbt6332@utulsa.edu)

Abstract 51 **EXPOSURE TO ARTIFICIAL LIGHT AT NIGHT INTERACTS WITH SOCIAL CONDITIONS TO AFFECT EGG-YOLK CAROTENOID INVESTMENT IN A MODEL GAMEBIRD SPECIES**

11:30 am EDT

Presenter: **PROFESSOR KEVIN J. MCGRAW**
Arizona State University, Tempe, AZ USA
(email: kjmcgraw@asu.edu).

Abstract 52. **EXPLORING THE DIVERSE ROLES OF RETINAL SIGNALING IN PLANTS**

11:39 am EDT

Presenter: ***DR.*** **RUPAK TIMILSINA**
UC San Diego, La Jolla, California USA
(email: rtimilsina@ucsd.edu)

11:48 am EDT **Session VIII-B – Questions and Discussion** (15 min)

Moderators: Profs. Matt Toomey and Kerim Eroglu

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12:03 pm EDT **Conclusion VICC 2022**

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2nd Virtual International Conference on Carotenoids

VICC 2022

International Carotenoid Society

April 12th, 13th, 14th

Dietary Sources of Carotenoids
&
Nutritional Supplementation

Considerations for developing an Australian lutein and zeaxanthin food composition database.

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Two carotenoids, lutein and zeaxanthin (L&Z) have been identified as having a role in long-term macular health, in particular in age-related macular degeneration prevention [1]. The importance of L&Z was highlighted when they were proposed as a candidate for dietary intake value recommendations in the United States [1]. In Australia, intake recommendations would be limited due to the lack of local food composition data. The Food Standards Australia New Zealand (FSANZ) database reports L concentrations for only 24 foods, and no Z [3]. Cultivar and climate conditions influence food carotenoid concentrations and should consequently be considered when building an L&Z database in a large country like Australia [4]. Therefore, the aim of this project was to investigate variability in L&Z concentrations of two Australian foods from samples grown in different locations, and compare values with reported FSANZ database values.

Broccoli (*Brassica oleracea var. italica*) and broccolini (*Brassica oleracea*) were chosen as L concentration is reported for both in the FSANZ database [3]. Six samples per food (a sample being a head of broccoli or bunch of broccolini) were sourced from local stores in Brisbane, Queensland, Australia. The broccoli samples were purchased between Feburary and March. Two samples of broccoli were grown in Tasmania, two in Queensland and two in Victoria (all Australian states). Three broccolini samples grown in Victoria were purchased in March, and 3 Queensland samples between May and June. The L&Z extraction method was adapted from methods described by Fanning et al. [5]. The edible food component was analysed for L&Z in triplicate by high performance liquid chromatography and photodiode array detection. The column was a Develosil 5µm RP-aqueous C30 140A, 250 x 4.6mm column run with isocratic mobile phase containing methanol (49.96%), acetonitrile (49.96%), and triethylamine (0.08%) at a flow rate of 1.2 mL/minute. Detection of L and Z at a limit of 0.09 µg/mL was performed at 445 nm [5]. Lutein and Z data were presented as the triplicate mean in µg/100g fresh weight for each individual sample. The L and Z combined mean ± standard deviation of the six samples per food was reported as µg/100g fresh weight.

The L concentrations of broccoli samples grown in Tasmania were 537 and 1442, in Victoria they were 886 and 2266, and in Queensland they were 994 and 885. The L mean concentration of all six samples was 1163 ± 614. In broccoli Z was below the detection limit. The broccolini concentrations grown in Victoria were 2688, 2790 and 1436 for L, and 61, 67, and 78 for Z. The concentrations of broccolini grown in Queensland were 2646, 2015 and 2114 for L, and 90, 49, and 41 for Z. The L mean concentration of all broccolini samples was 2277 ± 520, and 61 ± 18 for Z.

The L and Z concentrations of both foods were variable between and within the Australian states sampled. The FSANZ broccoli L concentration is 352.5 µg/100g (range <5.0 to 800.0 µg/100g), and 1417 µg/100g (range unavailable) for broccolini [3]. The mean L concentrations of both foods in this project were more than 50% higher than their respective FSANZ L values. The L concentrations measured in this project indicate that the current FSANZ L values for broccoli and broccolini may not be representative of the Australian food supply. This project did not investigate seasonal variation in L&Z concentrations, a limitation and avenue for future investigations.

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UNEXPECTED SIDE-EFFECTS OF ZEAXANTHIN BIOFORTIFICATION

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Introduction: Increasing zeaxanthin concentration in fruit and vegetables can be accomplished by manipulating the carotenoid pathway to increase flux towards zeaxanthin and away from other carotenoids. The risk of doing this is that other carotenoids may potentially play an important role in plant metabolism, with an over-reduction of these resulting in potentially unexpected or unwanted side-effects. High zeaxanthin in plants can be achieved by redirecting the carotenoid biosynthesis pathway towards the beta-branch which is usually achieved at the expense of the alpha-branch, which includes lutein, a carotenoid involved in protecting chlorophyll from excess light damage. High zeaxanthin can also be achieved by reducing the conversion of zeaxanthin to carotenoids further down the beta-branch of the pathway, such as violaxanthin and neoxanthin. A potential risk of this is a reduction in a plant's ability to produce abscisic acid, an apocarotenoid produced from these precursor carotenoids. Abscisic acid is a plant growth regulator necessary for controlling stomatal closure during water stress and maintaining seed dormancy within a fruit. The current study highlights some of the secondary side-effects of high zeaxanthin in a few varieties of orange capsicum, which have elevated levels of zeaxanthin.

Research and Methods: Leaves and fruits of eight orange and two red varieties of capsicum (*Capsicum annuum*) were quantified for violaxanthin, lutein and zeaxanthin. As these carotenoids (xanthophylls) have previously been reported to exist in an esterified form in capsicum, saponification was performed to convert them to free carotenoids. The subsequent carotenoid extracts were analysed by ultra-high performance liquid chromatography coupled with diode-array detection and mass spectrometry (UHPLC-DAD-MS). Phenological observations were recorded for each variety, including wilting, leaf/fruit damage, and plant vigour.

Results and discussion: From the leaf and fruit data, it was observed that two orange varieties (199-9 and DSP) had low concentrations of both lutein and violaxanthin. These varieties were observed to have poor plant vigour. The lower concentration of lutein may be responsible for this observation as lutein is known to play an important role in protecting chlorophyll. If chlorophyll is compromised, less energy is potentially available for plant development, hence varieties with very low concentrations of lutein may be stunted, as observed in 199-9 and DSP. By contrast, leaves of red varieties (Warlock, Plato) had sufficient concentration of lutein and violaxanthin and were observed to have an excellent plant vigour and no wilting. Other orange varieties which were observed to have comparable concentrations of violaxanthin and lutein to red varieties were similar in their phenology. One orange variety (Belle orange), however, behaved differently, displaying a lower concentration of both lutein and violaxanthin in fruit, but not in their leaves. Despite this, plant wilting, leaf/fruit damage and low plant vigour was observed in this variety. Although reduced concentration of lutein and violaxanthin are theoretically linked to the protection of chlorophyll and leaf wilting, respectively, it would appear that other factors may also be involved. From this study it appears, that it is important to be able to balance increasing zeaxanthin concentration with undesirable side-effects to the plant that could potentially be induced by reducing other carotenoids such as violaxanthin and lutein.

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BIOFORTIFYING MANGOES: FACTORS AFFECTING PRO-VITAMIN A CAROTENOIDS IN MANGO FLESH

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Introduction: The principal carotenoid present in mango (*Mangifera indica L.*) fruit flesh is trans-beta-carotene. Trans-beta-carotene is a major dietary precursor of Vitamin A, capable of producing two retinol molecules following cleavage. Apart from trans-beta-carotene, other pro-Vitamin A carotenoids exist, such as alpha-carotene, which when cleaved produce just a single retinol molecule. Although, mango fruit predominantly contain trans-beta-carotene, it is not unusual for varieties to contain a number of Vitamin-A precursors, the proportion of which can differ significantly between varieties and potentially impact on the amount of retinol that can be produced. In addition, total carotenoid production can vary greatly between mango varieties, further influencing their value as a source of Vitamin A. The current study analysed the carotenoid profile of 27 mango varieties and discusses the relative importance of the proportion of different pro-Vitamin A carotenoids present and total carotenoid concentration on potential retinol synthesis.

Materials and Methods: 26 varieties of mangoes from the Walkamin Research Facility (Queensland, Australia) germplasm collection and one commercial variety from a Brisbane supermarket were obtained and ripened at 23°C. Carotenoids were extracted and quantified from fully ripened mango fruits. Ripeness was based on the firmness of the pulp, and carotenoids analysed using ultra-high-performance liquid chromatography coupled with diode array detection (UHPLC-DAD).

Results and Discussion: The 27 varieties of mangoes varied in total carotenoid and proportion of different carotenoids. In all varieties, trans-beta-carotene was the predominant carotenoid present, usually constituting 50-85% of pro-Vitamin carotenoids. Other pro-Vitamin A carotenoids identified included trans-alpha-carotene, 13-cis-beta-carotene and 15-cis-beta-carotene. As trans-beta-carotene was consistently the predominant carotenoid present, and is able to yield two, rather than one retinol molecule following cleavage, its importance as a pro-Vitamin A carotenoid tended to well exceed that of the other pro-Vitamin A carotenoids present. In the varieties assessed, total carotenoid content had the greatest impact on trans-beta-carotene concentration, while the proportion of trans-beta-carotene was a secondary factor influencing retinol-producing potential.

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RELATIONSHIP BETWEEN KERNEL PROPERTIES AND CAROTENOID DEGRADATION RATE IN MILLED MAIZE

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Introduction: Yellow maize is the staple crop used as both food and feed, and for this purpose, the kernels are often stored milled. Maize is also a source of carotenoids, whose stability is strongly influenced by light, temperature and oxygen. Due to exposure to these influences, prolonged storage of milled kernels can lead to increased carotenoid degradation. Maize carotenoids are mostly located in the endosperm, and since physicochemical properties of the endosperm vary among genotypes [1], it is possible that some genotypes could have lower carotenoid degradation during storage. Therefore, in this study, milled kernels of different maize hybrids were subjected to prolonged storage at different temperatures, and carotenoid content was monitored over time. In addition, the physicochemical properties of maize were determined to evaluate their relationship with carotenoid stability.

Methods and Materials: Three commercial maize hybrids differing in kernel hardness were milled through an 8 mm sieve and stored for 90 days at -20, +4 and +25 °C. Carotenoid content was determined using the RP HPLC method in samples taken after 0, 7, 14, 21, 28, 42, 56, 78, and 90 days of storage. Carotenoid degradation followed first-order kinetics, which allowed the evaluation of the degradation rate (k) for individual and total carotenoids. Of the maize properties, the Stenvert index (SI), i.e., the kernel hardness, the content of amylose, zein, free, bound, and starch lipids, and the size distribution of starch granules were determined using methods adapted for maize. Statistical analysis of results was performed using SAS 9.4 software.

Results: Among maize carotenoids, lutein showed the highest stability (average k=0.00398 day^{-1}), while β-carotene was the most susceptible to degradation (average k=0.01087 day^{-1}). Carotenoid degradation was more pronounced when maize was stored at 25 °C (average for total carotenoids k_{TC}=0.00758 day^{-1}), while the degradation rate was lower when maize was stored at 4 °C or at -20 °C (average k_{TC}=0.00261 vs. 0.00396 day^{-1}). Hybrids with SI≈1.0 and SI≈1.1 exhibited a higher degradation rate of total carotenoids ($k_{25°C}$=0.00820 and 0.00770 day^{-1}, respectively), while greater carotenoid stability was enabled in the hybrid with the highest SI (1.2; $k_{25°C}$=0.00671 day^{-1}). The stability of the predominant lutein and zeaxanthin and total carotenoids correlated with other determined kernel properties ($P<0.05$). The results showed that hybrids with higher content of zein, free and bound endosperm lipids had lower carotenoid degradation rates (r=-0.431, -0.473 and -0.729, respectively). In addition, larger starch granules contributed to a higher degradation rate (r=0.688).

Conclusions/Discussion: The highest stability of carotenoids was ensured when milled maize was stored at +4 °C. As suspected, kernel properties had a significant effect on carotenoid stability. Our study suggests that hybrid with higher kernel hardness was able to maintain carotenoid stability better than hybrid with lower kernel hardness. In addition, our study provided new insights into the relationship between carotenoid stability and endosperm properties, particularly zein and endosperm lipid content and starch granule size, the values of which are opposite in hybrids with low and high kernel hardness [1,2].

Acknowledgements

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DID CAROTENOID CONSUMPTION AMONG BRAZILIANS CHANGE IN 10 YEARS?

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A carotenoid-rich diet may present a lower risk of developing non-communicable diseases. However, estimated intakes of carotenoids vary greatly on individual and national levels, and many populations do not consume the recommended amounts of fruits and vegetables, resulting in a dietary carotenoid intake gap. Thereby, this study aimed to assess the intake of the individual carotenoids and identify their major dietary sources by Brazilians. The food intake was obtained from the 2008-09 and 2017-18 Household Budget Survey from non-institutionalized individuals of both sexes, aged ten years and over. Carotenoid content of foods was identified using the Brazilian Table of Food Composition. Carotenoid intake from each fruit and vegetable was calculated by multiplying the content of each individual carotenoid by the daily amount of each consumed food and dividing by 100, and these values were added up to the yield individual and total carotenoid intakes, as well as their relative contribution.

The average intake of carotenoids by Brazilians was 31% higher in 2017-18 (3,675 µg/day) than in 2008-09 (2,803 µg/day). Individual carotenoid intake is shown in Fig. 1. The most consumed carotenoid in Brazil, in both periods, was β-carotene (38% and 43% of the total consumed carotenoids in 2008-09 and 2017-18, respectively). In addition, there was a higher consumption of carotenes (67% and 74% total carotenoid in 2008-09 and 2017-18, respectively), and non-provitamin A carotenoids (53% and 51% total carotenoid in 2008-09 and 2017-18, respectively).

The main contributors to carotenoid intake, in 2017-18, were: sweet potato (44%) for β-carotene, tomato (60%) for lycopene, salad (lettuce/arugula/watercress - 61%) for lutein, corn flour (69%) for zeaxanthin, pumpkin (31%) for α-carotene, and papaya (50%) for β-cryptoxanthin.

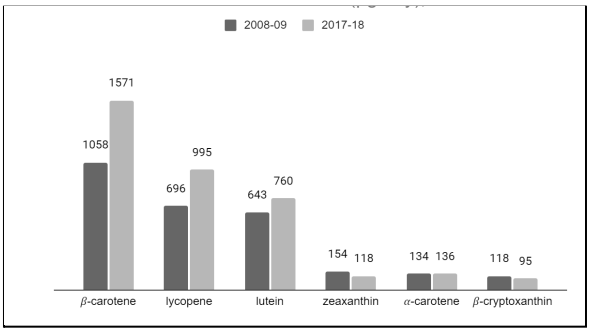

Fig. 1. Individual carotenoid intake (µg/day) in Brazilian dietary (2008-09 and 2017-18).

Carotenoid intake by Brazilians is lower than that observed for populations in developed countries, for example, Spain (6 mg carotenoid/day) and the United States (9 mg carotenoid/day). A higher intake of total carotenoid was observed in 2017-18. However, it is still not enough to reach the suggested value of prudent daily intake.

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Lemna – Rapidly Growing Zeaxanthin Hyperaccumulator

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We will briefly review the roles of xanthophylls (with a focus on zeaxanthin and lutein) in protection against radiation damage in organisms ranging from microorganisms to plants and humans as well as in counteracting non-resolving inflammation (linked to cognitive dysfunction and many diseases and disorders in humans). The reasons why zeaxanthin can be hard to come by in the human diet and present the aquatic plant *Lemna* (a duckweed) as an edible plant (traditionally consumed in Asia) with an unusual combination of desirable traits for human consumption. In contrast to land plants, *Lemna* accumulates exceptionally high levels of zeaxanthin while also growing very rapidly. Furthermore, *Lemna* has an unusually high content of other human micronutrients (especially antioxidants) as well as high-quality protein. We will describe the response of *Lemna's* content of carotenoids (especially lutein, zeaxanthin, and *b*-carotene) and the antioxidant vitamin E (tocopherol) as a function of light intensity and other aspects of the growth environment. These findings will be placed into the context of mechanistic interactions between carotenoids and vitamin E in hydrophobic microenvironments. Additional features will also be summarized that make *Lemna* an attractive candidate for (i) sustainable agriculture on earth as well as for (ii) spaceflight environments (in which ionizing radiation causes inflammation-related cognitive dysfunction and elevated disease risk for astronauts).

