1st Virtual International Conference on Carotenoids

VICC 2021
International Carotenoid Society
June 22, 23, 24, 25

Conference Organizers

John Landrum, Past-President ICS
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email: adrian.wyss@dsm.com

Comments from the Organizers

First and foremost, we wish to thank all of those who through their diligence and hard-work have done the fine research that is represented by the abstracts that have been submitted to VICC 2021. Without this exceptional science there would be no conference.

Secondly, the Organizers must thank Case Western Reserve University School of Medicine for their gracious technical support without which it is unlikely this project would have come to a successful fruition.

The original concept of VICC 2021 developed as it became clear that the continuing restrictions to travel due to COVID-19 would prevent the already once delayed International Carotenoid Symposium from occurring this summer. The ICS Counsel decided that creating a virtual conference would provide an opportunity for many to share the progress they have made in the past 18 months and more since the onset of the pandemic with colleagues around the world. It was felt this would be an especially welcome opportunity among graduate students and post-doctoral fellows who may soon be seeking positions and need to build awareness of their recent accomplishments. Our initial expectations for VICC 2021 were modest and we were overwhelmed by the enthusiastic response to our call for abstracts! With over sixty high quality abstracts from 27 different countries we have expanded the conference from three to four days and added an additional hour for presentations on each day, amounting to an increase in conference time from 9 to 16 hours (78%). Even after this increase in conference time we were still faced with a choice of declining excellent abstracts or limiting the presentation times. We chose the latter and the program here is the result of that decision.

We decided that VICC 2021 would not be an effort to supplant the in-person conferences that we all find so stimulating and informative but rather to enable each speaker to present a snapshot of their goals and accomplishments that would serve to initiate further communication in the days and weeks following VICC 2021. With that goal in mind, we have worked with our moderators encouraging them to reach out to the presenters in each session to work as a team and make every effort to enable the smooth transitions between speakers and sessions necessary to provide a showcase for each speaker's work. For their efforts in this regard we owe the moderators of each session a debt of gratitude.

To be sure, we are entering new territory using modern technology to create a conference that spans 24 time zones across the globe, coordinating speakers whose local times vary by as much as 19 hours (the conference begins at 4:30 am US Pacific Coast time when it will be 11:30 pm for participants in New Zealand!). Each speaker has been assigned an 8 minute window to deliver as tightly focused presentation that we hope will make the conference not only fast paced but also lively and exciting. We encourage participants to submit questions for the discussion periods that will follow after every 4-5 speakers. Moderators will select from among those to guide discussion.

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Tuesday June 22, 2021

7:30 am EST Welcome

7:35 am EST Session I – Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism

Moderators:

Professor Xiang-Dong Wang
Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA

Professor Nancy Moran
Department of Pediatrics–Nutrition, Baylor College of Medicine, 1100 Bates St., Houston, TX 77030

7:35:00 am EST Session I-A Antioxidant Properties & Modulation of Metabolism

1. ANTI-TUMORIGENIC EFFECTS OF β-CRYPTOXANTHIN VIA MODULATING CANCER CELL METABOLISM

Abstract 1

Presenter: Professor XIANG-DONG WANG
Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA. (email: xiang-dong.wang@tufts.edu)

2. STUDY ON THE SOLVENT DEPENDENCE OF ANTIOXIDANT CAPACITY OF SELECTED CAROTENOIDS

Abstract 2

Presenter: Dr. KATALIN BÖDDI
Departmentof Biochemistry and Medical.Chemistry, Medical School, Univ.of Pécs, Szigeti út12. H-7624 Pécs, Hungary (email: katalin.boddi@aok.pte.hu)

3. A DUNALIELLA SALINA EXTRACT COUNTERACTS SKIN ANTI-AGING UNDER INTENSE SOLAR IRRADIATION THANKS TO ITS ANTI-GLYCATION AND ANTI-INFLAMMATORY PROPERTIES.

Abstract 3

Presenter: Dr. FABIEN HAVAS
IFF Lucas Meyer, Yavne, Israel (fabien.havas@iff.com).

4. THE PROTECTIVE EFFECT OF CAROTENOIDS, POLYPHENOLS AND SEX HORMONES ON SKIN CELLS UNDER OXIDATIVE STRESS CONDITIONS

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Abstract 4

Presenter: Ms. AYA DARAWSHY
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Beersheva, Israel (email: ayadar@post.bgu.ac.il)

5. EFFECT OF PALM MIX CAROTENES (PMC) AGAINST OXIDATIVE STRESS INDUCED AGE-RELATED MACULAR DEGENERATION (AMD) IN HUMAN RETINAL PIGMENT CELLS

Abstract 5

Presenter: Ms. PUVANESWARI MEGANATHAN
Nutrition Unit, Product Development and Advisory Services Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. (email: puvaneswari@mpob.gov.my)

8:22 am EST **Session I-A – Questions and Discussion** (15 min)
Moderators: Prof. Xiang-Dong Wang and Prof. Nancy Moran

8:37 am EST **Break** (4 minutes)

8:41:00 am EST **Session I-B Antioxidant Properties & Modulation of Metabolism**
Moderators: Prof. Xiang-Dong Wang and Prof. Nancy Moran

6. CAROTENOID-MELATONIN CONJUGATES: SYNTHESIS AND ANTIOXIDANT CAPACITY

Abstract 6

Presenter: Dr. VERONIKA NAGY
Department of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary
Richmond University Medical Center, Department of Medicine, Staten Island, New York, US (email: vera.nagy@aok.pte.hu)

7. PROTECTIVE EFFECTS OF INDIVIDUAL AND COMBINED BETA-CAROTENE AND METFORMIN TREATMENT AGAINST OBESITY AND OBESITY COMORBIDITIES IN MICE

Abstract 7

Presenter: Professor M. LUISA BONET
Islands, Spain. (email: luisabonet@uib.es)
CIBER Fisiopatología de la Obesidad y Nutrición, Palma de Mallorca, Spain.
Institut d'Investigació Sanitària Illes Balears (IdISBa). Palma de Mallorca, Spain.

8. PLASMA CAROTENOID CONCENTRATIONS ARE INFLUENCED BY CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS

Abstract 8

Presenter: Professor RACHEL E. KOPEC
Human Nutrition Program, The Ohio State University, Columbus, OH (email: kopec.4@osu.edu)

9. PILOT FEASIBILITY AND VALIDITY ASSESSMENT OF REFLECTION SPECTROSCOPY-MEASURED SKIN CAROTENOID SCORE AS A BIOMARKER OF CAROTENOID INTAKE IN 4- AND 8-MONTH-OLD INFANTS

Abstract 9

Presenter: Professor NANCY E. MORAN
Department of Pediatrics–Nutrition, Baylor College of Medicine, 1100 Bates St., Houston, TX 77030 (email: Nancy.Moran@bcm.edu)

9:15:00 am EST **Session I-B - Questions and Discussion** (15 min)
Moderator: Prof. Xiang-Dong Wang and Prof. Nancy Moran

9:30:00 am EST **Break** (11 minutes)

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9:41:00 am EST Session II - Carotenoids and Health II: Supplementation & Bioavailability

Moderators:

Professor Paul Bernstein
Department of Ophthalmology and Visual Science, John A. Moran Eye Center, University of Utah, School of Medicine, Salt Lake City, UT.

Professor John Erdman
Department of Food Science and Human Nutrition, University of Illinois Urbana Champaign, Urbana, Illinois.

9:46:00 AM EST Session II-A Supplementation & Bioavailability

1. PRENATAL SUPPLEMENTATION OF ZEAXANTHIN PROMOTES BETTER RETINAL VASCULAR REGROWTH IN OXYGEN INDUCED RETINOPATHY (OIR) MODEL IN *BCO2-/- KO MICE*

Abstract 10

Presenter: Dr. RANGANATHAN ARUNKUMAR
Department of Ophthalmology and Visual Science, John A. Moran Eye Center, University of Utah, School of Medicine, Salt Lake City, UT. (email: Arunkumar.Ranganathan@hsc.utah.edu).

2. THE EFFECT OF MUCIN ON β-CAROTENE BIOAVAILABILITY CELL-BASED ASSAYS

Abstract 11

Presenter: Dr. LINA YONEKURA
Graduate School of Agriculture, Kagawa University, Miki, Kagawa, Japan (email: yonekura.lina@kagawa-u.ac.jp)

3. A PROTOCOL TO VALIDATE TWO FOOD FREQUENCY QUESTIONNAIRES DEVELOPED TO ESTIMATE LUTEIN AND ZEAXANTHIN DIETARY INTAKE.

Abstract 12

Presenter: Ms. NAOMI FITZPATRICK
School of Human Movement and Nutrition Sciences, The University of Queensland, Australia.(email: naomi.fitzpatrick@uq.net.au)

4. SUPERIOR BIOAVAILABILITY OF A NOVEL, PATENTED LUTEIN & ZEAXANTHIN MACULAR CAROTENOID FORMULATION

Abstract 13

Presenter: Dr. DESHANIE RAI
OmniActive Health Technologies, 67 E Park Place Suite 500, Morristown, NJ 07960, USA (email: d.rai@omniactives.com)

10:19:30 am EST **Session II-A – Questions and Discussion** (15 min)
Moderators: Prof. Paul Bernstein and Prof. John Erdman

10:34:00 am EST **Break** (4 minutes)

10:38:30 am EST **Session II-B Supplementation & Bioavailability**
Moderators: Prof. Paul Bernstein and Prof. John Erdman

5. LYCOPENE REDUCES FORMATION OF CHOLESTEROL CRYSTALS AND ACCELERATES THEIR DISSOLUTION

Abstract 14

Presenter: Dr. Ivan M. Petyaev
Lycotec, Cambridge, UK (email: Petyaev@lycotec.com)

6. ASSOCIATION OF PLACENTAL CAROTENOID STATUS WITH MATERNAL AND INFANTS' CAROTENOID STATUS IN THE LUTEIN AND ZEAXANTHIN IN PREGNANCY (L-ZIP) STUDY

Abstract 15

Presenter: Mr. EMMANUEL KOFI ADDO
Department of Ophthalmology, Moran Eye Center, University of Utah Health, Salt Lake City, UT, United States (email: Emmanuel.K.Addo@utah.edu)

7. EVALUATION OF THE BIOACCESSIBILITY OF A CAROTENOID BEADLET BLEND USING AN *IN VITRO* SYSTEM MIMICKING THE UPPER GASTROINTESTINAL TRACT

Abstract 16

Presenter: Dr. CHUN HU
Nutrilite Health Institute, 5600 Beach Blvd, Buena Park, California, USA 90621 (email: charles.hu@amway.com)

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8. KERNEL HARDNESS AFFECTS CAROTENOID DIGESTIBILITY IN COMMERCIAL MAIZE HYBRIDS

Abstract 17

Presenter: Professor DORA ZURAK
Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia (email: dzurak@agr.hr)

11:13:00 am EST **Session II-B – Questions and Discussion** (15 min)
Moderators: Prof. Paul Bernstein and Prof. John Erdman

11:28:00 am EST **Sessions I & II - Closing Comments** (5 minutes)

11:33:00 am EST **Close Session I & II**

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7:30 am EST Session III – Dietary Sources of Carotenoids and Nutritional Supplementation

Moderators:

Professor Tim O'Hare
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Health and Food Sciences Precinct, Coopers Plains Brisbane, Queensland 4108, Australia.

Professor Ralf Schweiggert
Geisenheim University, Institute of Beverage Research, Analysis and Technology of Plant-based Foods, Von-Lade-Strasse 1, 65366 Geisenheim, Germany

7: 34:30 am EST Session III-A - Dietary Sources of Carotenoids and Nutritional Supplementation

1. CHILLIES AS POTENTIAL CANDIDATES IN BREEDING FOR HIGH ZEAXANTHIN BELL-PEPPER VARIETIES

Abstract 18

Presenter: Ms. RIMJHIM AGARWAL
Centre for Nutrition and Food Sciences, QAAFI, The University of Queensland, St. Lucia, Australia (email: rimjhim.agarwal@uq.net.au)

2. LYCOPENE FROM ORANGE HEIRLOOM ('MOONGLOW') TOMATOES IS SUPERIOR TO RED TOMATOES IN INCREASING BONE BIOMECHANICS AND SUPPRESSING BONE TURNOVER IN A RAT MODEL OF OSTEOPOROSIS.

Abstract 19

Presenter: Mrs. UMANI S. WALALLAWITA
School of Food and Advanced Technology, Massey University, Palmerston North, 4442, New Zealand (email: u.walallawita@massey.ac.nz)

3. RED-FLESHED SWEET ORANGE MUTANTS: A NEW SOURCE OF LYCOPENE AND COLORLESS CAROTENES

Abstract 20

Presenter: Mr. JAIME ZACARÍAS-GARCÍA
Food Biotechnology Dep. Instituto Agroquímica y Tecnología de Alimentos (IATA-CSIC), Valencia, Spain (email: jaizagar@iata.csic.es)

4. ENGINEERED HIGH LEVEL PRODUCTION OF THE KETO- AND HYDROXYCAROTENOIDS, CANTHAXANTHIN, ZEAXANTHIN, AND ASTAXANTHIN, USING THE PURPLE BACTERIUM RHODOSPIRILLUM RUBRUM

Abstract 21

Presenter: Dr. CAROLINE AUTENRIETH
Department of Bioenergetics, Institute of Biomaterials and Biomolecular Systems, University of Stuttgart, Pfaffenwaldring 57, D-70569 Stuttgart, Germany
(email: caroline.autenrieth@bio.uni-stuttgart.de)

8:08:30 am EST **Session III-A – Questions and Discussion** (15 min)
Moderators: Prof. Tim O'Hara and Prof. Ralf Schweiggert

8:23:30 am EST **Break** (4 minutes)

8:27:30 am EST **Session III-B - Dietary Sources of Carotenoids and Nutritional Supplementation**
Moderators: Prof. Tim O'Hara and Prof. Ralf Schweiggert

5. BIOCHEMICAL CHARACTERIZATION OF BETA-CAROTENE CONTENTS IN SWEET POTATO (IPOMOEA BATATAS) CULTIVARS GROWN IN PAKISTAN

Abstract 22

Presenter: Dr. MUHAMMAD ZUBAIR KHAN
Department of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot, AJK, Pakistan (email: zubairgenes@gmail.com)

6. **RED AND YELLOW COLORED TAXUS ARILS: NATURAL SOURCES OF THE EXCEPTIONAL RETRO-CAROTENOIDS RHODOXANTHIN AND ESCHSCHOLTZXANTHIN AT HIGH ABUNDANCE**

Abstract 23

Presenter: Mr. ROLAND SCHEX
DSM Nutritional Products, Research and Development, P.O. Box 2676, 4002 Basel, Switzerland (email: roland.schex@dsm.com)

7. DO BLOOD-PLUMS (*PRUNUS SALICINA*) HAVE CAROTENOIDS?

Abstract 24

Presenter: Ms. GETHMINI KODAGODA
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia. k.kodagoda@uq.edu.au)

8. AVOCADOES AS A SOURCE OF DIETARY LUTEIN: IMPORTANCE OF TOTAL CAROTENOID SYNTHESIS TO ELEVATED LUTEIN CONCENTRATIONS.

Abstract 25

Presenter: Mr. TATSUYOSHI TAKAGI
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia. (email: t.takagi@uq.net.au)

9:01:00 am EST **Session III-B – Questions and Discussion** (15 min)
Moderators: Prof. Tim O'Hara and Prof. Ralf Schweiggert

9:16:00 am EST **Break** (11 minutes)

9:27:00 am EST **Session IV-A - Carotenoid Production by Microorganisms**

Moderators:

Professor Daniele Guiffrida
University of Messina, Department of Biomedical, Dental and Morphological and Functional Imaging Sciences, Viale Annunziata is. 524, Messina,Italy.

Professor Jaume Amengual
University of Illinois Urbana Champaign, Dept. of Food Science & Human Nutrition, 905 S GOODWIN AVE,Urbana, Illinois

9:31:30 am EST **Session IV-A - Carotenoid Production by Microorganisms**

1. EVALUATION OF ZEAXANTHIN PRODUCTION BY AN ANTARCTIC *FLAVOBACTERIUM* SP. USING CORN STEEP LIQUOR AS AN ALTERNATIVE NITROGEN SOURCE.

Abstract 26

Presenter: Ms. CAMILA RODRIGUEZ
Departamento de Bioingeniería, Instituto de Ingeniería Química, Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay (*email: crodriguez@fing.edu.uy*)

2. MAJOR CAROTENOID IN *Meiothermus ruber*: DEINOXANTHIN GLUCOSIDE ESTERS

Abstract 27

Presenter: Mr. RYO ASAKA
Department Molecular Microbiology, Tokyo University of Agriculture, Tokyo, Japan
(email: ryo_ask_0305@yahoo.co.jp)

3. ENHANCING BACTERIORUBERIN BIOSYNTHESIS IN WILD-TYPE HALOFERAX MEDITERRANEI R-4.

Abstract 28

Presenter: Ms. MICAELA GIANI
Biochemistry and Molecular Biology Division, Agrochemistry and Biochemistry Department, Faculty of Sciences, University of Alicante, Alicante, Spain. (email: micaela.giani@ua.es)
Multidisciplinary Institute for Environmental Studies "Ramón Margalef", University of Alicante, Ap. 99, E- 03080 Alicante, Spain.

4. FED-BATCH CAROTENOID PRODUCTION BY AN ANTARCTIC *FLAVOBACTERIUM* SP.

Abstract 29

Presenter: Ms. EUGENIA VILA
Departamento de Bioingeniería, Instituto de Ingeniería Química, Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay (email: mvila@fing.edu.uy)

5. ARTIFICIAL DIFFERENTIATION OF CHLOROPLASTS PROVIDES ENHANCED STORAGE CAPACITY FOR CAROTENOIDS AND OTHER NUTRITIONALLY VALUABLE ISOPRENOIDS IN LEAVES.

Abstract 30

Presenter: Mr. LUCA MORELLI
Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Spain
Institute for Plant Molecular and Cell Biology (IBMCP), Valencia, Spain (email: luca.morelli@cragenomica.es ;)

10:14:00 am EST **Session IV-A – Questions and Discussion** (15 min)
Moderators: Prof. Daniele Guiffrida and Prof. Jaume Amengual

10:29:00 am EST **Break** (4 minutes)

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10:33:30 am EST **Session IV-B - Carotenoid Production by Microorganisms**

Moderators: Prof. Daniele Guiffrida and Prof. Jaume Amengual

6. INTEGRATION OF AGROINDUSTRIAL WASTE IN THE CAROTENOIDS BIOPRODUCTION BY RED OLEAGINOUS YEAST

Abstract 31

Presenter: Mr. MIGUEL ÁNGEL VILLEGAS-MÉNDEZ
Department of Chemical Engineering, Autonomous University of Coahuila, Saltillo, Coahuila, México (miguel.villegas@uadec.edu.mx).

7. THERMALLY INDUCED (E/Z)-ISOMERIZATION AND CONTROLLED AGGREGATION OF RHODOXANTHIN TO MODULATE THE COLOR OF FORMULATIONS FOR FOOD AND BEVERAGES

Abstract 32

Presenter: Mr. ROLAND SCHEX
DSM Nutritional Products, Research and Development, P.O. Box 2676, 4002 Basel, Switzerland (email: roland.schex@dsm.com;)

8. ANTIOXIDANT ACTIVITY IN SUPRAMOLECULAR CAROTENOID COMPLEXES FAVORED BY NONPOLAR ENVIRONMENT AND DISFAVORED BY HYDROGEN BONDING

Abstract 33

Presenter: Professor YUNLONG GAO
College of Sciences, Nanjing Agricultural University, Nanjing 210095, China (email: yunlong@njau.edu.cn)

9. COOKIE WASTE AS ALTERNATIVE LOW-COST MEDIA FOR CAROTENOIDS BIOSYNTHESIS BY *RHODOTORULA* SP.

Abstract 34

Presenter: Ms. CASTRILLÓN-DUQUE, E. X.
Faculty of Biological Sciences, Autonomous University of Coahuila, Torreón, Coahuila, México (email: ecastrillon@uadec.edu.mx),

11:07:00 am EST **Session IV-B – Questions and Discussion** (15 min)

Moderators: Prof. Daniele Guiffrida and Prof. Jaume Amengual

11:22:00 am EST **Sessions III & IV - Closing Comments** (5 minutes)

11:27:00 am EST **Close Session III & IV**

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Thursday June 24, 2021

7:30 am EST Session V – Photoprotection and Photophysics

Moderators:

Professor Tomáš Polívka
Department of Physics, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Dr. Dariusz Niedzwiedzki,
Center for Solar Energy and Energy Storage, Department of Energy, Environmental and Chemical Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA.

7: 34:30 am EST Session V

1. HARNESSING NATURAL VARIATION IN PHOTOPROTECTION: RAPID NON-PHOTOCHEMICAL QUENCHING (NPQ) RELAXATION IN FERNS

Abstract 35

Presenter: Ms. NINA M. MARYN
Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720 (email: nina.maryn@berkeley.edu)

2. DIRECT PROBING OF THE (FORBIDDEN) S1 STATE OF CAROTENOIDS VIA TWO-PHOTON EXCITATION

Abstract 36

Presenter: Professor TOMÁŠ POLÍVKA
Department of Physics, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic (e-mail: tpolivka@jcu.cz)

3. SINGLET FISSION IN NATURALLY ORGANIZED CAROTENOIDS

Abstract 37

Presenter: Dr. MANUEL J. LLANSOLA-PORTOLES
Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France (email: manuel.llansola@i2bc.paris-saclay.fr).

4. TIME-RESOLVED SPECTROSCOPY OF TRAPPED ORANGE CAROTENOID PROTEIN

Abstract 38

Presenter: Mr. JAMES P. PIDGEON
Department of Physics and Astronomy, University of Sheffield, Sheffield, UK. (email: jppidgeon1@sheffield.ac.uk)

5. CONTROLLING CT STATES IN CHLOROPHYLL AND CAROTENOID COMPLEX

Abstract 39

Presenter: Dr. MINDAUGAS MACERNIS
Institute of Chemical Physics, Faculty of Physics, Vilnius University, Lithuania (email: mindaugas.macernis@ff.vu.lt)

8:17:00 am EST **Session V – Questions and Discussion** (15 min)
Moderators: Prof. Tomáš Polívka and Dr. Dariusz Niedzwiedski

8:32:00 am EST **Break** (4 minutes)

8:36:00 am EST **Session VI – Carotenoids and Environment**

Moderators:

Professor Eleanore Wurtzel
Department of Biological Sciences, Lehman College, The City University of New York, New York.

Professor Kevin McGraw
School of Life Sciences, Arizona State University, Tempe, Arizona.

1. UNRAVELING THE EVOLUTIONARY HISTORY OF CAROTENOID MODIFICATION WITHIN STICK AND LEAF INSECT CAMOUFLAGE (INSECTA: PHASMATODEA)

Abstract 40

Presenter: Mr. ROYCE T. CUMMING
Biology PhD Program, The Graduate Center, The City University of New York (CUNY), NY, USA; Dept. of Biological Sciences, Lehman College, CUNY, NY, USA; Richard Gilder Graduate School, The American Museum of Natural History, NY, USA (email: rcumming@gradcenter.cuny.edu)

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2. URBANIZATION ALTERS CAROTENOID METABOLIC AND ESTERIFICATION PROCESSES UNDERLYING SEXUALLY ATTRACTIVE MALE PLUMAGE COLORATION IN A COSMOPOLITAN NORTH AMERICAN BIRD SPECIES

Abstract 41

Presenter: Professor KEVIN J. MCGRAW
School of Life Sciences, Arizona State University, Tempe, AZ, USA 85287-4501 (email: kjmcgraw@asu.edu).

3. STRUCTURE ELUCIDATION OF THE NOVEL CAROTENOID GEMMATOXANTHIN FROM THE PHOTOSYNTHETIC COMPLEX OF GEMMATIMONAS PHOTOPHICA AP64.

Abstract 42

Presenter: Dr. NUPUR
Centre Algatech, Institute of Microbiology of the Czech Acad. Sci., 379 81 Třeboň, Czechia (email: nupur@alga.cz)

4. CAROTENOIDS IN THERMAL ADAPTATION OF PLANTS AND ANIMALS

Abstract 43

Presenter: Dr. IVAN M. PETYAEV
Lycotec, Cambridge, UK (email: Petyaev@lycotec.com)

9:14:30 am EST **Session VI – Questions and Discussion** (15 min)
Moderator: Prof. Eleanore Wurtzel and Prof. Kevin McGraw

9:29:30 am EST **Break** (11 minutes)

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8:40:30 am EST **Session VII – Carotenoid Biosynthesis & Biotechnology**

Moderators:

Dr. Antonio Meléndez Martinez
Universidad de Sevilla, Area of Nutrition and Food Science. Faculty of Pharmacy. Univers C/P. Garcia Gonzalez 2 Seville 41012, Spain

Professor Robin Ghosh
University of Stuttgart, Pfaffenwaldring 57, Stuttgart, Baden-Wuerttemberg, Germany.

1. CONTROL OF NEUROSPORAXANTHIN BIOSYNTHESIS IN THE FUNGUS *FUSARIUM FUJIKUROI* BY THE RING-FINGER PROTEIN CarS

Abstract 44

Presenter: Dr. M Carmen Limón
University of Sevilla, Faculty of Biology, Department of Genetics, Seville, Spain. M Carmen Limón (email: carmenlimon@us.es).

2. MANIPULATION OF CAROTENOID METABOLISM REDESIGN PLANT ARCHITECTURE, PLANT YIELD, AND METABOLISM IN CROP PLANTS

Abstract 45

Presenter: Dr. JUAN CAMILO MORENO BELTRAN
Max Planck Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg1 D-14476, Potsdam-Golm, Germany. (email: moreno@mpimp-golm.mpg.de)

3. HONEYSUCKLE RHODOXANTHIN SYNTHASE; FROM RED BERRY TO NOVEL BIOCHEMICAL PATHWAY

Abstract 46

Presenter: Dr. JOHN ROYER
DSM Nutritional Products, 60 Westview St, Lexington MA 02420.(email: john.royer@dsm.com)

4. A NOVEL CAROTENOID BIOSYNTHETIC ROUTE VIA OXIDOSQUALENE

Abstract 47

Presenter: Mr. YUSUKE OTANI
*Department of Applied Chemistry and Biotechnology, Chiba University, Chiba, Japan (*email: *y.otani@chiba-u.jp,)*

10:22:30 am EST **Session VII – Questions and Discussion** (15 min)

Moderators: Dr. Antonio Meléndez-Martinez and Prof. Robin Ghosh

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10:37:30 am EST **Break** (4 minutes)

10:41:30 am EST **Session VIII – Synthesis**

Moderators:

Professor Angel de Lera
Facultade de Quimica, University of Vigo, Vigo, Spain.

Dr. Hans-Richard Sliwka
Department of Chemistry, Norwegian University of Science, Trondheim N-7491 Norway.

1. DESIGNING NOVEL CAROTENOIDS FOR IMPROVED ANTIOXIDANT ACTIVITY – SYNTHESES AND DPPH / ABTS RADICAL ASSAYS

Abstract 48

Presenter: Professor SANGHO KOO
Department of Chemistry, Myongji University, Yongin, Gyeonggi-Do 17058, Korea (email: sangkoo@mju.ac.kr)

2. JOINING CAROTENOIDS AND POLYACETYLENES

Abstract 49

Presenter: Dr. HANS-RICHARD SLIWKA
Department of Chemistry, Norwegian University of Science and Technology, Trondheim (email: richard.sliwka@ntnu.no)

3. STEREOCONTROLLED SYNTHESIS OF 5,8-EPOXYCAROTENOIDS BY BIDIRECTIONAL HORNER-WADSWORTH-EMMONS REACTION

Abstract 50

Presenter: Dr. BELEN VAZ
Department of Organic Chemistry and Center for Biomedical Research (CINBIO), IBIV, Universidade de Vigo, 36310 Vigo, Spain. (email: belenvaz@uvigo.es)

11:11:00 am EST **Session VIII – Questions and Discussion** (15 min)
Moderators: Prof. Angel de Lera and Dr. Hans-Richard Sliwka

11:26:00 am EST **Sessions V, VI, VII & VIII - Closing Comments** (5 min)

11:31:00 am EST **Close Session V-VIII**

VICC 2021
International Carotenoid Society
June 22, 23, 24, 25

Friday June 25, 2021

7:30 am EST Session IX – Carotenoid and Apocarotenoid Biology

Moderators:

Professor Johannes von Lintig
Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA

Professor Salim Al-Babili
King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia.

7:39:30 am EST Session IX-A - Carotenoid and Apocarotenoid Biology

1. AN UPDATE ON THE PLANT APOCAROTENOID GROWTH REGULATORS ANCHORENE AND ZAXINONE

Abstract 51

Presenter: Professor SALIM AL-BABILI
Center for Desert Agriculture (CDA), Biological and Environment Science and Engineering (BESE), King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. (email: salim.babili@kaust.edu.sa).