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Skin Carotenoid Assessment Among School Children in the Hilly Region of Nepal

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Introduction: Vitamin A deficiency (VAD) is a major public health problem among children in Nepal. We conducted a cross-sectional study to assess skin carotenoid measurement as a rapid noninvasive screening tool for VAD among children in the hilly region of Nepal.

Methods: Based on the 49% prevalence of low skin carotenoid levels (<150 RS) among children of 8 to 12 years of age in our previous survey of VAD in Nepal, we enrolled 324 school children up to 7 years old from five public hospitals and a single private school in the hilly region of Nepal. Skin carotenoid levels were assessed using the Veggie Meter® (Longevity Link Corporation, Salt Lake City, Utah, USA) as a primary outcome. Detailed eye evaluations were conducted in subjects with low skin carotenoid scores (<150 RS) and those with night blindness. Factors associated with low skin carotenoids were assessed.

Results: Mean age of children was 5.12 ± 1.33 years old. Males were higher in number (53.5%). The skin carotenoid level was < 150 RS in 46.9% of children, between 150 and 200 RS in 23.5% of children, and >200 RS in 29.6% of children. Among the various schools, the skin carotenoid levels less than 150 RS ranged from 9.8% to 74.1%. Skin carotenoid scores of 150 to <200 ranged from 11.1% to 33.3%, and > 200 RS ranged from 7.4% to 60.8%. Among those with skin carotenoids <150 RS at the various schools, 17% were 3 years old, 28.3% were 4 years old, 28.3% were 5 years old, 20.4% were 6 years old, and 11.2% were 7 years old.

Conclusion: Nearly half of the total school children had skin carotenoid scores <150 RS, levels highly associated with VAD. Skin carotenoid levels were better in older children. In addition to enhancing awareness to increase consumption of green leafy vegetables and fruits, vitamin A supplementation may be required to combat vitamin A deficiency in children in the hilly region of Nepal.

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Carotenoid Production and Microorganisms

Impact of carbon sources on β-cryptoxanthin production and study of its anti-microbial effect on *E. coli*

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Kocuria marina DAGII was previously isolated in our laboratory during routine screening of pigment-producing microorganisms. It is a significant source of provitamin A carotenoid β-cryptoxanthin (β-CRX). Maximum β-CRX production was observed in the culture media where glucose and maltose were used as a carbon source at 56 h of incubation [1]. The objective of this study was to investigate the impact of carbon sources on β-CRX production, and the anti-microbial effect of β-CRX against *E. coli*.

K. marina DAGII has been cultured in three different nutrient conditions. β-CRX extraction has been done according to the protocol [1] with slight modifications. According to the protocol [2], the anti-microbial activity of β-CRX has been carried out against *E. coli* using Disc Diffusion and MIC (Minimum Inhibitory concentration) with slight modifications. The β-CRX yield was observed at 56 h of incubation.

Here it has been found that β-CRX yield was several-fold higher in cheese whey media compared to other two media.

Carbon source	β-CRX (mg/lit)
Glucose and Maltose	4.2
Glucose and Lactose	96.67
Cheese whey	131.82

In Disk diffusion at 8 mg/lit β-CRX concentration the zone of inhibition was found to be 8 mm , and the MIC was found at 8 mg/lit β-CRX concentration against *E. coli*.

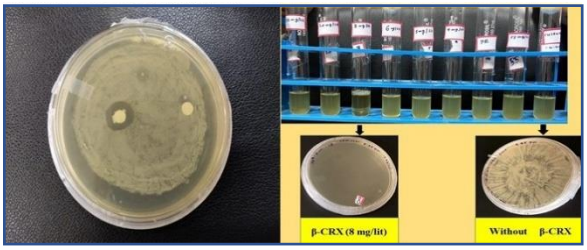

Fig.1. Disc Diffusion Fig.2. MIC

Cheese whey is the most potent carbon source for β-CRX production by *K. marina* DAGII. β-CRX can be explored as future nutrigenomics due to its several health benefits along with its anti-microbial activity.

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ENHANCED PRODUCTION AND ISOLATION OF A NOVEL CAROTENOID LOROXANTHIN FROM *SCENEDESMUS OBLIQUUS*

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Microalgae are known as a natural source of carotenoids and other beneficial bioactive compounds. Carotenoids have gained attention due to their potential health benefits. Some of the specific advantages of microalgae cultivation compared to traditional plant-based sources include faster cultivation, processing, and harvesting cycle and the ability to be cultured on waste materials. Despite these advantages, the large-scale and cost-effective manufacture of the carotenoids from microalgae is currently quite challenging for many reasons [1]. On the other hand, some microalgae produce novel carotenoids which have not been synthesized by some plants and/or other organisms. Loroxanthin is one of these unique carotenoids [2].

In the present study, loroaxanthin from *Scenedesmus obliquus* was detected (2.40±0.05 mg/g) and its production was induced (5.46±0.11 mg/g) by using $NaNO_2$ as a different nitrogen source. The biomass (0.20 g) was added $CaCO_3$ (0.20 g) and 10.0 mL of ethanol containing 0.01 % (w/v) pyrogallol. Then the extraction was realized by an ultrasonic bath for 15 minutes at 30^{o}C followed by a four-hour saponification procedure with 10% methanolic KOH. The amount of loroxanthin was determined by HPLC-DAD at 450 nm with a flow rate of 1.0 mLmin-1 using Waters YMC C_{30} Carotenoid column (4.6 x 250 mm, 5µm). After that, loroxanthin was isolated by prep-HPLC using a semi-prep column (Waters YMC C_{30} column: 10.0 x 250 mm, 5µm). The collected fractions were combined, evaporated, and dissolved in different solvents. The structural confirmation was performed with Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) equipped with an Atmospheric Pressure Chemical Ionization probe (APCI) and UV-vis spectroscopy.

Consequently, microalgal carotenoids production has excellent potential although there are major challenges to be overcome. Carotenoid production from microalgae is still an attractive and potentially rapidly growing field. For future studies, emphasis will be given to exploring the biological activities of loroxanthin.

Keywords: Microalgae, *Scenedesmus obliquus*, loroxanthin, isolation, prep-HPLC

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REGULATION OF CAROTENOID BIOSYNTHESIS BY STRESS IN THE FUNGUS *FUSARIUM FUJIKUROI*

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Fusarium fujikuroi is a fungal model to study the genetic regulation of the biosynthesis of neurosporaxanthin, one of the carotenoids accumulated in higher amount in its mycelium in response to light. The structural *carX, carB,* and *carRA* genes are clustered with *carO* opsin gene. This cluster is upregulated in the wild type when illuminated but also in overproducer strains in the dark. A representative overproducer is *F. fujikuroi* mutant SG39, selected based in its orange pigmentation after chemical mutagenesis. This strain contains a mutation in *carS* regulator gene, that codes for a putative E3-ubiquitin ligase [1]. In our laboratory, we are interested in determining if carotenoids are synthesized in this fungus in response to different abiotic stresses and if this is mediated by the CarS regulator.

As a control in our experiments, SG39 was transformed with the wild-type *carS* allele which restored the wild phenotype in the complemented strain. The wild type, the *carS* mutant (SG39), and the complemented strain (SG256) were cultured under different abiotic stresses that included nitrogen starvation, oxygen peroxide, and heat shock. Total carotenoids were extracted and expression of *carB, carRA,* and *carS* genes were analyzed by qRT-PCR in the three strains.

Results showed that the three stressing conditions provoked a higher production of carotenoids in the three strains grown in the dark, which in the control strains correlated with an upregulation of the structural genes and downregulation of *carS* gene. RNA-seq data showed that this regulatory gene suffers alternative splicing of an intron located in the 3' region, and we tested if this was affected under different stresses. PCR analysis of *carS* cDNA showed that the relative amounts of four spliced mRNAs variants found were influenced by temperature, with higher retention under heat shock of the *carS* intron.

The results suggest that enhanced carotenoid production under different stress conditions is mediated by changes in *carS* levels and, at least in the case of heat stress, there is another regulation at postranscriptional level of the *carS* gene.

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Investigating the role of HmgbC, a HMG-box family protein, in the regulation of carotenogenesis in *Fusarium*

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Fusarium fujikuroi is a phytopathogenic fungus that produces a wide variety of secondary metabolites that includes carotenoids. The *car* genes responsible for the biosynthetic steps of the carotenoid pathway in this fungus have been identified, and most of them are regulated by light and by the CarS repressor [1].

To identify specific regulators of *car* genes, a biotin-mediated pulldown of proteins binding to *car* promoters was performed in our laboratory (S. Nordzieke, unpublished). In this scrutiny, two proteins of the HMG-box family, HmgbC and HmgbB were found as possible candidates. The aim of this work is to understand the role of the HmgbC protein in the regulation of the *car* genes.
The *hmgbC* gene (*FFUJ_00508*) was deleted by homologous recombination in the wild-type strain IMI58289. For that, wild protoplasts were transformed with a linear replacing cassette containing the *hph* gene that confers resistance to hygromycin B, surrounded by upstream and downstream DNA fragments of the *hmgbC* gene. A set of hygromycin-resistant transformants was obtained and the replacement of *hmgbC* sequence by Hyg^r cassette in the different candidates was analyzed by PCR.

Preliminary phenotypic characterization suggests a more intense pigmentation in some of the transformants grown in the dark or under light conditions (Fig.1). This study will be followed by carotenoid analyses following protocols as described [2].

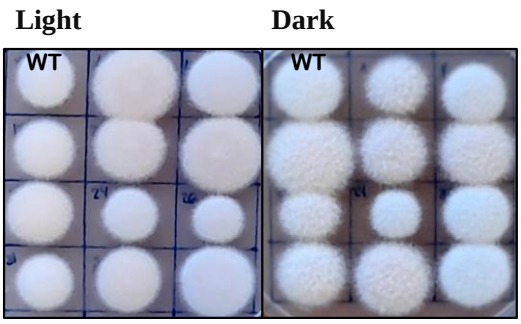

Figure 1. Aspect of colonies of wild type (WT) and 11 transformants grown on minimal medium for four days under light or in the dark at 30°C.

The correlation of the phenotype with *hmgbC* deletion is currently under investigation.

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Preliminary studies for the optimization of ultrasound-assisted extraction of microalgal carotenoids

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INTRODUCTION: The interest in carotenoids (CRS) in agro-food and health continues growing due to their versatility. Microalgae are the main primary producers of carotenoids in the trophic chain (1). Besides sustainable sources, the food industry increasingly requires the use of sustainable technologies, such as ultrasound-assisted extraction (UAE), which has been used to optimize the extraction of CRS from various sources (2). The aim of this study was to select the best solvent, among those allowed for food use in Europe, for the UAE of CRS from microalgae. In addition, dimethyl sulfoxide (DMSO) and ethyl lactate were also tested. for comparison. The effect of a milling ~~pretreatment~~ on the extraction was also evaluated.

MATERIALS AND METHODS: Freeze-dried *Chlorella vulgaris, C. sorokiniana* and *Dunaliella salina* were subjected to UAE with ethyl acetate, ethanol, acetone, hexane, methanol, dichloromethane, ethyl lactate, and dimethyl sulfoxide (DMSO). The prior application of micro-mill (30 Hz, 5 min) was also evaluated.

RESULTS: The highest CRS concentrations in the extracts from *C. vulgaris* were obtained with DMSO, methanol, and ethanol (concentrations 2.84, 2.21, and 1.16 mg/g, respectively). In order to select the best solvent among these three solvents, *C. sorokiniana* and *D. salina* were subjected to UAE with them (Table 1).

Table 1. Concentration of carotenoids (mg/g) in the extracts obtained by UAE.

	C. sorokiniana	***D. salina***
Methanol	1.12	2.32
Ethanol	0.88	2.07
DMSO	0.75	1.80

It was also found that, in general, a previous milling step led to significantly ($p < 0.05$) higher extractions. CONCLUSIONS/DISCUSSION: Methanol was the best food grade solvent tested for the UAE of CRS. Milling can improve the extractions probably due to the breakage of the cell wall.

Acknowledgments

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Applications:
Biotechnology
and
Analytical Methods

Characterization studies of provitamin A carotenoid β cryptoxanthin encapsulated liposomes

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Provitamin A carotenoid (β cryptoxanthin/ βcrx) was extracted from bacteria *Kocuria marina* DAGII cultivated on an optimized lactose media by using the procedure followed by [1]. βcrx was encapsulated in the liposome using soy-lecithin and tween80 (1:0.72 mass ratio). The encapsulation efficiency was calculated along with some characterization studies: particle size, zeta potential, FTIR analysis, and FESEM. Initial concentration 1% showed the highest encapsulation efficiency i.e., 99.82%, and other characterization studies also showed satisfactory results.

βcrx-producing bacteria were cultured for 56 hours (Fig.1A). The solvent extraction method was applied (methanol, and petroleum ether) to break the bacterial cells, βcrx was extracted and concentration was measured at 445 nm (Fig.1B). For liposome preparation, the Thin Film evaporation method was used [2]. βcrx was dissolved in chloroform, soy lecithin, and tween80. The initial concentration ($m_{\beta crx}/m_{lipids}$%w/w) of βcrx was selected at 1%,1.5%, and 2% respectively. A rotary vacuum evaporator produced the thin film and further, hydration media (0.01M phosphate buffer solution) was added. Probe sonication was done for 10mins (10secs on and 5secs off) and the liposomal solutions were kept at 4ºC temperature (Fig.1C).

Fig.1. βcrx encapsulated liposome formation from *Kocuria maria* DAGII culture

The particle size and zeta potential were 106.6nm and -34.2mv were recorded by dynamic light scattering. In FESEM, 25,0000x resolution showed a rough granular surface on liposome acted as resorption sites for encapsulation in the free liposome (Fig.2A). Whereas the same resolution showed shadows of the inner circular zone depicting entrapped particles in βcrx-encapsulated liposomes (Fig.2B).

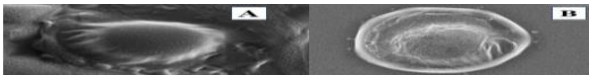

Fig.2. FESEM analysis at 25,000X resolution

In FTIR analysis, the presence of alcohol was observed at 3396.5cm^{-1} with other compounds in the encapsulated liposome. Which was absent in the free liposome. The absorption spectrum was similar with free liposome but encapsulation had only occurred due to O-H stretching because of the alcohol.

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CAROTENOID STABILITY OF MARIGOLD FLOWERS (*Tagetes erecta* L.) DURING STORAGE IN DIFFERENT CONDITIONS

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Introduction: Marigold (*Tagetes erecta* L.) is an annual medicinal plant of the *Asteraceae* family, widely known as a rich natural source of carotenoids contained in its orange and yellow flowers. Marigold flowers, rich in lutein and zeaxanthin, are used in the food, pharmaceutical, and cosmetic industries, as well as a feed additive in laying hen diet to achieve the desired egg yolk color. Different postharvest processes and storage conditions (temperature, light, oxidation, acidity, and moisture) can significantly alter plants' qualitative and quantitative carotenoid composition and, consequently, their bioavailability in food and feed [1,2]. The aim of this study was to investigate the effect of different storage conditions on the content and degradation rate of lutein (L) and total carotenoids (TC) in marigold flowers.

Methods and Materials: After collection, flowers were dried, and marigold meals were obtained by milling the whole flowers (A) or only the petals (B). The prepared meals were stored for 11 weeks under different conditions: 4 °C, 25 °C in the light and the dark, and 40 °C. Content of L and TC was determined using the RP HPLC method in samples taken once a week. The degradation of carotenoids was described by first-order kinetics, and the degradation rate (k) of L and TC was estimated. Statistical analysis was performed to evaluate the effects of meal preparation and storage conditions on the content and degradation rate of L and TC.

Results: Marigold meals differed in L and TC content ($P<0.05$), and their contents in the prepared meals were 5.88 and 13.63 mg/g DM in meal A and 4.91 and 10.353 mg/g DM in meal B, respectively. Storage conditions affected L and TC content in both meal types, and mean values were lowest in meals stored at 40 °C compared to other conditions (4.82 vs. 4.24 and 10.05 vs. 9.09 mg/g DM, respectively). Average degradation was 28% for meals stored at 4 °C and 25 °C in the dark, 33% at 25% in the light, and 50% at 40 °C. The degradation rate of both L and TC was influenced by meal preparation and storage conditions ($P<0.001$; Fig. 1).

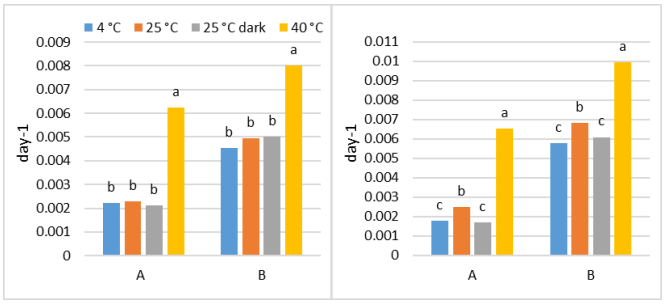

Fig. 1. The degradation rate of lutein (left) and total carotenoids (right) during storage of marigold flowers in different conditions.

Conclusions/Discussion: Milling only the petals resulted in a higher carotenoid content than milling the whole flowers due to the dilution effect of the green parts with lower carotenoid content. However, the meal prepared from whole flowers had lower degradation rates of both L and TC than the meal prepared from petals only. The optimal conditions for storing marigold meals were 4 °C or 25 °C in the dark, as light or elevated temperatures contribute to the carotenoid degradation. The similarity in the carotenoid degradation rates of meals stored at 4 °C or 25 °C in the dark was unexpected, and the results obtained suggest that exposure to oxygen is the most important factor in the stability of marigold flower carotenoids.

Acknowledgements
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Impact of High Pressure Processing on Extractability and Distribution of Bioactive Plant Ingredients in Kale

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1. Introduction

High pressure processing (HPP) is a non-thermal food preservation technique with minimal impact on food characteristics [1]. With regard to common pressure parameters up to 600 MPa, the impact of reduced pressure applications (< 100 MPa) on bioactive plant ingredients is less investigated. Since HPP was described as potential elicitor of plant secondary metabolic pathways at low pressure rates, the investigation of pressurized kale may contribute to further insights related to an impact on the content of carotenoids and vitamin E [2].

2. Experimental

A knife mill (Retsch Grindomix GM200) was used to crush kale leaves. HPP was performed at room temperature applying different pressures (10 MPa - 600 MPa) for 5 min - 40 min. After an extraction with MeOH/THF (50:50, *v/v*) with 0.1% of butylated hydroxytoluene, NP- and RP-HPLC with fluorescence and diode array detection were used to determine concentrations of vitamin E and carotenoids. Furthermore, light microscopy was used to evaluate the impact of HPP on cellular integrity (Figure 1).

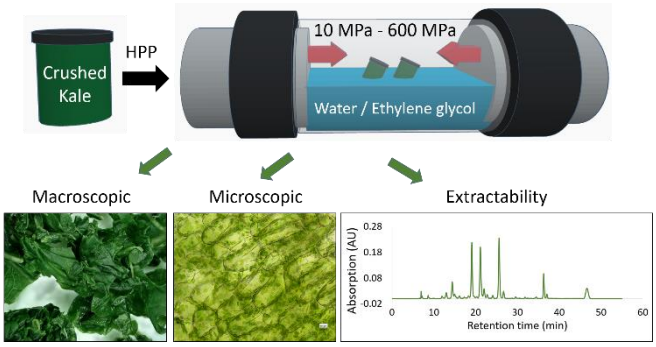

Figure 1: Crushed kale leaves were treated with HPP. Extraction and HPLC analysis and light microscopy were used to investigate impacts of HPP on bioactive plant ingredients and cell integrity.

3. Results and Discussion

Overall, 11 compounds were identified in kale including carotenoids, α-tocopherol and chlorophylls [3]. A significant reduction ($p \leq 0.05$, one-way ANOVA, Tukey-HSD post hoc) of total carotenoid content was only observed after treatments at 100 MPa (5 min, 10 min) as well as at 200 - 600 MPa (5 min). Concentrations of (*13Z*)- and (*15Z*)-β-carotene increased significantly after a 40 min treatment at 50 MPa. Surprisingly, increased concentrations of vitamin E by up to 105 % were determined after processing with pressure rates less than 100 MPa for 40 min. The formation and size dependence of carotenoid lipid globules were observed related to sample pre-treatment and different pressure parameters.

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PERCEPTION OF ADEQUACY OF FRUIT AND VEGETABLE INTAKE AND ASSOCIATED SKIN CAROTENOID STATUS AMONGST ADOLESCENTS

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Across all age groups in the US, adolescents have the poorest diet quality (DQ), partially due to inadequate intake of fruits and vegetables (FV). FV intake is vital as they may mitigate the risk of chronic diseases and early mortality. Improving FV intake may be accomplished using the Transtheoretical Model's (TTM) stages of change pillars, which places individuals into 1 of 7 stages based on their perception of adequacy of intake and estimated intake. However, perception of adequacy of intake and estimated intake may not align. Also, self-reported estimates of FV intake may not be accurate, further tampering with accurate stage placement. The objective of this study was to determine whether an indirect but objective measure of FV intake, skin carotenoid status (SCS), supports self-reported FV stage placement.

Data were collected from 4 high schools in central Florida in 2 waves (spring and fall 2021). Researchers cross sectionally examined the perception of adequacy of FV intake of adolescents using the TTM and linked their perception to estimated intake. Estimated intake was measured using self-reported FV intake from a food frequency questionnaire, the short Healthy Eating Index (sHEI), and SCS was measured thrice on the index finger via resonance Raman spectroscopy by a Veggie Meter™ machine. Researchers administered surveys to collect demographic traits. Based on perception, participants were put into 1 of 3 groups: did not perceive FV intake as adequate, perceived *either* fruit or vegetable intake as adequate, or perceived both FV as adequate. An ANOVA compared differences in mean (±SE) reported intake and SCS of groups.

Of the 311 adolescents included in this study, most were female (51%), white (57%), and aged 15 years (26%). The group that did not perceive their FV intake as adequate reported consuming less FV (2.0±0.26 C/day, $p<0.0001$) than the group that perceived *either* their fruit or vegetable intake as adequate (3.7±0.26 C/day). The group that perceived *either* their fruit or vegetable intake as adequate reported consuming less FV (3.7±0.29 C/day) than the group that perceived their FV as adequate (4.6±0.29 C/day, $p=0.003$). SCS was only different between groups that perceived intake of neither (203±10.52) or both (235±10.52) FV as adequate ($p=0.007$), and the group that perceived their intake of *either* fruits or vegetables (213±9.33) as adequate and the group that perceived their intake of FV as adequate ($p=0.046$).

SCS aligned with perception of adequacy of FV intake less frequently than reported FV intake. In general, SCS results supported reported FV intake; however, more research is needed to see if adolescents accurately report FV intake, since SCS varied by only 32 points at most between groups.

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Separately Mapping Zeaxanthin and *meso*-Zeaxanthin in the Human Fovea with Polarized Resonance Raman Microscopy

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Introduction: Zeaxanthin and *meso*-zeaxanthin are the dominant carotenoids in the human fovea. These two carotenoids are stereoisomers, sharing the same molecular formula but differing from each other only in the orientation of the hydroxyl group at the 3' position on one of the β-ionone rings. Conventional resonance Raman microscopy cannot distinguish zeaxanthin from *m*eso-zeaxanthin. Here, we investigated whether zeaxanthin and *meso*-zeaxanthin can be separately measured using polarized resonance Raman microscopy.

Methods and Materials: Our XploRA Plus confocal resonance Raman microscope is equipped with three polarized laser modes (vertical, horizontal, and circular) and three polarized Raman detection modes (vertical, horizontal, and non-polarized), generating nine polarized test conditions. To determine the optimal condition for the separation of zeaxanthin and *meso*-zeaxanthin, 100 µM zeaxanthin and 100 µM *meso*-zeaxanthin solutions in methanol were mixed with ratios of 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, and 0:1, respectively, and tested at nine polarized conditions with excitation of a 473 nm laser. Using the optimized conditions, we further measured the carotenoid distributions in the fovea of an 87-year-old female donor eye. A total zeaxanthin map was created by mapping the intensity of the V1 peak at 1528 cm^{-1}. Separate maps of zeaxanthin and *meso*-zeaxanthin were generated by LabSpec6 software's Classical Least Squares (CLS) fitting algorithm.

Results: The compositions of zeaxanthin and *meso*-zeaxanthin in the mixed carotenoid solutions measured under two polarized conditions--horizontal laser vs vertical Raman (H-V), and circular laser vs vertical Raman (C-V)--were significantly correlated with the known ratios of the mixed carotenoid solutions. The Raman signals of carotenoids under C-V condition were stronger than the H-V condition, serving as the optimized condition to measure carotenoids in the human fovea. The retinal distribution of total zeaxanthins exhibits a circle with a dot at its center. Further analysis revealed that zeaxanthin was localized mainly at the central dot area, while the *meso*-zeaxanthin was allocated on the circle.

Conclusion: This demonstrates that zeaxanthin and *meso*-zeaxanthin can be separately measured under polarized Raman conditions, which might offer new knowledge to understand the physiological role of macular carotenoids in the prevention of retinal disease.

Acknowledgments: This work was supported by the BrightFocus Foundation M201606, by NIH grants EY-11600, EY-14800, and by unrestricted departmental funds from Research to Prevent Blindness.

Carotenoid Biosynthesis & Biotechnology

MICROTOM TANGERINE TOMATO *CRTISO* MUTANT ROOTS SHOW INCREASED ACCUMULATION OF ACYCLIC CAROTENOIDS, LESS ABSCISIC ACID, DROUGHT SENSITIVITY, AND IMPAIRED ENDOMYCORRHIZAL COLONISATION

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Introduction: Heirloom golden tomato fruit varieties are highly nutritious due to the accumulation of tetra-*cis*-lycopene, which has a higher bioavailability with recognised health benefits in treating anti-inflammatory diseases compared to all-*trans*-lycopene isomers found in red tomatoes [1]. Loss-of-function in CAROTENOID ISOMERASE (CRTISO) rate-limits the isomerisation of tetra-*cis*-lycopene (prolycopene) into all-*trans* lycopene, leading to the accumulation of acyclic *cis*-carotenes in fruits from the MicroTom *tangerine* (*tang*^{mic}) variety [2]. Light can mediate the -*cis* to -*trans* switch in the presence of a photosensitiser, allowing photoisomerisation to proceed in photosynthetic leaf tissues [3]. We investigated if photoisomerisation of tetra-*cis*-lycopene can occur in the roots of *tang*^{mic} and correlated carotenoid profiles with mycorrhizal colonisation, abscisic acid (ABA) levels, and whole plant responses to drought stress in glasshouse grown plants.

Material and Methods: The research plan was first to quantify agronomical traits and carotenoid accumulation in leaves and roots of golden *tang*^{mic} and red WT tomato fruit varieties. Next, we quantified endogenous ABA levels in roots tissues. We inoculated the rhizosphere with spores of the ubiquitous *Rhizophagus irregularis,* after which root segments were stained to observe the presence of arbuscules and other mycorrhizal structures. Finally, both varieties were subject to drought stress, and the maximum efficiency of photosystem II (F_v/F_m) and relative water content were assessed to determine if carotenoid isomerisation rate-limited above ground physiological traits.

Results: *tang*^{mic} tomato plants grown in soil under glasshouse conditions displayed a reduction in height, number of flowers, fruit yield, and root length compared to wild-type (WT). Soil inoculation with *Rhizophagus irregularis* revealed fewer arbuscules and other fungal structures in the endodermal cells of roots in *tang*^{mic} tomato relative to WT. The roots of *tang*^{mic} tomato hyperaccumulated acyclic *cis*-carotenes and lacked xanthophylls required for ABA biosynthesis. In response to a water deficit, leaves from the *tang*^{mic} tomato plants displayed a rapid decline in the F_v/F_m compared to WT, indicating a defective root to shoot signalling response to the onset of drought.

Discussion/Conclusion: Photoisomerisation of tetra-*cis*-lycopene can be rate-limited in root tissues depending upon the transmission of light required to generate a chlorophyll-derived photosensitiser. The lack of xanthophylls biosynthesis in *tang*^{mic} tomato roots blocked ABA biosynthesis, which we attribute to impaired endomycorrhiza colonisation and insensitivity to the early perception of drought stress [4].

Acknowledgements: We thank Mahfuza Pervin for helping to generate phenotypic measurements. We thank Meena Mikhael from Western Sydney University (WSU) for assistance with the hormone analyses at the WSU Mass Spectrometry Facility.

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

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Xanthophylls are vital to plants by forming photosynthetic complexes in leaves and conferring attractive colors to the reproductive organs, flowers and fruits. They also have unique health and wellbeing benefits to humans, especially β-cryptoxanthin, lutein, and zeaxanthin. Therefore, there has been much effort to develop crops with elevated content of these xanthophylls in the human diet.

Despite the progress made in understanding carotenoid biosynthesis and sequestration, there remains the need to elucidate the molecular mechanisms underlying carotenoid hydroxylation and xanthophyll sequestration and storage. In pursuit of this goal, we have developed strategies, using both classical genetic and genetic engineering to produce valuable xanthophylls in tomato fruit through increasing their biosynthesis and elevating their accumulation in chromoplasts. We have developed a non-transgenic tomato line, named 'Xantomato', whose fruit accumulate non-esterified zeaxanthin at concentration of >50 μg/g fresh weight, which comprised ca. 50 percent of total fruit carotenoids[1]. It has been demonstrated that the esterification of carotenoids is associated with higher accumulation and stability in chromoplasts. The acyltransferase enzyme PALE YELLOW PETAL 1 (PYP1) of tomato has been implicated in accumulating of a high concentration of xanthophyll esters[2]. We have created transgenic tomato lines of the variety M82 expressing the flower-specific SlPYP1 in fruits. Although the overall carotenoid content of transgenic fruit was substantially higher than in the control M82, no xanthophyll esters were observed because wild type tomato fruit mainly accumulates carotenes. The effects of PYP1 are now being tested in zeaxanthin-accumulating tomato lines.

Acknowledgement: This research is supported by the Israel Science Foundation Grant No. 1930/18

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Metabolic engineering for crocin production in different plant, yeast and microalgal platforms

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Crocins are glycosylated apocarotenoid pigments synthesized by few plant taxa (Crocus, Buddleja, Gardenia). They have multiple roles in the prevention of chronic disease and their chemical synthesis is not possible, due to the abundance of chiral centers. We have characterized the crocin biosynthetic pathways in *Crocus sativus* and *Gardenia jasminoides*. In *Crocus*, their synthesis proceeds through a cleavage step by a CCD2, followed by dehydrogenation by an ER-localized ALDH7, glycosylation by one or more cytoplasmic UGTs, and vacuolar transport by ABCC-type transporters. In contrast to *Crocus*, the initial cleavage step leading to crocetin dialdehyde is mediated by CCD4 enzymes in *Gardenia* and *Bixa*.
We are using a panoply of biosynthetic enzymes with different substrate specificities and transporters, and different expression strategies (such as nuclear and plastid stable transformation, *Agrobacterium*- and virus-mediated transient expression) to achieve the production of large amounts of crocins in different platforms. Up to now, we achieved the highest levels in tomato fruits (>100 µg/g DW), with a glycosylation pattern comparable to *Crocus sativus* stigmas). Additional work, including optimization of precursor levels and subcellular sequestration, is under way to further improve the levels of these high value compounds in sustainable, edible products.

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CONSTRUCTION OF A MODULAR EXPRESSION SYSTEM FOR HIGH-LEVEL PRODUCTION OF CAROTENOIDS IN THE PURPLE BACTERIUM, *RHODOSPIRILLUM RUBRUM*

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Introduction: The gram-negative, non-sulphur purple bacterium, *Rhodospirillum rubrum*, is an excellent host for the high-level production of carotenoids, since, as a photosynthetic organism, it is a natural overproducer. In addition, the so far unique ability to express carotenoid-containing photosynthetic membranes in the dark (using a special medium [1]) at levels normally only observed under photosynthetic conditions is particularly attractive for large-scale production. The promoters for the genes for the carotenoid biosynthesis enzymes are regulated by the oxygen partial pressure and the reduction potential of the electron carriers in the photosynthetic membranes. Here, we demonstrate the potential of the promoter region for the *crtD* gene, encoding rhodopin desaturase, for producing high (photosynthetic) levels of carotenoids under dark, semi-aerobic conditions. The *crtD* promoter has been incorporated into an IncP1α-vector as a modular element for use in synthetic biology.