2. THE APOCAROTENOID ZAXINONE IS A POSITIVE REGULATOR OF STRIGOLACTONE AND ABSCISIC ACID BIOSYNTHESIS IN ARABIDOPSIS ROOTS

Abstract 52

Presenter: Mr. ABDUGAFFOR ABLAZOV
The BioActives Lab, Center for Desert Agriculture, Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia (email: abdugaffor.ablazov@kaust.edu.sa)

3. ACTIVITY-DEPENDENT PALMITOYLATION OF CAROTENOID CLEAVAGE OXYGENASES: A COMMON THEME?

Abstract 53

Presenter: Dr. T. MICHAEL REDMOND
Laboratory of Retinal Cell & Molecular Biology, National Eye Institute, NIH, Bethesda MD 20892 (email: redmondd@nei.nih.gov)

4. STRUCTURAL AND FUNCTIONAL ANALYSIS OF CAROTENOID OXYGENASE 2

Abstract 54

Presenter: Dr. SEPALIKA BANDARA
Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA; (email: sxb1081@case.edu)

5. HUMAN β-CAROTENE OXYGENASE 2 DEMONSTRATES ENZYMATIC ACTIVITY TOWARDS CAROTENOIDS AND APOCAROTENOIDS

Abstract 55

Presenter: Ms. LINDA D. THOMAS
Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA; (email: ldt29@case.edu)

8:17:00 am EST **Session IX-A – Questions and Discussion** (15 min)
Moderators: Prof. Johannes von Lintig and Prof. Salim Al-Babili

8:32:00 am EST **Break** (4 minutes)

8:36:00 am EST **Session IX-B – Carotenoid and Apocarotenoid Biology**
Moderators: Prof. Johannes von Lintig and Prof. Salim Al-Babili

6. REGULATION OF PLASMA CHOLESTEROL BY B-CAROTENE AND B-CAROTENE OXYGENASE 1 IN MICE

Abstract 56

Presenter: Ms. JOHANA CORONEL
Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL (email: acoronel@illinois.edu)

7. LACK OF BETA-CAROTENE 9',10' DIOXYGENASE (BCO2) IMPAIRS CARDIAC METABOLIC ADAPTATIONS IN ADULT FEMALE MICE

Abstract 57

Presenter: Ms. Chelsee Holloway
Endocrinology and Animal Biosciences Graduate Program;
Department of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Jersey;
(email: cth62@scarletmail.rutgers.edu)

8. LRAT COORDINATES THE NEGATIVE-FEEDBACK REGULATION OF INTESTINAL RETINOID BIOSYNTHESIS FROM β-CAROTENE

Abstract 58

Presenter: Dr. RAMKUMAR SRINIVASAGAN
Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH, USA (email: rxs787@case.edu)

9. A NOVEL ROLE OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR IN CAROTENOID BIODISTRIBUTION

Abstract 59

Presenter: Mr. WALTER CATALAN
Department of Food Science and Human Nutrition, University of Illinois Urbana Champaign, Urbana, IL, USA (email: walter24@illinois.edu)

10. IDENTIFICATION OF NOVEL FUCOXANTHIN CLEAVAGE METABOLITES AND ANTI-INFLAMMATORY ACTION AGAINST ACTIVATED MACROPHAGES

Abstract 60

Presenter: Dr. NAOKI TAKATANI
Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan. (email: n-takatani@fish.hokudai.ac.jp)

9:18:00 am EST **Session IX-B** **Questions and Discussion** (15 min)
Moderators: Prof. Johannes von Lintig and Prof. Salim Al-Babili

9:33:00 am EST **Break** (11 minutes)

VICC 2021
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8:44:00 am EST **Session X-A** **Applications of Biotechnology and Analytical Methodology**

Moderators:

Dr. Neal Craft
Eurofins Craft Technologies, Inc., 4344 Frank Price Church Rd Wilson, North Carolina.

Dr. Andre Duesterloh
DSM, Wurmisweg 576, Kaiseraugst, Switzerland.

1. USING POLARIZED RAMAN SPECTROSCOPY TO DISTINGUISH MESO-ZEAXANTHIN FROM ZEAXANTHIN

Abstract 61

Presenter: Mr. NATHAN GIAUQUE
The University of Utah, Moran Eye Center, 65 Mario Capecchi Dr, Salt Lake City, Utah, United States of America, 84132 (email: Nathan.Giauque@hsc.utah.edu)

2. EXTRACTION OF CAROTENOIDS FROM PITANGA A SCREENING WITH IONIC LIQUIDS, SURFACTANTS AND EUTECTIC SOLVENTS

Abstract 62

Presenter: Ms. BRUNA VITÓRIA NEVES
Department of Biosciences, Federal University of São Paulo, Street: Silva Jardim, 136, Zip code: 11015-136, Santos, São Paulo, Brazil. (email: brunavitorianeves@hotmail.com)

3. IMPACT OF HIGH PRESSURE PROCESSING ON BIOACTIVE PLANT INGREDIENTS IN KALE

Abstract 63

Presenter: Mr. MARIO SCHMIDT
Institute of Nutritional Sciences, Friedrich Schiller University Jena, 07743 Jena, Germany (email: mario.schmidt@uni-jena.de)

4. NEW TECHNIQUES TO RECOVER AND RECYCLE IONIC LIQUIDS USED AS SOLVENTS ON THE EXTRACTION OF CAROTENOIDS FROM AMAZON FRUITS

Abstract 64

Presenter: Mr. LEONARDO M. DE SOUZA MESQUITA
Department of Biosciences, Federal University of São Paulo (UNIFESP), Silva Jardim Street, 136, Vila Mathias, 11015-020, Santos, SP, Brazil, (email: mesquitalms@gmail.com)
CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

10:22:30 am EST **Session X-A – Questions and Discussion** (15 min)
Moderators: Dr. Neal Craft and Dr. Andre Duesterloh

10:37:30 am EST **Break** (4 minutes)

VICC 2021
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10:41:30 am EST **Session X-B** **Applications of Biotechnology and Analytical Methodology**

Moderators: Dr. Neal Craft and Dr. Andre Duesterloh

5. FREE- AND ESTERIFIED-CAROTENOIDS IN OILY AND NON-OILY FRUIT MATRICES: AN OPTIMISED PROCEDURE OF SAPONIFICATION FOR CAROTENOID QUANTIFICATION

Abstract 65

Presenter: Dr. HUNG TRIEU HONG
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia. (email: h.trieu@uq.edu.au)

6. UHPLC-MS BASED PLANT APOCAROTENOMICS ANALYTICAL PLATFORM

Abstract 66

Presenter: Dr. Jianing Mi
Center for Desert Agriculture (CDA), Biological and Environment Science and Engineering (BESE), King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. (email: salim.babili@kaust.edu.sa).

7. PRESSURE-MEDIATED REFLECTION SPECTROSCOPY DEMONSTRATES STRONG CRITERION VALIDITY AS A BIOMARKER OF FRUIT AND VEGETABLE INTAKE COMPARED TO PLASMA CAROTENOID CONCENTRATIONS: A US MULTI-CENTER CROSS-SECTIONAL STUDY ACROSS FOUR RACIAL/ETHNIC GROUPS.

Abstract 67

Presenter: Professor STEPHANIE B. JILCOTT PITTS
Dept. of Public Health, East Carolina University, 115 Heart Drive, Greenville NC 27834 (email: jilcotts@ecu.edu)

8. A CAROTENOID SPECTRAL LIBRARY COMBINING UV/VIS AND HIGH-RESOLUTION MS DATA

Abstract 68

Presenter: Dr. Richard Gössl
DSM Nutritional Products Ltd., Wurmisweg 576, Kaiseraugst, Switzerland.

11:16:00 am EST **Session X-B – Questions and Discussion** (15 min)

Moderator: Dr. Neal Craft and Dr. Andre Duesterloh

11:31:00 am EST **Sessions IX & X - Closing Comments** (5 min)

VICC 2021
International Carotenoid Society
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11:36:00 am EST **Summary and Concluding Remarks VICC 2021**

11:46:00 am EST **VICC 2021 Concludes**

Session IA Abstracts Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism

Abstract 1

ANTI-TUMORIGENIC EFFECTS OF β-CRYPTOXANTHIN VIA MODULATING CANCER CELL METABOLISM

XIANG-DONG WANG and Ji Ye Lim.

Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA. Email: xiang-dong.wang@tufts.edu; *jiye.lim@tufts.edu*

Introduction: Epidemiological studies have suggested that a high refined carbohydrate diet (HRCD) containing an excessive amount of sugar contributes to the development of nonalcoholic fatty liver disease (NAFLD) and liver cancer (hepatocellular carcinoma, HCC). Dietary carotenoid intervention represents a promising disease control strategy for the prevention of NAFLD and HCC development. Multiple avenues of mechanistic evidence from pre-clinical studies, indicating that the preventive effects of a pro-vitamin A carotenoid β-cryptoxanthin (BCX) were likely due to biological activities of its intact molecule and metabolites (retinoids and apo-carotenoids) produced by β-carotene-15, 15'-oxygenase (BCO1) and β-carotene-9', 10'-oxygenase (BCO2))[1]. Cancer cells have characteristic changes in their metabolic programs, including increased uptake of glucose and glycolysis, and production of lactate. Since this metabolic shifts supports tumor cells growth, survival and metastasis, we investigated whether β-cryptoxanthin treatment can inhibit HRCD-promoted HCC progression by modulating cancer cell metabolism in vivo.

Methods and Materials: Two-week-old male wild-type (WT) and BCO1$^{-/-}$/BCO2$^{-/-}$ double knock-out (DKO) mice were given i.p. injections of diethylnitrosamine to induce HCC. At six weeks of age, all animals were fed HRCD (66.5 % of energy from carbohydrate consisting of sucrose and maltodextrin) with or without BCX (10 mg/kg diet) for 24 weeks. Pathological and biochemical variables were analyzed in the liver. Data were analyzed by 2-factor ANOVA.

Results: Compared to their respective HRCD littermates, both WT and DKO fed BCX had significantly lower HCC multiplicity (58-60%), average tumor size (21-24%), and total tumor volume (51-58%), and the steatosis scores of NAFLD. BCX feeding increased hepatic vitamin A levels in WT mice, but not in DKO mice that showed a significant accumulation of hepatic BCX. The protein levels of lactate dehydrogenase, an important enzyme regulating glycolysis and HCC proliferation were significantly decreased by BCX supplementation in the liver tumors of WT and DKO mice. The chemopreventive effects of BCX were associated with increased p53 protein acetylation (active form of p53) and gluconeogenesis markers (phosphoenolpyruvate carboxykinase and glucose 6-phosphatase) and decreased protein levels of the hypoxia-inducible factor-1α and its downstream targets, matrix metalloproteinase 2/9 in tumors.

Conclusions/Discussion: The protective effects of dietary β-cryptoxanthin against NAFLD[2] and HCC[3] are achieved through different molecular mechanisms and genetic signaling pathways depend or independent on the carotenoid cleavage enzymes or with or without a significant alteration of vitamin A status. This study suggests that BCX supplementation modulates the activation of p53, hypoxic tumor microenvironment and glucose metabolism in the tumors, which may contribute to the inhibition of HCC progression and metastasis.

Acknowledgements: This work was supported by the USDA/ARS grant (8050-51000-096-02S).

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2) Lim JY, Liu C, Hu KQ, Smith DE, Wu D, Lamon-Fava S, Ausman LM, Wang X-D. Dietary β-cryptoxanthin prevents inhibits high-refined carbohydrate diet-induced fatty liver via differential protective mechanisms depending on carotenoid cleavage enzymes in male mice. *J Nutr*. 149: 1553–1564, 2019;

3) Lim JY, Liu C, Hu KQ, Smith DE, Wu D, Lamon-Fava S, Ausman LM, Wang X-D. Xanthophyll β-cryptoxanthin prevents inhibits high-refined carbohydrate diet-promoted hepatocellular carcinoma in mice. *Mol Nutr Food Res. 64, 1900949,* 2020

Abstract 2

Study on the solvent dependence of antioxidant capacity of selected carotenoids

a KATALIN BÖDDI, a Dalma Czett, a Attila Agócs, a József Deli, b Tibor Kurtán, b Sándor Balázs Király, c Ákos Kuki, c Tibor Nagy, a Veronika Nagy*

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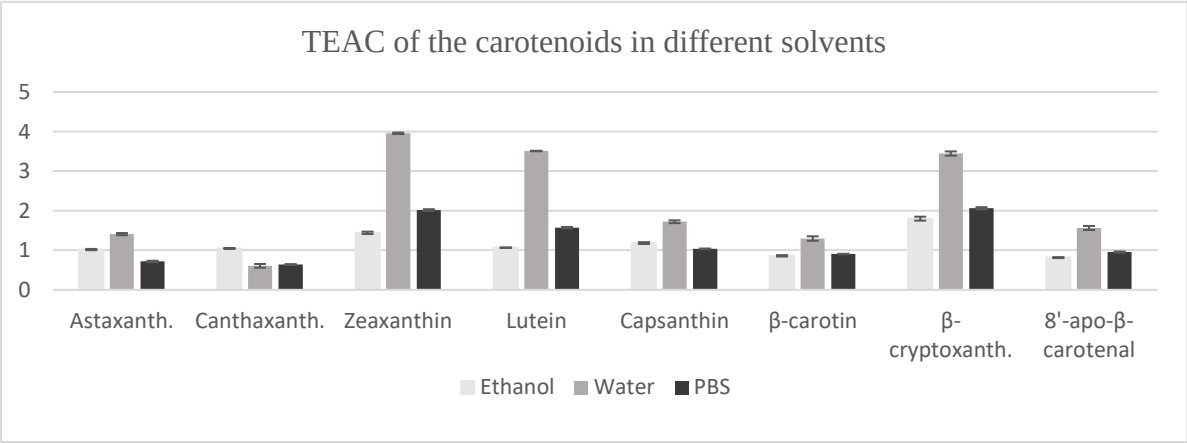
Introduction:
The carotenoids are hydrophobic compounds forming different type of aggregates in their aqueous solution with different antioxidant behavior.

Methods and Materials:
The antioxidant activity of the carotenoids was examined in different solvents by the ABTS method [1]. The different aggregates were studies with dynamic light scattering and circular dichroism spectroscopy.

Discussion
The carotenoids have increased antioxidant capacity in water compared to ethanolic or phosphate-buffered saline (PBS) solution, and there is a correlation between the TEAC values and the type or the size of the aggregates.



Acknowledgements:
This research was funded by Hungarian Scientific Research Fund (grants: NKFI K 115931 and 131493) and supported by PTE ÁOK-KA-2020-29. The authors are grateful to Mrs. Erika Jámbor, Mrs. Krisztina Sajti, Mrs. Judit Rigó and Mr. Roland Lukács for their skillfull assistance.

[1] I. Línzembold et al. *Molecules*, **25**, 636;(2020)

Abstract 3

A Dunaliella salina extract counteracts skin anti-aging under intense solar irradiation thanks to its anti-glycation and anti-inflammatory properties.

FABIEN HAVAS[1]; Shlomo Krispin[1]; Moshe Cohen[1]; Estelle Loing[2]; Morgane Farge[3]; Thierry Suere[3]; Joan Attia[3]*.

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**Corresponding Author*

Introduction: The glycation process is involved in both intrinsic (individual, genetic) and extrinsic (ultraviolet light, pollution and lifestyle) skin aging. UV-induced intracellular buildup of Advanced Glycation End products (AGEs) can damage skin proteins, cause the generation of reactive oxygen species, and trigger inflammatory responses – and is considered a major factor in skin aging.
Dunaliella salina is a halophile green unicellular microalga, notable for its adaptation to intense solar irradiation through its ability to produce large amounts of carotenoids, including beta-carotene and its colorless precursors phytoene and phytofluene. Carotenoids like beta-carotene may have anti-glycation benefits, however their intense coloration presents a challenge to cosmetic formulators – unlike the colorless phytoene and phytofluene. *Dunaliella salina* also produces valuable fatty acids and derivatives, including the ω-3 unsaturated fatty acid eicosapentaenoic acid (EPA). Here, we present results obtained with a supercritical CO_2 extract of *Dunaliella salina* rich in phytoene and phytofluene.

Materials & Methods: After harvesting, the alga is dried and extracted with supercritical CO_2, and the resulting oleoresin is taken up in a carrier oil (jojoba oil) and further purified to remove colored components, retaining the colorless carotenoids. The final extract is standardized for phytoene and phytofluene content.
The extract was tested *ex-vivo* on human skin explants, where its effect on glycation was evaluated by immunohistochemistry dosage of N^{ϵ}-(Carboxymethyl)lysine (CML), quantified by image analysis. Methylglyoxal was used to stimulate glycation. In a separate ex-vivo model, the extract's anti-inflammatory effect was evaluated by dosage of IL6 and IL8 (by ELISA) and NRF2 (by immunohistochemistry and image analysis).
This was followed with a 56-day double-blind, placebo-controlled clinical study on 25 female volunteers, aged 35-60, and submitted to intense and prolonged solar exposure (beach, during the summer months). Panelists applied a formulation containing 1% *Dunaliella salina* extract and a corresponding placebo, in a split-face manner. Glycation status was evaluated with the AGE reader (Diagnoptics Technologies, Netherlands). Anti-inflammatory effects were evaluated through microcirculation with concomitant histamine challenge (Periflux Laser Doppler flowmetry, Perimed, Sweden) and through evaluation of red spots (Visia-CR image analysis, Canfield, USA). Finally, wrinkles were evaluated by 3D image analysis (AEVA-HE, Eotech, France).

Results: In ex-vivo testing, the extract exhibited anti-glycation and anti-inflammatory activities: human skin explants exposed to methylglyoxal showed strongly reduced formation of N-ε-carboxy-methyl-lysine with concurrent treatment with the extract, by up to 68%. Explants treated with the extract showed significant reductions in production of key interleukins IL6 and IL8 (by 26% and 35%, respectively), and increased production of NRF2 (+19%).
The above data were borne out in the clinical trial, where the active significantly improved the skin's glycation scores; reduced its reaction to histamine (up to +32% in reaction onset time, and -13% in peak intensity, vs. placebo), and the occurrence of red spots (up to -26% vs. placebo); and, showed a significant anti-aging effect, through reduced wrinkle counts and volumes (up to -32% and -35% respectively, vs. placebo) (all with $p<0.05$).

Conclusion: These results demonstrate the value of this *Dunaliella salina* extract, rich in the colorless carotenoids phytoene and phytofluene and in the ω-3 unsaturated EPA, as an antiglycative, anti-inflammatory and anti-aging active ingredient, including in high solar irradiation contexts.

Abstract 4

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

Aya Darawshe, Marina Hanin, Hilla Ovadia, Joseph Levy and Yoav Sharoni
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Beer-sheva, Israel
ayadar@post.bgu.ac.il

introduction: Skin ageing is influenced by several factors including environmental and UV-exposure, hormonal changes and oxidative stress**.** Reactive oxygen species (ROS) induces inflammatory processes in the skin and cause severe damage to skin cells, which leads to cumulative alterations of skin structure and appearance called skin ageing. ROS such as H_2O_2 increase the production of matrix metalloproteinase (MMPs) in dermal fibroblasts, leading to collagen degradation. previous studies have shown that carotenoids and polyphenols activate the antioxidant defense system by inducing Nrf2 transcriptional activity (1,2). The aim of the current study was to examine the protective effect of dietary compounds, estradiol and their combination on skin cells under oxidative stress.

Research & Methods: We used Normal Human Dermal Fibroblasts (NHDF) and examined cell viability that was determined using the XTT cell proliferation kit. MMP1 and pro-collagen 1a1 levels were measured by ELISA assay as markers of skin damage. Expression of collagen 1a1 was measured by RT-PCR. ARE activity was determined by Reporter Gene Assay, and NQO-1 protein level was detected by Western blot assay. ROS level were determined by flow cytometry, using DCFH.

Results: Treatment of the cells with H_2O_2 led to cell death, increased secretion of MMP1 and reduced expression and secretion of pro-collagen 1a1. Pretreatment with tomato extract containing lycopene and tomato extract containing phytoene and phytofluene, and with estradiol, increased cell viability, reduced MMP1 secretion and increase pro-collagen1a1. These effects were associated with reduced ROS. Combinations of the tomato extract with rosemary extract containing Carnosic acid and with estradiol, increased the protective effects. The results suggest that tomato extract and rosemary extract cooperate with estradiol to protect dermal fibroblasts from oxidative damage, and thus may improve skin health and delay skin ageing.

References:

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2. Inoue Y, Shimazawa M, Nagano R, Kuse Y, Takahashi K, Tsuruma K, Hayashi M, Ishibashi T, Maoka T, Hara H. Astaxanthin analogs, adonixanthin and lycopene, activate Nrf2 to prevent light-induced photoreceptor degeneration. J Pharmacol Sci, 134(3), 147-157. 2017.

Abstract 5

Effect of Palm Mix Carotenes (PMC) against Oxidative Stress Induced Age-related Macular Degeneration (AMD) in Human Retinal Pigment Cells

PUVANESWARI MEGANATHAN[1], Chun-Wai Mai[2,3], Kanga Rani Selvaduray[1], Zaida Zainal[1] and Ju-Yen Fu [1]

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[2]State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Clinical Stem Cells Research Center, Department of Urology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, 200127, Shanghai, China.
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Abstract

Introduction: Oxidative stress is a condition caused by imbalance between accumulation of Reactive Oxygen Species (ROS) and their elimination. Consequently, excessive accumulation of ROS leads to chronic inflammation that may be the underlying root for many diseases as well as acceleration of the ageing process. A well-established example of a condition caused by ROS build-up is age-related macular degeneration (AMD) that is an irreversible eye condition commonly affecting the aged population worldwide. The pathogenesis of the disease is linked with excessive amount of ROS that causes apoptosis in the retinal pigment epithelium (RPE) and photoreceptors. Carotenoids are well established antioxidants in managing AMD either by intake of fruits and vegetables rich in carotenoids or via supplementation with beneficial carotenes or xantophylls. This study determined the efficacy of Palm Mix Carotenes (PMC) in modulating molecular targets implicated in oxidative stress induced inflammation (oxi-inflamm) and in conferring protection against AMD induced by oxidative stress in human retinal pigment epithelium (RPE) cells.

Material and Method: A panel of 17 human cancer cells and the non-cancerous human retinal epithelial cells (ARPE-19) were treated with palm carotene extract in order to determine whether PMC can induce cancer proliferation or antiproliferative effects. To determine whether ARPE19 can be protected from oxidative stress, ARPE19 cells was also pre-treated with PMC prior to exposure to hydrogen peroxide (H_2O_2) challenges, as a positive control to induce oxidative stress *in vitro*. PCR array study was conducted to evaluate the efficacy of PMC in modulating oxidative stress and its underlying molecular targets were also being studied using targeted reporter assays.

Results: The PMC was found neither inducing significant cancer proliferation nor cytotoxic to ARPE-19, suggesting PMC has no potent anticancer effect, and could be a safe therapeutic intervention. The protective PMC effect was observed at the concentration of 1.56-25 µg/mL in ARPE-19 cells challenged with 500 µM H_2O_2. In PCR array, PMC significantly reversed H_2O_2 induced damage in several key genes involved in AMD such as *TIMP* and *C5*. Reactome pathway analysis suggested immune system related pathways could be attributed to its oxidative stress protective effect. We confirmed PMC also showed similar activation as GW3965, a synthetic

agonist for Liver-X-Receptor (LXR) in target specific screening, suggesting its protective effect could be related to LXR, but not p53, NFκB and PPARγ.

Conclusion: The downregulation of key genes reported in AMD as well as activation of LXR indicate that PMC should be explored further as a nutraceutical in managing AMD by mitigating the ill-effects of oxidative stress and inflammation.

Acknowledgement:

We thank the Malaysian Palm Oil Board for funding this research.

[1] Age-Related Eye Disease Study Research Group. (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Archives of ophthalmology*, **119(10),** 1417-1436.

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Session IB Abstracts Carotenoids and Health I: Antioxidant Properties & Modulation of Metabolism

Abstract 6

CAROTENOID-MELATONIN CONJUGATES: SYNTHESIS AND ANTIOXIDANT CAPACITY

Dalma Czett[a], Katalin Böddi[a], VERONIKA NAGY[a], Anikó Takátsy[a], József Deli[a], Paul Tone[b], Attila Agócs[a,*]

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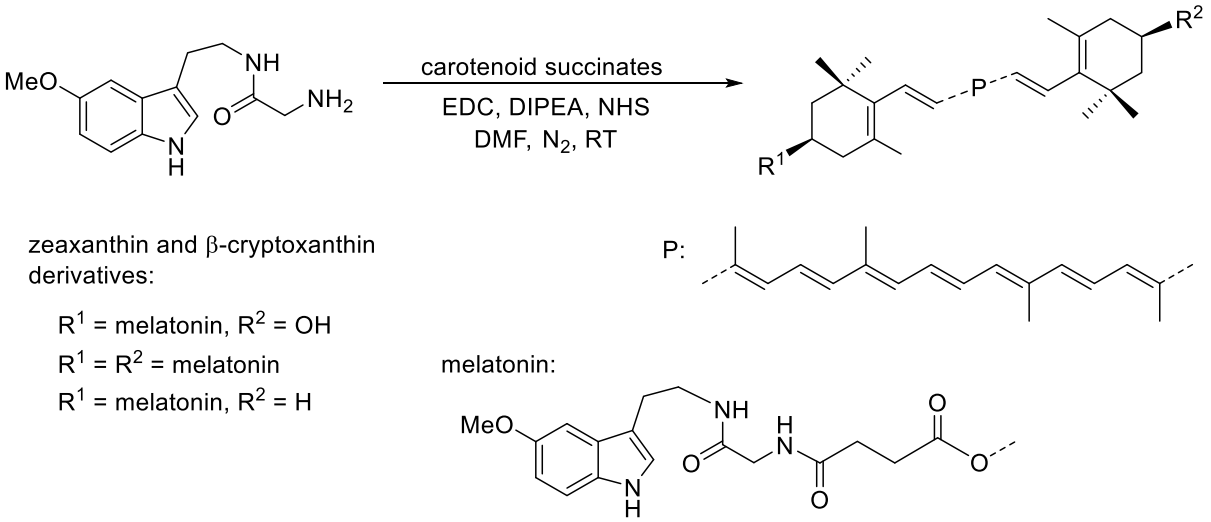
**Corresponding Author (email: attila.agocs@aok.pte.hu)*

Introduction: Melatonin is one of the best natural antioxidants and radical scavengers. It is a small size amphiphilic substance, fairly soluble both in fats and in water, preferentially located at hydrophilic/hydrophobic interfaces, and can easily cross all the anatomic barriers and distribute throughout the cell. In this work we present the covalent coupling of carotenoids with melatonin. The conjugation could improve the cell membrane penetration of the carotenoid due to melatonin moiety, hence these hybrid bifunctional molecules could show enhanced intracellular antioxidant activity.

Methods and Materials: Antioxidant behavior was measured with TEAC/ABTS and the FRAP methods in different solvents.

Results and Discussion: Carotenoid succinates were synthesized from hydroxy carotenoids and were coupled to a commercially available derivative of melatonin via amide bond in acceptable to good yields. Succinylation highly increased the water solubility of the carotenoids, while the conjugation with melatonin resulted in more lipophilic derivatives. Antioxidant behavior was determined for the carotenoids, the carotenoid succinates, and the conjugates with melatonin. In these measurements, a strong dependence on the quality of the solvent was observed. TEAC values of the new derivatives in phosphate-buffered saline were found to be comparable to or higher than those of parent carotenoids, however, synergism was observed only in FRAP assays.



Acknowledgements:
This research was funded by Hungarian Scientific Research Fund (grants: NKFI K 115931 and 131493) and supported by PTE ÁOK-KA-2020-29.

Abstract 7

PROTECTIVE EFFECTS OF INDIVIDUAL AND COMBINED BETA-CAROTENE AND METFORMIN TREATMENT AGAINST OBESITY AND OBESITY COMORBIDITIES IN MICE

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Introduction: The combination of pharma drugs with natural bioactive compounds is a potential strategy to simultaneously tackling the same or different health/therapeutic targets in cumulative or synergistic manners, resulting in more effective treatments, decreased drug doses or an improved patient's response to treatment. We aimed to investigate if physiological/low doses of beta-carotene and metformin are effective in ameliorating diet-induced obesity and its comorbidities, and whether their combination can counteract unwanted effects of obesogenic high-fat diet feeding more effectively than the individual components.

Research and methods: Forty-five C57BL/6 adult male mice pre-habituated to a normal fat diet (NFD, 10% energy as fat) were distributed into 4 groups (9 animals/group): normal fat (NF), high fat (HF), beta-carotene supplemented (BC, 3 mg/kg/day), metformin supplemented (MET, 100 mg/kg/day), and combination of BC and MET supplemented (BC+MET). The supplements were orally administered daily; the animals in the NF and HF groups received the vehicle. After one week of supplementation, the HF, BC, MET, and BC+MET groups were shifted to an HF diet (HFD, 45% energy as fat) for four weeks, while the NF group remained on the NFD. Biometric and glucose control-related parameters were monitored, and tissues were collected for gene and protein expression and morphology/ immunohistochemistry analyses.

Results and Discussion: Cumulative food intake was not different between groups. HFD-induced increases in body weight gain and inguinal white adipose tissue (WAT) adipocyte size were attenuated selectively in the BC+MET group, in which a redistribution towards smaller adipocytes was noted. HFD-induced increases in fasting blood glucose, insulin and HOMA-IR index were attenuated in the BC group and, more robustly, the BC+MET group, but not the MET group. Rectal temperature by the end of the HFD period was increased in the BC group relative to the HF group, paralleling increased UCP1 protein levels in brown adipose tissue. Liver total lipid content tended to be lower in the groups receiving BC, especially the BC+MET group, in which the hepatic expression of autophagy-related genes (*Becn1, Foxo3a, Atg7*) and of *Pgc1a* was maximal. Transcriptional and morphological analyses of WAT did not reveal signs of browning. However, in the retroperitoneal (visceral) WAT depot, MET and especially the BC+MET combined treatment repressed lipogenic *Pparg* and *Glut4* gene expression, and counteracted an HFD-induced downregulation of the expression of lipid catabolism-related genes (*Cpt1b* and *Ppara*). The results sustain beneficial effects of low doses of BC and metformin against obesity and its metabolic burden, and specific benefits of the BC+MET combination.

Acknowledgements: Supported by the Spanish Government (grant AGL2015-67019-P to A.P.). Authors are members of EU Cost Action EUROCAROTEN (Action CA15136).

Abstract 8

PLASMA CAROTENOID CONCENTRATIONS ARE INFLUENCED BY CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS

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Introduction: Chemotherapy upregulates inflammatory processes, as measured by circulating concentrations of pro-inflammatory cytokines and their signaling lipids.[1] Previous studies in breast cancer survivors have reported an inverse correlation between dietary and total plasma carotenoid concentration and oxidative stress (as determined via urinary 8-hydroxy-2′-deoxyguanosine concentrations),[2] and a reduced risk of breast cancer reoccurrence.[3] However it is not known whether breast cancer treatment itself influences plasma carotenoid concentrations. Objective was to measure the change in circulating concentrations of carotenoids and fat-soluble vitamins in free-living breast cancer patients both before and after treatment.