Methods and Materials: A plasmid, pRKCAG53, which was derived from the IncP1α-vector pRK404, was constructed to incorporate the *crtD* gene and flanking regions. The plasmid and *crtD* elements were re-designed to incorporate modular elements in a similar way to those employed in the BioBricks® project, but codon usage-modified to be compatible with the *R. rubrum* background. pRKCAG53 and its derivatives were introduced into *R. rubrum* by triparental conjugation, and selected by the appropriate antibiotic resistance.

Results: We showed that pRKCAG53 can be used to complement the *crtD* deletion mutant, ST4 [2], by growing small cell cultures (100 ml) semi-aerobically in the dark, and extracting and determining the carotenoids by spectroscopy and HPLC-MS analysis [3]. Several modifications of the promoter were performed to delineate the minimal length required for achieving high-level carotenoid expression.

Conclusions/Discussion: pRKCAG53 is a modularly designed expression plasmid which allows the expression of high levels of carotenoids in *R. rubrum*. The *crtD* gene has also been re-designed to facilitate gene fusion with other carotenoid genes from other sources.

Acknowledgements:

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ENGINEERING HIGH LEVELS OF SAFFRON APOCAROTENOIDS IN TOMATO

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Introduction: The apocarotenoids from saffron are high-value compounds used in the food and pharmaceutical industries. Saffron production is very labor-intensive as well as non-sustainable as it is highly dependent on environmental conditions, leading to high costs and restricting the utilization of saffron by other industrial sectors. The saffron apocarotenoids are derived from the cleavage of zeaxanthin at 7,8:7',8' positions, in a reaction catalyzed by a specific carotenoid cleavage dioxygenase enzyme (CsCCD2L). As result of this cleavage reaction, two molecules of 6,6-trimethyl-4-hydroxy-1-carboxaldehyde-1 cyclohexene (HTCC) and one molecule of crocetin are produced (Fig. 1). These compounds acquired water-solubility by glucose molecules added to their skeletons by the action of glucosyltransferases; CsUGT2, which added glucose to the crocetin molecule, and UGT709G1 that is involved in the glucosylation of HTCC producing picrocrocin (Fig. 1). With the advances of metabolic engineering and its progression into synthetic biology, it is now possible the low-cost production of rare metabolites, such as saffron apocarotenoids in other host. Tomato (*Solanum lycopersicum*) is a popular and highly consumed food worldwide, being used as a model crop for biotechnological applications. In this study, tomato was selected for the introduction of the saffron pathway because the fruit accumulates high levels of carotenoids as substrates, and there are well established downstream processes, including concentrates and juices for human consumption. The apocarotenoids of saffron might provide the characteristic flavor and aroma of the saffron spice, and their solubility will enhance the nutraceutical properties of tomato juice.

Research & Methods: The initial plan of the research was to introduce combinations of fruit-specific promotors with the apocarotenogenic genes from saffron in tomato. Characterization of the apocarotenoid composition of the obtained tomatoes will be described.

Results and Discussion

By using a combinatorial approach in which CsCCD2L was introduced with CsUGT2 and CsUGT709 under the control of different promoters, a maximum crocins accumulation in line O1 11 up to 14.48 mg/g DW was reached, which has been the highest level reported for saffron apocarotenoids in heterologous systems as a result of transient or stable expression. Additionally, apocarotenoid-enriched tomatoes showed high antioxidant activity and exerted high effectiveness in Alzheimer's disease reduction, as revealed by using a *Caenorhabditis elegans* model.
Overall, this study demonstrates the potential and feasibility of using tomatoes as a biotechnological platform for the simultaneous production of pro-nutritional lipophilic and rare high-valuable hydrophilic apocarotenoid compounds that are beneficial for both industry and human health.

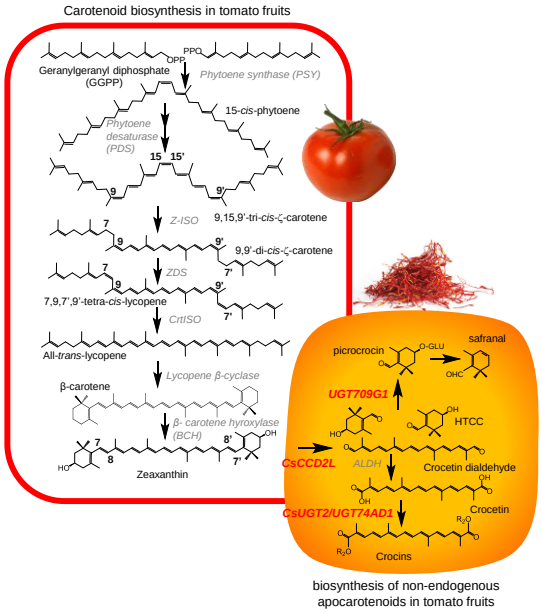

Fig. 1. Representative scheme of the carotenoid pathways in tomato and the crocins and picrocrocin pathways from saffron that were introduced into the tomato fruit. Introduced enzymes are highlighted in red. Z-ISO, ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotene isomerase; ALDH, aldehyde dehydrogenase.

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Establishment of Yeast-based Microbial Cell Factory for Efficient Biosynthesis of Violaxanthin

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Violaxanthin, a plant-derived epoxidated carotenoid, not only exhibits broad spectrum of bioactivities, but also functions as a key precursor of valuable phytochemicals, such as abscisic acid. However, insufficient supply from natural resources and traditional production methods hindered its wide applications for commercial and research purposes. Recently, much interest focuses on utilizing microbial cell factories for heterologous biosynthesis of natural carotenoids[1].

Here, we engineered *Saccharomyces cerevisiae*, a well-studied model microbial host, to develop an efficient violaxanthin production platform. To functionally reconstitute the bioproduction of violaxanthin in yeast, we first introduced a pair of previously identified β-carotene hydroxylases (BCH1 and BCH2) and zeaxanthin epoxidase (ZEP) from *Arabidopsis thaliana* into a β-carotene producing yeast strain SYL23 (Fig.1a). Although violaxanthin was successfully detected, there was still much intermediate zeaxanthin accumulated due to the low enzymatic activity. We then employed several engineering strategies to optimize the violaxanthin production. Enzyme modifications, such as lipid bodies compartmentalization and N-terminal truncation of chloroplast transit peptides, were first applied to AtBCH1/2 and AtZEP, which resulted in improved conversion of these plastid-localizing enzymes expressed in yeast. We also demonstrated that co-expression of the plant ZEP redox partners (FD3/RFNR1)[2] could largely boost the violaxanthin yield. The overall performance of the engineered yeast strain was further enhanced by increasing the expression level of the modified AtZEP and up regulating the production of upstream isoprenoid precursors. We were able to establish a violaxanthin-producing yeast strain at a titer of 1.98 mg/L in shaking flask (Fig.1b).

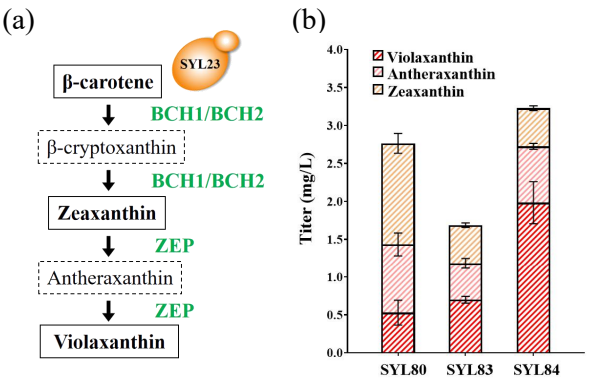

Fig1.(a) Overview of violaxanthin biosynthetic pathway in engineered *S. cerevisiae*; (b) Carotenoids titers in different engineered yeast strains.

The establishment of the microbial violaxanthin producing platform set the foundation for discovery and biosynthesis of other less explored carotenoids.

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Photophysics and Chemistry

Effect of non-conjugated functional group to the optical properties of carbonyl carotenoid.

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Introduction: Siphonaxanthin (Sx) contains an asymmetric carbonyl group attached to the conjugated chain, which induce the intramolecular charge transfer (ICT) characters strongly coupled to the singlet excited states, resulting in efficient energy transfer in photosynthetic systems. Recently we discovered a 19-deoxy Sx (dS) that is biosynthetic precursor of Sx and accumulates only when the green algae are exposed to intense illumination. Since the chemical structures of dS, Sx and its ester siphonein (Sn) are identical in the conjugation system, they were expected to be energetically identical. However, Sn has been reported to exhibit stronger ICT properties than Sx, suggesting the non-conjugated functional groups may affect the electronic structure (1). In this study, we compared the spectroscopic properties of dS with those of Sx and Sn and discussed the effect of non-conjugated groups on the excited-state properties.

Materials and Methods: Stereoselective synthesized Sx and dS were used after further purification by HPLC. Isolated Sn from *C. fragile* was purified by HPLC. The purity was evaluated by HPLC to be >99%. Each sample was dissolved in 16 polar solvents and 6 non-polar solvents, and the absorption spectra were measured at room temperature. Peak maxima were determined by the second derivative and vibrational structure was reproduced by three Gaussians, and ICT band was extracted as difference from the observed value.

Results & Discussion: In polar solvents, the asymmetric broadening at lower energy region was observed in the order, Sn>Sx>dS. This indicates that the 19-hydroxymethyl group contributes somewhat to the induction of ICT properties. In non-polar solvents, the absorption spectrum of dS was shifted significantly to the short wavelength side than that of Sx and Sn. In other words, the effective conjugation length of dS was shown to be shorter than that of Sx and Sn. This may be because the rotation of the carbonyl group to the conjugated plane is not prevented in dS.

Acknowledgement

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MODELING EXCITED STATES AND RAMAN SPECTRA FOR CAROTENOID AND THEIR COMPLEXES

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Introduction: Carotenoids (Cars) have linear conjugated isoprenoid chain affords them an intense absorption in the blue-green range, and the colours they confer on fruits, flowers and animals are at the basis of complex signaling processes. In photosynthetic organisms, they are implicated in the harvesting of solar photons while natural carotenoids display a large structural diversity, and more than 1100 molecular species have been now identified. There are more than several photophysics models of Cars: three-state model involving $S_2(1^1B_u^+)$, $S_1(2^1A_g^-)$ and $S_0(1^1A_g^-)$; four-state model involving $S_2(1^1B_u^+)$, $1^1B_u^-$, $S_1(2^1A_g^-)$ and $S_0(1^1A_g^-)$; additional four-state model involving $S_2(1^1B_u^+)$, $S_y(nA_g^+)$, $S_1(2^1A_g^-)$ and $S_0(1^1A_g^-)$; and others. They all are used to interpret the excited state dynamics of Cars with Chls, containing Q_y and Q_x states, and this shows that it is more complicated than it can be expected. Additional states for the red absorption shift is proposed to arise from an intramolecular charge transfer (ICT) character of the second excited state, generated by the electron-rich keto group also [1-3]. Large scale computations provide more detailed information which allow better understand carotenoid properties which can be expected in real life biological systems.

Research & Methods: The Car was chosen vaucheriaxanthin, fucoxanthin, lutein, beta-carotene and diadinoxanthin. All structures were optimized separately. Careful orbital analysis allowed us to label each calculated excited states. We chose the B3LYP and CAM-B3LYP functionals with cc-pVDZ basis sets for the present study which are available in the Gaussian package. Study was done using a combination of Raman and absorption spectroscopy and density functional theory (DFT) modelling, including Car–Parrinello molecular dynamics (CPMD) simulations as it is implemented in NwChem package together with PACKMOL software.

Results and Discussion: The Cars specific bond were analysided by suing energetic surface scan methodology by fixing all the other atoms. This methodology allows to identify additional local minima as possible conformers (Fig. 1). Using such unstable structures, they were studyed by changing local environments such as with water models (Fig. 2). The stabilized new structures provide new properties in excited states or Raman activities (Fig. 3) in various Cars. In order to find stability of investigated structures the CPMD simulations are performed. Additional results with Fx suggest that due to fluctuations and interactions with the environment the new conformers can appear what can participate in explaining the S_{CT} states in Cars.

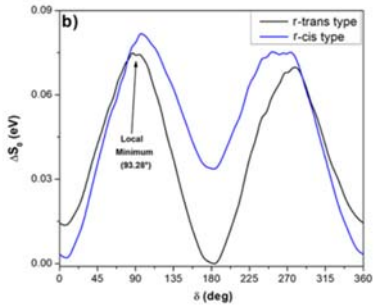

Fig.1. Energetic surface scan methodology for diadinoxanthin [1]

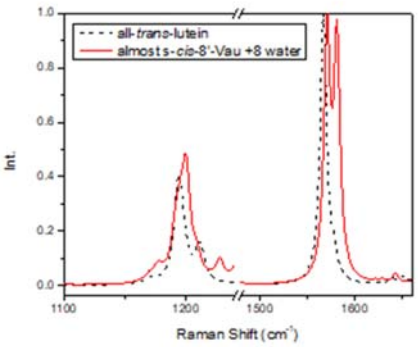

Fig.2. Vaucheriaxanthin: Arrows indicate the two major C=C bond stretching vibrations, $\nu_{1\text{-}1}$ and $\nu_{1\text{-}2}$.[3]

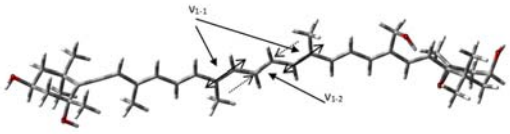

Fig.3. Vaucheriaxanthin and lutein Raman activity model [3]

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CORONA AND **C**AROTENOIDS – MORE IN COMMON THAN INITIAL LETTER C?

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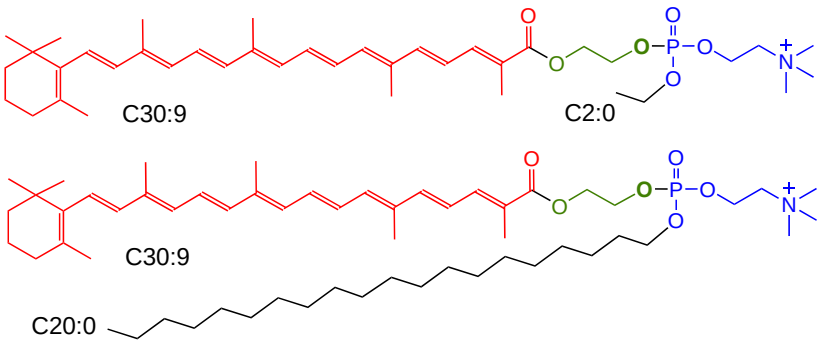
Introduction: Native nucleic acids (NA) are continually released within our cells. However, the introduction of foreign NA (DNA, mRNA, siRNA) into cells (transfection) is impeded by many barriers. The cell wall is an obvious obstacle. Most transfections rely on transporters to deliver NA to cells. Safe viruses, analogous to "small vans"**,** can be loaded with only a limited NA cargo. Viruses are now extensively used as mRNA carriers (e.g. Johnson-Johnson, Astra Zeneca, Sputnik V). Introduction of larger NA loads into cells calls for "heavy good vehicles", which are assembled by chemists. Chemical transfection is based on packing NA onto cationic or ionizable lipids. These lipids aggregate in water to nm- and µm-liposomes. The NA charged liposome (lipoplex) enters the cell (lipofection); in the cytosol the lipoplex is discharged and NA moves to the cellular compartments: DNA goes into the nucleus replacing a deleterious gene, mRNA causes the ribosomes to generate proteins (actually, a specific mRNA initiates the ribosomes to release the spike protein of COVID), siRNAs silence or knock out native mRNA generating disease-causing proteins. Two of the NA lipid-carriers are now produced in copious amounts (BiontechPfizer, Moderna). Lipofection can rely on a vast number of compounds. A review article (2021) presents the structures of 526 lipids, of which 5 contain carotenoids [1]. Other carotenoid lipids have been added [2]. The combination of rigid and flexible chains and the color of the lipids could confer potential advantages for transfection. Lipids packed with DNA and siRNA were investigated *in vitro* and *in vivo* for efficiency, cytotoxicity, liposome and lipoplex size, and structure activity relationship (SAR). Carotenoid-selena-Au-nano-particles have been synthesized for size-controlled formation of liposomes. [3]. C30:9 carotenoic acid, besides being associated with NA-transfection, has antiviral activities [4].
Results: Transfections with carotenoid lipids were comparable to those with reference lipids. The carotenoid lipid with the longest rigid polyene and longest flexible alkyl chain revealed minimal cell toxicity. Double chain lipids were found to be more active than single chain lipids. Lengthening of the saturated chain enhanced the efficiency. Transfection in eye cells improved with a short polyene and a long alkyl chain. Simple or no technological assistance located the transfections. SAR was not found. Liposomes of selected dimensions were obtained with carotenoidlipid-Au nanoparticles.
Conclusion: Carotenoid lipids efficiently transfected NA (DNA, siRNA) into cells. The color of the lipids allowed tracking NA delivery by direct observation. Charged with spike mRNA, **C**arotenoid lipids with C30:9 fatty acid could, in principle, be applied to combat **C**orona. In addition, C30:9 **c**arotenoic acid acts effectively as an antiviral drug against **C**orona [4].



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Stereocontrolled synthesis of lipofuscin pigment A2E, a pyridinium bisapocarotenoid

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Diseases affecting ophthalmological health have a great impact on society and have been the focus of scientific activity in different fields during the 20th and 21st centuries. Among them, age-related macular degeneration stands out for its incidence, a pathology classified by the World Health Organization (WHO) as the third leading cause of blindness in the world, for which there is no effective treatment.

Recent studies have shown that this disease is related to the inhibition of the visual cycle, due to the accumulation of lipofuscin pigments in the retinal pigment epithelium. The lipofuscin pigments that have been isolated and identified include A2E, a pyridinium bisapocarotenoid, and two of its isomers, *iso*-A2E and *iiso*-A2E, which have a major influence on the onset of visual degeneration. However, their direct role in the disease is not really known, so their biological properties, as well as their effects on the retina, are being studied and need to be clarified in order to develop new therapies. Thus, it is essential to have simple and efficient mechanisms that allow the preparation of sufficient quantities of these pigments to facilitate chemical and biological studies for a better understanding of this disease.[1,2]

This work focuses on the stereocontrolled synthesis of the lipofuscin pigment A2E using modern organic synthesis tools (Figure 1).

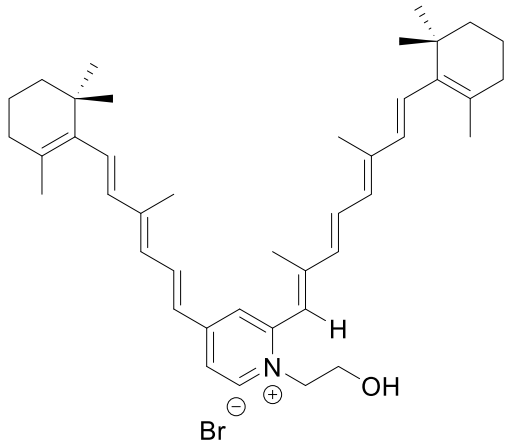

Figure 1. Structure of lipofuscin pigment A2E.

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Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism

The protective effect of carotenoids, polyphenols and sex hormones on skin cells under oxidative stress

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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 which occurs during ageing. ROS activate the nuclear factor-kappa B (NFκB) transcription systems leading to inflammatory processes and increased production of matrix metalloproteinase (MMPs) by skin cells, which leads to collagen degradation. Several studies have shown the protective role of estrogens and of various phytonutrients including carotenoids and polyphenols on skin health. The aim of the current study was to examine the damage caused by ROS that originate in the mitochondria due to its dysfunction, or by H_2O_2, and to examine the protective role of lycopene, rosemary extract and estradiol. Human dermal fibroblasts and keratinocyte were used to determine ROS levels and their effect on cell viability, MMP1 and pro-collagen secretion as markers of skin damage. Rotenone was used to cause mitochondrial disfunction which leads to ROS production, cell death, upregulation of MMP1 secretion and decreased collagen secretion. This was accompanied by activation of the antioxidant response element/Nrf2 (ARE/Nrf2) and NFκB transcriptional activity. Pretreatment with dietary compounds such as tomato extract containing lycopene and rosemary extract and estradiol reduced ROS level, and MMP1 secretion and increased cell viability and pro-collagen secretion. These effects can be partially explained by the increased activity of the ARE/Nrf2, and the decreased activity of NFκB transcriptional activities. This study indicates that carotenoids and sex hormones protect skin cells from ROS-induced damage and may improve skin health and appearance.

Dietary beta-carotene supplementation to *Isx*$^{-/-}$ dams during late gestation and lactation impacts offspring responses to a high-fat diet in adulthood

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Introduction: Perinatal nutrition impacts future metabolic health and susceptibility to obesity. Vitamin A, mainly as retinoic acid, has anti-obesity effects in adult rodents [1]. In seeming paradox, oral supplementation of a moderate excess vitamin A directly to rat pups throughout the suckling period led to greater subsequent diet-induced adiposity gain [2]. We here aimed to get further insight into the activity of vitamin A in imprinting complex metabolic phenotypes. Intestine-specific homeobox (ISX)-knockout mice lack the transcriptional repressor of intestinal beta-carotene (BC) absorption and conversion to retinoids [3]. We used *Isx*$^{-/-}$ mice as a model to increase maternal vitamin A supply to the newborn.

Methods: We compared the *Isx*$^{+/-}$ offspring of *Isx*$^{-/-}$ dams fed a vitamin A sufficient (4000 IU/kg diet) diet or a BC-enriched diet (25 mg BC/kg diet) during the last week of gestation and the entire lactation period (from here on, VAS and BC offspring). A subset of VAS and BC offspring was harvested at weaning (postnatal day 21), and a second subset at adult age, following 13 weeks of feeding a normal fat diet (NFD, 10% energy as fat) or a high fat diet (HFD, 45% energy as fat) with regular vitamin A content. The study was conducted in female and male animals.

Results: Hepatic retinyl ester and retinol levels were higher in the BC offspring than the VAS offspring at weaning, as expected. HFD-induced increases in body weight, white adipose tissue (WAT) mass, WAT adipocyte area, and adiposity index were greater in male than in female animals, and were attenuated (males) or suppressed (females) in the BC offspring as compared with the VAS offspring. HFD-induced changes in inguinal WAT DNA content, when considered together with changes in adipocyte size and size distribution, suggested female sex and maternal BC supplementation favored adipocyte hyperplasia over hypertrophy on an HFD. On the NFD, however, adult BC offspring of both sexes had a higher proportion of enlarged adipocytes in inguinal WAT as compared with VAS offspring. HFD-induced increases in blood glucose and triglyceride levels were evidenced in the male animals only, and were blunted in the BC males.

Conclusions/Discussion: Results suggest that a good mother's vitamin A status and enhanced vitamin A supply through the milk may have protective effects against obesity development in the offspring.

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Antioxidant, antihyperglycemic and antihyperlipidemic activity of the haloarchaeal carotenoid bacterioruberin

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Introduction: Haloarchaea are extremophilic microorganisms that inhabit hypersaline ecosystems. The constant exposure to oxidants such as ionic strength and radiation has influenced the development of different coping mechanisms, including carotenoid production. The major carotenoids produced by haloarchaea are bacterioruberin (BR) and its derivatives. BR has been described as a potent antioxidant due to its structure consisting of a hydrocarbon chain with 50 carbon units, 13 conjugated double bonds and 4 hydroxyl groups. Nevertheless, there are few studies characterizing its antioxidant activity and exploring potential pharmaceutical and nutraceutical applications.

Research & Methods: This line of research explored the antioxidant capacity of carotenoids extracts obtained from *Haloferax mediterranei* R-4 cells grown under different nutritional conditions. To elaborate a complete antioxidant activity study a multitarget approach that used different tests (DPPH, ABTS, FRAP and β-carotene bleaching assays) was applied. Furthermore, their ability to inhibit α-glycosidase, α-amylase and lipase enzymes was assessed to determine if they could be used as an antihyperglycemic and antihyperlipidemic agents. All carotenoid extracts have been analyzed by HPLC-MS to find out if the differences observed regarding the properties are due to an alteration of the composition of the extracts.

Results & Discussion: This study shows how a variation in the type and concentration of carbon source in the cell culture can modify the antioxidant activity of the resulting carotenoid extract, probably due to a change in the carotenoid composition. More specifically, higher levels of glucose or starch in the culture media result in a stronger antioxidant, antiglycemic, and antilipidemic activity. The carotenoid extract which showed the lowest IC_{50} value for all tests was the one obtained from the cell culture containing 2.5% glucose.. These assays contribute to the characterization of these rare haloarchaeal carotenoids and give us a hint about their usefulness to reduce postprandial hyperglycemia and hyperlipidemia, which are considered risk factors for diabetes and obesity, respectively.

In conclusion, the concentration and type of carbon source influence the carotenoid production in *Haloferax mediterranei* and, in consequence, the properties of the carotenoid extracts obtained.

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Abstract

Tissue lycopene accumulation in transgenic mice lacking one or both carotenoid cleaving enzymes

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Introduction:

Lycopene is one of the most abundant carotenoids found in the body and with its antioxidant properties has led researchers to explore its potential as a therapeutic modality for various diseases. Its metabolic pathways are largely undefined, but studies indicate that both β-carotene oxygenase 1 (BCO1) and β-carotene oxygenase 2 (BCO2) are involved in the cleavage of lycopene [1][2]. This study aims to evaluate the impact of gender and ablation of β-carotene oxygenase 1 (BCO1), β-carotene oxygenase 2 (BCO2) or both on tissue accumulation in lycopene dosed transgenic mice. Previous work from our laboratories suggests that BCO2 appears to be the primary carotenoid cleavage enzyme for lycopene *in vivo*.

Methods and Materials:

Three-week old C57BL/6 male and female mice (wild type [WT], *Bco1*-/-, *Bco2*-/-, *Bco1*-/- X *Bco2*-/- double knock out [*DKO]*) were divided into groups based on genotype (n=8 per group/per gender) and fed a powdered AIN 93G control diet for 2 weeks. Then the mice were gavaged daily for 2 weeks with 1mg of lycopene dissolved in cottonseed oil. At the end of two weeks the mice were fasted overnight and sacrificed. Liver, serum, and extra-hepatic tissues were harvested. Tissues were preserved in liquid nitrogen and stored at -80 until analysis. Lycopene concentration was measured with high-performance liquid chromatography. Data analyses were performed using one-way ANOVA.

Results:

Except for serum, female mice had higher lycopene tissue accumulation, irrespective of genotype. On a concentration basis, liver, duodenum and adrenal lycopene were higher than other tissues. As expected, serum and tissues of DKO mice accumulated the highest lycopene. DKO mice had significantly higher lycopene than *Bco1*$^{-/-}$ mice in the liver ($p<.002$), heart ($p<.004$), adipose ($p<.03$), and the testes ($p<.004$). Compared to *Bco2*$^{-/-}$ mice, DKO mice had greater accumulation in the serum ($p<.001$), intestine ($p<.04$), heart ($P<.0001$), kidneys ($p<.0001$), adipose ($p<.04$), and testes ($p<.0001$). Liver ($p<.007$) and adrenal (ns) tissues in *Bco2*$^{-/-}$ mice had higher levels of lycopene than *Bco1*$^{-/-}$ mice whereas *Bco1*$^{-/-}$ mice had significantly higher levels in the kidneys ($p<.001$) and tended to have greater accumulation in other tissues.

Conclusions:

Accumulation of lycopene in tissues depended upon gender, genotype and tissue type. The abolition of both enzymes and mice of a female sex generated a higher accumulation. We are probing whether tissue-specific expression levels of BCO1, BCO2 or carotenoid transport proteins explain differential tissue accumulation across genotypes.

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β-Carotene Mitigates Liver Inflammation During Atherosclerosis Regression

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Introduction:
Atherosclerosis is characterized by the development of lipid-rich lesions within the arterial walls. These lesions are typically composed by atherogenic lipoproteins, mostly low-density lipoproteins (LDL), and monocyte-derived macrophages. The LDL receptor (LDLR) is essential for LDL uptake into the tissues, and its absence results in the development of atherosclerosis. On the other hand, the recovery of the LDLR expression in experimental models leads to atherosclerosis regression. Thus, the genetic manipulation of LDLR levels enables us to study the development and regression of atherosclerosis in mice. We showed that the conversion of β-carotene to vitamin A delays atherosclerosis progression in mice and our preliminary data suggest that β-carotene accelerates atherosclerosis regression. We hypothesize that β-carotene accelerates atherosclerosis regression by reducing liver inflammation.

Materials and Methods:
We utilized a reversible model of atherosclerosis consisting in the transient reduction of LDLR expression by using mRNA interference. We fed these mice a vitamin A-deficient Western diet (WD-VAD) for 16 weeks. After this period, we harvested baseline mice, while the rest were subjected to atherosclerosis regression for three weeks by recovering LDLR expression to normal levels. These mice were fed either WD-VAD or WD containing β-carotene. Livers were harvested for RNA sequencing and histological analyses.

Results:
RNA sequencing indicates that atherosclerosis regression reduces liver inflammation, an effect that is enhanced by β-carotene. Inflammation was associated with a reduction in hepatic macrophage markers. These findings were confirmed by histological quantification and western blotting analyses. Baseline mice showed a 2-fold and 3-fold increased number of macrophages in comparison to regression control (VAD) and β-carotene, respectively.

Conclusions/Discussion:
Our data show that atherosclerosis regression reduces liver inflammation, and that these effects are enhanced by the supplementation of β-carotene.

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We thank the National Institutes of Health Grant (NIH-1R01HL147252-01) and the US Department of Agriculture (W4002, Multistate project).

β-carotene enhances atherosclerosis resolution by reducing inflammation and increasing plaque stability

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Introduction: Atherosclerosis is the underlying cause of most cardiovascular diseases, and its development is driven by elevated plasma cholesterol. Human studies suggest that plasma β-carotene levels have an inverse association with circulating cholesterol and the risk of developing atherosclerosis. We recently showed that β-carotene conversion to vitamin A delays atherosclerosis progression in a mouse model. However, it is unknown if β-carotene or its vitamin A derivative(s) affect atherosclerosis resolution. In this study, we aimed to determine the impact of β-carotene on atherosclerosis resolution in a reversible murine model.

Materials and Methods: We injected antisense oligonucleotide to transiently block the low-density lipoprotein receptor (LDLR ASO) expression in wild-type mice fed a Western diet deficient in vitamin A (WD-VAD) for 16 weeks to induce atherosclerosis. After this period, we harvested a subset of mice (baseline group), while the remaining mice underwent atherosclerosis regression by interrupting LDLR ASO injections. To study the role of β-carotene on atherosclerosis regression, we maintained a subset of mice on WD-VAD. The remaining mice were fed with WD-VAD containing 50mg/kg of β-carotene (WD- β-carotene) for three weeks before sacrifice. Plasma and aortic roots were collected to analyze the size and composition of atherosclerosis lesions.

Results and Discussion:
We observed that baseline mice showed a 96% reduction of LDLR hepatic expression accompanied by a three-fold increase in plasma total cholesterol ($p<0.005$) when compared to both our regression groups. Histological analyses showed a reduction of macrophage content in both regression groups; however, the reduction was more pronounced in WD- β-carotene fed mice. Additionally, we found an increased amount of collagen content in the plaques of both regression groups and was more prominent in WD- β-carotene fed mice (42%) compared to our baseline (30%). Overall, our results suggest that β-carotene enhances atherosclerosis resolution by decreasing inflammation (macrophage content) and increasing plaque stability (collagen content).

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ROLE OF LECITHIN-RETINOL ACYLTRANSFERASE IN TRIGLYCERIDE SECRETION

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Introduction: Lecithin-retinol acyltransferase (LRAT) is involved in the esterification of retinol in the liver and other tissues. Studies suggest that the disruption of the LRAT gene leads to the formation of retinoic acid. Wild-type mice are resistant to vitamin A deficiency, even when fed a vitamin A-deficient diet (VAD) for several months. However, *Lrat*$^{-/-}$ mice fed VAD rapidly develop vitamin A deficiency after just 6 weeks. We previously showed that retinoic acid exposure in mice and cell culture reduces hepatic lipid secretion. We hypothesize that *Lrat*$^{-/-}$ mice fed a high vitamin A diet have lower triglyceride secretion than mice fed a vitamin A-deficient diet. The objective of this study was to determine whether high levels of vitamin A consumption in *Lrat*$^{-/-}$ mice result in lower triglyceride secretion than in vitamin A deficiency.