Research & Methods: Serum samples were collected from patients (n=34) immediately prior to standard adjuvant and neo-adjuvant chemotherapy for breast cancer, and 4 months following chemotherapy commencement. Patient multivitamin and non-steroidal anti-inflammatory (NSAID) drug use was noted at both visits. Lipophilic extracts were analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to quantify α- and β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin, retinol, α-tocopherol and phylloquinone. Linear mixed models were developed to assess the relationship between the main factors (i.e. chemotherapy, multivitamin, and NSAID use), and their interaction effects, on serum carotenoid and fat-soluble vitamin concentrations. Random effects included a fixed intercept for each subject.

Results and Discussion: Chemotherapy was significantly associated with reduced serum concentrations of α-carotene (P = 0.053) and retinol (P = 0.042), with a trend observed for reduced β-carotene (P = 0.076) and phylloquinone (P = 0.082). There was no main effect of multivitamin or NSAID use on any analytes investigated. An interaction effect was observed for chemotherapy*multivitamin use, with increased concentrations of serum retinol (P = 0.004) and lycopene (P = 0.004), and a trend observed for zeaxanthin (P = 0.087) for those who took multivitamins. Chemotherapy*NSAID use was also significantly associated with a trend in increased serum lutein (P = 0.061) for those who consumed NSAIDS. Our results suggest randomized, controlled trials of multivitamin use and/or provitamin A carotenoid-rich food consumption merit further investigation in patients undergoing chemotherapy treatment.

Acknowledgements

This research was supported by The Ohio State University Stefanie Spielman Breast Cancer Center Kroger Fund, a Pelotonia Fellowship awarded to D.B., NIH R01CA189947, and sample analyses partially supported by NIH P30 CA016058, OSU, and OSUCCC.

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Abstract 9

Pilot Feasibility and Validity Assessment of Reflection Spectroscopy-measured Skin Carotenoid Score as a Biomarker of Carotenoid Intake in 4- and 8-month-old Infants

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Introduction: Skin carotenoid measurement by reflection spectroscopy offers a rapid, non-invasive assessment of carotenoid intake and status. However, feasibility and validity have not been established in infants. We hypothesize that skin carotenoid measurement of infants' heels and fingers will be feasible and valid.

Methods and Materials: Skin carotenoid scores (SCS) were measured by a modified, portable reflection spectroscopy device (Veggie Meter, Longevity Link) at the index finger and heel of the foot in infants at 4- (n=21) and 8- (n=9) months-of-age. Four-month-old infant plasma (n=11) and mothers' breastmilk (n=16) carotenoid concentrations (alpha- and beta-carotene, lutein and zeaxanthin, beta-cryptoxanthin, and total lycopene) were measured by HPLC-PDA, while 8-month-old infant carotenoid (alpha- and beta-carotene, lutein and zeaxanthin, beta-cryptoxanthin, and total lycopene) intake was estimated from 7-day food diaries. Feasibility was defined as a time-to-acquire triplicate measures of <300 sec. Validity was established in 4-month-olds if SCS were moderately correlated (r>0.5) with breastmilk carotenoid concentrations during exclusive milk-feeding and with infant plasma carotenoid concentrations, and in 8-month-olds if SCS were moderately correlated with total dietary carotenoid intake.

Results: Twenty-one infants (52% female) were enrolled (47% non-Hispanic white, 29% Hispanic white, 14% African American, and 10% Asian). Due to the pandemic, there was high loss to follow-up. Triplicate heel and finger SCSs were feasibly acquired in 56±11 to 87±32 seconds in 4- and 8-month olds. Mean heel and finger SCSs were highly correlated within-subjects in 4- and 8-month-olds (r=0.81, *P*=1x10^-5; r=0.90, *P*=7.6 x 10^-4, respectively). Finger and heel SCSs were valid correlates of 4-month-old total plasma carotenoid concentrations (r=0.77, P=0.006 and r=0.66, P=0.026, respectively, n=11). Finger SCS of exclusively milk-fed 4-month-olds was significantly correlated with breastmilk carotenoid concentration (r=0.66, *P*=0.006), while heel SCS weakly, non-significantly correlated (r=0.40, *P*=0.13). Finger and heel SCS were significantly correlated with daily total carotenoid intake in complementary-fed 8-month-olds (r=0.83, *P*=0.005 and r=0.80, *P*=0.009, respectively).

Conclusions: This pilot suggests that 4- and 8-month-old infant skin carotenoids can be feasibly measured by reflection spectroscopy and that finger SCS are correlated with both infant plasma and maternal breastmilk carotenoid concentrations at 4-months and total carotenoid intake at 8-months-of-age. Skin carotenoid measurement by reflection spectroscopy should be further investigated to facilitate the assessment of infant dietary intake and health outcomes.

Funding Sources: Texas Children's Hospital, USDA-ARS

Session IIA Abstracts Carotenoids and Health II: Supplementation and Bioavailability

Abstract 10

PRENATAL SUPPLEMENTATION OF ZEAXANTHIN PROMOTES BETTER RETINAL VASCULAR REGROWTH IN OXYGEN INDUCED RETINOPATHY (OIR) MODEL IN *BCO2$^{-/-}$ KO MICE*

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Introduction: Retinopathy of prematurity (ROP) is an ocular pathology affecting premature infants and is one of the leading causes of juvenile blindness worldwide. Premature infants at risk for ROP are known to be severely carotenoid deficient because they miss out on the placental transfer of carotenoids from their mother during the third trimester. Carotenoids are not typically part of preterm maternal nutrition formula. We, therefore, hypothesized that prenatal maternal supplementation of macular carotenoids prior to preterm birth could alleviate ROP pathology. To study the possible beneficial effect of carotenoids on prenatal and postnatal feeding in a mouse model of ROP using oxygen-induced retinopathy (OIR), we used β, β-carotene-9', 10'-oxygenase 2 (*Bco2$^{-/-}$*) knockout (KO) mice because, unlike wild-type mice, they can accumulate macular pigments in their retinas in a manner similar to humans.

Materials and Method: Female *Bco2$^{-/-}$* KO mice on a C57BL6/J background were divided into 2 groups (N=3/group). Group1: Female *Bco2$^{-/-}$* KO mice fed with zeaxanthin chow (~2.6 mg of carotenoid/mouse/day; DSM Kaiseraugst) at E0 day (confirmed with vaginal plug) as prenatal supplementation. Group 2: Female *Bco2$^{-/-}$* KO mice fed with zeaxanthin chow at P0 day (the day mouse litters born) as postnatal supplementation. On P7th day, group 1 and 2 *Bco2$^{-/-}$* KO mouse litters along with their nursing mothers were exposed to 75% oxygen for 5 days (until P12) in a ProOx model P360 hyperoxia chamber (Biosphere, Ltd.) to initiate OIR and then returned to room air (21% oxygen) for another 5 days (until P17) for vascular regrowth and neovascularization. Mice sacrificed at different time points (N=4 pups/time point) (P12, P14 & P17) were used for isolectin-stained retinal whole mounts. The central avascular area (AVA) and total retinal area was measured using Image J (v. 2018). The percentage of AVA over total retinal area was calculated.

Results: Prenatal supplementation with zeaxanthin to *Bco2$^{-/-}$* KO mice shows (23.4 ± 1.2) retinal AVA at P12 and (11.2 ± 0.7) retinal AVA at P14. Whereas, postnatal supplementation with zeaxanthin shows (28.6 ± 1.5) retinal AVA at P12 and (15.0 ± 0.9) retinal AVA at P14. The blood vessels were completely revascularized at P17 in both prenatal and postnatal supplementation groups. The central AVA is one of the pathological phenotypes of OIR. The lower AVA values in the prenatal supplemented group were significantly different ($p \leq 0.05$) compared to the postnatal zeaxanthin supplementation.

Conclusions: Prenatal supplementation of zeaxanthin promotes better retinal vascular regrowth compared to postnatal supplementation in oxygen induced retinopathy (OIR) model in Bco2$^{-/-}$ KO mice. This result supports the hypothesis that prenatal maternal supplementation of carotenoids could alleviate ROP pathogenesis. However, further detailed study is warranted.

Acknowledgment: NIH-EY011600, Research to prevent Blindness

Abstract 11

THE EFFECT OF MUCIN ON β-CAROTENE BIOAVAILABILITY CELL-BASED ASSAYS

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Introduction: The human intestinal mucosa is covered by a mucus layer that is mainly constituted by mucins. These glycoproteins possess hydrophilic and hydrophobic domains, so they can interact with carotenoids or with components of the bile salt micelles that carry them upon digestion. The mechanisms involved in the interaction and how much these factors influence carotenoid bioavailability remains to be elucidated. In this study we evaluated the effect of a natural mucus layer on β-carotene uptake using a human enterocyte/goblet cell co-culture model, and investigated the possible mechanisms underlying the mucin-β-carotene interaction using model systems.

Methods and Materials: Caco-2 and HT29-MTX cells (at 8:2 ratio) were cultured for 3 weeks. Mucin layers in Caco-2/HT29-MTX monolayers were visualized by scanning electron microscopy (SEM) and by fluorescence microscopy after acridine orange staining. β-Carotene in artificial micelles were incubated with Caco-2 and Caco-2/HT29-MTX monolayers and cell uptake was quantified. Micelle size was measured by small angle X-ray scattering, and mucin-β-carotene (2.5~80 μM) interactions were investigated by fluorescence and UV-vis spectroscopy.

Results/Discussion: SEM micrographs showed a continuous mucus layer on the top of Caco-2/HT29-MTX monolayers. The characteristic low pH of mucin was confirmed by the orange fluorescence overlaying the green-stained monolayers. β-Carotene uptake from taurocholate micelles were usually reduced by 40-60% in the mucus-covered Caco-2/HT29-MTX, compared to the mucus-free (control) Caco-2 monolayer, except that from phosphatidylcholine-containing micelles which remained unchanged. Micelle sizes (27.5~35.9 nm) did not seem to be directly related to changes in β-carotene uptake in the presence/absence of mucus. UV-vis spectrum of mucin/β-carotene solutions showed an extra peak at 508 nm, indicating the formation of β-carotene-mucin composites or β-carotene aggregates. Quenching of mucin's tryptophan fluorescence with increasing carotenoid concentrations was observed, with Ksv decreasing as temperature increased, suggesting the formation of complexes between β-carotene and mucin. The calculated *r* ranged between 0.50~0.68 nm, which were near the critical FRET distance (R_0, 0.47~0.48 nm) indicatng that FRET energy transfer between the two molecules was possible and again, confirming the complex formation between mucin and β-carotene. Thermodynamic parameters (ΔH, ΔS and ΔG) indicated spontaneous interaction of hydrophobic nature.

Conclusions: Mucin reduces the cell uptake of β-carotene micelles in cell-based assays, probably by hydrophobic interaction between β-carotene with the mucin backbone, and poses a significant barrier for intestinal absorption.

Acknowledgements: We thank Tomohiro Fujita, Takahiro Tanaka, Go Namba for their technical assistance. This work was supported by JSPS KAKENHI Grant Number 15K18699.

Abstract 12

A protocol to validate two food frequency questionnaires developed to estimate lutein and zeaxanthin dietary intake.

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Introduction: Two carotenoids, lutein (L), zeaxanthin (Z), are selectively deposited in the macula. L and Z may protect the macula through absorption of damaging blue light, and also have direct and indirect antioxidant activity. Not synthesised endogenously, L/Z must be obtained from the diet or supplementation [1]. Increasing macula L/Z concentrations is of significant interest in the prevention of macular conditions, and thus many clinical trials have investigated prescriptive dietary or supplemental L/Z intake [2,3]. To date, trials have inconsistently monitored habitual dietary L/Z intake, a potential confounding variable. Habitual dietary L/Z intake is difficult to monitor due to the lack of specific and validated tools. A food frequency questionnaire (FFQ) specifically aimed at monitoring dietary L/Z intake was developed in two formats. One format has a recall timeframe of a week, and the second a month. This study aims to validate the two formats of the questionnaire by comparing L/Z intake reported from the two FFQs with the report from multiple 24-hour diet recalls over a period of one month.

Methods: Fifty healthy Australian adults will report their dietary intake over four consecutive weeks using three diet recall tools: two 24-hour recalls on the online Automated Self-Administered 24-Hour Dietary Assessment Tool® each week, one recall timeframe FFQ each week, and one monthly recall timeframe FFQ at the end of week 4. The two FFQs list the same 90 foods items, including fruits, vegetables, grains, egg, nuts, seeds and some discretionary foods. The United States Department of Agriculture food composition database will be used to calculate dietary L/Z intake from reported foods in all three tools [4]. In addition, the Australian Food, Supplement and Nutrition Database 2011-13 will be used to calculate the mean estimated energy intake from the eight 24-hour diet recalls [5]. Participant basal metabolic rate with be estimated using participant reported age, weight and height, and physical activity status. The ratio of mean estimated energy intake over basal metabolic rate will be compared to the Goldberg cut-offs to determine accuracy of participant reporting and identify any misreporting [6]. The sum of L/Z intake reported in each the eight 24-hour recalls, the monthly FFQ, and the combined four weekly FFQs will be converted to an average daily intake of L/Z (mg/day), and compared.

Results and Discussion: Validity of the FFQs will be determined by degree of agreement between the three tools using a Bland-Altman plot analysis [7]. The degree of agreement between mean daily L/Z intake measured by the weekly FFQ and eight 24-hour recalls FFQs will be tested. This testing will also be repeated between the monthly FFQ and eight 24-hour diet recalls. Results will determine the validity of these new L/Z specific tools. These tools will be valuable to accurately monitor fluctuations in habitual dietary L/Z intake in future studies.

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Abstract 13

Superior Bioavailability of a Novel, Patented Lutein & Zeaxanthin Macular Carotenoid Formulation

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Objectives: Enhancing the bioavailability of lutein (L) and zeaxanthin (Z), the only dietary carotenoids exclusively deposited in the macula and associated with visual and cognitive benefits, has gained significant scientific attention. In response to this nutritional need, we developed and patented a unique LZ formulation designed for enhanced absorption (Test) . The objective of this study was to measure its bioavailability in a pharmacokinetic clinical study in comparison to a commercially available formula (Reference).

Methods: This randomized, double-blind, parallel study involved ninety healthy, adult human volunteers. Volunteers consumed a single-dose of the Test or Reference product, each comprising of 10 mg L and 2 mg Z immediately after a breakfast meal. Blood samples were collected prior to dosing @ -48.00, -24.00 & 0.00 hrs and subsequently at 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 20.00, 24.00, 48.00 and 72.00 hrs post-dose. L and Z levels were measured in serum using a validated HPLC method. The primary outcomes of the study included Cmax, AUC0-72, AUC0-T. Secondary outcomes included AUC0-12, AUC0-24, AUC0-48, AUC0-inf, Tmax and t1/2. All subjects stayed within the study facility three days before administration of the study products and for three days post-dose.

Results: All subjects completed the study with 100% compliance and no drop-outs. Baseline-corrected serum L and Z levels were significantly higher in the Test group vs. the Reference group with a 2-fold and 1.5-fold greater absorption of L and Z levels at all time points. Moreover, Cmax, AUC0-72 and AUC0-T for serum L levels were 2.5, 2.9 and 3.2-folds respectively greater in the group receiving the Test formula. Similarly, the Cmax, AUC0-72 and AUC0-T for serum Z were also significantly greater with the Test formula. Finally, the Cmax and AUC parameters were significantly higher than the 80-125% criteria established by the FDA for bioequivalence confirming the superior bioavailability of Test product compared to Reference product.

Conclusions: These clinical findings support the superior bioavailability of this novel and uniquely designed L and Z formulation. This can be an advantageous offering for individuals looking to quickly improve their L and Z status and enhance their vision protection and performance.

Funding Sources: OmniActive Health Technologies Limited

Keywords: bioavailability, lutein, zeaxanthin, carotenoids, vision

Session IIB Abstracts Carotenoids and Health II: Supplementation and Bioavailability

Abstract 14

Lycopene reduces formation of cholesterol crystals and accelerates their dissolution

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Introduction: Cholesterol crystals are one of the main culprits behind the rupture of atherosclerotic plaque and resultant thrombosis, the process responsible for the development of heart attack and ischemic stroke, the world's leading causes of mortality. Today there are no drugs or treatment able to target these crystals.

Research and Methods: Effect of lycopene on the growth of cholesterol crystals was investigated. A study was also made on the effect of this carotenoid on dissolution of already formed cholesterol crystals *in vitro* and in human atherosclerotic plaques.

Results and Discussion: It was found that lycopene was directly able to disrupt, slow down and facilitate reversal of the cholesterol crystallisation process. In *in vitro* experiments, lycopene delayed the time of the beginning of the process by 8 fold and its rate of this process itself by 5 fold. In addition it was able to reduce the cholesterol mass involved into crystallisation by 60%, fig. 1.

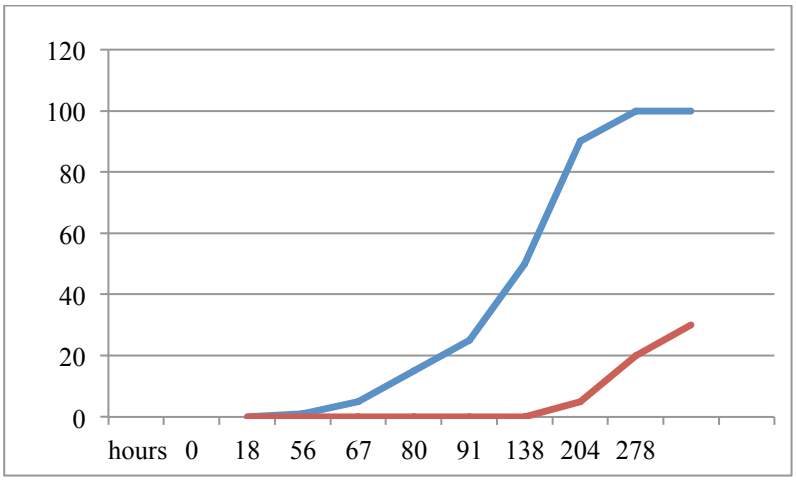

Fig.1 Kinetics of cholesterol crystal growth; vertical axis – percentage of crystallisation
blue – control cholesterol, red - in presence of 0.1% of trans-Lycopene

Even when the cholesterol crystals were formed in the presence of lycopene their size was significantly smaller than when the crystals grew undisrupted, fig. 2.

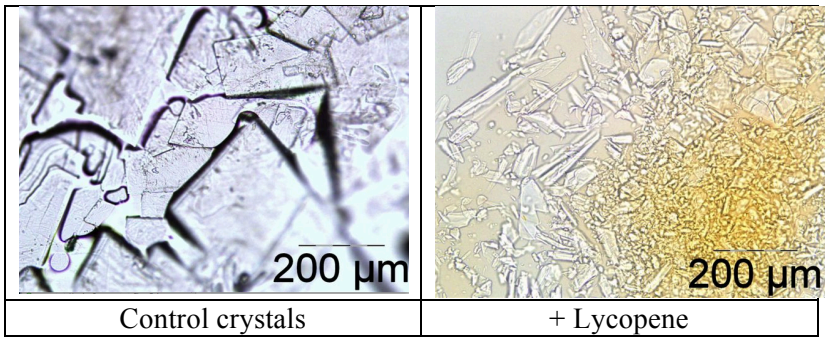

Fig 2 Size of cholesterol crystals reduced by *trans*-Lycopene, *in vitro* - light microscopy

This effect could be observed in a ratio of 1 molecule of lycopene to 1,000-10,000 molecules of cholesterol, which indicates that these two molecules can create a thermodynamically favourable complex, which triggers a long-range ordering transition in their folding.

Abstract 15

Association of placental carotenoid status with maternal and infants' carotenoid status in the Lutein and Zeaxanthin in Pregnancy (L-ZIP) study

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Introduction: Lutein (L) and zeaxanthin (Z) are dietary xanthophyll carotenoids that uniquely localize in the central retina of humans and are collectively called the macular pigments (MP). MP attenuates the harmful effect of blue light through its antioxidant and anti-inflammatory properties, improving visual and cognitive function. MP's accumulation in the brain and the eye is believed to occur before birth and is detectable immediately after birth, suggesting a possible physiological and protective role in early visual and cognitive development. Presently, maternal and infants' carotenoid status throughout pregnancy is poorly understood. Therefore, we sought to investigate the relationship between placenta, postpartum maternal, and newborn infants' carotenoid status.

Methods and materials: In this prospective, single-site, double-masked, randomized, active-controlled trial (ClinicalTrials.gov identifier: NCT 03750968; still recruiting), participants were randomized (1:1 allocation) either to the carotenoid or the control group for a period of 6 to 8 months. The carotenoid group received standard prenatal vitamins plus 10 mg L and 2 mg Z softgel, whereas the control group received standard prenatal vitamins with a softgel containing only safflower oil (without 10 mg L and 2 mg Z). Serum, skin, and ocular carotenoids of participants were measured at first trimester (baseline), second trimester, third trimester, and postpartum. Also, placenta samples, umbilical cord blood, and infants' skin carotenoids were assessed 0-2 weeks after birth. Serum, placenta, and umbilical cord blood carotenoids were assessed using high-performance liquid chromatography, skin carotenoid status measured using resonance Raman spectroscopy, and MP assessed with dual-wavelength autofluorescence.

Results: The present analytic sample comprised masked data of 13 mother-infant pairs who had completed the L-ZIP study. Placental L+Z levels significantly associated with umbilical cord blood L+Z levels ($r = 0.77$, $p = 0.004$), infant's skin carotenoids ($r = 0.58$, $p = 0.039$), maternal serum L+Z levels ($r = 0.63$, $p = 0.029$), and maternal skin carotenoids ($r = 0.65$, $p = 0.008$). Maternal serum L+Z levels significantly associated with umbilical cord blood L+Z levels ($r = 0.80$, $p = 0.002$). Infants' skin carotenoids significantly correlated with umbilical cord blood L+Z levels ($r = 0.62$, $p = 0.024$). Maternal skin carotenoids correlated with serum L+Z levels ($r = 0.63$, $p = 0.020$).

Conclusion: Our results demonstrate a significant positive correlation between placenta, maternal, and infant's carotenoid status. These findings imply that augmenting maternal carotenoid status through prenatal carotenoid supplementation may provide beneficial effects to newborn infants.

Acknowledgment: This study was supported by grants from the National Institutes of Health (EY029857 and EY014800; Bethesda, MD, USA) and an unrestricted grant from Research to Prevent Blindness (New York, USA) to the Department of Ophthalmology and Visual Sciences, University of Utah, Salt Lake City, Utah, USA.

Abstract 16

Evaluation of the Bioaccessibility of a Carotenoid Beadlet Blend Using an *in vitro* System Mimicking the Upper Gastrointestinal Tract

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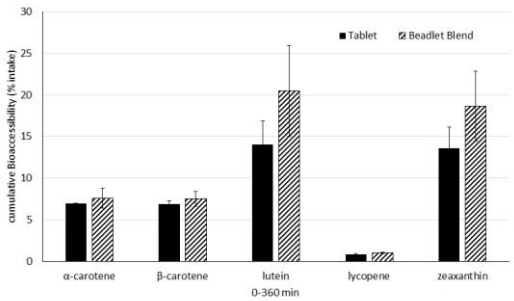
Introduction: Carotenoids are a major class of colorful phytonutrients that naturally occur in fruit, vegetables and algae that are also important in human nutrition. The majority of population does not consume the recommended amounts of fruits and vegetables resulting in a dietary carotenoid intake gap. One way to help close this phytonutrient dietary intake gap is through supplementation, and the various commercially available forms and doses of carotenoid supplement often contain a mixture of six primary carotenoids and there are many dietary factors that affect their interaction and bioavailability. Therefore, we present here a test of the release profile of a blend of carotenoid beadlets designed to separate individual carotenoids with a gut simulation system (TIM), an *in vitro* model capable of testing materials through a complete digestion process, therefore predicting digestibility and bioaccessibility within the human intestinal tract.

Materials and Methods: A blend of six carotenoids (at a fixed ratio) were designed with a sequential release profile. The carotenoid beadlet blend was further compressed into tablet form. Both a free beadlet blend and tablet were digested in the TIM system with a high-fat standard meal. The jejunum filtrate, ileum filtrate, ileum effluent and residue were collected from each correspondent compartment within a fixed time interval, followed by quantification using HPLC. The bioaccessibility of each carotenoid was then calculated.

Results and Discussion: Bioaccessibility peaks of each individual carotenoid were observed over approximately 3-4 hours in the order of lutein and zeaxanthin (120-180 min), followed by lycopene, and finally α- and β-carotene at between 240 and 300 min when tested as either a free beadlet blend or when the blend was compressed to tablets.

The result of cumulative bioaccessibility for both the beadlet blend and the tablets was 7-20% (shown in figure) which was similar to previously reported values using this model.



The total recovery of carotenoid from all digestion compartments ranged from 70-90%, except for lycopene where nearly 50% was unrecoverable under these test conditions.

Acknowledgement: We appreciate Katja Lang, Annet Maathuis and Susann Bellmann of the TIM Company for help in experimental design and equipment operation along with Dr. Xiang-Dong Wang and Kang-Quan Hu of Tufts University for their efforts in analysis of lycopene and its derivatives.

Abstract 17

KERNEL HARDNESS AFFECTS CAROTENOID DIGESTIBILITY IN COMMERCIAL MAIZE HYBRIDS

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Introduction: Yellow maize is known as a source of a variable amount of carotenoids, a diverse family of yellow-orange pigments contributing to vitamin A, antioxidant and pigmentation status of animals. The rate and extent of carotenoid bioavailability depend on the degree of grain digestibility, which is related to the structure of the starch-protein matrix in the endosperm, determined by hybrid genotype [1]. Since carotenoids of maize grain are located in the endosperm, the endosperm matrix structure will possibly affect the digestibility of carotenoids. The physical properties of maize grain also depend on the structure of the starch-protein matrix in the endosperm, and of them, grain hardness is related to starch digestibility kinetics [2]. Thus, carotenoid digestibility could also be related to hardness, and this study aimed to relate digestibility of individual [lutein (L), zeaxanthin (Z), α- (αCX) and β-cryptoxanthin (βCX), and β-carotene (βC)] and total carotenoids (TC) to hardness in commercial maize hybrids.

Methods and Materials: The study was conducted on 105 commercial maize hybrids grown at the same location. Hardness of the collected hybrids was determined using the Stenvert test; milling time, milling column height and coarse-to-fine ratio in milled grain were used to calculate the Stenvert index based on the one-third contribution of each variable. The RP HPLC method was used to quantify the carotenoid profiles in the grain and digesta after *in vitro* digestion according to the INFOGEST protocol. Based on total carotenoid content, hybrids were classified into 5 groups (C1, C2, C3, C4 and C5; <15, 15-20, 20-25, 25-30, and >30 μg/g DM, respectively), and based on Stenvert index, into 3 groups (S1, S2, and S3; <1, 1-1.2 and >1.2, respectively). The effects of carotenoid content and hardness on carotenoid digestibility were tested using the MIXED procedure of SAS 9.4 statistical package.

Results: The TC content of the collected hybrids ranged from 12.56 to 36.85 μg/g DM, and the average contents of L, Z, αCX, βCX, and βC were 7.66, 10.84, 0.93, 1.62 and 0.69 μg/g DM, respectively. The digestibility of individual and TC was not affected by carotenoid content, while hardness affected the digestibility of L, Z, βC and TC ($P<0.05$). The highest digestibility of L, Z, βC and TC was obtained in the S3 group (70, 59, 54, and 60%, respectively), followed by the S1 (62, 48, 41, 50%, respectively) and S2 (55, 45, 29, and 46%, respectively) groups. In all Stenvert groups, the digestibility of carotenoids decreased in the order: L > Z > βC > αCX > βCX.

Conclusions/Discussion: In the present study, digestibility of individual and total carotenoids was not related to their grain content but to the hardness of maize hybrids. Commercial hybrids with the highest hardness had the highest carotenoid digestibility, which is consistent with the fact that starch, the main component of maize endosperm, is digested more rapidly from grain with hard endosperm than from those with soft endosperm [2]. In all Stenvert groups, xanthophylls L and Z were digested to the greatest extent. The results suggest that hardness of maize grain represents the agronomic trait related to carotenoid digestibility, and the selection of harder maize hybrids could contribute more carotenoids to vitamin A, antioxidant and pigmentation status of animals.

Acknowledgements

This work has been fully supported by Croatian Science Foundation under the project ColourMaize (IP-2019-04-9063). The work of doctoral student Dora Zurak has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation.

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Session IIIA
Dietary Sources of Carotenoids and Nutritional Supplementation

Abstract 18

Chillies as potential candidates in breeding for high zeaxanthin bell-pepper varieties

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Introduction: Zeaxanthin, an orange-coloured carotenoid, has been associated with slowing progression of macular degeneration [1], the leading cause of blindness in developed countries. Zeaxanthin is obtained from dietary sources but compared to other carotenoids such as beta-carotene or lutein, it is a relatively uncommon carotenoid in the Western diet. Orange capsicums (*Capsicum annuum*), also known as bell peppers, have been specifically reported to be an excellent source of zeaxanthin, with a greater concentration of zeaxanthin than any other dietary vegetable. Apart from this general observation, very little has been reported about specific varieties of orange capsicums, or orange-coloured chillies. It is currently unknown if zeaxanthin is responsible for the orange colour of these chilli varieties, and if the concentration of zeaxanthin significantly exceeds that of orange capsicum. As chillies can be readily crossed with capsicums, the potential exists to utilise this more diverse germplasm to further increase the concentration of zeaxanthin in orange capsicums through conventional breeding.

Material and Methods: Carotenoid profiles of 33 varieties and breeding accessions of capsicum/chilli (orange, red, and yellow varieties sourced from *C. annuum, C. baccatum* and *C. chinense)* were determined and their zeaxanthin content quantified. As zeaxanthin and other 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). External standards were used for the quantification of zeaxanthin and the other principal carotenoids present. Total carotenoid content was estimated using carotenoid equivalent concentrations of the principal carotenoid in each variety.

Results and Discussion: Among the 33 varieties tested, only the orange capsicums demonstrated zeaxanthin (53-79%) as their principal carotenoid. Although several chilli varieties were orange coloured, this colour appeared to be due to an accumulation of other orange carotenoids, such as beta-carotene or beta-cryptoxanthin. Alternatively, orange colour was associated with the presence of a low concentration of the red carotenoid pigments, capsanthin and capsorubin, in conjunction with yellow carotenoids, such as violaxanthin or lutein. By contrast, yellow and red coloured chillies had a low percentage of zeaxanthin, ranging from 7-10% for yellow and 0.9-6% for red chilli varieties. From this study, it appears that none of the chillies examined (orange coloured or otherwise) would provide a good source of dietary zeaxanthin and are unlikely to be a potential parental donor.