Methods and materials: To this end, we fed 6-week-old male and female *Lrat*$^{-/-}$ mice with either high vitamin A (23 IU/G) or VAD for eight weeks. Mice were maintained at 24°C on a 12 h light/dark cycle and had free access to food and water. After an overnight fasting period, mice were given an intraperitoneal injection of 1,000 mg/kg pluronic F127 poloxamer-407 (Sigma-Aldrich) to inhibit lipoprotein clearance from plasma. Blood samples were collected every hour from the tail to determine triglyceride secretion rate using commercially available kits.

Results: Compared to VAD *Lrat*$^{-/-}$ mice, we observed a significant reduction in triglyceride secretion in high vitamin A fed *Lrat*$^{-/-}$ mice. This effect was already evident just after two weeks of intervention. *Lrat*$^{-/-}$ mice did not show significant changes in triglyceride secretion rate upon feeding whether high vitamin A or VAD for 4, 6, and 8 weeks.

Conclusions/Discussion: Overall, our findings provide insights into the role of LRAT and vitamin A in the secretion of triglycerides in mice. These results agree with the effects of direct exposure to retinoic acid on the reduction of hepatic lipid secretion.

Acknowledgments

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Carotenoids and Health II: Supplementation and Bioavailability

CAROTENOID DIGESTIBILITY IN COMMERCIAL MAIZE HYBRIDS IS AFFECTED BY GRAIN PROPERTIES

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Introduction: Carotenoids in yellow maize contribute to antioxidant and pigmentation status and some of them to vitamin A status in animals. The bioactivity of carotenoids requires their prior release from the dietary matrix. Therefore, the structure of the maize kernel, determined by genotype, has been identified as a key factor governing carotenoid digestion. In addition, there is genetic variation in starch digestibility in maize [1], so the factors that affect starch digestibility are likely to affect the availability of all compounds in the starch-protein matrix of the kernel endosperm. Therefore, the objective of this study was to evaluate the potential relationship between grain properties and *in vitro* digestibility of individual [lutein (L), zeaxanthin (Z), α- (αCX) and β-cryptoxanthin (βCX), and β-carotene (βC)] and total carotenoids (TC) in commercial maize hybrids.

Methods and Materials: A total of 104 commercial maize hybrids were analysed. The carotenoid profile was determined using the RP HPLC method, and the same method was used to determine the amount of digested carotenoids after *in vitro* INFOGEST procedure. Standard methods were used to determine the chemical composition of maize kernels [crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), starch, and sugars]. The content of zeins and amylose was determined using methods adapted to maize. The size distribution of starch grains was determined in extracted starch. The relationship between grain properties and carotenoid digestibility was determined using the CORR procedure of the SAS 9.4 statistical package.

Results: The amount of digestible TC ranged from 27.33 to 85.69%, and the average amounts for L, Z αCX, βCX, αC, and βC were 62.19, 50.42, 31.99, 30.50, 31.28, and 42.49%, respectively. Digestibility of total and individual carotenoids correlated negatively ($P<0.05$) with the content of amylose, zeins, and the ratio of amylose to amylopectin in maize grains. Positive correlations ($P<0.05$) were found with CP, amylopectin and NDF contents.

Conclusions/Discussion: The digestibility of starch is negatively affected by amylose and zeins in maize [1], and this negative effect is transferred to carotenoids due to their localization in the starch-protein matrix. Moreover, the observed negative correlations were to be expected since these traits may reduce lipid digestibility. Unexpectedly, CP and NDF content correlated positively with carotenoid digestibility. Since hemicellulose accounts for most of the NDF in maize, it could increase digesta viscosity and inhibit droplet aggregation in the stomach phase [2,3]. Except for zeins, which account for 53% of CP in tested hybrids, other grain proteins probably had the same effect [4]. The results suggest that maize grain properties affect carotenoid digestibility and that the selection of hybrids with certain properties could increase the utilization of carotenoids.

Acknowledgements
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Bioaccessibility of carotenoids in baby food

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Feeding in early childhood is extremely important in the epigenetics of the individual and, consequently, influences health in the present and throughout life. Carotenoids, especially provitamin A, play a fundamental role in the human health, helping in bone growth, eyes, and skin health, in addition to acting as antioxidants in various reactions. The aim of this work was to determine the bioaccessibility of total and individual carotenoids in baby food.

Five industrialized baby fruit purees commercialized in the city of Campinas-SP were analyzed: assorted fruits (AF); tropical fruits (TF); apple, mango, and carrot (AMC); papaya and orange (PO); and grape and banana (GB). Exhaustive extraction of carotenoids was carried out with a mixture of ethanol and hexane (4:3). To determine the carotenoid bioaccessibility, *in vitro* digestion was performed using the Infogest 2.0 method, the micelles were separated by centrifugation, and the carotenoids were extracted from the micelles with diethyl ether. Carotenoids' separation and quantification were performed by HPLC-DAD, and the identification was confirmed by HPLC-DAD-MS with a single quadrupole analyzer. The separation of carotenoids was achieved on a C_{30} YMC column, with a linear gradient of methanol and MTBE.

The results showed a significant difference in the bioaccessibility of total carotenoids among all the samples ($p < 0{,}005$), varying between 1.1% and 15.5% in GB and TF, respectively.

All-*trans*-β-carotene was the most prevalent carotenoid, being found in all the samples. The bioaccessibility of this carotenoid ranged from 4.9% (AF) to 24.1% (AMC). Furthermore, the all-*trans*-β-carotene showed the highest bioaccessibility when compared to the other carotenoids present in the samples, such as 9- and 13-*cis*-β-carotene, and all-*trans*-α-carotene. All-*trans*-α-carotene was identified in three samples and its bioaccessibility ranged from 5.2% to 13.8% in GB and AMC, respectively. The 13-*cis*-β-carotene was founded in four samples and its bioaccessibility ranged from 5.0% (AF) to 10.5% (AMC). The 9-*cis*-β-carotene was identified in three samples and the bioaccessibility ranged from 5.0% (AF) to 10.7% (TF). Finally, all-*trans*-β-cryptoxanthin was identified in AF and PO, from which this xanthophyll was 4.3% and 7.6% bioaccessible, respectively.

The bioaccessibility of total carotenoids in the baby food samples varied according to their composition. The baby food containing oat, for example, showed the highest bioaccessibility. The baby food composition also influenced the carotenoid profile diversity and the bioaccessibility of each carotenoid individually. The all-*trans*-β-carotene, for example, showed higher bioaccessibility in the baby foods that containing mango as the main ingredient, the same being observed for its 9- and 13-*cis*-isomers. These results demonstrated the importance of evaluating the bioaccessibility of carotenoids in baby food, mainly due to the fruit composition and the interaction among the ingredients, which can affect bioaccessibility positively or negatively.

Acknowledgements

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Trait Stacking Simultaneously Enhances Mineral and Provitamin A Carotenoid Bioaccessibility in Biofortified *Sorghum bicolor*

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Abstract

Introduction: Vitamin A, iron, and zinc deficiencies represent major dietary inadequacies in Sub-Saharan Africa and disproportionately affect women and children. Biotechnology strategies have been tested to individually improve carotenoid or mineral content and/or bioaccessibility in relevant cereal crops such as sorghum (*Sorghum bicolor*). However, approaches combining carotenoid enhancement and reducing the mineral limiting antinutrient phytate in the same event have not been thoroughly evaluated. **Materials and Methods:** Two sorghum transformation constructs containing HGGT, to increase vitamin E accumulation and stabilize provitamin A carotenoids during grain storage, CRTI, to increase provitamin A biosynthesis, PSY1 or CRTB, to increase flux through the carotenoid pathway, and PhyA, to decrease phytate, were engineered to produce transgenic events. These sorghum events were processed into model porridges and evaluated for carotenoid and mineral content as well as bioaccessibility. **Results:** All transgenic events produced markedly higher amounts of carotenoids compared to corresponding null segregants and wild-type control (Tx430). A steeping step prior to porridge production to pre-activate phytase drastically reduced phytate content, altered the profile of inositol phosphate conversion products, and reduced molar ratios of phytate to iron and zinc; preventing the chelation of minerals by phytate and enhancing their bioaccessibility. The subsequent release of minerals did not affect micellarization efficiency and the bioaccessible fraction of provitamin A carotenoids were over 2300% greater in transgenic events compared to compared to corresponding null segregants and wild-type control; providing 53.7% of a 4–8-year-old child's vitamin A estimated average requirement. **Conclusions:** These data suggest that a combination of strategies to enhance micronutrient content and bioaccessibility are feasible and warrant further assessment in human studies.

Acknowledgements: We thank Sydney Corbin, Jacob Fitzgerald, Micaela Hayes, Zulfiqar Mohamedshah, and Candace Nunn for their assistance during *in vitro* digestion and carotenoid extraction experiments. This work was supported in part by the U.S. Department of Agriculture, Agricultural Research Service. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy. USDA is an equal opportunity provider and employer.

ASSOCIATION BETWEEN SKIN CAROTENOID LEVELS AND BMI WITH FAMILY FUNCTIONING AND PEDIATRIC QUALITY OF LIFE IN CHILDREN FROM LOW-INCOME HOUSEHOLDS

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Introduction: Increased fruit and vegetable (F/V) intake in children is associated with positive home environments (e.g., high family cohesion) and lower body-mass index (BMI). However, the relationship between F/V intake and family functioning or pediatric quality of life in families from low-income households has not been examined. This study examined the cross-sectional relationship between F/V intake, as measured by skin carotenoids, along with child BMI with family functioning and pediatric quality of life in children 2-8 years from low-income households during a community garden intervention.

Methods: We analyzed data from a community garden intervention (2018-2019) that provided tools and resources for families from low-income households to grow fruits and vegetables. Families with a child age 2-8 years old were invited to provide anthropometric measurements and complete the surveys to assess family functioning (Family Environment Scale [FES]), pediatric quality of life (PedsQL), and F/V intake (skin carotenoids through resonance spectroscopy). Bivariate regression analysis was used to determine the associations between F/V intake, family functioning, pediatric quality of life, and BMI using SAS 9.4.

Results: Data were analyzed from 85 children (47% female) with an average age of 5.7 (SD 2.2) years across six different community sites, with a median household income of $34,080. Skin carotenoid scores were associated with higher FES scores (β=0.0001, p=0.03) but not PedsQL or BMI. Child BMI was negatively associated with total FES scores (β=-2.86, p=0.03) and PedsQL (B=-0.79, p=0.01) (Table 1).

Conclusions: F/V intake is positively associated with family functioning while child BMI is associated with poor family functioning and decreased pediatric quality of life. Future work will examine the possible mechanisms between F/V intake and changes in BMI mediated through the home environment.

Acknowledgments/Funding: We would like to thank the participants and community liaisons from Buckeye ISA and the Kellogg Foundation who supported the funding of this project.

Table 1: Relationship between Skin Carotenoids (SC) and BMI with Family Functioning and Pediatric Quality of Life

Predictor	**Associations with SC** (β, p-value)	**Association with BMI** (β, p-value)
Family Functioning [a]	0.0001, 0.03	-2.86, 0.03
Cohesion	0.00003, 0.15	-3.67,0.28
Expression	0.00003, 0.07	-1.61, 0.60
Conflict	-0.00003, 0.16	7.31, 0.003
Quality of Life [b]	0.0001, -0.52	-0.79, 0.01
Physical	0.0002, 0.49	-0.31, 0.14
Emotional	0.0001, 0.68	-0.70, 0.01
Social	0.0001, 0.77	-0.59, 0.005
School	0.0002, 0.30	-0.44,0.12

Values presented are Beta coefficients
[a] Measured by Family Environment Scale
[b] Measured by Pediatric Quality of Life Survey Parent Report
[c] Calculated based on height and weight measurements obtained objectively. BMI calculated using CDC standard equation

Role of the Low-density lipoprotein receptor in the uptake and excretion of carotenoids

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Introduction: Over the years, it has been shown that high levels of carotenoids in the plasma are inversely correlated with the incidence of cardio metabolic diseases such as atherosclerosis. Carotenoids are bioactive compounds mainly synthesized by photosynthetic organisms. One of the most well known carotenoids is β-carotene. The limiting step in the conversion of β-carotene into vitamin A is the enzyme β-carotene Oxygenase 1 (BCO1). Mice do not accumulate β-carotene as humans do. Therefore, mice lacking BCO1 will accumulate β-carotene. There are several transporters involved in the biodistribution of carotenoids; one of them is low-density lipoprotein receptor (LDLR). This receptor is expressed in different parts of the body such as the intestine and the liver. The objective of this study is to determine the role of LDLR in the uptake and elimination of β-carotene in tissues.

Materials and methods: In this study, we compared age and sex-matched *Bco1*$^{-/-}$ and *Ldlr*$^{-/-}$*/Bco1*$^{-/-}$ mice fed with a Standard diet or a Western diet containing 50 mg/kg of β-carotene (WD-β-carotene) for 12 weeks. After 12 weeks, we changed the diet for β-carotene-free diets for four days. We collected feces every 12 hours for four days. We measured β-carotene in tissues and feces by High-Performance Liquid Chromatography (HPLC).

Results: HPLC results showed that fecal and intestinal β-carotene in *Bco1*$^{-/-}$ mice were 2.5-fold higher than those observed in *Ldlr*$^{-/-}$*/Bco1*$^{-/-}$ mice. These results were comparable between Standard diet and Western diet-fed mice. On the contrary, plasma β-carotene levels were increased in *Ldlr*$^{-/-}$*/Bco1*$^{-/-}$ mice than *Bco1*$^{-/-}$ control mice.

Conclusion: Our data indicate that LDLR expression mediates fecal carotenoid elimination.

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Tissue Distribution of Lutein in Neonatal Sprague-Dawley Rats Reared by Mothers Consuming a Normal- or a High Fat Diet

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Objective: Lutein, the most abundant carotenoid in the eye and brain of infants, is critical for their visual and cognitive development. Lutein cannot be synthesized *de novo* and newborns must obtain it from breast milk or infant formula. Due to its lipophilic nature, a high adiposity may affect the tissue distribution of lutein and compromise its availability in key organs by increasing its storage in fatty tissues. The aim of the present study was to assess the distribution of lutein in neonatal rats reared by mothers consuming a normal- or a high fat diet.

Methods: Pregnant Sprague-Dawley rats were randomized to a normal fat diet (NFD, 25% kcal from fat) or a high fat diet (HFD, 50% kcal from fat) both with 0.3% of lutein during their gestation and lactation. At postnatal day 6 (P6) and P11, rat pups (n=7/group/time) were euthanized. Blood, liver, stomach, small intestine, eye, brain, spleen, kidney, lung, visceral white adipose tissue (WAT), brown adipose tissue (BAT), and subcutaneous white adipose tissue (SWAT) were collected. The concentration of lutein in the serum, milk separated from stomach, and all the other tissues were measured by UPLC with a photodiode array detector.

Results: No significant difference in maternal lutein intake was found between the NFD and the HFD group. At both P6 and P11, a significantly ($P < 0.05$) lower lutein concentration was noted in the milk samples separated from the stomach of HFD pups compared to that in NFD pups (Fig. 1). At P6, HFD pups showed a significantly higher lutein concentration in the serum, spleen, lung, and WAT than the NFD group and a significantly lower concentration in the liver. At P11, the HFD group exhibited a significantly lower lutein concentration in the brain, eye, and BAT, but a significantly higher concentration in the WAT.