Acknowledgements: This study was funded in part by 'Naturally Nutritious' (Horticulture Innovation Ltd., project HN15001).

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Abstract 19

Lycopene from orange heirloom ('Moonglow') tomatoes is superior to red tomatoes in increasing bone biomechanics and suppressing bone turnover in a rat model of osteoporosis.

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Introduction: Lycopene is a carotenoid that exists in all-*trans*- and *cis*- forms in nature. In red tomatoes >90% of lycopene is *trans*-lycopene, whereas in orange heirloom tomatoes such as the 'Moonglow' variant >90% of lycopene is the more bioavailable *cis*- lycopene. Lycopene has shown protective effects against bone loss. Therefore, this study compared red and 'Moonglow' tomato lycopene on turnover markers and biomechanical properties of bone in a rat model of osteoporosis.

Methods and Materials: Female Sprague-Dawley rats underwent no surgery (sham) or ovariectomy (OVX) surgery at age 16 weeks to induce osteoporosis. Sham and OVX control groups received no dietary supplement; 'post-red' and 'post-Moonglow' received tomato for 8 weeks post-surgery; 'pre-red' and 'pre-Moonglow' received tomato for 8 weeks prior to plus post-surgery (N=15/group). Rats were fed tomato powder containing 0.172 mg lycopene (~ 0.35 mg lycopene/kg body weight/day). The bone turnover markers c-terminal telopeptide of collagen type I (CTX-1) and osteocalcin (OC) in the serum, and bone biomechanical properties, were evaluated.

Results: OVX significantly increased CTX-1 (bone resorption) and OC (bone turnover) compared to sham. 'Pre-red' (247.0 ± 17.4 ng/ml), 'pre-Moonglow' (260.2 ± 10.1 ng/ml), and 'post-Moonglow' (260.3 ± 10.4 ng/ml) had significantly reduced serum osteocalcin levels compared to OVX control (337.6 ± 19.6 ng/ml). Serum CTX-1 was not significantly different among OVX tomato treatment groups. Femur break stress was significantly higher in 'post-Moonglow' group (94.8 ± 2.8 N/mm^2)) compared to OVX control (91.3 ± 2.3 N/mm^2). 'Post-Moonglow' group had the highest values for break force, break stroke, break strain and energy compared to OVX control although the difference did not reach statistical significance.

Conclusions/Discussion: Both red and 'Moonglow' tomatoes reduced biomarkers of bone resorption and turnover in rats with simulated post-menopausal bone loss, but the effect was more consistent with 'Moonglow' feeding. In contrast to the similar studies with doses of all-*trans*-lycopene (15-45 mg/kg BW/day), the tendency for bone loss with low doses of 'Moonglow' is lower even after menopause-initiated osteoporosis. These results demonstrate that 'Moonglow' *cis*- lycopene is superior to red tomato *trans*- lycopene in improving biomechanical properties of bone and reducing bone turnover that occurs due to oestrogen loss.

Acknowledgements: We thank the Heritage Food Crops Research Trust (HFCRT) Wanganui, New Zealand; Plant and Food Research, Palmerston North, New Zealand; Aimee Hamlin (Small

Animal Research Unit, Massey University); Massey technical laboratory staff; Professor Jessica Cooperstone (Ohio State University).

Abstract 20

RED-FLESHED SWEET ORANGE MUTANTS: A NEW SOURCE OF LYCOPENE AND COLORLESS CAROTENES

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Introduction: Accumulation of lycopene is an unusual feature in Citrus fruit, limited to a few cultivars of grapefruits, pummelos, lemons and oranges. The high antioxidant capacity of lycopene would confer to lycopene-accumulating citrus fruits additional health-related benefits and promising marketing opportunities. In this research results, two new red-fleshed sweet orange genotypes are reported on: 'Kirkwood', a mutant of Navel orange characterized by precocious maturity and highly appreciated for fresh consumption, and 'Ruby', belonging to the late maturity 'Valencia' orange, the main variety in many producing countries, especially for juice production. Both spontaneous mutants have been recently established in Spain under Mediterranean agronomic conditions to offer consumers new orange varieties with added healthy value.

Research & Methods: This study aimed to shed light on the biochemical and molecular mechanisms of lycopene accumulation in 'Kirkwood' and 'Ruby' orange mutants. For this purpose, we carried out a comprehensive characterization of carotenoids content and composition (HPLC-DAD) and analyzed the expression of 24 genes of carotenoids metabolism (qRT-PCR). In addition, ABA and related catabolites were measured via LC-HRMS. The analyses were performed from samples of the peel and pulp of both red-fleshed oranges varieties as well as their respective parental genotypes, during fruit development and maturation.

Results and Discussion: From the early stages of fruit development the total carotenoids were substantially higher in the pulp of both red-fleshed oranges compared to the parental genotypes. Phytoene and phytofluene were the major carotenoids in 'Kirkwood' and 'Ruby', accounting for more than 90% of total carotenoids in all maturation stages. Accumulation of lycopene in the red-fleshed oranges occurred from early fruit development and significantly increased throughout maturation. The presence of δ-carotene, a rare carotene in Citrus fruits, was detected in the red oranges. By contrast, the concentration of β,β-xanthophylls, the major carotenoids in ordinary oranges, was lower in the red-fleshed fruits. Interestingly, marked differences in carotenoids composition were also detected in other fruit tissues and leaves of the red-fleshed genotypes compared to the parental. These results lead us to postulate that a partial blockage in the cyclization of lycopene to β-carotene occurs in mutant fruit. This assumption is reinforced by the reductions of ABA and ABA-GE levels in the pulp of red-fleshed fruits during fruit development and maturation. However, the transcriptomic analyses of carotenoid metabolism genes did not reveal substantial differences in transcripts abundance between red-fleshed and parental fruits that may explain the alteration in the carotenoid complement in the mutants.

Altogether, biochemical and molecular data indicated:

- The red-fleshed phenotype of 'Kirkwood' and 'Ruby' is mainly due to the accumulation of lycopene and colourless carotenes, phytoene and phytofluene.
- The unusual accumulation of carotenoids in mutant fruits start early in the fruit development and is not related to the maturation process.
- The mutation appears to affect cyclization of lycopene into β-carotene.
- The mutation is not linked to differences in the transcription of carotenoids biosynthesis genes, being more likely related to alterations in factor/s associated with or essential for lycopene β-cyclase activity.

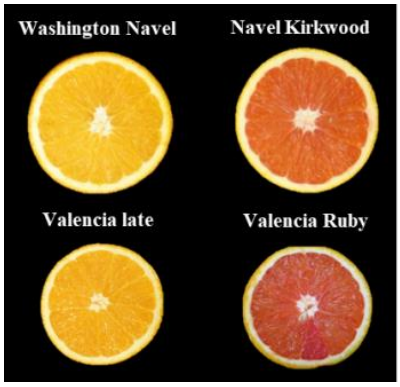

Fig.1. Internal aspect of the red-fleshed orange mutants Navel Kirkwood and Valencia Ruby and their respective parental genotypes, Washington Navel and Valencia late.

Acknowledgements
JZ is the recipient of a FDEGENT/2018/007 predoctoral scholarship from Generalitat Valenciana. This work was supported by a research grants RTI2018-095131-B-I00 of the Ministry of Science and, Innovation (Spanish Government), PROMETEO/2020/027 (Generalitat Valenciana) and the EUROCAROTEN COST Action CA15113. Authors acknowledged the Fundación ANECOOP for the provision of fruits and Citrus Rosso for its continuous support

Abstract 21

ENGINEERED HIGH LEVEL PRODUCTION OF THE KETO- AND HYDROXYCAROTENOIDS, CANTHAXANTHIN, ZEAXANTHIN, AND ASTAXANTHIN, USING THE PURPLE BACTERIUM *RHODOSPIRILLUM RUBRUM*

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Introduction:
Biotechnological production of keto- and hydroxycarotenoids such as astaxanthin, canthaxanthin, and zeaxanthin is of considerable interest due to their value as colourants and nutriceuticals. Here, we demonstrate that the introduction of the re-designed genes *crtW* (encoding β-carotene ketolase) and *crtZ* (encoding β-carotene hydroxylase) from *Paracoccus* sp. N81106 (formerly *Agrobacterium auranticum*) into a β-carotene-producing strain of the purple, photosynthetic bacterium *Rhodospirillum rubrum*, allows the production of keto- and hydroxycarotenoids at high levels.

Methods and Materials:
The synthetic *crtW* and *crtZ* genes were completely re-designed (codon optimization for the high GC-rich *R. rubrum* background, optimal ribosomal-binding Shine-Dalgarno sequences) for heterologous expression in a β-carotene-producing *R. rubrum* host strain. Expression was performed with a low copy IncP1α-replicon plasmid under control of the *lac* promoter.

Results:
The carotenoids produced were characterized by UV-VIS spectroscopy and UHPLC, and shown to contain large amounts of canthaxanthin and zeaxanthin, with lesser amounts of astaxanthin. We estimate the total amount of carotenoids produced to be about 5.7 mg/g DW cells. The precursor, β-carotene, was not detectable. The carotenoids were produced under dark, semi-aerobic conditions, using a special defined medium (M2SF) which allows maximal expression of photosynthetic pigment-containing membranes in the absence of light.

Conclusions/Discussion:
We have now demonstrated that the purple bacterium *R. rubrum* can be used to produce high levels of plant keto- and hydroxycarotenoids. Furthermore, we have evidence that the carotenoid can be incorporated into the photosystem, thereby opening the door to unique studies of structure-function. The dark, semi-aerobic process is also readily amenable to up-scaling at the industrial scale.

Acknowledgements:
This research was funded by the German Federal Ministry of Education and Research (BMBF grant nos. 0315282, 031A171A, and 031B0835A).

Session IIIB Abstracts Dietary Sources of Carotenoids and Nutritional Supplementation

Abstract 22

BIOCHEMICAL CHARACTERIZATION OF BETA-CAROTENE CONTENTS IN SWEET POTATO (*IPOMOEA BATATAS*) CULTIVARS GROWN IN PAKISTAN

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Introduction: Sweet potato [*Ipomoea batatas* (L.) Lam], belongs to the Convolvulaceae family and occupies the seventh position among the top food crops of the world [1]. In comparison with other tuber crops, sweet potato contains more carbohydrates, many minerals, and more protein estimates than other vegetable crops [2]. It also contains higher levels of pro-vitamin A, vitamin C, and minerals than rice or wheat [3]. Despite of the huge economic importance, the sweet potato production of Pakistan is much low [4]. Reports on nutritive composition, genetic variation, and other bioactivities are very much lacking. Current study focused sweet potato local cultivars, in order to estimate their potential variation in in terms of Beta-Carotene (Pro-vitamin A) contents.

Materials & Methods: Plant material for sweet potato high yielding cultivars/clones or genotypes was collected from local farmers of Punjab through personal field visits. The material included sweet potato tubers for four cultivars only. Selection of cultivars was based on the color of tubers viz., purple, orange, yellow and white. Estimations of beta-carotene were carried out according to the AOAC official method for nutrient analysis with some modifications as per experimental requirements [5]. Retinol equivalent was used to finally estimate the vitamin A from analysed beta-carotene values.

Results and Discussion: The analytical estimations of beta-carotene among the four cultivars of sweet potato are given in **table 1**. The data were measured and presented as mean values of three experimental replicates (Mean ± SD; n = 3) by routinely used statistical software viz; SPSS and MS Excel.

First the beta-carotene was calculated using the following formula:

Beta carotene µg/100g = Absorbance × V × D × 100 × 100/W × Y

Retinol equivalent was used to finally estimate the vitamin A using the relation:

1 µg beta-carotene = 0.167 µg RE.

Table 1 Estimated vitamin A contents deduced from Beta carotene composition of sweet potato tuberous roots of selected cultivars

Vitamin (mg/100g)	Ib1 (Purple)	Ib2 (Orange)	Ib3 (Yellow)	Ib4 (White)
Vitamin A	0.70±0.001	0.72±0.001	0.60±0.002	0.20±0.001

Vitamin A equivalents in tuberous roots ranged from maximum in Ib2 (0.72±0.001), an orange fleshed cultivar to the minimum (0.20±0.001) in Ib4, a white fleshed cultivar.

CONCLUSION

In current study, we were able to first time report that sweet potato cultivars with colored fleshed tuberous roots accumulated more amounts of Beta-carotene and hence the vitamin A.

Acknowledgements

We thank the Higher Education Commission (HEC) Pakistan for providing funding under SRGP to conduct this study.

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Abstract 23

Red and yellow colored *Taxus* arils: Natural sources of the exceptional *retro*-carotenoids rhodoxanthin and eschscholtzxanthin at high abundance

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Introduction: Rhodoxanthin and eschscholtzxanthin belong to the exceptional group of *retro*-carotenoids that scarcely occur in nature compared to ubiquitous carotenoids like lycopene, β-carotene, and lutein [1]. In particular, rhodoxanthin has gained scientific attention owing to its extraordinary (*E/Z*)-isomeric ratios [2] and the potential to function as purple or red colorant in water-dispersible formulations for food applications, e.g., in confectionary and beverages [3]. We found both *retro*-carotenoids to dominate the pigment profiles of yellow- and red-fleshed yew arils of a series of *Taxus* spp. cultivars [4].

Research & Methods: We characterized carotenoid profiles of yellow- and red-colored arils obtained from various cultivars of *Taxus* × *media* Rehder and *Taxus baccata* L. (Taxaceae). Comprehensive HPLC-DAD-ESI/APCI-MS^n analyses were used to first identify and then quantitate free carotenoids and xanthophyll esters in seven defined cultivars. Light micrographs of fresh *Taxus* aril tissues were assessed to gather first insights into the appearance of chromoplasts in yew arils and the respective type of carotenoid deposition [4].

Results & Discussion: In total, 43 carotenoids were identified in yellow- and red-fleshed *Taxus* arils. Red arils were characterized by five eschscholtzxanthone esters and, at high abundance, nine (*E/Z*)-isomers of rhodoxanthin, being predominantly represented by (all-*E*)-, (6*Z*)-, and (6*Z*,6′*Z*)-rhodoxanthin in ratios of up to 1:2:1. By contrast, *retro*-carotenoids with oxo-groups were not present in the carotenoid profiles of the yellow-colored arils of *T. baccata* 'Lutea' where free and esterified eschscholtzxanthin (*E/Z*)-isomers were prevailing. Abundant in-source fragment ions of eschscholtzxanthin were detected owing to the spontaneous loss of water or the cleavage of fatty acyl moieties at C-3 and C-3′, resulting in a specifically intricate assignment of numerous eschscholtzxanthin (*E/Z*)-isomers and their esters. As illustrated in Fig. 1, the in-source fragmentation may be favored by product ions with an extended resonance-stabilized electron system across the entire conjugated double bond system. Further minor carotenoids were β-carotene, lutein, violaxanthin, zeaxanthin, and various esters of β-cryptoxanthin and lutein [4].

High total carotenoid concentrations of 17.0–58.8 µg/g fresh weight (FW) were found in all *Taxus* arils assessed. The unusual *retro*-carotenoids rhodoxanthin (19.5–51.4 µg/g FW) and eschscholtzxanthin (14.8–17.4 µg/g FW) were found at strikingly high abundance. Light micrographs of fresh tissue of yellow- and red-colored yew arils both showed small, round-shaped chromoplasts in assembled clusters. Polarized light microscopy indicated that the carotenoids in yellow arils were deposited in a highly ordered or even crystalline state, whereas carotenoids in red arils were characterized by a presumably non-ordered or low-ordered deposition state [4].

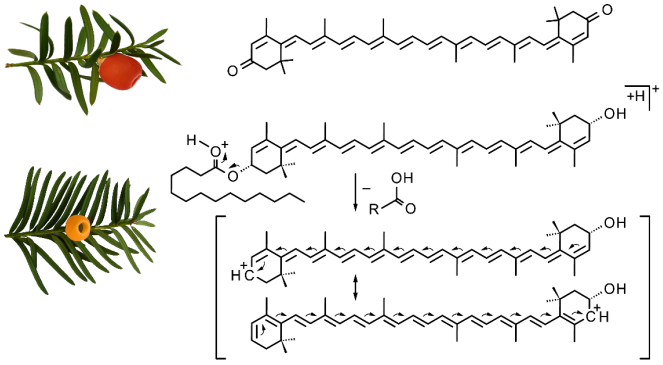

Fig.1. Red and yellow *Taxus* arils and molecular structures of rhodoxanthin and the protonated molecule $[M + H]^+$ of eschscholtzxanthin myristate, resulting in resonance-stabilized in-source fragment ions in the APCI(+) mode. Modified from Schex et al. [4].

Acknowledgements
We sincerely thank Bettina Wüstenberg (DSM Nutritional Products) and the Hohenheim Gardens (Stuttgart, Germany) for providing authentic reference standards of carotenoids and *Taxus* aril samples, respectively.

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Abstract 24

Do blood-plums (*Prunus salicina*) have carotenoids?

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Introduction

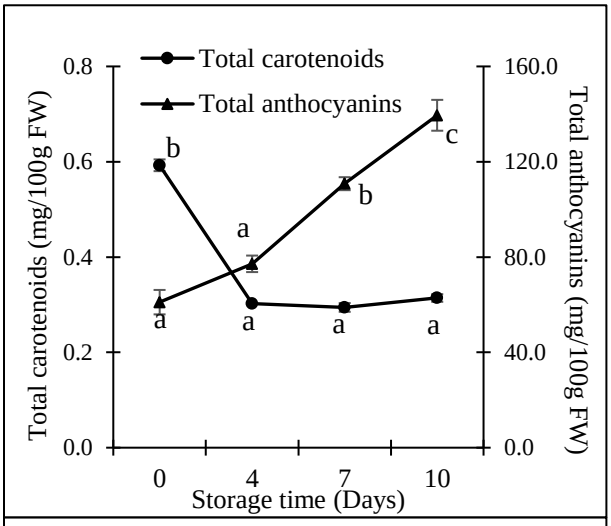
'Blood plums' (red/black peel and red flesh) are popular due to their high anthocyanin content. The red/purple color of these plums (flesh and peel) is used as an indicator of maturity as well as the anthocyanin content. Apart from anthocyanins, carotenoids are also present in blood plums, but their colour is largely masked by the deep red/purple colour of the anthocyanins. This study was conducted to identify the carotenoids in the high-anthocyanin 'Queen Garnet Plum' (QGP), a Japanese blood plum cultivar, and how the concentration of these carotenoids change during ambient temperature storage.

Materials and Methods

Mature QGP obtained from a commercial grower in Queensland, Australia were stored at 23ºC for 10 days and on day 0, 4, 7 and 10, changes in the carotenoid profile and total anthocyanin concentration were determined.

Results and Discussion

The principal carotenoids identified in QGP were α-carotene, β-carotene and lutein. While total anthocyanin concentration of the QGP was observed to increase 2.3-fold ($p<0.05$) during the storage period, total carotenoids exhibited a significant initial decline ($p<0.05$) from 0.59 to 0.30 mg/100 g FW (day 0 to day 4), after which it remained unchanged ($p>0.05$). It is of interest to note, that although the QGP is renowned for its high anthocyanin concentration, its initial carotenoid content was higher than in yellow-fleshed plum cultivars such as 'Angeleno', 'Black Amber' and 'Amber Jewel' [1].



Total carotenoids and total anthocyanins in QGP. Data are represented as mean ± SE (*n*=6-10). Different letters within each curve indicate significant differences at α=0.05.

Conclusion

The QGP, which is renowned for its dark colour and high anthocyanin content, also contains considerable amounts of carotenoids, comparable to yellow-fleshed plums. The nutritional impact of this needs to be investigated further.

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Acknowledgement

This research was funded by 'Naturally Nutritious' (Horticulture Innovation Australia Ltd., project HN 15001) and the University of Queensland. QGP fruit were supplied by Nutrafruit Pty Ltd.

Abstract 25

Avocadoes as a source of dietary lutein: Importance of total carotenoid synthesis to elevated lutein concentrations.

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Introduction: Avocado (*Persea americana*) is a subtropical/tropical fruit high in mono-unsaturated fatty acids but is also a moderate source of lutein [1], an important dietary macula carotenoid. Although leafy green vegetables generally contain higher concentrations of lutein, the presence of oil in the avocado potentially increases the bioavailability of lutein, due to its fat-solubility. Numerous cultivars of avocado exist; however, the Australian Industry is currently dominated by Hass and Shepard, and to a lesser degree by Wurtz, Sharwil and Pinkerton. Although the concentrations of lutein in some of these cultivars have been previously assessed [1] factors influencing the content of lutein have been much less studied. This study investigated the effects of total carotenoid concentration and the proportion of lutein making up the carotenoids present on the final lutein concentration of these five avocado cultivars.

Materials and Methods: Five locally grown cultivars of avocadoes (cvs. Hass, Shepard, Wurtz, Sharwil and Pinkerton) were purchased from the market in Brisbane, Queensland, Australia. Carotenoids were extracted and quantified from fully ripened avocado fruit, based on the firmness of the pulp, using ultra-high-performance liquid chromatography coupled with diode array detection (UHPLC-DAD).

Results and Discussion: Total carotenoid content and the proportion of different carotenoids varied between the five avocado cultivars. Shepard was found to have the highest carotenoid content (7.26 mg/100g DW), followed by Sharwil (7.13 mg/100g DW), Pinkerton (4.44 mg/100g DW), Wurtz (4.39 mg/100g DW) and Hass (3.58 mg/100g DW), respectively. The principle carotenoids identified in all cultivars were as lutein, followed by neoxanthin, except in Sharwil in which the concentration of neoxanthin and lutein-5,6-epoxide was similar. Interestingly, the ratio of alpha-branch carotenoids (e.g. lutein, lutein-5,6-epoxide) to beta-branch carotenoids (e.g. neoxanthin, violaxanthin) also varied greatly between avocado cultivars. The highest alpha-beta ratio (1.54) was observed in Wurtz, and the lowest (1.01) in Shepard. Although a high alpha-beta ratio tended to favour a higher proportion of lutein (42%) relative to carotenoids, the factor that predominantly influenced lutein concentrations was total carotenoid synthesis. Cvs. Shepard and Sharwil, which had the highest total carotenoid concentration (>7 mg/100g DW), also had the lowest proportions of lutein (30-31%) present. Despite this, their lutein concentrations were the highest observed in the current trial. This study demonstrated that high total carotenoid content was more important in providing high lutein concentrations than a high relative proportion of lutein, and that this could be a focus for future improvements in avocado.

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Session IVA Abstracts Dietary Sources of Carotenoids and Nutritional Supplementation

Abstract 26

Evaluation of zeaxanthin production by an Antarctic *Flavobacterium* sp. using Corn Steep Liquor as an alternative nitrogen source.

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Introduction: Zeaxanthin (3,3´-dihydroxy-β-carotene) is an oxygen-containing carotenoid that performs important physiological functions [1]. Microbial production of zeaxanthin has advantages over traditional methods. However, the cost of the culture medium is critical to make its production at large scale economically viable. Corn steep liquor (CSL) is a main by-product of corn starch processing which has been used as a low-cost source of nutrients for biotechnological production in a variety of products [2]. It could replace complex nitrogen and nutrients sources as peptone and yeast extract (YE). Among microbial sources of zeaxanthin, bacterial species belonging to *Flavobacterium* are well-known as zeaxanthin producers [3].
In this work, the effect of CSL as a substitute for yeast extract and peptone on zeaxanthin production by an Antarctic *Flavobacterium* sp. was studied.

Materials and methods: *Flavobacterium* sp. P8 strain was isolated from saltwater samples collected at King George Island in Antarctica in December 2014 [4]. The addition of CSL as a substitute for yeast extract, peptone, or both in a previously optimized medium, was carried out in 1 L-Erlenmeyer flasks with 100 mL of culture at 20°C and 200 rpm for 72 h. The different nutrient additions are shown in figure 1. Assays were by triplicate. Biomass and glucose quantification, and carotenoid extraction were previously described in [5]. The chromatographic method used for carotenoid quantification has been previously described in detail in [6]. Analysis of variance was performed via InfoStat/Estudiantil version to determine the statistical significance of data differences ($p \leq 0.05$).

Results and Discussion: Zeaxanthin production for each run is presented in Figure 1. The maximum zeaxanthin concentration reached was 3123 ± 562 µg/L when peptone was replaced by CSL. This result meant a 5-fold improvement when compared to optimized medium for zeaxanthin production (YE 7 g/L and peptone 7 g/L) by *Flavobacterium* sp. P8 described in [4]. Moreover, zeaxanthin concentration was 98% of total carotenoids. Zeaxanthin accumulation was 833 ± 5 µg/g when medium contained CSL and thiamine. The ANOVA showed that there were not significant differences between using CSL with or without YE in zeaxanthin accumulation. Thiamine supplementation further increased zeaxanthin production, this could be explained because it has been reported that CSL is thiamine deficient [2]. Thiamine is a B-complex vitamin which has a major role in carbohydrate metabolism. However, the maximum biomass concentrations were obtained in run 1 (CSL (4 g/L) +YE (7 g/L)), and run 5 (Peptone (7 g/L) +YE (7 g/L)). Results indicate that CSL is a promising nitrogen and nutrient source for zeaxanthin production by *Flavobacterium* sp. P8 that could replace YE and peptone, with significantly higher zeaxanthin content and reducing culture medium costs. Next studies will be aimed to upscale the process to bioreactor.

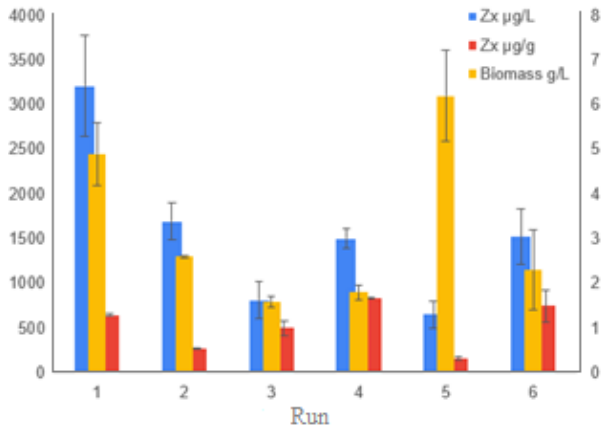

Fig. 1. Biomass (g/L), zeaxanthin accumulation (µg/g) and concentration (µg/L) at 72 h of cultivation in shaken flasks at 20°C. Runs: 1) CSL (4 g/L) +YE (7 g/L), 2) CSL (4 g/L) + Peptone (7 g/L), 3) CSL (4 g/L) +YE (2 g/L), 4) 3) CSL (8 g/L) + Thiamine (0.3 mg/L), 5) Peptone (7 g/L) +YE (7 g/L) and 6) CSL (8 g/L)

Acknowledgements: The authors would like to thank to Instituto Antártico Uruguayo (IAU) and Agencia Nacional de Investigación e Innovación (ANII) for their financial support (FMV_3_2018_1_148809).

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Abstract 27

MAJOR CAROTENOID IN *Meiothermus ruber*: DEINOXANTHIN GLUCOSIDE ESTERS

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Introduction: *Meiothermus ruber* is a thermophilic bacterium, and its color is red. Its pigment was reported to be carotenoid of 1'-β-glucopyranosyl-3,4,3',4'-tetradehydro-1',2'-dihydro-β,ψ-caroten-2-one (meiothermo-xanthin) after saponification [1].

In this study, we reidendified the pigment to be deinoxanthin glucoside esters, and found the reported carotenoid was artifact of saponification.

Methods and Materials: *M. ruber* DSM 1279^T was cultured in the thermus medium for 24 hours at 50 °C and with agitation 150 rpm. The cells were harvested by centrifugation, and the pigments were extracted with acetone-MeOH (7:2). Major carotenoids were purified using silica gel TLC developed with DCM: EA: Acetone=1:2:1 and C_{18}-HPLC. After purification, UV/Vis spectrum, molecular mass and NMR spectra were measured.

The purified carotenoids were saponified with 10% KOH-MeOH for overnight at room temperature. Its UV/Vis and mass spectrum were measured.

Results and Discussion: The purified carotenoids showed four C_{18}-HPLC peaks, and their UV/Vis spectra were identified with broad peak at 480 nm and shoulder at 507 nm. The molecular masses were 884, 912, 940 and 968 respectively, ^{1}H-NMR spectra were also measured.

With combination of these data, the major pigments were identified to be deinoxanthin glucoside esters (Fig. 1), and esterified fatty acids were C9:0, C11:0, C13:0 and C15:0, respectively.

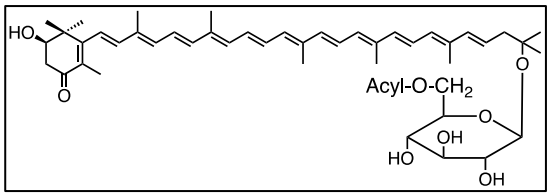

Fig. 1. Structure of deinoxanthin glucoside esters

When the purified carotenoids were saponified, molecular mass was changed 726, which was compatible with meiothermoxanthin not deinoxanthin glucoside (744). Consequently, meiothemoxanthin was artifact of saponification.

Genome DNA sequence of *M. ruber* is publish, and all of carotenoid synthesizing genes were found.

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Abstract 28

Enhancing bacterioruberin biosynthesis in wild-type *Haloferax mediterranei* R-4.

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Introduction

Haloarchaea are extremophilic microorganisms which require high salt concentrations for their survival. They usually inhabit hypersaline environment and therefore, are frequently exposed different sources of oxidative stress such as elevated sun radiation or high ionic strength. The synthesis of antioxidant pigments is one of their main strategies to endure these conditions.
Haloarchaea produce mainly the rare carotenoid bacterioruberin (BR) and its derivatives. BR presents a particularly high number of carbon units (C_{50}) and double bonds, which make this carotenoid a superior radical scavenger than C_{40} carotenoids.
The outstanding antioxidant activity of BR has recently raised interest of many researchers due to its potential applications on biomedical and biotechnological industries. Several factors are involved in the regulation of carotenoid biosynthesis in haloarchaea, as for example salinity, temperature, and C/N ratio.