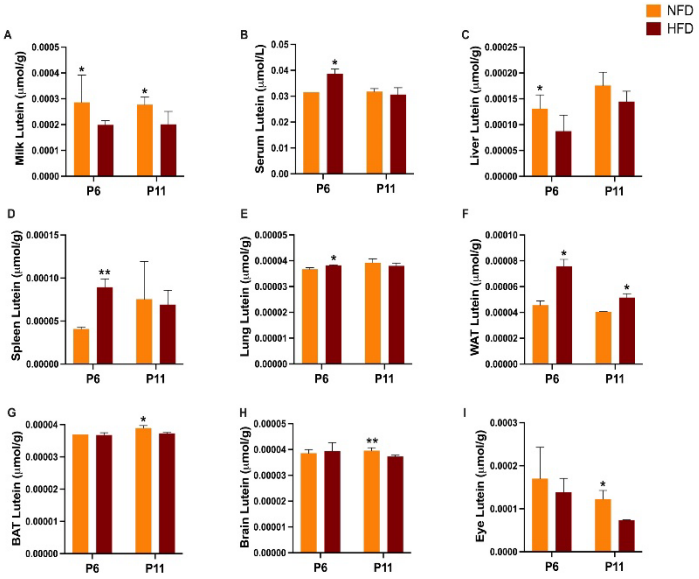

Figure 1. Concentrations of total lutein in milk from stomach (A), serum (B), liver (C), spleen (D), lung (E), visceral white adipose tissue (F), brown adipose tissue (G), brain (H), and eye (I) of rat pups. Bars show mean ± SEM, significant difference between NFD and HFD was indicated by *.

Conclusions: Neonatal rats reared by mothers fed an HFD received a lower level of lutein in milk and exhibited different tissue distribution compared to pups in dams consuming an NFD. At P11, the HFD group had a significantly lower lutein concentration in the organs where lutein plays a critical role, i.e., eye and brain, accompanied with a higher concentration in the adipose tissue. The present study was the first to provide evidence that maternal HFD consumption affected or even potentially compromised the availability of lutein to its target organs in neonatal offspring.

Acknowledgments: This study was supported by the College Start-up Fund awarded to LT. The authors thank BASF North America for their generous donation of lutein (Xangold®) for the study.

Biopolymer Stabilized Emulsions Improved Storage Stability and in vitro Bioaccessibility of Lutein

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Objective: Lutein plays a critical role in the visual and cognitive development of infants. However, the application of lutein as a potential nutraceutical is limited by its low stability and poor water solubility. While various encapsulation systems have been developed for lutein to enhance its stability and bioavailability, few utilized bio-based polymers that are safe to use in infant foods. The aim of the study was to develop a novel emulsion system for lutein using food-grade colloids, octenylsuccinylated (OS) starch and gum Arabic (GA), as emulsifiers, which could improve the stability and bioaccessibility of lutein.

Methods: Lutein oil-in-water emulsions were prepared using two types of OS starch, capsule TA® (CTA) and HI-CAP®100 (HC), and one type of GA, TICAmulsion® 3020 (TM). Lutein was dissolved in olive oil and then mixed with the aqueous biopolymer dispersions at 70% oil volume fraction using a homogenizer. The stabilities of the emulsion were assessed by measuring droplet size and distribution, changes of droplet size, and lutein retention at 25 and 45 °C after a week of storage. The *in vitro* bioaccessibility of lutein was measured using a simulated *in vitro* gastrointestinal model. Free lutein was used as control.

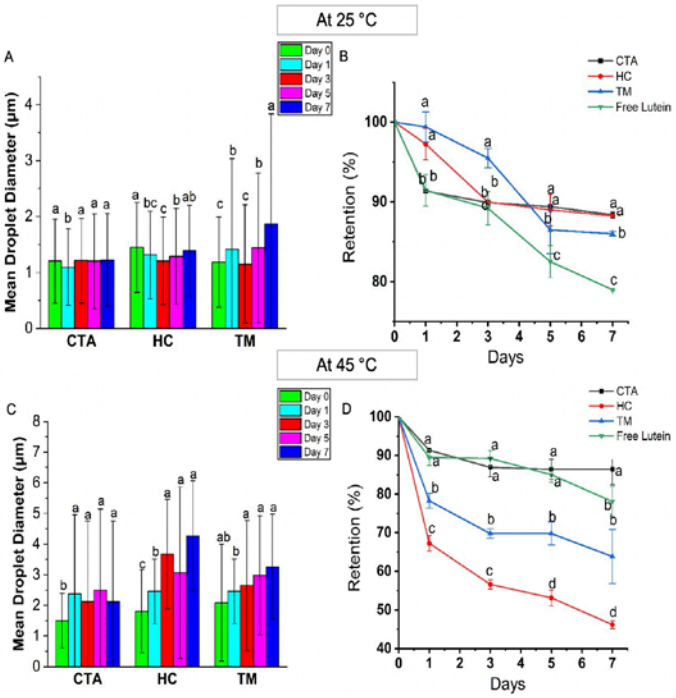

Figure 1. Changes of droplet size and lutein retention in lutein emulsions stabilized by capsule TA®, HI-CAP®100, and TICAmulsion® 3020 during storage at 25°C (A & B) and at 45°C (C & D) for a week. Free lutein as the control group. Significant differences among samples at each time point are indicated by difference letters (a > b > c > d, $p < 0.05$)

Results: The mean droplet size of lutein emulsions stabilized by CTA, HC, and TM were 1.19 ± 0.75, 1.45 ± 0.80, and 1.18 ± 0.8 μm, respectively. After a week of storage at 25 °C, the particle size stabilized by OS starches did not change significantly, while GA-stabilized emulsion showed 1.58-fold larger droplet size than fresh sample ($P < 0.05$) (Fig. 1). Lutein retention in the control and emulsions stabilized by CTA, HC, and TM were 79%, 88%, 89%, and 86% at day 7, respectively. After a week of storage at 45 °C, the lutein emulsions stabilized by CTA, HC, and TM showed 1.34-, 2.38-, and 1.55- fold larger particle size compared to fresh samples ($P < 0.05$). The retention of lutein in free lutein and emulsions were 78%, 86%, 46%, and 63%, respectively. The *in vitro* bioaccessibility of lutein emulsions were 1.95-, 1.46-, and 1.27- fold higher than that of free lutein ($P < 0.05$).

Conclusion: Lutein emulsion stabilized by OS starch CTA had the best overall stability in droplet aggregation, color retention, and *in vitro* release. The oil-in-water emulsion stabilized by biopolymers could be promising carriers for lutein to expand their application in infant foods.

Acknowledgements: This study was supported by the Louis and Evelyn Knol Nutrition Graduate Research Support Fund awarded to YZ. The authors thank BASF North America for their generous donation of lutein (Xangold®) for the study.

SYSTEMATIC REVIEW OF WORLDWIDE INFANT BLOOD AND HUMAN MILK CAROTENOID CONCENTRATIONS

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Introduction: Dietary carotenoids may support infant vitamin A status, and cognitive and visual functions, but infant carotenoid exposures are not well defined. The purpose of this study is to address a knowledge gap in worldwide estimates of human milk and infant blood carotenoid concentrations across lactation and feeding stages, respectively.

Methods and Materials: Systematic reviews of articles published in English describing infant blood and human milk carotenoid concentrations in healthy populations were conducted in three databases. Concentration data from healthy participants not currently receiving a carotenoid intervention, for which the postpartum time of specimen collection was clear, and measurements were acquired by HPLC were included for analysis. Mean ± 95% confidence intervals of infant blood (serum/plasma) carotenoid species concentrations were calculated within feeding stage [newborn, exclusive milk feeding (<6 mo), complementary feeding (6-12 mo)], and by milk type (human milk, infant formula); and milk carotenoid species concentrations were calculated by lactation stage [colostrum (<5 d), transitional (5-14 d), and mature (>14 d)].

Results: Infant blood carotenoid concentrations came from 47 articles published from 1989-2021. An array of major dietary carotenoids are observed in infant blood across newborn, exclusively human milk-feeding, and complementary feeding stages, with more abundant species being beta-carotene (6.1±3.7, 15.7±8.5, 5.7±2.9 ug/dL), lutein (3.6±1.1, 9.7±2.7, 9.9±2.3 ug/dL), and lycopene (2.0±0.4, 7.7±2.7, 5.4±1.6 ug/dL), respectively. Human milk carotenoid data came from 65 articles, published from 1994-2020. An array of major carotenoids are observed in human milk, with more abundant species in colostrum, transitional, and mature milk being beta-carotene (18.4±2.8, 6.9±3.3, 2.5±0.5 ug/dL), lutein (14.6±2.6, 9.1±1.1, 5.7±0.6 ug/dL), beta-cryptoxanthin (20.1±4.8, 3.3±0.4, 3.1±0.9 ug/dL) and lycopene (26.9±5.9, 2.9±0.5, 2.4±0.3 ug/dL), respectively.

Conclusion/Discussion: Infants are exposed to an array of major dietary carotenoids. These population estimates of milk and infant blood carotenoid concentrations can be used to contextualize research findings and to design nutritionally relevant carotenoid interventions to study the role of carotenoids in infant nutrition and health.

Acknowledgements: We thank Amy Sisson of the Texas Medical Center Library for her guidance in building the systematic review strategy.

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The Relationships Between Postpartum Maternal and Infant Ocular and Systemic Carotenoid Status in the Lutein and Zeaxanthin in Pregnancy (L-ZIP) Trial

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Introduction: Lutein (L) and zeaxanthin (Z), collectively known as the macular pigment (MP), are dietary xanthophyll carotenoids that preferentially accumulate in the human macula where they protect the eye from photo-oxidative damage. Detectable amounts of MP are present in the brain and eye at birth, indicating a transfer from the mother via the placenta. This transfer puts mothers at risk of systemic and ocular carotenoid depletion that may impact their eyes and overall health. Therefore, we investigated whether prenatal carotenoid supplementation counteracts maternal carotenoid depletion, particularly in the third trimester, and if it can enhance maternal and infant systemic and ocular carotenoid status when the baby is born.

Methods: In this controlled prospective trial (ClinicalTrials.gov identifier: NCT03750968), 47 participants were randomized 1:1 to receive standard-of-care daily prenatal vitamins with or without 10 mg of L and 2 mg of 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 the newborns' carotenoid levels in the umbilical cord blood, skin, and eyes using similar techniques as above but optimized for infants.

Results: Postpartum maternal macular pigment optical density at 9^0 eccentricity ($MPOV9^0$), serum L+Z concentrations, and skin carotenoids significantly increased in the carotenoid groups compared to the control group ($p<0.0001$, for all). Infants whose mothers were in the carotenoid group had a statistically significant increase in umbilical cord blood L+Z concentrations and skin carotenoid levels relative to the control group ($p<0.0001$, for all). Postpartum maternal serum L+Z levels were significantly associated with umbilical cord blood L+Z concentrations ($r=0.73$, $p<0.0001$) and infants' skin carotenoids ($r=0.65$, $p<0.0001$). Postpartum maternal skin carotenoid levels were significantly correlated with infants' skin carotenoids ($r=0.51$, $p=0.0008$) and umbilical cord blood L+Z concentrations ($r=0.70$, $p<0.0001$). Postpartum maternal $MPOV9^0$ was significantly associated with infants' skin carotenoids ($r=0.36$, $p=0.0313$) and umbilical cord blood L+Z concentrations ($r=0.43$, $p=0.0150$). Analysis of infant macular pigment levels is still in progress.

Conclusion: Our findings show that prenatal carotenoid supplementation improves both maternal and infant postpartum carotenoid levels, which could positively affect both the mother's and her infant's eye and general health.

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Carotenoid
and
ApoCarotenoid Biology

STUDYING THE ROLES OF APOCAROTENOIDS IN TOMATO BY GENOME EDITING OF THE CAROTENOID CLEAVAGE DIOXYGENASE (CCD) GENES

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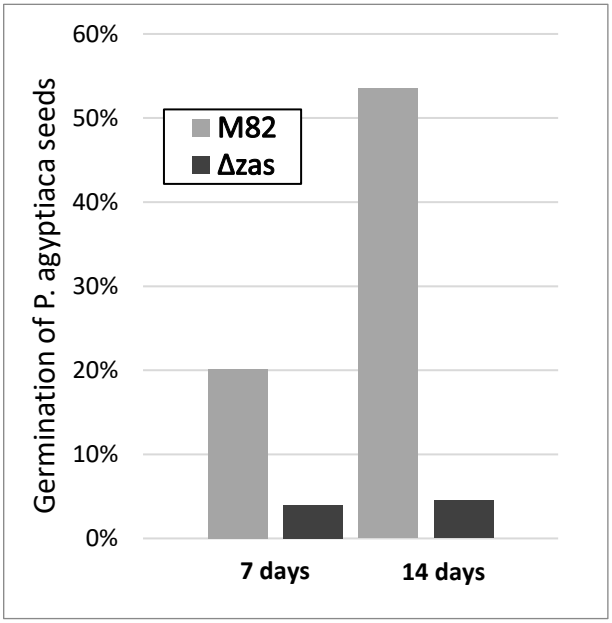
Apocarotenoids are carotenoid derivatives produced through oxidative cleavage of double bonds in the backbone of the carotenoid molecule. In plants, the metabolic conversion of carotenoids by carotenoid cleavage dioxygenases (CCDs) leads to the production of a variety of apocarotenoids. Some of them are volatiles that provide scents and flavor to flowers and fruits and others function as hormones such as abscisic acid (ABA) and strigolactones (SLs) or signaling molecules such as β-cyclocitral, an abiotic stress signal and growth regulator, zaxinone that is involved in root growth and development, and anchorene, a diapocarotenoid that functions in root development [1].

The overall goal of our research is to elucidate the specific roles of CCDs and their apocarotenoid products in tomato (*Solanum lycopersicum*). The tomato genome has seven CCDs genes. We focus on CCD1A, CCD1B, CCD4A, CCD4B and "CCD-Like". To this end, we apply a reverse genetics approach by creating CCD-deficient mutants using CRISPR-Cas9 editing and characterization of these plants.

Knockout of both CCD4A and CCD4B, separately or together, increased the levels of total carotenoids in leaves and fruits, suggesting the involvement of these enzymes in controlling the steady-state concentration of carotenoids in these organs. However, there was no significant change in total fruit yield or plant weight in field trials.



Based on sequence similarity to rice, the CCD-like of tomato was identified as a zaxinone synthase (ZAS) homolog. Tomato plants lacking a functional ZAS have smaller roots and exhibit more branching. The level of strigolactones in roots, quantified based on a bioassay of *Phelipanche aegyptiaca* seed germination, was >90% lower than in control plants due to lower expression of the DWARF27 gene, which encodes the first committed step in strigolactone biosynthesis (fig.1). The results suggest the roles of ZAS in root development possibly through the regulation of strigolactone biosynthesis.

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Retinol as a regulator of energy homeostasis during embryogenesis

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Beta-carotene is the main dietary precursor of vitamin A, an essential regulator of mammalian embryogenesis, mainly owing to the transcriptional regulatory action of its active form, retinoic acid. Retinol (vitamin A alcohol) instead has long been thought to lack intrinsic biological activities and serves exclusively as a precursor of bioactive vitamin A forms. However, evidence exists in the literature indicating that retinol per se, not retinoic acid, also regulates mammalian development, even though the mechanism(s) underlying this function has never been clarified[1,2]. We identified a protein kinase Cδ (PKCδ) signaling pathway that requires retinol (vitamin A) as a cofactor and is involved in the regulation of fuel utilization in mitochondria. The mitochondria-localized PKCδ forms a signaling complex (PKCδ signalosome) with the adapter p66Shc, cytochrome c and retinol that stimulates the conversion of pyruvate to acetyl-coenzyme A by the pyruvate dehydrogenase complex (PDHC) and regulates glucose flux, ultimately modulating mitochondria respiration and ATP production[3,4].

To investigate the potential role of the PKCδ signalosome in maintaining energy homeostasis during embryogenesis, we generated a mouse strain in which only the retinol-binding site of PKCδ was mutated (*PKCδki-/-*). *PKCδki-/-* mice display early embryonic lethality. Specifically, a severely reduced Mendelian ratio of PKCδki-/- mice from PKCδki+/- crosses was observed after gestational day 10. This finding strongly supports the involvement of retinol and PKCδ as regulator of mitochondrial energy metabolism, given the high-energy requirement during organogenesis and the dependence of the developing embryo on oxidative phosphorylation upon establishment of the placenta (i.e. around day 9-10 of gestation).