Research & Methods

In this study, the halophilic archaea *Hfx. mediterranei* was grown under aerobic conditions in a medium containing inorganic salts (10-25% w/v) in combination with different carbon sources (glucose (0.5-2.5% w/v), starch (0.5-2.5% w/v)) or oxalacetate (0.5%) in order to evaluate the effect on BR production. The response to the sudden exposure of an oxidative stress due to H_2O_2 (0-75mM) was also assessed.

Results and discussion

The highest concentration of BR (84.41 mg/L) was observed with 12.5% of inorganic salts and 2.5% of glucose. However, under these conditions a notable decrease of pH was observed (<5.5), which may be limiting cell growth. Starch was also very efficient inducing BR production (71.33 mg/L) without pH drop. Oxalacetate, although presented the best [BR]/biomass ratio, limited growth and BR production. Furthermore, *Hfx. mediterranei* successfully tolerated until 25 mM H_2O_2, leading to a higher production of the pigment when compared to the control. Therefore, BR was very efficient scavenging the strong oxidizer H_2O_2.
In conclusion, BR synthesis can easily be enhanced modifying several parameters during growth which is useful to produce it at a higher scale. Currently, we are preliminarily evaluating the antioxidant effect of BR in breast cancer human cell lines to assess potential biomedical applications.

Acknowledgements

RTI2018-099860-B- 316 I00/Ministerio de Economía y Competitividad and VIGROB-309/Universidad de Alicante
Predoctoral fellowship grant for M.G. (G.N. ACIF/2019/043)/Generalitat Valenciana

Abstract 29

Fed-batch carotenoid production by an Antarctic *Flavobacterium* sp.

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Introduction: The production of carotenoids by microbial sources is a field of increasing interest since consumers are shifting to the use of natural and environmentally friendly products. However, bacterial production of carotenoids is still not competitive compared to chemical synthesis due to the high cost and lower yields. Antarctic heterotrophic bacteria are known to synthesize carotenoids as a response to environmental stress (fluctuating temperatures and high UV-B radiation) [1], being a novel interesting source for industrial production.

The aim of this study was to evaluate the production of carotenoids by an Antarctic *Flavobacterium* sp. P8 in fed-batch bioreactor.

Methods and Materials: *Flavobacterium* sp. P8 strain was isolated from saltwater samples collected at King George Island in Antarctica in December 2014 [2]. Fed-batch carotenoid production was evaluated in a bioreactor Biostat A Plus (Sartorius) with 3 L of medium at 20°C, pH 7 and airflow of 1 vvm. The agitation rate was regulated to ensure a minimum concentration of dissolved oxygen (DO) at 10% saturation. When glucose concentration reached 10 g/L, a pulse feed (composition: 400 g/L glucose, 140 g/L peptone and 140 g/L yeast extract) was added. Samples were taken periodically to monitor cell growth, glucose consumption and carotenoid production. Biomass and glucose quantification, and carotenoid extraction were previously described by Vila et al. [3]. The chromatographic method used for carotenoid quantification has been previously described in detail by Delgado-Pelayo and Hornero-Méndez [4].

Results and discussion: Carotenoids production by *Flavobacterium* sp. P8 were growth associated. The strain was capable to consume 50 g/L of glucose at 81 h of cultivation. The maximum total carotenoid concentration reached was 8.4 ± 0.2 mg/L at 60 h. Zeaxanthin concentration was 25% of total carotenoids and β-cryptoxanthin and β-carotene were 22% and 54% respectively. Zeaxanthin content (µg/g) remained constant during the culture. However, β-cryptoxanthin increased from 21 ± 8 µg/g to 115 ± 1 µg/g and β-carotene from 18 ± 4 µg/g to 289 ± 6 µg/g in 60 h.

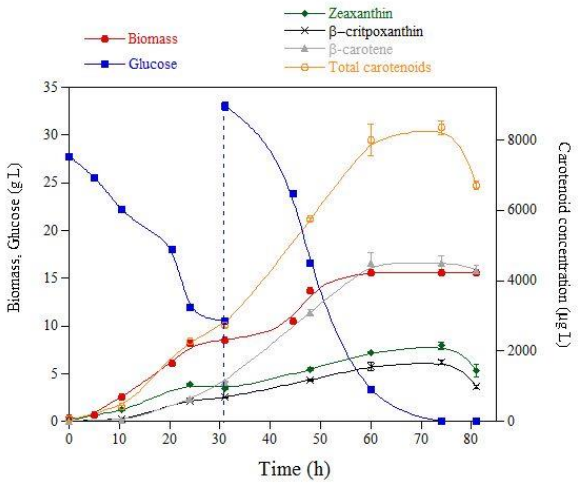

Fig.1 Production profiles in fed - batch mode at 20 ° C and 10% pO_2 by *Flavobacterium* sp. P8.

Conclusions: *Flavobacterium* sp. P8 is a promising strain for carotenoids production. However, the results showed an incomplete conversion of intermediates to zeaxanthin. It could be caused by a high oxygen consumption rate demanded for growth and cellular maintenance making it not available for carotenoid hydroxylation. The low intermediate conversion could be also associated to exhaustion of other nutrients related to this pathway. Further studies will aim to enhance oxygen transport to satisfy cellular demand to reach higher zeaxanthin conversion and study media components which could act as reaction promoters.

Acknowledgements

The authors would like to thank to Comisión Académica de Posgrado (CAP) for the postgraduate scholarship given to Eugenia Vila, Instituto Antartico Uruguayo (IAU) and Comisión Sectorial de Investigacion Cientifica (CSIC) for financial support (CSIC INI 230).

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Abstract 30

Artificial differentiation of chloroplasts provides enhanced storage capacity for carotenoids and other nutritionally valuable isoprenoids in leaves.

Session: Biotechnology and industrial production of carotenoids

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Plants synthesize carotenoids in plastids. Carotenoids are essential in chloroplasts, but their maximum levels are detected in specialized plastids named chromoplasts typically found in non-green pigmented organs such as flower petals and ripe fruits. We recently developed a system that allows the artificial transformation of leaf chloroplasts into carotenoid-rich chromoplasts [1]. These artificial chromoplasts display intricate membrane formations and more plastoglobules, which are potential accumulation site for carotenoids. In this work, we used plastid fractionation methods to confirm that carotenoids are stored in the membrane compartments formed in artificial leaf chromoplasts. In particular, β-carotene (the main pro-vitamin A carotenoid) and phytoene accumulated mostly in the plastoglobules while xanthophylls (mainly lutein) were most abundant in the membrane fractions. Interestingly, plastoglobules-associated plastidial isoprenoids of nutritional interest such as tocopherols (vitamin E) and phylloquinones (vitamin K) were also found to accumulate at much higher levels in synthetic leaf chromoplasts. Treatments known to promote plastoglobules differentiation led to further increases in the levels of these metabolites. Further increases in carotenoids, tocopherols and phylloquinones in leaves were accomplished by genetic engineering strategies, including the introduction of cytosolic pathways [2].

Acknowledgements: LM was supported by "la Caixa" Foundation (ID 100010434) with the PhD INPhINIT fellowship LCF/BQ/IN18/11660004. LM stay at the University of Neuchatel was supported by the EMBO Short-Term fellowship 8907.

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Session IVB Abstracts Dietary Sources of Carotenoids and Nutritional Supplementation

Abstract 31

Integration of agroindustrial waste in the carotenoids bioproduction by red oleaginous yeast

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Introduction: The global concern of the massive accumulation of residues generated from the food supply chain has emphasized the agroindustrial waste valorization to reduce their disposal's negative impact on the environment. Therefore, recent research focuses on the proposal of sustainable and feasible methodologies that aim to both obtain high-value compounds and reduce emissions through biochemical conversions. Carotenoid synthesis by yeast is an alternative to the growing market demand for natural pigments. Thus, the integration of renewable feedstock obtained from agroindustry residues could help the process profitability and sustainability [1]. This work aims to evaluate the performance of enzymatic hydrolysates as culture media in the carotenoid synthesis of red yeast.

Methods: Residues were collected from local farms (Coahuila, Mexico). Brewer's spent grain (BSG), pasta processing waste (PPW), and bakery waste (BW) was hydrolyzed using crude enzymatic extracts and used as the whole fermentation media adjusted to 20 g/L of total sugars. The strain *Sporidiobolus roseus* CFGU-S005 kindly provided by Universidad Nacional de San Agustin de Arequipa (Arequipa, Perú) was used for carotenoids production, which was carried out at flask level at 24 °C, 180 rpm for 120 h. The evaluated responses were carotenoids yield per biomass (Yx, µg/g), carotenoids production (P, µg/L). Carotenoids, total sugar and FAN content were measured by spectrophotometric methodologies,

Results and Discussion: Among the evaluated agroindustrial strains in the crude enzymatic hydrolysis, the higher total sugar obtained (30 g/L) was obtained with PPW hydrolysate, while a maximum of 38% total kjendahl nitrogen to FAN conversion yield was attained using BSG hydrolysate. The yeast *S. roseus* showed a great performance in carotenoids production using the crude enzymatic hydrolysates as whole culture media. The BW hydrolysate resulted in higher carotenoid production in the comparison study (Fig 1). Since it has been suggested that nitrogen content in the medium can trigger the secondary metabolites synthesis such as carotenoids [2], the lower FAN content in BW hydrolysate might enhance the intracellular synthesis of carotenoids in *S. roseus*. Nonetheless, the high conversion yield of sugar in the PPW hydrolysis allows exploring C/N ratios in further experiments.

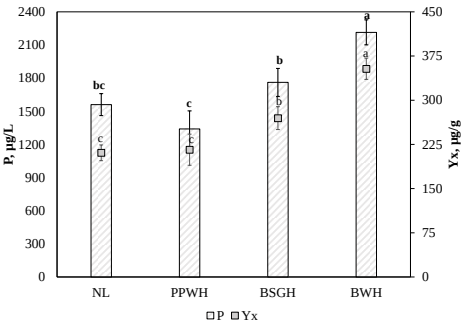

Figure 1. Carotenoid production (P) and carotenoid yield per cell dry weight (Yx) in *S. roseus* fermentation in hydrolysates.

Acknowledges: Author Villegas-Mendez MA acknowledges Conacyt-México for the financial support provided for conducting his PhD (750450-2019) study.

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Thermally induced (*E/Z*)-isomerization and controlled aggregation of rhodoxanthin to modulate the color of formulations for food and beverages

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Introduction: Until now, conveying vibrant red color shades to foods and beverages with colorants other than carmine and azo dyes is intricate. Carotenoids obtained from microorganisms, plants, or produced by chemical synthesis are promising alternatives [1]. For instance, rhodoxanthin is a barely studied *retro*-carotenoid that may function as red colorant owing to its exceptional π-electron system with 14 conjugated double bonds that absorbs light at longer wavelengths than those of most other carotenoids [2, 3]. In addition to the molecular structure, isomerization and aggregation of carotenoids are frequently occurring phenomena having substantial impact on their UV/vis spectra and color hues [2, 4].

Research & Methods: We elucidated the kinetic and thermodynamic fundamentals of rhodoxanthin (*E/Z*)-isomerization reactions using computational modeling. (*E/Z*)-ratios of dissolved rhodoxanthin were monitored during isothermal heating at 40, 50, 60, and 70 °C in ethyl acetate and compared to those of the non-*retro*-carotenoid canthaxanthin [5]. Lyotropic aggregation of rhodoxanthin at varying (*E/Z*)-ratios was induced in acetone/water mixtures at ratios of 75:25, 50:50, and 25:75 (*v/v*) prior to recording UV/vis spectra and CIE-L*a*b* color values. High-performance wet-milling and a solvent-based emulsification process were used as micronization techniques to prepare techno-functional rhodoxanthin formulations for their later application in food and beverages [6].

Results & Discussion: A mathematical procedure was established to obtain kinetic and thermodynamic parameters of six equilibrium reactions that interlink the (all-*E*)-configured molecule with mono-, di-, and tri-(*Z*)-isomers of rhodoxanthin using multi-response modeling. Rate constants with respect to the reaction from (all-*E*)- to (6Z)-rhodoxanthin, i.e., the rotation at the exocyclic double bonds, were 11–14 times higher than those of common (*E/Z*)-isomerization reactions, e.g., at position C-13,14 of canthaxanthin. The product favored equilibrium reaction among (all-*E*)- and (6Z)-rhodoxanthin was characterized by negative Gibbs free energies ranging from −1.6 to −2.2 kJ mol^{-1}, being very atypical for carotenoids at the applied temperatures and, therefore, providing novel insights into the (*E/Z*)-isomerization characteristics of exocyclic double bonds as distinct feature of *retro*-carotenoids [5].

Lyotropic aggregation of rhodoxanthin at (all-*E*)-shares of 20 and 73% in acetone/water mixtures yielded red-orange (CIE-h° = 44°) to pink (9°) and red (29°) to purple (−7°) color shades, respectively. As illustrated in Fig. 1, wet-milled and emulsified rhodoxanthin formulations imparted purple (CIE-h° = −6°) and red color hues (33–37°) in aqueous dispersion, respectively. Therefore, we established technological measures to produce water-dispersible rhodoxanthin formulations that revealed great potential for conveying purple and red color shades to foods and beverages as potential alternatives to azo dyes and carmine [6].

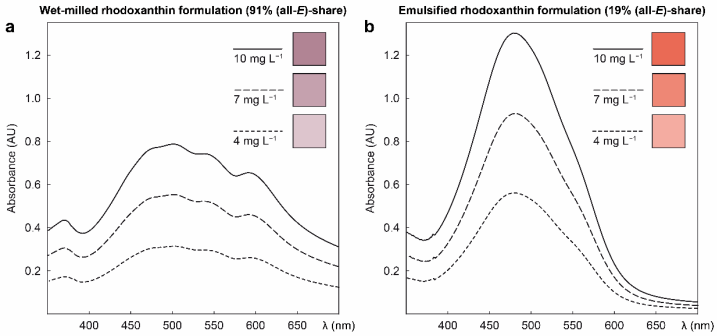

Fig.1. UV/vis spectra of wet-milled (a) and emulsified (b) rhodoxanthin formulations at total rhodoxanthin concentrations of 4, 7, and 10 mg L^{-1} in aqueous dispersion. Modified from Schex et al. [6].

Acknowledgements
We are thankful to Alain Mauranne and Hansjoerg Grass (DSM Nutritional Products) for preparing techno-functional formulations of rhodoxanthin.

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Abstract 33

Antioxidant Activity in Supramolecular Carotenoid Complexes Favored by Nonpolar Environment and Disfavored by Hydrogen Bonding

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Introduction: Carotenoids are well-known antioxidants. They have the ability to quench singlet oxygen and scavenge toxic free radicals preventing or reducing damage to living cells. We have found that carotenoids exhibit scavenging ability towards free radicals that increases nearly exponentially with increasing the carotenoid oxidation potential. With the oxidation potential being an important parameter in predicting antioxidant activity, we focus here on the different factors affecting it. This presentation describes how the chain length and donor/acceptor substituents of carotenoids affect their oxidation potentials but, most importantly, presents the recent progress on the effect of polarity of the environment and orientation of the carotenoids on the oxidation potential in supramolecular complexes. The oxidation potential of a carotenoid in a nonpolar environment was found to be higher than in a polar environment. Moreover, in order to increase the photostability of the carotenoids in supramolecular complexes, a nonpolar environment is desired and the formation of H-bonds should be avoided [1].

Research & Methods: Antioxidant activities of carotenoids and supramolecular carotenoid complexes were determined by the radical-scavenging abilities of these complexes, which were examined by electron paramagnetic resonance (EPR). The oxidation potentials of carotenoids were measured in CH_2Cl_2 by cyclic voltammetry (CV). The photostability of carotenoids was determined by the amount of the radical cations produced at 77 K following photoirradiation. The oxidation potentials of carotenoids in solvents with various polarities were calculated by density-functional theory (DFT) method.

Results and Discussion: The antioxidant activity of a carotenoid is exponentially dependent on the oxidation potential of the carotenoid. The oxidation potential of a carotenoid is related to the conjugation length and donor/acceptor substituents of the carotenoid. Carotenoids with shorter conjugation lengths and containing electron-withdrawing groups have higher oxidation potentials. This conclusion is important for the selection or synthesis of carotenoids in the pharmaceutical, food and cosmetic industries. The polarity of the environment for a carotenoid in a supramolecular carotenoid complex also significantly affects the oxidation potential. The oxidation potential in a non-polar environment is much higher than in a polar environment. The difference in the oxidation potential of a carotenoid in a non-polar solvent with dielectric constant (ε) of about 2 versus that in a very polar solvent with ε of about 80, can be as large as 0.6 V [2].

These results are independent of the symmetries and chain lengths of the studied carotenoids, and are basically applicable to all drugs, nutrients or cosmetics containing conjugated systems. However, the position and orientation of a carotenoid in a complex are to be considered in the determination of the radical scavenging ability as some positions or orientations may block the access of the radical to the most acidic protons of the carotenoid. The chemical bonds or anchoring modes of carotenoids in drug delivery systems significantly affect the photostability and absorption of the carotenoids. The photoinduced charge separation efficiency of a carotenoid H-bonded to a host depends on whether the carotenoid acts as the H-bond donor or acceptor. The efficiency is much higher for the carotenoid acting as the H-bond donor than acceptor. For a carotenoid physisorbed on the surface of a host, the efficiency can be very low, especially if the environment is nonpolar, which results in much higher photostability. The different anchoring modes of a carotenoid on the surface of a semiconductor affect the degree of conjugation of the carotenoid, the driving force for ET and the mixing between the carotenoid's LUMO and the conduction bands of the semiconductor, which in turn affect the photoinduced charge separation efficiency and photostability of the carotenoid. The maximum absorption wavelength of a carotenoid is also different for various anchoring modes, which can be exploited to adjust the color of the carotenoid in a product.

Acknowledgments

This work was funded by the Faculty Research Rewards Program at the Nanjing Agricultural University (Y.G.) and by the Faculty Research Seed Grants (FRSG) Program at Valdosta State University (A.L.F.). It was also funded by the National Science Foundation with EPR instrument grants CHE-0342921 and CHE-0079498 (L.D.K.) and by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Sciences, U.S. Department of Energy, grant DEFG02-86ER-13465 for lab supplies.

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Abstract 34

Cookie waste as alternative low-cost media for carotenoids biosynthesis by *Rhodotorula* sp.

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Introduction: Yeasts are a promising carotenoids biofactory due to their high growth rate and cost-efficiency. However, the main bottleneck during biotechnological production of carotenoids is the medium cost, then agroindustrial waste emerges as alternative. Low-cost substrates are been recently used for the microbial synthesis of carotenoids [1] The aim of the present work was to assess the potential of a low-cost substrate based on cookie waste for carotenoids production by yeast.

Research & Methods: The strain of *Rhodotorula* sp., was donated from the Department of Food Science (Autonomous University of Coahuila, Saltillo) and it was conservated in YM agar at 4 ° C. Three days before use, the strain was reactivated in YM at 25 °C. Assays were performed on shaker in 25mL flasks, as carbon source Cookie-waste (CW), which was provided by a local supplier (Saltillo, México) and Corn steep liquor (CSL) was used as nitrogen source and yeast-malt (YM) medium was used as control, with 10% *Rhodotorula* sp., inoculum, for 144 hours**,** at 25 ºC. A sample was taken every 24 hours. The carotenoids were recuperated and quantified following the methodology described by Martinez *et al.* 2020 [2].

Results and Discussion: In flasks, the highest carotenoids yield per biomass was carried out at 120h, 79,952µg/g, carotenoids production at 120h, 1215,27 µg/L (**Fig. 1).** The carotenoids production is similar to the described by Dias-Rodrigues TV *et al.*, 2019 [1] those in 216h using sugar cane molasse as carbon source and CSL as nitrogen source obtained a productivity of 1248,5 µg/L. CW has potential as a culture medium for the production of carotenoids.

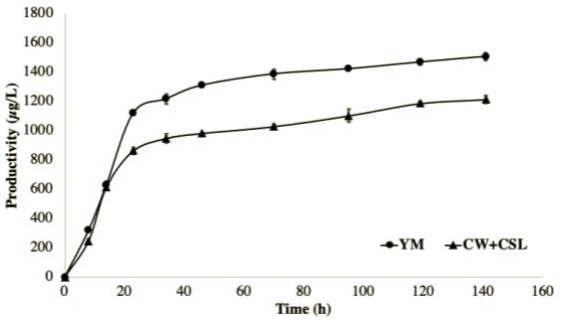

Figure 1. Kinetics of carotenoids productivity by-using cookie-waste as substrate by *Rhodotorula* sp.

Ackowledgements
To CONACYT Mexico for master grant to Elizabeth X. Castrillón Duque (No. 762005-2020).

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Session V Abstracts Photoprotection and Photophysics

Abstract 35

Harnessing Natural Variation in Photoprotection:
Rapid Non-Photochemical Quenching (NPQ) Relaxation in Ferns

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Photosynthesis is a precarious process that brings excited chlorophylls into close proximity with molecular oxygen, which can generate reactive oxygen species. Photosynthetic light harvesting must be continuously regulated to adjust to changes in ambient light conditions in natural habitats and crop canopies. High light exposure rapidly induces non-photochemical quenching (NPQ) mechanisms that are important for photoprotection, but NPQ competes with photochemistry and can require several minutes to relax after transitions back to low light. It has been shown that accelerating the relaxation kinetics of NPQ can improve photosynthesis and biomass productivity in a model crop. A pulse amplitude modulated (PAM) fluorometric survey of over 100 species of land plants was conducted to identify species with rapid (<1 min) NPQ relaxation. Three fern species were identified that exhibit nearly instantaneous induction and relaxation of NPQ under fluctuating light conditions. They also have 2-3x higher NPQ capacity than the model angiosperms *Arabidopsis thaliana* and *Nicotiana benthamiana*. These results were corroborated by time-correlated single photon counting spectroscopy using similar light regimes. High-performance liquid chromatography was used to analyze the levels of chlorophyll and carotenoid pigments during dark acclimation, high light treatment, and recovery in the dark. The fern species have 2-3x higher carotenoid content than leaves of model angiosperms, and they specifically accumulate the photoprotective xanthophylls zeaxanthin and antheraxanthin in the dark, prior to NPQ induction. Additionally, the NPQ in the two xerophytes was not significantly impaired when treated with an inhibitor of zeaxanthin formation, dithiothreitol. This combined evidence suggests that the ferns are primed to induce pH-dependent NPQ, known as qE, but relaxation is not dependent on the conversion of zeaxanthin back to violaxanthin in the dark. These species, therefore, are excellent candidates for discovery of new mechanisms of rapid NPQ relaxation that could lead to increases in crop productivity.

Abstract 36

Direct probing of the (forbidden) S_1 state of carotenoids via two-photon excitation

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The forbidden nature of the carotenoid S_0-S_1 transition implies that the properties of the S_1 state are typically studied after population of the S_1 state from the S_2 state that is readily accessed via allowed S_0-S_2 transition. In this approach, the S_1 state is always prepared as a hot state that cools down on the sub-picosecond time scale. Further, other forbidden excited states may participate in the S_2-S_1 relaxation process, which complicates identification of the excited-state related solely to the S_1 state as the signals originating from different states often overlap.

We have developed an experimental set-up allowing to measure transient absorption spectra of carotenoids after two-photon excitation (2PE), thereby exciting the lowest excited state S_1 directly. First, we have compared 2PE and 1PE data of three carotenoids, lycopene, β-carotene, and neurosporene, in solution. Compared to 'standard' 1PE, the transient absorption spectra of all three carotenoids are broader after 2PE, suggesting larger conformational disorder induced by direct excitation of the S_1 state. Also, 2PE generates slightly longer S_1 lifetimes compared to 1PE, which is most likely due to increased magnitude of the S* signal after 2PE. The difference in the S* signal magnitude is explained by a different subset of conformers excited by 1PE and 2PE.

Further, we have applied femtosecond transient absorption spectroscopy following two-photon excitation (2PE) to the protein binding both carotenoids and chlorophylls, LHCII of higher plants. We have determined contributions of carotenoids and chlorophylls to the 2PE signal obtained after 2PE at 1210 and 1300 nm. At both excitation wavelengths, the transient absorption spectra exhibit a shape characteristic of excited chlorophylls with only minor contribution from carotenoids. We have compared the 2PE data measured for LHCII with those obtained from 2PE of a lutein/chlorophyll *a* mixture in acetone. We estimate that, although the 2PE cross section of a single carotenoid in acetone is ~1.7 times larger than that of a Chl *a*, due to the 1:3.5 carotenoid:Chl ratio in LHCII, only one third of the absorbed 2PE photons excites carotenoids in LHCII in the selected excitation range.

Abstract 37

SINGLET FISSION IN NATURALLY ORGANIZED CAROTENOIDS

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Introduction: The bright colours of many flowers and fruits originate from the carotenoids accumulated in specialized plastids in the plant cell, termed chromoplasts. The main function of the chromoplast is thought to be secondary signaling, attracting animals to flowers and fruits to aid pollination and seed dispersal, respectively. We discovered recently that carotenoids aggregates in chromoplasts are capable to perform singlet fission. The natural variability offers a great platform to understand this phenomenon, the parameters controlling it and its role (if any) in chromoplasts.

Research & Methods: We investigated chromoplasts from tomatoes and daffodils containing aggregated lycopene, and violaxanthin/lutein, respectively. We also created artificial aggregates of carotenoids and compared them with their natural counterpart. We determined carotenoid aggregation parameters, absorption properties, vibrational properties using resonance Raman, and singlet fission efficiency by transient absorption spectroscopy form the fs-to-µs range.

Results and Discussion: We found that carotenoid organization has a large influence on the absorption, on the yield of singlet fission, and on the yield of long-living triplet generation. Absorption spectra of lycopene aggregates (artificial and natural in tomatoes) are largely distorted, with a hypsochromic shift of the main band absorbing in the UV, and a bathochromic shift of the vibronic bands in the visible part peaking at 568 nm accompanied with a tail reaching 700 nm. These lycopene aggregates produce singlet fission and generate long living triplet states living in the microsecond. Interestingly, the chlorophyll presence in the aggregates stabilizes the carotenoids triplet states. In daffodil chromoplasts, the carotenoids present are violaxanthin:lutein (4:5). The absorption spectra of the aggregates is barely distorted respect to isolated carotenoids with small redshift and increased intensity of 0-1 and 0-2 electronic transitions. We observed that these aggregates have efficient singlet fission but that the triplet states recombine quickly in the ps scale. We interpret these different results in function of two parameters: (1) excitonic states produced by the organization of the aggregates and (2) perturbations of the excited states created by imperfections in the organization of carotenoids or dopants like chlorophylls. We hypostatize that higher degree of disorder (different nature) favors the generation of long living triplets.

Acknowledgements: This work was supported by the French Infrastructure for Integrated Structural Biology (FRISBI) ANR-10-INBS-05, and the CNRS 80PRIME interdisciplinary program (CAROFISSION).

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Abstract 38

Time-resolved spectroscopy of trapped orange carotenoid protein

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Introduction: Orange carotenoid protein (OCP) is a carotenoid-binding photoprotective protein found in cyanobacteria. The photoprotective mechanism involves light-induced transition from an inactive (orange; OCPo) form to an active (red; OCPr) form, with an associated large conformational change in the protein. Back-transition from OCPr to OCPo occurs in darkness. The phototransition mechanism is under debate, with the carotenoid S* electronic state recently suggested to be a trigger.[1] Characterising the optical properties of both OCPo and OCPr will give insight into the mechanism. However, previous spectroscopic studies have relied either on taking low temperature measurements to prevent transitions[2] or analysis techniques such as target analysis to isolate the OCPo and OCPr contributions.[3] These techniques can leave associated artefacts. We present room-temperature time-resolved spectroscopic measurements on isolated OCPo and OCPr in sugar films, as well as OCPo and monomeric carotenoids in solution.

Materials and methods: The OCPo and OCPr protein conformations are trapped in optically transparent sugar (sucrose-trehalose) films, and transitions are completely prevented at room temperature. The samples are characterised by steady-state and time-resolved spectroscopy on timescales from picoseconds to milliseconds.

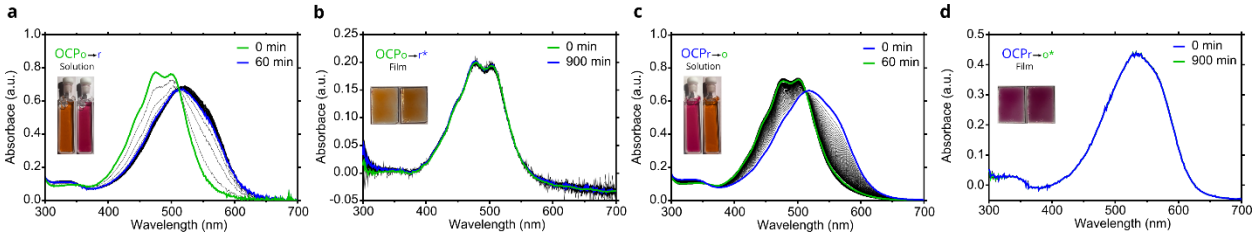

Fig.1. Steady-state absorption spectra taken at 22°C in 1-minute intervals (black lines) showing OCP transitioning in solution (a, c) and not transitioning in sugar films (b, d). OCPo (a, b) is under constant white-light illumination (1600 µmol photon s^{-1} m^{-2}), and OCPr (c, d) is in complete darkness.

Results and discussion: Films of trapped OCPo (OCPr) left under strong white light (complete darkness) at room temperature for several hours shows no change in the UV-vis absorption spectra (Fig.1). Similarly, no transition is observed in the films after experiments using high-fluence pulsed lasers. Excitation wavelength-dependent transient absorption measurements and literature data do not support the S* electronic state as a trigger for the OCPo to OCPr phototransition. We suggest instead that the transition is triggered by carotenoid photoisomerisation occurring on the triplet potential energy surface.

Acknowledgements: The authors thank [c]Dimitri Chekulaev and [b]David G. Bossanyi for assistance with laser systems and spectroscopic setups. J.P.P. thanks the EPSRC Doctoral Training Partnership Scholarship for studentship support.

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Abstract 39

CONTROLLING CT STATES IN CHLOROPHYLL AND CAROTENOID COMPLEX

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Introduction: Carotenoids (Cars) are responsible for protecting against excessive light by quenching excited states of chlorophylls (Chls) or for transferring energy to chlorophylls in the photosynthesis of microorganisms and plants [1]. The knowledge on the excited-state properties of cars is still limited as well as the mechanism of energy flow from Cars to Chls [2]. 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. Also, there was shown there are flexibility up to 20° of Car-Chl pairs in fluctuating environment of the protein [3]. In this study we modelled dimer complexes between carotenoid and chlorophyll by describing additional S_{CT} states which should be involved in the photophysics models also.

Research & Methods: The complexes of Car-Chl pairs were maid artificially by putting them as there are located in crystallography data (PDB code: 1PPR). The Car was chosen fucoxanthin as artificial structure and peridinin as it is in crystallography. Before making the dimers, all structures were optimized separately. Careful orbital analysis allowed us to label each calculated excited state as Chl Q_x, Q_y states or Car S_2 ($1^1B_u^+$) and CT state between Car and Chl. We chose the B3LYP and CAM-B3LYP functionals with cc-pVDZ basis sets for the present study which are available in the Gaussian package.

Results and Discussion: The Car-Chl pairs of fucoxanthin calculations showed there were S_{CT} states below $1^1B_u^+$ type state only at one specific complex according crystallography data: it was labeled as 624. The S_{CT} major configuration was transition between HOMO-LUMO where HOMO was orbital of Car and LUMO was orbital of Chl. The 621, 622 and 623 complexes had three lowest excited states: Q_y, Q_x and $1^1B_u^+$. The same results were for peridinin complexes. The S_{CT} state for fucoxanthin in 624 complex was at 2.53 eV while it's S_2 was at 2.62 eV. The S_{CT} state for peridinin in 624 complex was at 2.62 eV while it's S_2 was at 3.09 eV. It is widely known the scaling factor is required in order they could be comparable to experimental values. The S_{CT} states position in 624 complexes was related to positioning between Car and Chl.

Next step was to study the energetic surface of the 624 complexe with fucoxanthin (Fig. 1). The changes distances between Car-Chl pair parts had clear dependents on S_{CT} positioning according the $1^1B_u^+$ state. The Car was moved in X and Y plane according Chl plane what resulted there was clear surface range were S_{CT} energy positions were below the $1^1B_u^+$ state. The distances were from the 5Å to 1Å from the primary position than it is described in 624 complexes.

The results suggest that the Car-Chl pairs in fluctuating environment of the protein has ability to control the S_{CT} positioning according the $1^1B_u^+$ state and it at low light can disappear while at high light the S_{CT} should be involved in the photophysics models also.

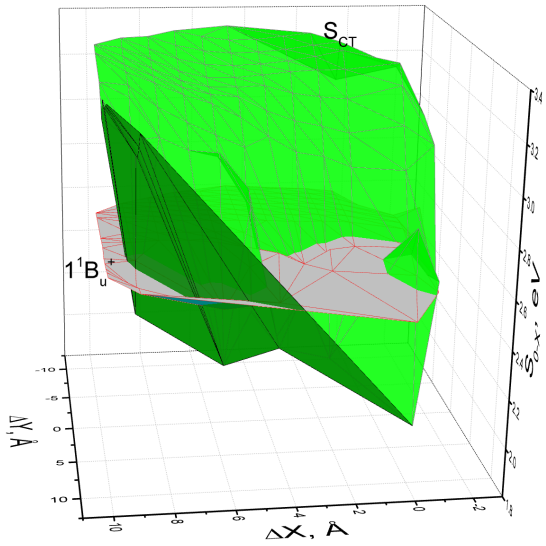

Fig.1. Energetic surface of the 624 complex with fucoxanthin and chlorophyll

Acknowledgements
M.M. and G.K. acknowledges the support by Research Council of Lithuania (grant no. S-MIP-20-47). Computations were performed on resources at the supercomputer "VU HPC" of Vilnius University in Faculty of Physics location.

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Session VI Abstracts Carotenoids and Environment

Abstract 40

UNRAVELING THE EVOLUTIONARY HISTORY OF CAROTENOID MODIFICATION WITHIN STICK AND LEAF INSECT CAMOUFLAGE (INSECTA: PHASMATODEA)

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Introduction: The stick and leaf insects (Phasmatodea) are masters of camouflage with 3,000+ species nearly all mimicking leaves, twigs, and moss (see an example, **Fig 1**). With ~150+ million years of evolutionary history revolving around camouflage, our central hypothesis is that the evolutionary trajectories of these insects have been influenced by the ability of the phasmids to use plant carotenoids for creating camouflage necessary to match the forest surrounding. While several labs are exploring the genetic basis of the morphological innovations, the chemical basis of camouflage coloration is unknown. Therefore, we began studying the role of plant-derived carotenoids in driving phasmid mimicry of plants.

Fig 1 Camouflaging leaf insect.

Methods & Materials: Carotenoids were extracted from two distantly related phasmid species (a stick insect (*Heteropteryx dilatata*) and a leaf insect (*Phyllium philippinicum*), both of which were green in color, but have drastically differing morphologies) as well as the sole host plant species on which the insects were reared (bramble; *Rubus* sp.), using n-hexane: acetone: ethanol (2:1:1 [v/v]) containing 0.01 % (w/v) 2, 6-di-tert-butyl-4-methylphenol (BHT). Extracted carotenoids were suspended in methyl-tert-butyl ether and a saturated NaCl solution was used to remove the water-soluble impurities. HPLC analyses of extracts were carried out using a Waters 2690 Separations Module, with a model 996 photodiode array detector to obtain peak spectra, and a C30 carotenoid column to separate compounds.

Results and Discussion: Phasmid insects were reared on the same plant species, bramble, to compare carotenoid profiles. HPLC analysis revealed that the insects shared carotenoids, such as β-carotene, with the host plant. However, the phasmids also accumulated unique profiles of other unidentified carotenoids that were not detected in the host plant and that were unique to the phasmid species. These results suggest that phasmids can sequester carotenoids obtained from the host plant and modify plant-derived carotenoids in a manner that is specific to the morphologically-variant lineages. Our *future directions* will aim to relate the ability to modify carotenoids with the evolutionary success of different lineages throughout stick and leaf insect history.

Acknowledgements: We thank the Long Island Aquarium for assistance in breeding phasmids; the AMNH, Lehman College CUNY Graduate Center for support to RC and the project; and Lehman College and Huazhong Agricultural University, China, to support KZ at Lehman College.

Abstract 41

Urbanization alters carotenoid metabolic and esterification processes underlying sexually attractive male plumage coloration in a cosmopolitan North American bird species

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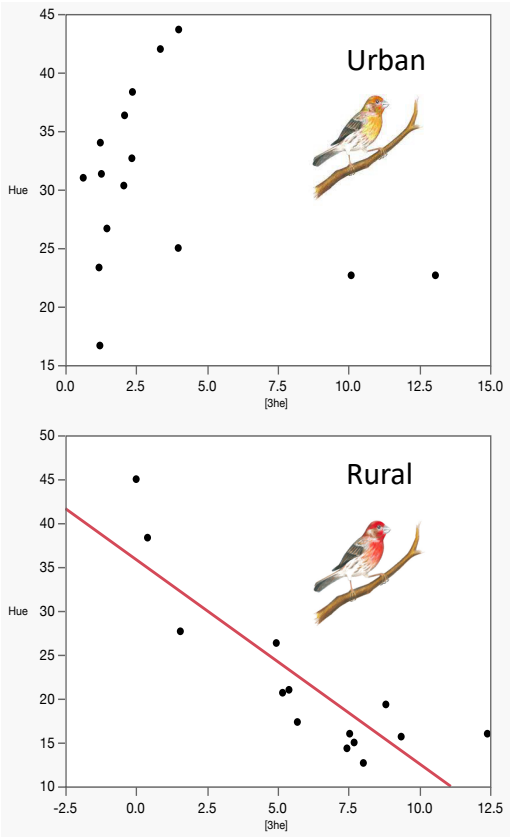
Introduction: Degree of expression of carotenoid-based colors in animals can often reveal the underlying quality of individuals, thus making these colors ideal subjects for studying honest signaling as well as for tracking health and viability of wild populations in relation to rapid environmental changes. Urban development continues to accelerate across the globe, altering natural habitats and often leading to declines in wildlife health and in population extinctions. We have previously shown in several studies that the sexually selected carotenoid-based plumage coloration of house finches (*Haemorhous mexicanus*) is significantly more red in males inhabiting natural areas than in males from urban and suburban areas. However, the carotenoid-specific physiological mechanism(s) underlying such urban impacts are presently unresolved.

Research & Methods: We captured 90 house finches – ca. 15 of each sex from each of a natural (desert park), suburban (residential backyard), and urban (ASU-Tempe campus) study area – during their feather-growth (molt) period (August 2020), and from each bird plucked a small patch of colorful feathers (for digital photography) and drew blood. From photographic measurements of carotenoid-based plumage coloration (hue) and HPLC analyses of circulating carotenoid levels during molt, we investigated (1) if and how circulating carotenoid colorants of plumage differed across urban/suburban/rural habitats, and (2) if the relationship between circulating red carotenoids and the redness of growing feathers was disrupted in urban birds.



Results & Discussion: We found that the primary red, metabolically derived carotenoid (3-hydroxy-echinenone; 3HE) produced for sexually attractive plumage in male house finches is more concentrated in blood samples from molting rural finches, compared to urban and suburban birds, but we also found esterified 3HE in circulation and its levels differed more dramatically as a function of urbanization; esterified 3HE was present in the plasma of nearly all molting rural males, but absent or very low in concentration in nearly all molting urban and suburban males. Moreover, we found that the relationship between circulating 3HE (whether free or esterified) concentration and plumage redness was statistically significant for rural males, but not significant for urban males (Fig. 1). These results implicate two key endogenous processes – carotenoid metabolism and esterification – as driving factors explaining why urban males fail to attain the attractive carotenoid plumage colors of natural males; further work is needed to understand specific environmental and/or physiological (e.g. gene expression, enzymes, fatty-acid esterification) challenges underlying urban limitations in these natural carotenoid products.

Acknowledgements: We thank South Mountain Regional Park managers for permission to study finches there. This work was supported by the National Science Foundation under grant number DEB-1832016, Central Arizona-Phoenix Long-Term Ecological Research Program (CAP LTER).

Abstract 42

Structure elucidation of the novel carotenoid gemmatoxanthin from the photosynthetic complex of *Gemmatimonas phototrophica* AP64

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Introduction: Bacterial phylum Gemmatimonadetes was formally established in 2003, with *G. aurantiaca* as a type species. Despite of its common presence in soil and freshwater habitats, only few Gemmatimonadetes strains have been cultured in laboratory. *G.phototrophica* [1] and *G.groenlandica* [2] are the only two phototrophic strains which represents the phototrophic species in Gemmatimonadetes phylum. *G.phototrophica* contain bacteriochlorophyll *a* as a main light-harvesting pigment and more than 10 different carotenoids [1]. Most of these carotenoids are not bound to the photosynthetic complexes (PS) and probably have only a photoprotective role [3]. Our aim is to characterize unknown carotenoid isolated from the PS of *G.phototrophica.*

Experimental: The unknown carotenoid was isolated from purified photosynthetic complexes of *G. phototrophica* using reverse phase chromatography. Its chemical structure was elucidated using the techniques i.e. HPLC-HRMS/MS, Orbitrap HRMS, Raman, FTIR and NMR spectroscopy.

Results and Discussion: The PS complexes contained an unknown major carotenoid with a unimodal broad absorption spectrum (490 nm) in methanol which showed a three-peak characteristic after reduction in $NaBH_4$ (λmax 422, 447, 475 nm) signalizing the presence of a formyl group adjacent to the conjugated system (Fig.1). The HPLC-APCI-HRMS and orbitrap-HRMS analysis identified a main molecular ion $[M+H]^+$ at *m/z* 613.4264 (Δ= -0.9 ppm) and 611.4106 (Δ= -0.7 ppm) which implied the carotenoid summary formula $C_{41}H_{56}O_4$. The follow up fragmentation analysis documented the presence of methoxy, formyl and carboxyl group. Also the loss of water molecule observed. The functional groups were also identified in the FTIR spectrum at 3378 cm^{-1} corresponds to the hydroxyl group, 1685 cm^{-1} (conjugated carbonyl functional group) and 1741 cm^{-1} (C=O stretch). Raman spectroscopy confirmed the presence of 11 conjugated double bonds. The entire structure was elucidated and supported by the correlation experiment of ^1H-^{13}C HSQC and ^1H-^{13}C HMBC. Therefore, we can conclude the C40 carotenoid from PS is novel, acyclic, linear, containing 11 conjugated double bonds further substituted by methoxy, carboxyl and aldehyde group named as Gemmatoxanthin. Its IUPAC- IUBMB semi-systematic name is 1'-Methoxy-19'-oxo-3',4'-didehydro-7,8,1',2'-tetrahydro- Ψ, Ψ carotene-16-oic acid .

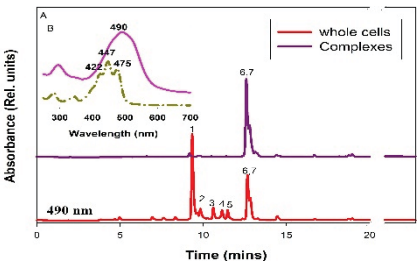
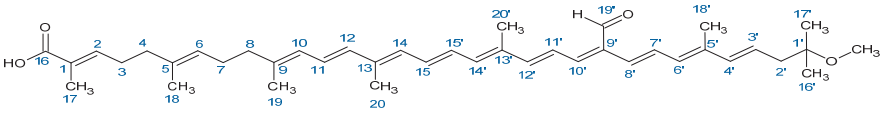


Figure 1. A. HPLC analysis of carotenoids extracted from *G. phototrophica* (red) cells and the purified complexes (dark pink). 1 and 2, (2S,2'S)-oscillol-2,2'-di-(α-L-rhamnoside); 3-5, unknown carotenoids; 6-7, unknown photosynthetic keto-carotenoids. **B.** absorption spectra of peak 6 (solid pink line) and it's reduced from (dashed green line).



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Abstract 43

Carotenoids in Thermal Adaptation of Plants and Animals

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Introduction: It has been reported that carotenoids can change the viscosity of lipid membranes, which affect their different functions. This may help some bacteria and algae, for example, to adapt and function in cold temperatures. Here we present data demonstrating that carotenoids can work not only as lipid antifreezers, but can also increase the lipid thermal energy storing capacity in plants and animals.

Research and Methods: Thermal conductivity and viscosity of plant and animal lipids with fatty acids of different saturation, length and composition were measured. Four carotenoids in different doses were blended into these oils and fats: two carotenes, lycopene and b-carotene, and two xanthophylls, lutein and astaxanthin. The lipid phase transition and temperature monitoring were analysed from -28°C to +80°C.

Results and Discussion: In freeze-thawing experiments it was found that carotenoids were able to not only significantly delay reduction of the temperature in the lipid phase of plant and fish oils, but also keep them warmer overnight. When frozen oils were thawed, carotenoids to could accelerate their melting and further warming up.
In heat exposure experiments, carotenoids were able not only to increase the rate of absorption of the thermal energy but also warm up oils and fats to a significantly higher level. This allowed the accumulated heat to last substantially longer than in lipids without carotenoids.
In all these experiments different carotenoids performed to a different degree and in a dose-dependent manner. Some were better as antifreezers, others were better in the facilitation of heat capacitation and storage. It could be suggested that having more than one carotenoid complementing each other would further be able to expand the range of lipid adaptation to changes of their temperature.
Since lipids are not only structural cellular components but also energy storage and insulating tissues, our results indicate that the presence of carotenoids in plants and animals can increase their ability to adapt and function in different temperature environmental variations such as those of day-night or seasonal, or at different altitudes.

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Abstract 44

CONTROL OF NEUROSPORAXANTHIN BIOSYNTHESIS IN THE FUNGUS *FUSARIUM FUJIKUROI* BY THE RING-FINGER PROTEIN CarS

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Introduction: The fungus *Fusarium fujikuroi* is well known as a producer of different secondary metabolites. Our group uses this microorganism as a model to study the biosynthesis of carotenoids and its genetic regulation. Among the carotenoids produced by this species, it stands out the xanthophyll neurosporaxanthin (NX). Its production is stimulated by light through the action of more than one photoreceptor, of which the White Collar protein WcoA plays a major role. The genes encoding phytoene synthase/carotene cyclase and phytoene desaturase, *carRA* and *carB*, are clustered with a gene involved in retinal biosynthesis, *carX*, and a rhodopsin gene, *carO*. The cluster is upregulated by light and it is overexpressed in the dark in strains with mutations in the gene *carS*, encoding a RING finger protein with features resembling E3 ubiquitin ligases. This communication summarizes the current knowledge on the function of the CarS protein, and the potential use of *carS* mutants for neurosporaxanthin overproduction for biotechnological purposes. Under appropriate growth conditions, *carS* mutants may accumulate up to 7 mg/g of NX, making it suitable to obtain this xanthophyll in large amounts for assays of its antioxidant properties.

Research & Methods: *F. fujikuroi carS* mutants were selected based in their orange pigmentation after chemical mutagenesis. The *carP* mutants were generated by targeted deletion of the long non-coding RNA and their wild allele was ectopically integrated at its *locus* to complement the mutation of the gene. Moreover, double *carPcarS* mutants were also generated after deletion of the lncRNA in an overproducer background. The *carS* gene was introduced under the constitutive *gpdh* promoter and under a doxycycline inducible promoter using the Tet-on system, both in the wild type and the *carS* strains. Gene expression was achieved by qRT-PCR and RNA-seq in illuminated mycelia and compared to controls grown in the dark.

Results and Discussion: Transformants with constitutive expression of the *carS* gene showed an albino phenotype that demonstrates a repressor role of this regulator. In the strains with the *carS* gene controlled by the Tet-on system their carotenoid biosynthesis decreased after the addition of doxycycline, which was explained by a downregulation in the transcription of *carB* and *carRA* genes. Mutants with a deletion of the *carP* lnc-RNA were albino, which correlated with the downregulation of the *car* genes and the increase in the expression of the *carS* gene. These mutants were only complemented when *carP* gene was re-introduced at its original *locus* but not in the ectopic mutants. Transcriptomic data of *carS* and *carP* mutants grown in dark and illuminated will be shown. Results suggest that there could be an interplay between the *carS* and *carP* at RNAs.

Acknowledgements: This work was funded by the Spanish Government with project BIO2015-69613-R from Ministerio de Economía y Competitividad, and Project RTI2018-101902-B-I00 from Ministerio de Ciencia e Innovación (MCI), Agencia Estatal de Investigación (AEI), and Fondo Europeo de Desarrollo Regional (FEDER). Spanish grants included support from European Regional Development Fund.

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Abstract 45

MANIPULATION OF CAROTENOID METABOLISM REDESIGN PLANT ARCHITECTURE, PLANT YIELD, AND METABOLISM IN CROP PLANTS

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Introduction: Carotenoids act as accessory pigments in photosynthesis and play a key photoprotective role. Because they are also of major nutritional importance for animals, carotenogenesis is a target for improving both photosynthesis and the nutritional value of plants. Although carotenoids are important precursors of phytohormones and signaling molecules, previous genetic manipulations had little if any reported effect on general plant development and biomass production, but resulted in specific modifications in carotenoid content. Unexpectedly, the expression of the carrot lycopene β-cyclase (*DcLCYB1*) in *Nicotiana tabacum* cv. Xanthi not only resulted in increased carotenoid accumulation, but also in faster plant growth, early flowering and increased biomass. In this study, we focus on demonstrate our hypothesis: The perturbation of the carotenoid metabolic flux by influencing *LCYB* expression impacts positively/negatively plant yield.

Research & Methods: To validate our hypothesis, we generated nuclear lines (overexpression and RNAi) with increased/reduced *LCYB* expression levels. Lines were characterized at molecular and phenotypical level (in three climate conditions) to confirm the stability of the phenotype. At molecular level, qRT-PCR and UHPLC were used to quantify expression levels of key genes from different plastidial pathways, to quantify pigment and hormone content in our lines. At physiological/phenotypical level we performed photosynthetic measurements to investigate photosynthetic efficiency and biomass experiments to asses and quantify plant development and plant yield, respectively. For the ultimate confirmation of our hypothesis, we used previously published *LCYB*-expressing lines from different plant and bacterial organisms.

Results and Discussion: We observed an increase in plant growth and accelerated development for the nuclear lines. By contrast, RNAi lines showed reduced growth and delayed development. We found that high *DcLCYB1* expression levels, trigger a molecular cascade of processes inside the plastid that led to the increase in plant yield (up to ~23% increase) and photosynthesis in tobacco. Reduced *LCYB* expression levels mimic the molecular cascade observed for the nuclear *DcLCYB1* lines (but all processes downregulated) leading to reduced tobacco biomass (~65% reduction). In our tobacco lines changes in growth, development and photosynthesis can be explained by modifications in hormone content (abscisic acid/ABA and gibberellins/GA) but also due to other isoprenoids. In conclusion, *LCYB* expression affect other key genes from several plastid pathways which is reflected in the accumulation of the metabolites produced on those pathways (e.g. ABA, GA). Thus, leading to an increase/decrease in plant yield and photosynthesis. In order to answer one of the remaining open questions in our research: is this effect specific from the *DcLCYB1* gene or can other *LCYB*s trigger the same effect in food crops? We follow our experimental pipeline using available *LCYB*-expressing tomato lines (transplastomic and nuclear) and ultimately proved our hypothesis by showing altered biomass partitioning (up to 77% in fruits) and enhanced stress tolerance due to alterations in their hormone accumulation. Our results uncover the missing link between carotenoids, biomass, yield, and stress tolerance in plants, which was hidden for more than 20 years. Given that carotenoid pathway is present in all plants, we anticipate a broad application of this strategy in different crops, and specially in cereal crops (e.g., rice) that, in general, do not accumulate high levels of carotenoids but whose yield must be greatly increased by 2050.

Abstract 46

HONEYSUCKLE RHODOXANTHIN SYNTHASE; FROM RED BERRY TO NOVEL BIOCHEMICAL PATHWAY

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Introduction: Rhodoxanthin is a vibrant red carotenoid found across the plant kingdom and in certain birds and fish. It is a member of the atypical retro class of carotenoids which contain an additional double bond and a concerted shift of the conjugated double bonds relative to the more widely occurring carotenoid pigments. While the biosynthetic routes to the major plant carotenoids are well established, the enzymes and pathway(s) to retro-carotenoids have remained elusive.

Methods and Materials: We used transcriptomics and proteomics to discover a honeysuckle beta carotene hydroxylase enzyme (LHRS) that is responsible for rhodoxanthin production in red berries and that confers rhodoxanthin biosynthetic capability when expressed in a rhodoxanthin-lacking plant expression system and a beta-carotene–accumulating bacterial expression system. Site directed mutagenesis was used to identify a small number of amino acids that are responsible for rhodoxanthin activity in LHRS and confer rhodoxanthin-synthesizing activity to a typical tomato beta-carotene hydroxylase. We used MS analysis to identify intermediates in rhodoxanthin accumulating *E. coli* cultures.

Results and Discussion: Here, we identify LHRS (*Lonicera* hydroxylase rhodoxanthin synthase), a variant beta-carotene hydroxylase (BCH)–type integral membrane diiron enzyme that mediates the conversion of beta-carotene into rhodoxanthin. We identify residues that are critical to rhodoxanthin formation by LHRS. Substitution of only three residues converts a typical BCH into a multifunctional enzyme that mediates a multistep pathway from beta-carotene to rhodoxanthin via a series of distinct oxidation steps in which the product of each step becomes the substrate for the next catalytic cycle. We propose a biosynthetic pathway from beta-carotene to rhodoxanthin.

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Abstract 47

A Novel carotenoid biosynthetic route via oxidosqualene

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Introduction: The triterpene carotenoids are biosynthesized via 4,4'-diapophytoene or dehydrosqualene (DSQ) in nature (**route-1**). DSQ (the C_{30} counterpart of phytoene) is the custom-made precursor for carotenoids, and sorely used for the construction of carotenoid pigments. Recently, we reported that *Staphylococcus aureus* carotenoid desaturase CrtN can convert squalene (SQ) into DSQ [1, 2], enabling the biosynthesis of numbers of carotenoids derived from SQ (**route-2**), the precursor of sterols and hopanoids. In this presentation, we report the construction of another route for carotenoid *via* oxidosqualene (OSQ), the direct precursor of plant and animal triterpenoids including steroids and saponins.

Methods: Squalene epoxidase (SQE) from *Arabidopsis thaliana*, squalene synthase (SQS) from human, and CrtN were co-expressed in *Escherichia coli*. This strain was cultivated at TB medium, and then products were analyzed by HPLC/MS.

Results and Discussion: In natural triterpenoid pathways, one side of the SQ is epoxidated by SQE into OSQ, which is the common substrates of a number of the oxidosqualene cyclases, to form a variety of complex structures. Very recently, we happened to discover that the co-expression of SQE with squalene synthase and CrtN in *E. coli* resulted in the accumulation of novel, epoxidated carotenoid pigment with a molecular mass of 418 ($C_{30}H_{42}O$) with unique absorption spectrum (413, 435, 464 nm in methanol, putative number of conjugated double bonds = 9). Unlike other carotenoid desaturases, CrtN has been demonstrated to be highly promiscuous, acting on various compounds with isoprenyl substructures [1-4]. Based on this, we propose another route for carotenoid pigment (**route 3**). In this presentation, we report our recent effort to expand, evolve, and use this new carotenoid pathway for biotechnological applications.

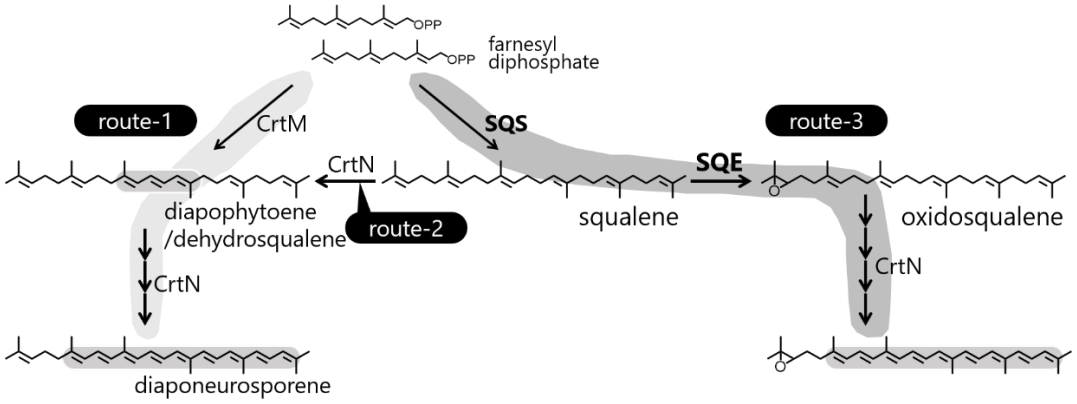

Figure 1 Three routes toward triterpenoid carotenoids

Acknowledgements: This work was supported by a Grant-in-Aid for JSPS Fellows.

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Session VI Abstracts Carotenoid Synthesis and Chemistry

Abstract 48

DESIGNING NOVEL CAROTENOIDS FOR IMPROVED ANTIOXIDANT ACTIVITY – SYNTHESES AND DPPH / ABTS RADICAL ASSAYS

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Introduction: Carotenoids are structurally characterized by the conjugated polyene chains, which exhibit anti-oxidant activity by scavenging reactive oxygen species. These health-benefit nutraceuticals find wide applications in colorant, food additive, cosmetics, and drug industries. Synthesis of the polyene chain of carotenoids mostly relied on the Wittig olefination reaction. Sulfone-mediated method, known as Julia olefination, has been applied only to the production of retinol derivatives. We extended the synthetic repertoires of the carotenoid polyene chains by use of organo-sulfur chemistry. [1]

Isoprene was utilized as the starting material. Various building blocks containing a sulfone group were devised for the construction of the carotenoids. Benzothiazolyl (BT) sulfone **2** containing an acetal moiety was devised for iterative chain extension of apocarotenoids from C_{10} dial **1**, and crocetin **4** was efficiently prepared. [2] A C_5 unit **3** containing BT-sulfone and diethyl phosphonate moieties was developed for the sequential olefination reactions for carotenoids, which firstly reacted with C_{10} dial **1** to give C_{20} bis(diethyl phosphonate) **5**. [3] We envisaged that the above extended C_{20} polyene building blocks would allow us to design and synthesize diverse novel carotenoid compounds with improved anti-oxidant activity in a highly efficient manner.

Objectives of Research: The plan was to develop novel carotenoids **6** from the above C_{20} building blocks by attaching terminal benzene rings of diverse substituents of different electronic nature and/or steric bias to facilitate or deter an effective conjugation with the polyene chain, which would affect the anti-oxidant activities of the new carotenoids. Another plan of this research was to devise apo-carotenals to compare the anti-oxidant activities with those of the full-sized novel carotenoids.

Results and Discussion: BT-sulfone **3** with a phosphonate moiety reacted with C_{10} dial **1** to produce C_{20} bis(diphosphonate) **5**, which provided novel carotenoids upon olefination with various benzaldehydes (Ar-CHO). Electron-donating or electron-withdrawing substituents were attached at the *para*-position of the benzene ring for maximize or minimize the conjugation effect. Alkyl-substituents were introduced at *ortho*-, *meta*-, and *para*-positions to determine the steric bias generated by the substituent. High throughput screening and hierarchical clustering analysis were applied to the novel carotenoids for evaluation of their antioxidant activities utilizing DPPH and ABTS assays. [3] All the electronic and steric effects generated by the substituents in the benzene ring on the effective conjugation (or anti-oxidant activity) were fully elucidated.