Seahorse analysis of mouse embryo fibroblast (MEF) derived from PKCδki$^{+/+}$ and PKCδki$^{+/-}$ embryos showed that the respiration rates of PKCδki$^{+/-}$ MEF cells were reduced compared to the PKCδki$^{+/+}$ MEFs. This reduction was not due to mitochondrial numerical deficiency. Moreover, acute production of reactive oxygen species (ROS) measured by fluorescence spectroscopy was attenuated in PKCδki$^{+/-}$ MEF compared to WT cells. Overall, our preliminary findings indicate that inactivation of the PKCδ retinol-binding site compromises mitochondrial energy metabolism, already in heterozygosity. They support our hypothesis that retinol and PKCδ may have an important role during embryogenesis in regulating respiration and ATP production.

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The synthetic retinoid fenretinide inhibits the conversion of β-carotene to vitamin A in mice

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Introduction: Fenretinide is a synthetic retinoid with pleiotropic benefits for human health that is currently utilized in clinical trials for cancer, and cystic fibrosis. However, fenretinide reduces plasma vitamin A levels by interacting with retinol-binding protein 4 (RBP4), which often results in reversible night blindness in patients treated with this compound. Cell culture and in vitro studies show that fenretinide binds and inhibits the activity of β-carotene oxygenase 1 (BCO1), the enzyme responsible for endogenous vitamin A formation. We hypothesized that fenretinide inhibits vitamin A formation via BCO1 *in vivo*, thereby affecting systemic vitamin A levels of mice independent of its effects on vitamin A transport via RBP4.

Methods and Materials: Six-week-old male and female wild-type and *Rbp4*$^{-/-}$ mice were fed a vitamin A-deficient diet for 14 days followed by a vitamin A-deficient Western diet supplemented with 50 mg of β-carotene/kg body weight as the lone source of vitamin A for 10 days. During this intervention, mice received 30 mg/kg fenretinide suspended in olive oil or the same volume of olive oil (vehicle control) daily by oral gavage. We monitored body weight progression and food intake during the entire experiment. Feces were collected every other day during the 10-day treatment period to assess β-carotene absorption. During sacrifice, final body weight and selected tissues were collected. Plasma was collected by cardiac puncture. We quantified retinoids and carotenoids by HPLC, as well as changes in gene expression by RT-PCR.

Results and Discussion: Our data show that fenretinide decreases systemic vitamin A levels. In both wild-type and *Rbp4*$^{-/-}$ mice, we observed approximately 2-fold higher plasma β-carotene accompanied by significantly higher tissue β-carotene and significantly lower tissue retinoids in fenretinide-treated mice. These results were consistent with the inhibitory effects of fenretinide on BCO1 previously observed *in vitro* and were independent of β-carotene absorption.

Conclusions: Fenretinide supplementation for 10 days alters blood and tissue vitamin A status in wild-type mice fed a β-carotene-rich diet. These changes largely remain in *Rbp4*$^{-/-}$ mice, suggesting that fenretinide modulates vitamin A formation by inhibiting BCO1 activity in vivo.

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Characterization of mammalian carotenoid cleavage dioxygenases: Heterologous expression, purification, enzyme assays, and substrate selectivity.

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Introduction: Carotenoid cleavage dioxygenases (CCDs) are nonheme iron enzymes that catalyze the double bond processing of carotenoids and their apocarotenoid metabolites. Beta-carotene oxygenase 1 (BCO1) and Beta-carotene oxygenase 2 (BCO2) are critical enzymes in carotenoid metabolism of mammals. Functional analysis of CCDs requires robust recombinant protein expression, purification methods, and enzyme activity assays. CCDs convert hydrophobic substrates and; therefore, substrate solubility and delivery play significant roles in the functional analysis of CCDs. These limitations have hampered the understanding of the biological roles of these enzymes and let to controversial outcomes in studies.

Methods and Materials: We established method to expresses CCDs as maltose binding protein fusion proteins. We established enzymes assays for the recombinant enzyme for diverse carotenoids and apo-carotenoid substrates. Detergents were selected according to the solubility of the substrate and their interaction with the recombinant enzymes. We also established methodology to analyze the substrate and products of the assays by high-performance liquid chromatography.

Results and Discussion: We report methods for the heterologous expression of mammalian carotenoid processing enzymes in *E. coli* and the biochemical characterization of the recombinant enzymes. Our protein expression and purification optimization resulted in soluble and active enzymes. We analyzed BCO1 and BCO2 substrate specificity with various carotenoid and apo-carotenoids. Based on the substrate selectivity and structural analyses we made predictions how the enzymes interacted with their substrates. We scrutinized these predication by site directed mutagenesis and identified critical amino acids for substrate selectivity of the two enzymes. This work enhances the knowledge of mammalian carotenoid metabolism and clarifies the role of the two mammalian CCDs in this process.

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Carotenoid accumulation in ISX/BCO2 DKO serves as a macula pigment mouse model

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Introduction: Carotenoids are lipophilic pigments whose light-absorbing property and physiology functions in organisms are attributed to their conjugated double-bond system. Within the central region of the human retina, zeaxanthin, mesozeaxanthin, and lutein, collectively referred to as the macula pigment are enriched. Macula pigment reduce chromatic aberration and have been shown to be photoprotective against damaging blue light[1]. Clinical studies revealed that supplementation with these pigments can reduce the risk of the age-related macular degeneration (AMD). As a result, health professionals advocate for carotenoid supplementation for patients experiencing AMD. While such recommendations are based on the relationship between carotenoid intake, blood, and tissue concentrations, studies in animal models indicate that this relationship is influenced by β-carotene oxygenase 2 (BCO2) activity and the transcription factor ISX. BCO2 converts carotenoids into more polar metabolites and ISX affects carotenoid absorption in the intestine[2,3].

Methods: We generated an ISX/BCO2 double KO (DKO) mouse line by crossing the *Isx* and *Bco2* genes into the genetic background of B6(Cg)-Tyrc-2J/J mice. These mice display an albino background and are susceptible to light damage. We subjected 4-week old DKO mice to dietary intervention with zeaxanthin supplemented diets at 50mg/kg and 250mg/kg concentrations. Non-supplemented diet was used as the control. These mice were kept on the diets for 4-weeks. High performance liquid chromatography (HPLC) was used to determine the level of zeaxanthin and retinoid metabolites. Optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO), and electroretinogram (ERG) were used to assess retinal morphology and function.

Results & Discussion: We aimed to generate and validate a macula pigment mouse model that accumulates ocular carotenoids such as zeaxanthin within the eyes. Eyes and other tissues extracted from DKO mice contained higher levels of dehydro-zeaxanthin than the parent carotenoid, zeaxanthin, indicating a metabolic conversion of zeaxanthin into its oxidized form. HPLC analysis showed that the concentration of ocular retinoid metabolites was not affected by zeaxanthin accumulation. OCT, SLO, and ERG showed healthy retinal layers and normal eye function in all dietary groups. Therefore, we have established a macula pigment mouse model to study the protective roles of ocular carotenoids.

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Title: Genetic dissection of the vitamin A delivery pathways to ocular tissues in mice

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Introduction: Vitamin A deficiency or excess are associated with blinding and inflammatory diseases. The eyes are supplied with vitamin A derived from either the extrinsic lipoprotein-dependent or intrinsic retinol binding protein-dependent pathway which distribute vitamin A from intestinal enterocytes or from hepatic stores. The mechanisms underlying the regulation of these distribution pathways remain not entirely understood. We employed a genetic dissection approach in transgenic mice to investigate the effects of the different pathways on ocular vitamin homeostasis and photoreceptor function.

Methods and Materials: We utilized mice harboring mutations in intestine-specific transcription factor (ISX) or stimulated by retinoic acid gene 6 (STRA6) which have been identified as gatekeepers of the extrinsic and intrinsic pathways, respectively. Mice were supplemented with either preformed vitamin A (4,000 IU/kg) or β-carotene (25 mg/kg) for 7 weeks. Retinoid concentrations were measured by HPLC, and retinoid-dependent processes were evaluated by gene and protein expression analyses. To assess visual function, scotopic and photopic electroretinograms were recorded in mice that were dark adapted overnight. Rhodopsin and M-opsin levels were evaluated by immunohistochemistry in retinal cross sections and whole mounts.

Results: We observed that ISX-deficiency increased utilization of both preformed and pro-vitamin A in mice. Peripheral tissues of acquired higher levels of retinoids in a STRA6-dependent manner, but ocular vitamin A concentrations remain comparable to that of WT mice. Ocular retinoid levels were significantly lower in *Stra6*$^{-/-}$ compared to WT and *Isx*$^{-/-}$ mice. Photopic responses and M-opsin staining were significantly reduced in *Stra6*$^{-/-}$ mice. Interestingly, genetic deletion of ISX partially rescued ocular vitamin A deficiency in *Isx*$^{-/-}$*/Stra6*$^{-/-}$ double-knockout mice. Photopic ERG responses were significantly improved in *Isx*$^{-/-}$*/Stra6*$^{-/-}$ double-knockout mice compared to that of the *Stra6*$^{-/-}$ counterparts. This was accompanied by a significant recovery of cone photoreceptors. Conditions of vitamin A excess resulted in rescue of vision, but this advantage came at the expense of a massive accumulation of vitamin A in other tissues and nonspecific vitamin A distribution.

Conclusions: Our analyses across the different genotypes revealed STRA6 is critical for maintaining ocular vitamin A homeostasis and cone photoreceptor function. The partial rescue of vision observed in *Isx*$^{-/-}$*/Stra6*$^{-/-}$ double-knockout mice was associated with hyper-vitaminosis A in most peripheral tissues, demonstrating that an eye-specific delivery pathways of the essential nutrient is critical for vision.

Funding: This work was supported by the National Eye Institute (EY028121; EY011373) and the T32 Visual Science Training Grant (EY007157).

MECHANISMS OF CAROTENOID-BASED SPECTRAL FILTERING IN THE AVIAN VISUAL SYSTEM

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Introduction: Vision is essential to the lives of birds and its importance is reflected in the sophisticated form and function of avian photoreceptors. Bird color vision is mediated by four single-cone photoreceptor subtypes that express visual pigment opsins sensitive to red, green, blue, or violet/ultraviolet portions of the light spectrum. These visual pigments are coupled with carotenoid-pigmented cone oil droplets that filter light and fine-tune receptor sensitivity. Within each cone subtype, the spectral filtering of oil droplets is precisely matched to the spectral sensitivity of the visual pigment opsin through the selective accumulation of specific types and amounts of carotenoid, including metabolically derived apocarotenoids and C4-ketocarotenoids.

Methods: To determine mechanisms mediating the specific accumulation of carotenoids, we used fluorescence-activated cell sorting to isolate to enrich populations of each cone subtype. We then compared gene expression profiles among subtypes to identify candidate enzymes and transporters. We screened the function of these candidates with heterologous expression assays.

Results and Discussion: We found that while the transcriptomes of the cone photoreceptors subtypes are strikingly similar, each subtype expresses a distinct suite of carotenoid metabolizing enzymes and transporters that mediate the specific pigmentation of the droplet. We will present in vitro functional evidence supporting these candidates and discuss the role of these candidates in both color vision and plumage coloration.

Acknowledgments: This work was supported by the University of Tulsa and the National Science Foundation (IOS 2037739).

EXPOSURE TO ARTIFICIAL LIGHT AT NIGHT INTERACTS WITH SOCIAL CONDITIONS TO AFFECT EGG-YOLK CAROTENOID INVESTMENT IN A MODEL GAMEBIRD SPECIES

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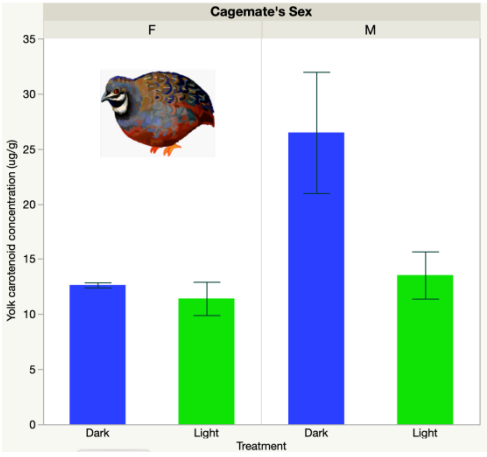
Introduction: Human impacts on wildlife continue to expand globally, and this now includes exposure to artificial light at night (ALAN) in built environments. ALAN can alter both survival and reproduction in animals, but comparatively less is known about effects on specific reproductive investments, such as in egg quality and quantity in oviparous species. We conducted an overnight light-exposure manipulation in captive king quail (*Excalfactoria chinensis*) to examine effects of ALAN on various components of egg investment – concentrations of carotenoid pigments (which can boost offspring growth and health) in egg yolk as well as egg quantity and mass. Birds of this social species also were co-housed with either a male or female, which permitted us to examine consequences of cagemate sex on egg traits. We hypothesized that ALAN exposure during early life would impair egg quantity and quality but that more and higher-quality eggs would be laid by females co-housed with males.
Research and Methods: We hatched 41 fertilized, artificially incubated king quail eggs shipped to us from commercial hatcheries. Hatchlings were housed first in two plastic brooding chambers for 2 weeks and then in larger wire cages in randomly assigned pairs of birds for a one-week acclimation phase and then for the duration of the 5.5-week experiment. Birds were sexed (22 female, 19 male) by integumentary coloration at sexual maturity. From weeks 4-9.5 of life, quail either continued receiving no night lighting (n = 10 females in the control group) or they were exposed to weak (ca. 0.3 lux) blue light (n = 12 ALAN-treated females) that was shone directionally at each cage (distance approximately 0.3 m) throughout the entire subjective night (18 h light:6 h dark; chosen for optimal growth and survival of hatchlings; Landry, 2015). Females started laying eggs at week 7 of life, and we monitored egg-laying progress for 2.5 weeks. We weighed each egg upon detection, counted total number of eggs laid per female, and for the first and last egg laid in the clutch we extracted egg yolk carotenoids and analyzed them using high-performance liquid chromatography.
Results and Discussion: Lutein and zeaxanthin were the dominant carotenoids in king quail eggs (46% and 41% of total), with beta-cryptoxanthin being a minor component (13% of total). Cagemate sex and ALAN treatment significantly affected yolk carotenoid concentrations in first-laid eggs, such that females housed with males and under a natural light/dark cycle laid the most carotenoid-rich egg (Fig. 1). We found no effects of cagemate sex or ALAN treatment on levels of yolk carotenoids in last-laid eggs or on onset of egg laying, however. Interestingly, light and social treatments also affected egg mass and number of eggs laid, such that ALAN-stimulated females laid more, smaller eggs, but only when housed with another female. These findings highlight the complex social and anthropogenic environmental factors that can alter key fitness parameters like offspring quantity and quality.



Abstract

EXPLORING THE DIVERSE ROLES OF RETINAL SIGNALING IN PLANTS

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Introduction: Retinaldehyde (retinal), a derivative of β-carotene metabolism [1], is the precursor of all retinoid molecules including retinoic acid [2], a major bioactive metabolite in vertebrates as well as invertebrates [3], and is critical for a number of biological processes, including embryogenesis [4], organogenesis [5], regeneration [6], and light perception [7]. Although plants are long known as the major source of β-carotene, the production of retinoid compounds as well as the functions of retinal signaling in plants, mediated by retinoid-binding proteins (RBPs) [8], have only been recently identified. Recent reports have shown that plants can make retinoids [9] and are critical for initiation of lateral root organogenesis in *Arabidopsis* [10]. We strived to further investigate retinal signaling, its components, and its roles in development and physiology of evolutionarily distant species.

Methods and materials: To investigate retinal signaling in plants, we used a fluorescent chemical probe called merocyanine aldehyde (MCA) that fluoresces upon binding to RBPs, thus enabling live and dynamic investigation of their properties, including cellular localization and trafficking, in genetically tractable as well as non-tractable plant species [10]. We further applied exogenous treatments of retinal and D15, a chemical inhibitor of β-carotene metabolism [10].

Results: Here we report that retinal signaling is existent as well as critical in vascular as well as nonvascular primitive plants like moss. We report that inhibition of β-carotene metabolism in moss, *Physcomitrella patens*, causes halted growth and development of gametophytic body implying that retinal signaling is ancient and evolutionarily conserved. Retinoids have been reported as a potent anti-aging compound as well as involved in p53-induced apoptosis after DNA damage in animals [11]. Our investigations suggest that retinal signaling has a role in genome protection and genotoxin-induced stem cell death in the plant meristem as well.

Conclusion: Taken together, our results demonstrate that retinal signaling is conserved in the diverse life forms and essential for various critical aspects of plant development and physiology.

Acknowledgements

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