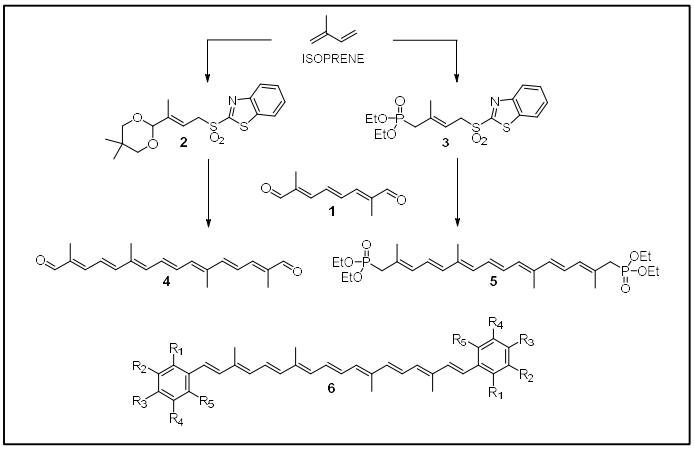

Fig.1. Synthetic plan for the novel carotenoids **6**.

Apocarotenoids are secondary metabolites in nature by oxidative degradation of natural carotenoids. We were interested in the effect of the formyl conjugation of the intermediate radical species, generated by ROS quenching. Various apo-carotenals were prepare, and their anti-oxidant activities were compared with the corresponding full-sized novel carotenoids by DPPH / ABTS radical scavenging assays. All the above results will be summarized in the online presentation.

Acknowledgements
This work was supported by the National Research Foundation of Korea (NRF) through the grant funded by the Korean government, Ministry of Science and ICT (NRF-2020R1A2C1010724) and partly by Basic Science Research Program funded by the Ministry of Education (NRF-2020R1A6A1A03038817).

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Abstract 49

JOINING CAROTENOIDS AND POLYACETYLENES

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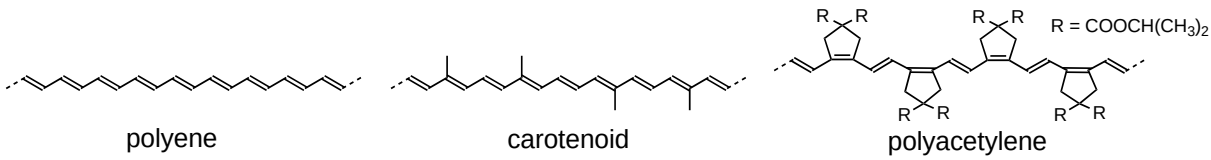
Introduction: Both Paul Karrer (1889–1971) and Richard Kuhn (1900–1967) became progenitors of the chemistry of conjugated polyenes when they independently determined the structure of *β*-carotene as a compound with a methyl-branched polyene chain. Whereas Kuhn was interested in polyenes, branched or unbranched, Karrer concentrated on carotenoids. Karrer's book "Carotenoids" (1948) initiated the separation of carotenoids from polyenes, and the cultivation, by each field, of its own jargon: investigators of methyl branched compounds developed the carotenoid nomenclature, and the polyenes came to be called polyacetylenes. Regardless of the family, the shift, with increasing N (number of double bonds), of the wavelength of maximum absorption towards longer wavelength, and its approach to a limiting value λ_∞ are fundamental topics in spectroscopy, molecular electronics and material sciences. In 1937, Kuhn set the limit of polyene chain extension to $N = 15$. Karrer located, in 1951, the ceiling of *β*-carotene lengthening at $N = 19$. Ignoring experimental limits, theorists extended the polyene chain to $N = \infty$ and determined λ_∞ = 536–913 nm. In 1999, the synthesis of polyacetylenes with $N = 1100$ was proclaimed, which rose to $N = 100000$ in 2010. Yet, syntheses of long polyacetylenes are adversely affected by the inability to obtain products with N_{exact}. Subsequent studies have revealed that the declared high number of conjugated C=C bonds is yet to be attained.
Results: Therefore, Karrer's $N = 19$ constraint persisted to 2012 when the longest polyene (N =27) was synthesized.[1] In 2020, λ_{max} of pure "short" polyacetylenes (N=5-23) were extrapolated together with the λ_{max} of zeaxanthins (N = 11-27). Both polyacetylenes and carotenoids reached the same $\lambda_\infty \approx 623$ nm.[2][3]
Conclusion: With the identical, inadvertently found λ_∞, both carotenoids and polyacetylenes were un-noticedly again united as polyenes. There is also no longer any reason to maintain the disjunction of carotenoids and polyacetylenes below $N = \infty$. Combining the chemistry of polyacetylenes and carotenoids predicts achieving long polyenes with an exactly quantifiable number of double bonds.



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Abstract 50

Stereocontrolled Synthesis of 5,8-Epoxycarotenoids by Bidirectional Horner-Wadsworth-Emmons Reaction

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Introduction. Carotenoids are an important group of natural pigments, that are involved in photosynthesis and photoprotective structural arrangements, acting also as antioxidants, and hold potential as chemopreventive agents in humans.[1] Nature has modulated these activities by subtle structural changes, often located at the terminal cyclohexenyl ring and its proximal double bonds. A particular modification common to all carotenoid furanoxides (traditionally called 5,8-epoxides) is the presence of one (or two) furanoxide ring at C5 and C8 (Figure 1).

Methods and Materials The aim of this work is the stereo- and enantioselective synthesis of all-*trans* C_{40}-carotenoids with dihydrofuran skeletons, namely symmetrical aurochrome and auroxanthin and non-symmetrical equinenone-5',8'-epoxide, based on a one-pot or stepwise bidirectional Horner-Wadsworth-Emmons (HWE) reaction of a C_{15}-5,6-epoxydienylphosphonate and a central C_{10}-trienedial **4**

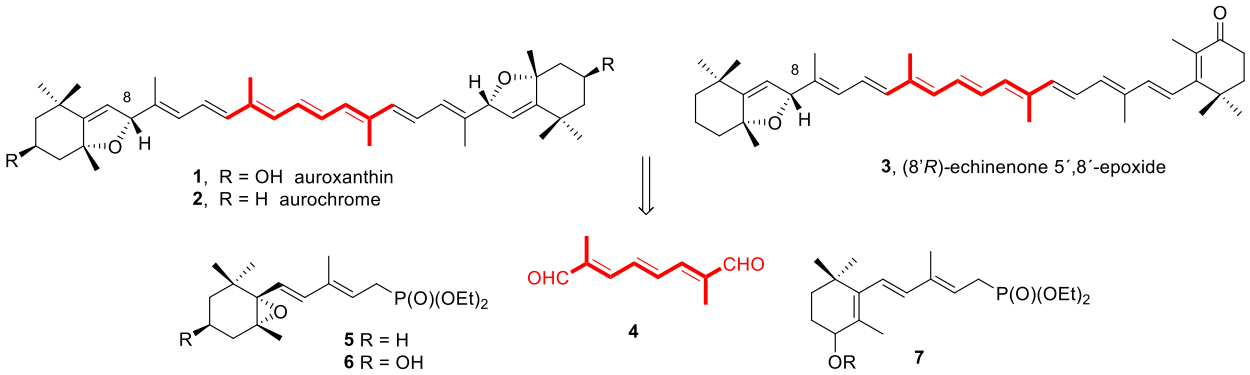

Fig. 1. Proposed synthetic scheme for the synthesis of carotenoid furanoxides.

Results and Discussion: This strategy, based on a $C_{15} + C_{10} + C_{15} = C_{40}$ pattern, operates through the concomitant HWE reaction and stereoretentive C5,C6-epoxide ring-expansion to the C5,C8-dihydrofuran promoted by the basic media. Unexpectedly, if previously formed, the C_{15}-5,8-dihydrofuranylphosphonates proved to be unreactive under the HWE reaction conditions

Acknowledgements: Spanish MINECO (PID2019-107855RB-I00) and Xunta de Galicia (GRC ED431C 2017/61); ED-431G/02-FEDER "Unha maneira de facer Europa" to CINBIO, Galician Research Center 2016-2019 (contract to A.R.).

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Session IXA Abstracts Carotenoid and Apocarotenoid Biology

Abstract 51

An update on the plant apocarotenoid growth regulators Anchorene and Zaxinone

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Introduction: Carotenoids are a source for regulatory metabolites, including plant hormones, such as abscisic acid (ABA) and strigolactones (SLs) [1]. Besides these known growth regulators, several lines of evidence suggest that plants rely on yet unidentified carotenoid-derivatives to regulate their growth, development and carotenoid biosynthesis itself [1]. Recently, we have reported on the C_{18}-apocarotenoid zaxinone and the C_{10}-dialdehyde anchorene as two novel carotenoid-derived growth regulators [2, 3]. Anchorene acts as a specific signal regulating the outgrowth of a less investigated type of Arabidopsis roots, the anchor roots, while zaxinone is a candidate for a novel plant hormone/precursor thereof required for normal plant growth and development. We identified anchorene by testing the effect of known and presumed carotenoid cleavage products on Arabidopsis root growth and architecture, and confirmed its presence as a natural metabolite. Further screening unraveled a structural isomer of anchorene, called iso-anchorene, as a further diapocarotenoid with growth-regulating activity [4]. Zaxinone is a common plant apocarotenoid. We identified a rice carotenoid cleavage dioxygenase, Zaxinone Synthase (ZAS), which catalyzes the formation of zaxinone *in vitro*. A corresponding mutant shows retarded growth and elevated levels of SLs. Application of zaxinone to this mutant partially rescued the growth phenotypes and decreased SL content and release. Treatment of wild-type seedlings promoted their growth and decreased their SL biosynthetic activity [2]. These results indicate the importance of zaxinone for rice development as well as its application potential in agriculture. However, our understanding of the mechanisms underlying the growth-promoting activity of zaxinone is still limited. In addition, exploiting the potential of zaxinone in fundamental science and agricultural application is constrained by the difficult synthesis of this compound and its commercial non-availability.

Research & Methods: We investigated the effect of iso-anchorene on Arabidopsis root meristem and auxin homeostasis [4]. For this purpose, we employed several Arabidopsis marker lines and used LC-MS analytic to investigate the presence of iso-anchorene in Arabidopsis. We developed easy to synthesize and efficient zaxinone mimics [5]. The activity of synthesized compounds was determined by phenotyping treated rice seedlings, expression analysis of SL biosynthetic genes, LC-MS based quantification of SLs, and *Striga* Bioassay.

Results and Discussion: We demonstrate that iso-anchorene is a growth inhibitor with specific impact on the growth of primary roots and that it exerts its inhibitory effect by decreasing cell division rates in the root apical meristem. Furthermore, we show that iso-anchorene activity involves changes in local auxin concentrations. Finally, we confirm that iso-anchorene is a natural Arabidopsis metabolite. To pave the way for further investigation of zaxinone biology, we developed highly efficient mimics of zaxinone (MiZax) with simple structure. For this purpose, we first analyzed the structure-activity relationship of zaxinone by testing the biological activity of a series of apocarotenoids that differ from zaxinone in several structural features. Based on these results, we developed phenyl-based compounds that can be produced in a simple method and with high yield. Activity assessment showed that the synthetic compounds MiZax3 and MiZax5 can rescue the retarded growth phenotype of the *zas* mutant, and decrease SL biosynthetic activity, similar to zaxinone.

Acknowledgements

This work was supported by baseline funding and a Competitive Research Grant (CRG2017) given to Salim Al-Babili from King Abdullah University of Science and Technology, the Bill and Melinda Gates Foundation (grant OPP1194472), and by grants from the Core Research for Evolutional Science and Technology(CREST) Program and the SATREPS Program of the Japan Science andTechnology Agency (JST) given to Tadao Asami.

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Abstract 52

THE APOCAROTENOID ZAXINONE IS A POSITIVE REGULATOR OF STRIGOLACTONE AND ABSCISIC ACID BIOSYNTHESIS IN ARABIDOPSIS ROOTS

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Introduction: Carotenoids are precursors of many regulatory metabolites in plants as well as in other organisms. For instance, the carotenoid-derived plant hormones strigolactones (SLs) and abscisic acid (ABA) are important signal molecules in regulating plant growth, development, and stress response. Recently, zaxinone was identified as a novel apocarotenoid metabolite that promotes growth and development and negatively regulates SL biosynthesis and release in rice [1]. Rice contains the enzyme Zaxinone Synthase (ZAS) that was shown to catalyze zaxinone production *in vitro* [1]. However, zaxinone is a common plant metabolite and is present in plant species including Brassicales species, such as *Arabidopsis thaliana*, which lack ZAS orthologues. This raises the question about the biological activity of zaxinone in such plants.

Research & Methods: In this study, we investigated the biological activity of zaxinone in Arabidopsis, focusing on its possible effect on SL and ABA biosynthesis. In this regard, we quantified the content of both hormones and determined the transcript levels of corresponding biosynthetic genes in Arabidopsis roots upon zaxinone treatment. For SL quantification, we also conducted *Striga* seed germination bioassay [2].

Results and Discussion: Recently, we have reported that the apocarotenoid zaxinone, synthesized by the zaxinone synthase (ZAS), is a regulatory metabolite suppressing strigolactone biosynthesis in rice [1]. In contrast to rice, we show here that zaxinone application led to an increase in SL biosynthesis through enhancing the transcript levels of the SL biosynthetic enzymes MORE AXILLARY GROWTH 3 (MAX3) and MORE AXILLARY GROWTH 4 (MAX4) in Arabidopsis roots [1,2]. Moreover, zaxinone application triggered the expression of stress related genes and alleviated ABA content by inducing the expression of ABA biosynthetic genes, including *ABA1*, *ABA2*, and 9-*cis*-epoxy carotenoid dioxygenases (NCED2, NCED3 and NCED9) in Arabidopsis roots (Fig. 1). In addition, the increase in ABA biosynthesis upon zaxinone application caused a reduction in hypocotyl elongation. These results suggest that zaxinone acts in Arabidopsis as a stress signal that enhances the level of stress related hormones, rather than being a growth promoting compound as observed in rice. The current findings provide new opportunities to broaden our knowledge about biological role of zaxinone in non-mycorrhizal plant species, such as Arabidopsis, which lack ZAS homologs.

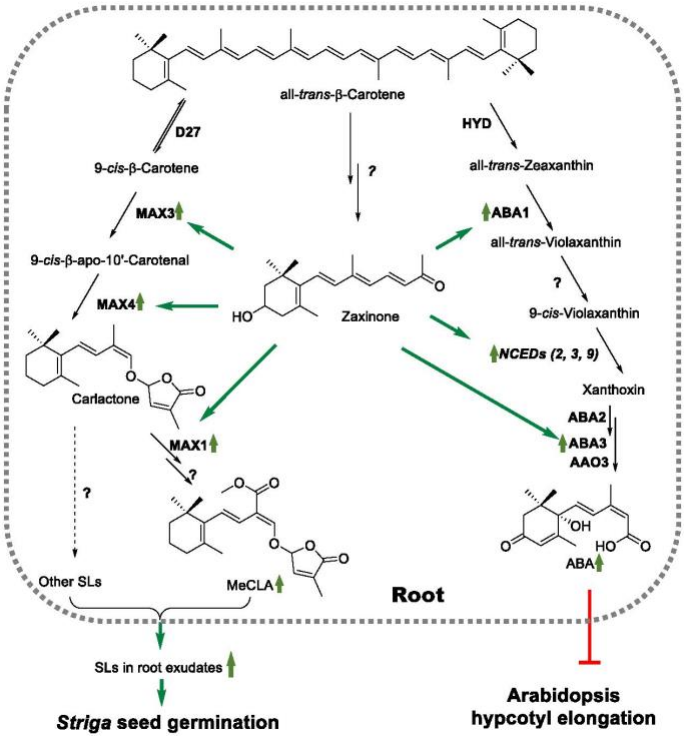

Fig. 1. Proposed model of zaxinone effect on SL and ABA biosynthesis in Arabidopsis root. Application of zaxinone caused an increase in the production of the SL (methyl carlactonoate, MeCLA) in Arabidopsis roots by enhancing the expression of genes involved in its biosynthesis, including *MAX3* and *MAX4*. High MeCLA production results in higher SL content in root exudates and, hence, higher activity in inducing *Striga* seed germination. In addition, zaxinone application led to an increase in the transcript level of ABA biosynthetic genes, i.e. *ABA1*, *ABA3* and *NCED2*, *3*, *9*), giving rise to a higher root ABA content and reduced hypocotyl elongation [2].

Acknowledgements
This work was supported by baseline funding and a Competitive Research Grant (CRG2017) given to Salim Al-Babili from King Abdullah University of Science and Technology.

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Abstract 53

ACTIVITY-DEPENDENT PALMITOYLATION OF CAROTENOID CLEAVAGE OXYGENASES: A COMMON THEME?

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Introduction: Carotenoid cleavage oxygenases (CCOs) are a family of carotenoid cleaving enzymes widespread in all kingdoms of life. One member, RPE65 retinoid isomerase, a divergent outlier that neither has a carotenoid as substrate (it uses all-*trans* retinyl esters) nor does it do oxidative cleavage of carbon:carbon double bonds (rather an O-alkyl cleavage of retinyl ester), is well-known to be cysteine-palmitoylated, and this is associated with a -PDPCK- motif common to the majority of metazoan CCOs. We have shown that RPE65 palmitoylation is function-dependent, modulated by the presence of active lecithin:retinol acyltransferase (LRAT). We wished to see if this was a particular feature of RPE65 or if it was common to other CCOs.
Materials and Methods: Constructs expressing different CCOs were developed for transfection into HEK293F and COS7 cells. Confocal microscopy was used to study localization of BCO2 in different organelles. Phospholipid large unilamellar vesicle (LUV)-based nanocarriers were developed to deliver xanthophylls into cells. CCO palmitoylation was detected by the acyl-resin assisted capture (acyl-RAC) method. Ligand docking modelling was carried out using Autodock Vina using models developed on Swiss-Model and I-Tasser servers.
Results: We found that in the absence of its β-carotene substrate, BCO2 was palmitoylated. However, in the presence of 0.2 μm β-carotene, BCO2 lost its palmitoylation and also showed partial re-localization from mitochondria to nuclei. As BCO2 also cleaves xanthophylls, we developed a novel non-disruptive (i.e., no detergents) method to deliver xanthophylls to cells using limonene to assist solubilization of xanthophylls in bilayers of appropriately sized phospholipids. Using these, we found that when treated with 0.15 μM lutein, BCO2 is also depalmitoylated with partial nuclear localization (38±0.04%), while zeaxanthin (0.45 μM) and violaxanthin (0.6 μM) treatment induced depalmitoylation and protein translocation to a lesser degree (20±0.01% and 35±0.02% respectively). This suggests that loss of palmitoylation upon substrate binding promotes BCO2 shuttling to the nucleus. However, the secreted BCOLs and cytosolic ACOLs (both lacking the -PDPCK- motif) lacked evidence of palmitoylation.
Conclusions: Like RPE65, BCO2 shows evidence of palmitoylation, but this is lost in presence of its substrate. The translocation of mBCO2 into the nucleus in the presence of β-carotene and various xanthophylls suggests this as a possible mechanism for transport of carotenoids and/or carotenoid cleavage products to the nucleus to affect transcriptional regulation, such as activation of cellular oxidative stress response genes. The apparent lack of palmitoylation of BCOLs and ACOLs suggests that CCO palmitoylation is dependent upon presence of the -PDPCK- motif.
Acknowledgements: This research was funded by the Intramural Research Program of the National Eye Institute, NIH and by National Science Foundation (CHE-1709921). Access to vSANS and NSE instruments was provided by the Center for High-Resolution Neutron Scattering, a partnership between the National Institute of Standards and Technology (NIST) and the National Science Foundation under Agreement No. DMR-2010792. We acknowledge the support of NIST, U.S. Dept. of Commerce, in providing the neutron research facilities used in this work.
Conflicts of Interest: The authors declare no conflict of interest.

Abstract 54

Structural and functional analysis of Carotenoid oxygenase 2

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Introduction: Apocarotenoids share a common structure and play important roles as signaling molecules and chromophores in all kingdoms of nature. Modifications of their ionone rings and variations in chain length contribute to the large diversity of these compounds. Apocarotenoids have been implicated in modulating cellular signaling pathways across different species. However, the mechanism that controls the activity of these dietary compounds remain undefined.

Methods and Materials: We studied the biology and chemistry of the enzyme β-carotene oxygenase-2 (BCO2) in the metabolism of long-chain β-apocarotenoids using recombinant murine BCO2. We utilize structure modeling, site-directed mutagenesis, together with high-performance liquid chromatography analysis to scrutinize the enzymatic properties of BCO2.

Results and Discussion: Our analyses show that the BCO2 plays a critical role in the apocarotenoid metabolism. Recombinant mouse BCO2 cleaved the alcohol, aldehyde, and carboxylic acid of an apocarotenoid substrate. Chain length variation (C20 to C40) and ionone ring site modifications of the apocarotenoid substrate did not impede the catalytic turnover by BCO2. Structural modeling identified Asn132 and Phe525, two highly conserved amino acids in the substrate tunnel of BCO2, as critical for the binding of the carbon backbone of an apocarotenoid and the positioning of the scissile C9, C10 double bond to the ferrous iron in the active center. Site-directed mutagenesis, Asn132Leu, and Phe525Leu impeded catalytic turnover by a mutant BCO2 variant. Mice deficient for BCO2 displayed impaired metabolism and altered signaling responses when supplemented with apocarotenoids. Thus, our analysis established BCO2 as an apocarotenoid scavenger with broad substrate specificity that prevents inadvertent side reactions of these dietary compounds with endogenous signaling pathways.

Acknowledgements

We wish to thank Dr. A. Wyss (DSM) for the gift of 3-hydroxy-β-12′-apocarotenal and zeaxanthin enantiomers. We are grateful to Dr. H. Ernst (BASF) for the gift of the different β-apocarotenoic acids. This research was funded by the NIH grants R01EY020551 and T32EY007157.

Abstract 55

Human β-carotene oxygenase 2 demonstrates enzymatic activity towards carotenoids and apocarotenoids

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Introduction: Carotenoids are lipophilic pigments with light-absorbing property that conduct various physiological functions in organisms. The enzyme β-carotene oxygenase 2 (BCO2) eccentrically cleaves carotenoids into more polar metabolites. Studies in vertebrates revealed that BCO2 controls carotenoid homeostasis and is involved in the pathway for vitamin A production. However, it is controversial whether BCO2 function is conserved in humans, due to a 4-amino acid long insertion caused by a splice acceptor site polymorphism. Therefore, we examined the conservative nature of human BCO2 enzymatic activity towards carotenoids and apocarotenoids.

Methods: We expressed recombinant isoforms of human BCO2 and mouse BCO2 to determine the biochemical factors that influence enzymatic activity. We performed both cell-based enzymatic assays and *in vitro* enzymatic activity assays to examine differences in enzymatic activity between various isoforms. High performance liquid chromatography (HPLC) was used to determine BCO2's capability to cleave these substrates and identify the products that were generated.

Results & Discussion: We show that human BCO2 splice variants, BCO2a and BCO2b, are expressed as pre-proteins with mitochondrial targeting sequence (MTS). When expressed in ARPE-19 cells, the MTS of BCO2a directed a green fluorescent reporter protein to the mitochondria. Removal of the MTS increased solubility of BCO2a when expressed in *Escherichia coli* and rendered the recombinant protein enzymatically active. Introduction of the 4-amino acid insertion into mouse *Bco2* did not impede the enzyme's catalytic proficiency. In addition, we showed that the chimeric BCO2 displayed broad substrate specificity and converted carotenoids into two ionones and a central C14-apocarotendial by oxidative cleavage reactions. Thus, our study demonstrates that human BCO2 is a catalytically competent enzyme. Consequently, our data on BCO2 becomes broadly applicable in human biology with important implications for the physiology of the eyes and other tissues.

Acknowledgments: We thank Dr. Min Hyung Kang (Ophthalmology, Case Western Reserve University) for sharing retinal samples for RNA isolation. This research was funded by NIH grants RO1EY020551 and T32EY007157.

Session IXB Abstracts Carotenoid and Apocarotenoid Biology

Abstract 56

Regulation of plasma cholesterol by β-carotene and β-carotene oxygenase 1 in mice

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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 is associated with circulating cholesterol and the risk of developing atherosclerosis. Whether β-carotene or its vitamin A derivative(s) are responsible for this association remains unclear. In this study, we aimed to determine the impact of dietary β-carotene and the role of β-carotene oxygenase 1 (BCO1), the enzyme responsible for β-carotene cleavage to vitamin A, on circulating cholesterol levels and atherosclerosis development.

Materials and Methods: In the first study, we compared congenic wild-type and *Bco1*$^{-/-}$ mice fed a Western diet deficient in vitamin A containing 50 mg/kg of β-carotene (WD- β-carotene). In the second study, we compared low-density lipoprotein receptor (LDLR)-deficient (*Ldlr*$^{-/-}$) mice and *Ldlr*$^{-/-}$*Bco1*$^{-/-}$ fed WD- β-carotene or the same diet without β-carotene (WD-control).

Results and Discussion:
In the first study, we observed that *Bco1*$^{-/-}$ mice accumulated >20-fold greater plasma β-carotene than wild-type control mice fed the same diet, and 2- fold increase in circulating total cholesterol ($p<0.01$) and non-HDL cholesterol ($p<0.01$). In the second study, we observed that *Ldlr*$^{-/-}$ mice fed WD- β-carotene presented a reduced atherosclerotic lesion size ($p<0.05$) that correlated with reduced plasma cholesterol levels ($p<0.05$) in comparison to mice fed WD-control. *Ldlr*$^{-/-}$*Bco1*$^{-/-}$ mice fed WD- β-carotene accumulated β-carotene in plasma and atherosclerosis lesions but failed to show differences in plaque size or lipid parameters in comparison to *Ldlr*$^{-/-}$*Bco1*$^{-/-}$ mice fed WD-control. Overall, our results suggest that BCO1 activity and β-carotene contribute to circulating cholesterol levels, linking vitamin A formation with the risk of atherosclerosis progression.

Acknowledgments
We thank the American Heart Association and the National Institute of Health Grants.

Abstract 57

Lack of beta-carotene 9',10' dioxygenase (BCO2) impairs cardiac metabolic adaptations in adult female mice

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Introduction: As the postnatal heart grows it also undergoes critical metabolic adaptations to use mainly lipids rather than carbohydrates as energy-providing substrates. Abnormal growth patterns and lack of metabolic flexibility are pathological responses of the heart that lead to cardiovascular diseases (CVD). Dietary intake of carotenoids, such as β-carotene - the most abundant dietary precursor of vitamin A - have been linked to the prevention of CVD. In the heart, β-carotene can be metabolized to retinoids and other apo-carotenoids by the action of β-carotene 9',10'-dioxygenase (BCO2), which is the only β-carotene cleavage enzyme expressed in the adult heart. We found that *Bco2*$^{-/-}$ (mutant) female mice have significantly smaller hearts than wild-type (WT), both pre- and post-puberty. It is well known that aberrations in heart growth is accompanied by underlying cardiac metabolic adaptations as well. Therefore, we aim to elucidate the role of BCO2 in cardiac metabolic adaptations in the adult heart. We hypothesize that loss of BCO2 impacts postnatal cardiac metabolic reprogramming.

Methods and Materials: Age matched WT and *Bco2*$^{-/-}$ female mice raised on a chow diet were used to determine differences due to loss of BCO2. Cardiac mRNA and protein expression of retinoid and metabolic regulatory genes were measured. HPLC and LC/MS detected cardiac retinoids (vitamin A and its derivatives) levels.

Results and Discussion: Expression of cardiac foetal genes (*BNP, α-Skeletal actinin* and *β-MyHc*), typically upregulated during embryogenesis or under pathological conditions in the adult, were significantly increased in the mutant. *Bco2*$^{-/-}$ females also showed impaired exercise capacity, displaying a ~40% reduction in running distance compared to WT. These data indicate functional cardiac defects in the mutant females under stress conditions. Additionally, from a cardiac energetic perspective, we found that *Bco2*$^{-/-}$ females displayed impaired cardiac metabolic flexibility that we could link to retinoic acid insufficiency in this organ. We found that *Bco2*$^{-/-}$ mice have reduced retinoic acid levels in the heart due to increased oxidative stress in this organ. Such reduced retinoic acid levels downregulated Pdk4 expression and hence enhanced PDH activity. Thus, as expected, we demonstrated that the heart of the female mutant mice relies mainly on glucose, rather than lipids, as an energy providing substrate. Indeed, for instance, cardiac expression of *Glut1* and *Mct1*, key glucose and lactate transporters, respectively, were significantly higher in *Bco2*$^{-/-}$ females, while triglyceride levels were lower in the heart and serum. These and other findings provide novel critical insights into a potential role of the retinoid and carotenoid metabolic pathways in regulating adult heart function.

Sources of Funding: NIH/NHLBI F31 (1F31HL143930-01)

Abstract 58

LRAT coordinates the negative-feedback regulation of intestinal retinoid biosynthesis from β-carotene

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Introduction:
There is increasing recognition that dietary lipids can affect the expression of genes encoding their metabolizing enzymes, transporters, and binding proteins. This mechanism plays a pivotal role in controlling tissue homeostasis of these compounds and avoiding diseases. The regulation of retinoid biosynthesis from β-carotene (BC) is a classic example for such an interaction. The intestine-specific homeodomain transcription factor (ISX) controls the activity of the vitamin A-forming enzyme β-carotene oxygenase-1 in intestinal enterocytes in response to increasing concentration of the vitamin A metabolite retinoic acid. However, it is unclear how cells control the concentration of the signaling molecule in this negative-feedback loop. We demonstrate in mice that the sequestration of retinyl esters by the enzyme lecithin:retinol acyltransferase (LRAT) is central for this process.

Materials and Methods:
Wild type (WT) and *Lrat*$^{-/-}$ were subjected to dietary intervention with vitamin A sufficient diet (VAS) and β-carotene (BC) diet for various time periods. BC and retinoid levels were analyzed in different tissue such as intestine, liver, WAT, lymph nodes. *Isx*$^{-/-}$ *Lrat*$^{-/-}$ double knock out mice were generated to study the role of ISX in the control of vitamin A homeostasis. Marker gene for retinoid metabolism were determined using qPCR analysis. Pharmacological treatment with the pan-RAR antagonist (AGN193109) was used to study the contribution of retinoid signaling in the control of vitamin A biosynthesis.

Result and Discussion
Using genetic and pharmacological approaches in mice, we observed that in LRAT deficiency, the transcription factor ISX became hypersensitive to dietary vitamin A and suppressed retinoid biosynthesis. The dysregulation of the pathway for retinoid biosynthesis resulted in BC accumulation and vitamin A deficiency of extrahepatic tissues. Pharmacological inhibition of retinoid signaling and genetic depletion of the *Isx* gene restored retinoid biosynthesis in enterocytes of LRAT-deficient mice. Together, our study indicates that the catalytic activity of LRAT coordinates the negative-feedback regulation of intestinal retinoid biosynthesis and maintains optimal retinoid levels in the body. Thus, our study provides a framework how vitamin A homeostasis is controlled in a mammal and indicates a critical role of LRAT in this process.

Acknowledgment
The authors thank A. Wyss (DSM) for the gift of BC beadlets. The research was supported by grants from the National Eye Institute (EY020551, EY028121, and EY011373).

Abstract 59

A novel role of the low-density lipoprotein receptor in carotenoid biodistribution

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Introduction: Carotenoids are plant pigments synthesized by photosynthetic organisms. Among them, β-carotene is crucial for animals because it is the main precursor of vitamin A. The limiting step of β-carotene conversion to vitamin A is β-carotene oxygenase 1 (BCO1), and mice lacking this enzyme accumulate β-carotene in tissues like humans. There are several transporters implicated in the cellular uptake and distribution of β-carotene. Among them, we focused on the low-density lipoprotein receptor (LDLR), a membrane protein expressed in several tissues. The objective of this study is to determine the role of LDLR in tissue β-carotene uptake and biodistribution.

Methods and materials: We compared age and sex-matched *Bco1*$^{-/-}$ and *Ldlr*$^{-/-}$/*Bco1*$^{-/-}$ mice fed a Western diet containing 50 mg/kg of β-carotene (WD- β-carotene) for 12 weeks. We measured β-carotene and vitamin A levels in the liver, adipose tissue, plasma, and intestine by High-Performance Liquid Chromatography (HPLC).

Results: HPLC results showed that the adipose tissue and intestine of *Bco1*$^{-/-}$ mice were two-fold higher than those observed in *Ldlr*$^{-/-}$/*Bco1*$^{-/-}$ mice. Hepatic and circulating levels of BC were increased in *Ldlr*$^{-/-}$/*Bco1*$^{-/-}$ mice than *Bco1*$^{-/-}$ control mice. These changes occurred independently of differences in food intake.

Conclusion: Our data show that LDLR participates in the tissue distribution of β-carotene in mice.

Aknowledgments: We thank the National Institutes of Health Grant (R01 HL147252) and US Department of Agriculture (W4002)

Abstract 60

Identification of novel fucoxanthin cleavage metabolites and anti-inflammatory action against activated macrophages

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Introduction: Marine carotenoid fucoxanthin (Fx) showed anti-obesity and anti-inflammatory effects. Fx is known metabolized to amarouciaxanthin A (AmxA) and fucoxanthinol (FxOH) in mice. On the other hand, in recent study, carotenoid cleavage products, apocarotenoids, such as β-apocarotenals and apolycopenals, have been found in human and rodents. Furthermore, novel carotenoid cleavage enzyme β-carotene-9',10'-dioxygenase was identified in mammals. However, there are little knowledge of apocarotenoids from xanthophylls, especially marine carotenoids. In this study, we identified Fx cleavage products, apo-fucoxanthinoids, in Fx fed mice and investigated anti-inflammatory effect leading to the prevention against life-style related diseases.

Methods and Materials: (*In vivo study)* Male C57BL/6J and KK-A^y mice (4-week old) were housed at 23 ± 1°C and at 50 % humidity with a 12 h light/12 h dark cycle. After acclimation for 1 week by feeding a standard diet MF, AIN-93G-based 0.2% Fx-containing diet was fed for 1 week. These mice were dissected under anesthesia and then each tissue was collected. Total lipids were extracted by Folch method and analyzed using HPLC and LC-MS. (*In vitro study)* Murine macrophage-like RAW264.7 cells were treated with apo-fucoxanthinoids and then induced inflammation by lipopolysaccharide (LPS). After LPS stimulation, total RNA was extracted from the cells, and mRNA expression levels of inflammatory factors were quantified by RT-qPCR method. Inflammatory cytokine IL-6 and nitric oxide (NO) levels in the culture media were analyzed by ELISA and Griess method, respectively. Inflammatory signaling pathways were analyzed by western blotting.

Results and Discussion: We successfully found paracentrone and apo-10'-fucoxanthinal as well as FxOH and AmxA in the serum and each tissue of both Fx fed mice (**Fig. 1**).

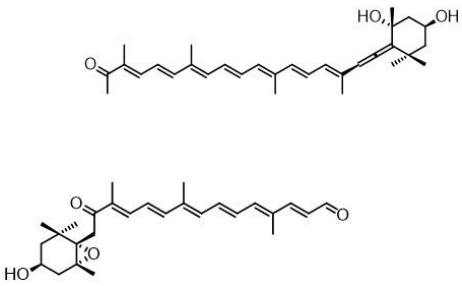

Fig. 1 Chemical structure of paracentrone (Upper) and apo-10'-fucoxanthinal (Lower)

These apofucoxanthinoids suppressed IL-6 and NO secretion in LPS-stimulated RAW264.7 cells through down-regulation of IL-6 and NO synthase gene mRNA expression. Since paracentrone and apo-10'-fucoxanthinal also suppressed IκB phosphorylation and NF-κB p65 nuclear translocation, it was suggested that apo-fucoxanthinoids showed anti-inflammatory action through inhibition of NF-κB signaling pathway.

Acknowledgements: This work was supported by Grants - in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (M.H. 18H02274).

Session XA Abstracts Carotenoids: Biotchnological and Analytical Methods

Abstract 61

USING POLARIZED RAMAN SPECTROSCOPY TO DISTINGUISH MESO-ZEAXANTHIN FROM ZEAXANTHIN

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Introduction: Carotenoids are important biomolecules in the human eye that act mainly as antioxidants to scavenge and reduce the retinal reactive oxygen species (ROS). Zeaxanthin, *meso*-zeaxanthin, and lutein are the dominant carotenoids found in the human retina, and they are configurational isomers. To understand these three carotenoids' functions more fully, it is necessary to distinguish their spatial distribution in the human retina. Using confocal resonance Raman spectroscopy, we recently have distinguished the retinal distribution of lutein from zeaxanthin and *meso*-zeaxanthin [1]; however, the differential distributions of zeaxanthin and *meso*-zeaxanthin remain unavailable. The goal of this work is to investigate if zeaxanthin can be differentiated from *meso*-zeaxanthin with polarized Raman spectroscopy in *in vitro* assays.

Research & Methods: 100 µM of zeaxanthin and *meso*-zeaxanthin methanol solutions were prepared and allowed to dry on microscope slides. A confocal Raman microscope (Horiba XploRA Plus) with a 473-nm blue laser was used to focus on the crystals of each sample to obtain spectra. Various parameters were tested on the samples using the Raman microscope with different polarization settings on the laser (circular, vertical, and horizontal) and different settings on the detector (accepting vertical, horizontal, or both). The results were compared to find the optimal settings for distinguishing the compounds.

Results and Discussion: The only difference between zeaxanthin and *meso*-zeaxanthin is a change in orientation of one hydroxyl group making zeaxanthin chiral and *meso*-zeaxanthin achiral. There are therefore differences in intensities of certain vibrational modes if polarized light is used in Raman spectroscopy, due to polar, Raman-active vibrational modes being more active in *meso*-zeaxanthin, a symmetric achiral molecule. These effects will only be seen if there is a fixed orientation in the molecules, which can be achieved by using crystalized samples or by human retina samples, in which macular carotenoids are immobilized on carotenoid-binding proteins such as GSTP1 and StARD3 [3,4]. It was found that using circularly polarized light for excitation with a vertically polarized setting on the Raman detector gave the biggest difference in the ratio of the v1 and v2 vibrational modes in zeaxanthin and *meso*-zeaxanthin. The ratios of v1/v2 under these conditions for zeaxanthin and *meso*-zeaxanthin are 1.04 and 1.19, respectively.

This result suggests that the v1/v2 ratio may be used to distinguish zeaxanthin and *meso*-zeaxanthin, facilitating the differentiation of the spatial distribution of zeaxanthin and *meso*-zeaxanthin in the human macula.

Acknowledgements: This work was supported by NIH grants EY-11600 and EY-14800, by Lowy Medical Research Foundation, and by unrestricted departmental funds from Research to Prevent Blindness.

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[3] B. Li, P. Vachali, J.M. Frederick, Bernstein PS (2011). Identification of StARD3 as a lutein-binding protein in the macula of the primate retina. Biochemistry, 50 (13), 2541-9.

Abstract 62

Extraction of Carotenoids From Pitanga a Screening with Ionic Liquids, Surfactants and Eutectic Solvents

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1. Introduction

Carotenoids are natural pigments that are present mainly in vegetables, and are responsible for the yellow, orange and red colorations. These natural pigments can are obtained by chemical synthesis, being that carotenoids are specifically extracted from petroleum derivatives[2]. The pursuit for methods that have a negative impact on the environment has been widely studied in recent years, and among them we have grenn solvents, inckuding ionic liquids, eutectic solvents and surfactants. These solvents are considered alternatives because they are not volatile at room temperature and non-flammable, thus presenting less risk of exposure compared to conventional organic solvents and consequently less damage to the envyroment [3]. Fruit of Brazilian biodiversity, the Pitanga (*Eugenia Uniflora* L) is currently underexplored, though ia knok to have a high amount of fibers, and a high content of total carotenoids 5880.98 ± 434.5 µg / 100 g, making it an excellent food matrix [1]. Thus, the objective of this work was to perform a screening with 8 green solvents in order to identify sustainable and safe alternatives for the extraction of carotenoids.

2. Experimental

Four ionic liquids (IL), four surfactants and four conventional organic solvents (VOS) were tested. The ultra-sonic a system extraction a power 70%, sonication time 5 min, centrifugation 4000 rpm, time 30 min room temperature. Absorbance in spectrophotometer 449 nm b-Cryptoxanthin. The quantification of carotenoids was determined by high performance liquid chromatography (HPLC-PDA-MS/MS).

3. Results and Discussion

Twelve extraction solvents were tested. the major carotenoids identified were Lutein, B-cryptoxanthin monoesters, rubixanthin mono and diesters and all trans lycopene. Under the conditions evaluated, [C6min] [CL] Ionic liquid and TWEEN 20 250mM surfactant solution were the ones who had a better result, as shown in figure 1. Surfactants are recognized as important solvents in the extraction of hydrophobic substances. The results demonstrate that the surfactant solution had a higher result than conventional solvents. The efficiency of surfactants solutions is already known in the literature. As noted in this work, extraction with these solvents is very promising since the benefits are numerous, its efficiency of extraction of carotenoids is very interesting and thought-provoking.

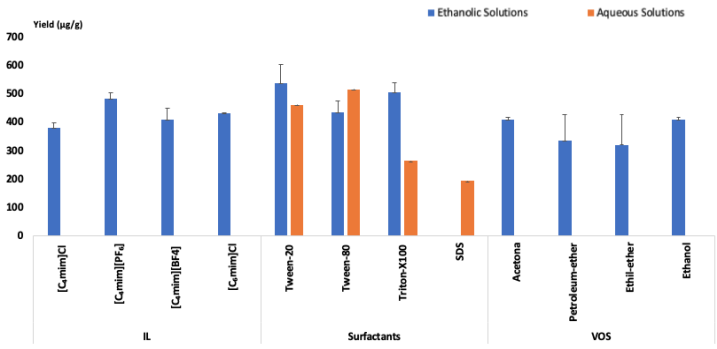

Figure 1. Screening for extraction of carotenoids from Pitanga with ionic liquids and surfactants.

[1] K.R. Biazotto, L.M.S. Mesquita, B.V. Neves, A.R.C. Braga, M.M.P. Tangerina, W. Vilegas, A.Z. Mercadante, V.V. De Rosso. *J. Agri. Chem.,* **67** (2019) 1860-1876.

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[3]D.C. Murador, , A.R.C. Braga, P.L.G Martins, A.Z. Mercadante, V.V. De Rosso. *Food. Reser. Inter.*, **126** (2019) 108-653.

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Abstract 63

Impact of High Pressure Processing on Bioactive Plant Ingredients in Kale

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1. Introduction

High pressure processing (HPP), a non-thermal food preservation technique, is characterized by a minimal impact on sensory, nutritional and functional food characteristics [1]. In contrast to numerous studies about the influence of HPP on general food quality aspects, the impact on lipophilic bioactive plant ingredients such as carotenoids and vitamin E has been less investigated. In this study, kale was chosen as representative food matrix with high contents of α-tocopherol and various carotenoids for treatment with high hydrostatic pressure.

2. Experimental

A knife mill (Retsch Grindomix GM200) was used to crush kale leaves. HPP was performed at room temperature applying different pressures (200 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 (Figure 1). Furthermore, the bioaccessibility of nutrients in kale samples was investigated by applying a modified *in-vitro* digestion model [2].

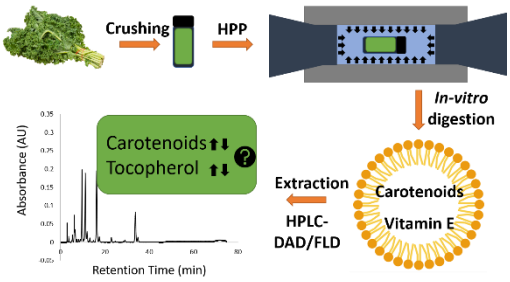

Figure 1: Crushed kale leaves follow HP-treatments. Extraction and HPLC analysis of digested samples provide information about bioaccessibility.

3. Results and Discussion

We identified 11 compounds in kale including carotenoids, α-tocopherol and chlorophylls. Nutrient extractabilities followed increasing and decreasing trends regarding elevated pressure rates and extended treatment periods, which may indicate competitive pathways of HP-related changes in concentration of ingredients. *In-vitro* digestion of HP-treated samples resulted in increased bioaccessibilities within constant pressure regimes and extended treatment length, which might be related to a disrupted cell integrity (Figure 2). Further insights in antioxidative capacity of HP-treated kale samples will be presented.

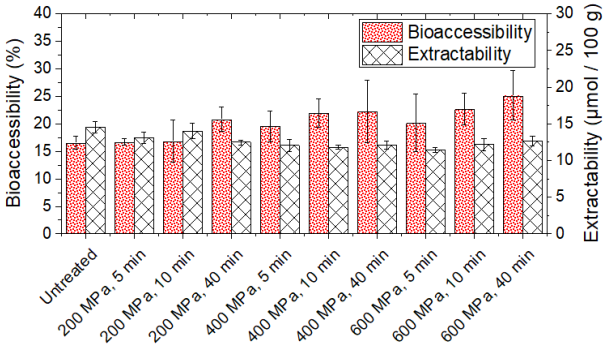

Figure 2: Effect of HPP parameters on bioaccessibility and extractability of *(all-E)*-lutein in kale.

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Abstract 64

New techniques to recover and recycle ionic liquids used as solvents on the extraction of carotenoids from amazon fruits

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1. Introduction

Ionic Liquids (ILs) have been used as alternative solvents to replace volatile organic solvents (VOS) in the extraction of bio-based compounds [1]. Conventionally, carotenoids are obtained by chemical synthesis or extraction using VOS, both considered non-eco-friendly processes. In addition, to meet the demands of the food sector, new methodologies are desirable to replace synthetic dyes. In this sense, based on circular economy and biorefinery concepts [2], we have developed new alternative processes mediated by ILs to obtain natural carotenoids from *Bactris gasipaes* fruit, carotenoids-rich biomass from the Amazonian forest, which is usually considered as a waste.

2. Research & Methods

To obtain natural carotenoids, ethanolic and aqueous solutions of different ILs were evaluated for the extraction of carotenoids (imidazolium, ammonium and phosphonium families). Two processes were developed with the best ILs. Variables of extraction, namely, time (T_{min}), solid-liquid ratio ($R_{(S/L)}$), concentration (C_{IL}), and performed the recovery of the IL (without the use of VOS) were optimized, as well as its recycling in a new extraction process. In the end, and after proper optimization, the processes were evaluated for their environmental impact using a carbon footprint as the main output.

3. Results and Discussion

In the first process, we developed an ultrasound-assisted extraction (T_{mim}: 12×4, $R_{(S/L)}$: 1; C_{IL}: 80 mM) performed using ethanolic solution of 1-butyl-3-methylimidazolium-tetrafluoroborate ([C4mim][BF4]) with recovery of 143 $\mu g_{carotenoids} \cdot g_{dried\ biomass}^{-1}$ (94% purity). The IL was recovered by thermal precipitation at -80 °C, proving its success for at least 10 new cycles of extraction while decreasing the process carbon footprint by 50% compared with the conventional method using acetone (Figure 1A). In the second process, an orbital-shaking homogenization in aqueous-solutions of 1-decyl-trimethylammonium bromide was used ($[N_{1,1,1,10}]Br$ - T_{mim}: 8.5, $R_{(S/L)}$: 0.75, C_{IL}: 140 mM). The recyclability of the solvents, as well as the high efficiency (88.7 ± 0.9 $\mu g_{carotenoids} \cdot g_{dried\ biomass}^{-1}$ with 99.9% purity) and the low environmental impact of the integrated process (33% compared with the conventional method), were demonstrated (Figure 1B). Thus, this work is giving an answer regarding the development of strategic eco-friendly processes that can be applied to other raw materials and to extract other carotenoids. Besides, both processes developed in this work had better yields of extraction compared with VOS-mediated processes, highlighting that new strategies are desirable in order to obtain eco-friendly and safer-carotenoids.

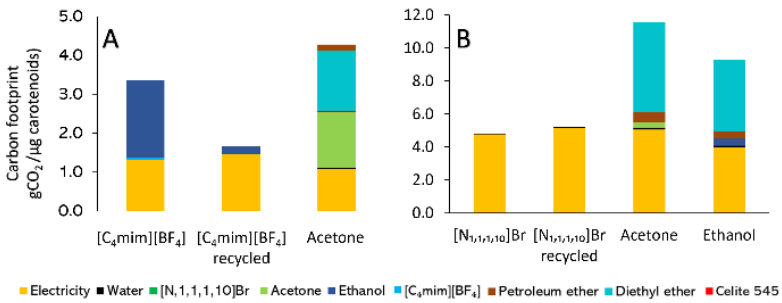

Figure 1. Carbon footprint of the scenarios investigated in this work.

Acknowledgements

Fundação de Amparo à Pesquisa Do Estado de São Paulo (FAPESP #2016/2324-8; #2016/18910-1).

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Session XB Abstracts Carotenoids: Biotchnological and Analytical Methods

Abstract 65

Free- and esterified-carotenoids in oily and non-oily fruit matrices: an optimised procedure of saponification for carotenoid quantification

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Introduction: Carotenoids are a class of fat-soluble compounds widely associated with natural yellow-to-red colour in fruit and vegetables. Carotenoids exist in nature as free and esterified forms with fatty acids. The latter increases their lipophilic nature, stability and colour intensity. Esterification of carotenoids is common in capsicums/chillies, and also in high oil fruit, such as avocadoes. Their carotenoids are normally extracted in conjunction with other undesirable compounds (chlorophylls, lipids and fatty acids) present in the fruit-cell matrix, and can interfere with equipment detection. In addition, cis−trans isomerization, thermal degradation of carotenoids following extraction, and a strong tendency for carotenoids to remain bound to fatty acids and cell plant matrices are common analytical challenges in carotenoid profiling and accurate quantification. The present study optimised carotenoid extraction and saponification procedures for accurately determining the carotenoid content of both avocado and capsicum fruit.

Materials and Methods: Queensland-grown avocadoes (cv. Hass) were purchased from a local supermarket in Brisbane, Queensland, Australia, while chillies (cv. Bulgarian) were harvested at the Gatton Research Facility, Queensland, Australia. These representative species were used to optimise the saponification procedure for extraction and quantification of esterified carotenoids in oily and non-oily fruit matrices. Free zeaxanthin and β-carotene were added as exogenous spikes to a white chilli (carotenoid-free) sample at three concentrations (0.3. 2.0, 10.0 mg/L). The recoveries of carotenoids during extraction and saponification and the carotenoid content in Hass avocado and Bulgarian chillies were determined by ultra-high-performance liquid chromatography coupled with diode array detection and a triple quadrupole mass spectrometer (UHPLC-DAD-MS/MS).

Results and Discussion: The optimised saponification and extraction method showed a high recovery (94.4 - 106.2%) for both chillies and avocado at low, medium and high concentrations of zeaxanthin and β-carotene. The current recovery of carotenoids after saponification was considerably higher than that reported in previous studies: 50% recovery of β-carotene and other carotenoids [1]. The presence of phosphate buffer to minimise the formation of 'soap' (de-esterification products) with free carotenoids was quintessential to minimising losses of carotenoids after the saponification procedure. The current method provides an important improvement in the extraction and saponification methodology for analysing carotenoids in highly esterified matrices.

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Acknowledgement

This study was funded in part by 'Naturally Nutritious' (Horticulture Innovation Ltd., project HN15001).

Abstract 66

UHPLC-MS BASED PLANT APOCAROTENOMICS ANALYTICAL PLATFORM

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Introduction: Carotenoids represent a large class of isoprenoids generally consisting of a C_{40}-backbone equipped with an extended conjugated double bond system, and are synthesized by all photosynthetic organisms and many heterotrophic microorganisms. The oxidative breakdown of carotenoids leads to the formation of a diverse family of apocarotenoids (APOs), which is either triggered by reactive oxygen species or catalyzed by enzymes, e.g., CAROTENOID CLEAVAGE DIOXYGENASE. Emerging studies have shown that APOs are essential for plants, as this group of metabolites includes phytohormones, e.g. abscisic acid and strigolactones, signaling molecules and growth regulators, such as zaxinone, anchorene, and β-cyclocitral. Besides the large number of the carotenoid precursors, further enzymatic modifications, such as hydroxylation and glycosylation, give rise to the large diversity and complexity of plant APOs [2]. Thus, it can be assumed that plants contain a great number of APOs and their derivatives, which are interwoven via numerous metabolic pathways and networks. Moreover, these biosynthetic pathways and networks are dynamic and reflect changes in the environment and growth conditions, such as exposure to biotic and abiotic stress. Determining the pattern of APOs and elucidating the pathways involved is crucial for understanding their biological function. Such demands have catalyzed the emergence of a new branch of metabolomics, apocarotenomics, which enables the large-scale study of APOs using the principles of analytical chemistry. Mass spectrometry (MS), largely due to its analytical power and rapid development of new instruments and techniques, has been widely used in studies on APOs, and greatly accelerated advances in the field.

Research & Methods: In this work, we present an ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) based apocarotenomics analytical platform integrating multiple analytical methods for the profiling of APOs from minute amounts of dried plant material (less than 25 mg). This plant apocarotenomics approach includes, 1) The rapid and effective ultrasonic-assisted extraction (UAE) with methanol including 0.1% butylated hydroxytoluene; 2) The fast sample purification with solid-phase extraction (SPE) and chemical derivatization (N^2,N^2,N^4,N^4-Tetraethyl-6-hydrazinyl-1,3,5-triazine-2,4-diamine), which is used for the detection of instable and low abundant diapocarotenoids (DIALs); 3) Multiple analytical methods for APOs, hydroxylated apocarotenoids (OH-APOs), DIALs, and glycosylated apocarotenoids (GAPOs) based on high-performance chromatographic separation and high-resolution hybrid quadrupole-Orbitrap MS [1-3]. This apocarotenomics approach was used in the comprehensive profiling of APOs and their derivatives from tomato fruits.

Results and Discussion:

Using our apocarotenomics approach, we identified and quantified a total 74 of APOs and their derivatives from tomato fruits, including 38 volatile, non-volatile, and hydroxylated APOs with chain lengths ranging from C_{10} to C_{30}, 30 of DIALs with C_5 to C_{24}, and six of C_{13} GAPOs (Figure 1). These results show the high structural diversity of tomato APOs, confirming that the apocarotenomics strategy can significantly facilitate the apocarotenoid analysis as previously shown for GAPOs present in carotenoid-accumulating *E. Coli* cells [2] and DIAL detection in green tissues [3].

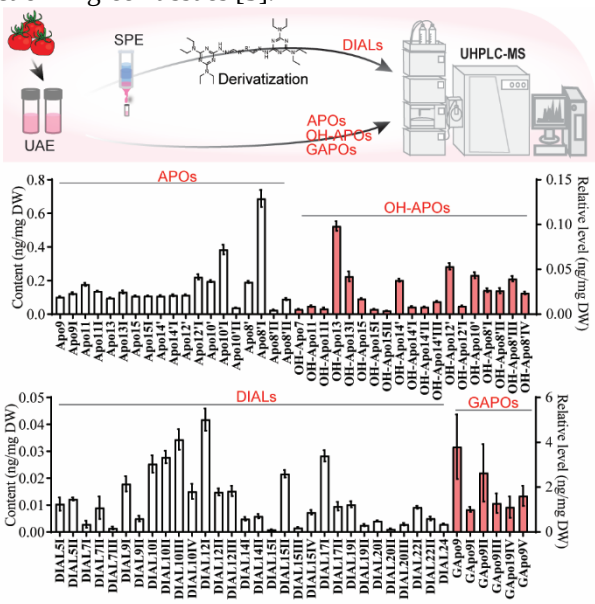

Fig.1. UHPLC-MS based apocarotenomics analytical platform and its application on the comprehensive profiling of APOs, OH-APOs, DIALs, and GAPOs from tomato fruit extract.

Taken together, our approach allowed comprehensive analysis of apocarotenoids and derivatives from various complex matrices, paving the way for elucidating their roles in human health and plant growth and development.

Acknowledgments

This work was supported by the King Abdullah University of Science and Technology

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Abstract 67

Pressure-mediated reflection spectroscopy demonstrates strong criterion validity as a biomarker of fruit and vegetable intake compared to plasma carotenoid concentrations: A US multi-center cross-sectional study across four racial/ethnic groups.

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Abstract: Introduction: Non-invasive skin carotenoid measurement may offer a rapid biomarker for dietary fruit and vegetable intake. The purpose of this study was to examine the criterion-related validity of pressure-mediated reflection spectroscopy across four racial/ethnic groups, using plasma carotenoids as the primary criterion measure and self-reported fruit and vegetable (FV) intake and carotenoid intake as the secondary criterion measure. Methods and Materials: We recruited adults who self-identified as non-Hispanic Black (n = 61), Asian (n = 53), non-Hispanic White (n = 70), or Hispanic (n = 29) in North Carolina and Minnesota between October 2019-March 2020. Eligibility criteria included: BMI between 18.5-34.9 kg/m^2, not pregnant/lactating, no self-reported chronic diseases. Fasting blood samples were measured by HPLC-PDA for beta- and alpha-carotene, lutein, zeaxanthin, lycopene, and beta-cryptoxanthin. Total skin carotenoids were assessed by pressure-mediated reflection spectroscopy (the Veggie Meter ®). Self-reported carotenoid and FV intake were assessed using the semi-quantitative Harvard Food Frequency Questionnaire. We used bivariate correlations and adjusted multivariate linear regressions to examine associations between skin carotenoids, log2-transformed plasma carotenoids, and F, V and carotenoid intake, as well as heterogeneities by race/ethnicity. Results: There were moderate to strong bivariate correlations between skin and plasma carotenoids in all four racial/ethnic groups, ranging from 0.64 (non-Hispanic Black) to 0.80 (Hispanic). The overall correlation among the entire sample was r=0.71. There were low to moderate correlations between skin carotenoids and self-reported FV intake, ranging from 0.24 (non-Hispanic Black) to 0.53 (non-Hispanic White), with an overall correlation of r=0.35. In adjusted regression models, skin carotenoids remained associated with plasma carotenoids, and FV and carotenoid intake. Some individual plasma and dietary carotenoids (e.g. beta-carotene) were more strongly associated with skin carotenoids than others (e.g., lycopene). Race x skin carotenoid interactions were not statistically significant. Conclusions: Plasma and skin carotenoids were significantly correlated in all four racial/ethnic groups. Future work is needed using feeding studies to understand the sensitivity of the Veggie Meter® in response to increased intake of carotenoid-rich FV. Acknowledgements: NIH-NHLBI (R01HL142544) and USDA/ARS (CRIS 3092-51000-059-NEW2S; NEM).

Abstract 68

A carotenoid spectral library combining UV/VIS and high-resolution MS data

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Introduction: Historically physico-chemical data on carotenoids have been collected in books and monographs. The renowned "Handbook" collected for the first time systematically actual spectroscopic data (UV/VIS, MS and CD) rather than only references to the primary literature (1). Due to the analog nature, this rich data set cannot be retrieved by computerized systems. The objective of the current work was to capture compound information and spectra of carotenoids in a digital format which facilitates fast computer-assisted searching and processing of the data. The format comprises a chemical database with a carotenoid spectrum library at its core. Its key features are the seamless integration of high quality UV/VIS spectra and high-resolution MS spectra

Research & Methods: Solutions of authentic carotenoid standards were subjected to UHPLC analyses using a generic carotenoid profiling method to acquire reference spectra. The system was equipped with a photo-diode array detector and a high-resolution QTOF mass spectrometer. The method recorded the UV/VIS spectrum, parent mass spectrum (MS^1) and product ion spectra (MS^2) at several collision energies. The curated spectra and retention times were then deployed to the database. Samples from diverse sources have been analyzed by the generic method and the obtained analytical data was searched as query spectra against the library. The hits were ranked according to highest similarity.

Results: Currently 165 MS and 60 UV/VIS spectra from 49 authentic standards were deployed to the constantly growing library.

Conclusions/Discussion: Spectral library searching has become a mature method to annotate tandem mass spectra in proteomics and metabolomics. For carotenoid analysis UV/VIS spectroscopy is another indispensable technique. The developed approach fully integrates both techniques into a new carotenoid spectrum database. Query spectra obtained from complex samples can be searched and matched against a large number of library spectra fast and effectively and false positive identifications is minimized. Applicability is shown with some examples of real world samples and an outlook will be given.

Acknowledgments:
We thank Raymond Ramaker for excellent technical support and Guido Wahl for invaluable advice on the database application.

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