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N°16, July 2020

NEWSLETTER

EUROCAROTEN

EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

WELCOME

We are pleased to welcome you to the 16th issue of the EUROCAROTEN newsletter.

In this issue, we would like to introduce you to Dr Gianfranco Diretto, Research Scientist at Italian Agency for New Technologies, Energy and Sustainable Development (ENEA).

Also, read about canthaxanthin, the Carotenoid of the Month in this issue. Furthermore, in Carotenoids in our daily life rubric, read how autumn leaves could be used a valuable source of carotenoids in text by Häkkinen Suvi T, Ritala Anneli and Nohynek Liisa from VTT Technical Research Centre of Finland Ltd, Finland, and about establishment of carotenoid analysis capacity in Africa in text by Tawanda Muzhingi from International potato centre, Kenya.

In "Think Tank Information" rubric, read about Publications by EUROCAROTEN ECIs and young researchers: Lisa Schüler, Mohammed Iddir and Cristina Tudor, and in "Working Group News" rubric, find out summarized progress report of WG3, Nutrition and Health, given by Dr Torsten Bohn.

Also, you can find more information about EUROCAROTEN COST Action on its COST website http://www.cost.eu/COST_Actions/ca/CA15136 and on our website www.eurocaroten.eu.

Yours sincerely,
Anisa Peçuli,
Ng'andwe Kalungwana,
Kristina Kljak

Subscription to the e-mailing list is available via the EUROCAROTEN website.

Send your comments and proposals to info@eurocaroten.eu.

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NEWS FROM THE ACTION

CAROTENOIDS IN OUR DAILY LIFE

NEWS FROM THE ACTION

EXTENSION OF EUROCAROTEN

Given the extraordinary situation caused by the Covid-19 pandemics, the Management Committee approved to request a 6-month extension of EUROCAROTEN. This extension has been eventually approved by COST, so the support of COST to the Action will continue up to 16 October 2020.

Special Issue „Natural and Synthetic Antioxidants as Food Additives" in Antioxidants (IF = 5.014)

EUROCAROTEN member Prof. Serkos A. Haroutounian will be guest editor of special issue of Antioxidants "Natural and Synthetic Antioxidants as Food Additives". Deadline for manuscript submissions is 31 October 2020.

For more information, visit the following link: https://www.mdpi.com/journal/antioxidants/special_issues/Natural_Synthetic_Antioxidants_Additives.

Special Issue about Natural Colorants in Molecules (IF = 3.060)

This issue will cover a wide range of research areas concerning natural colorants. Research or review articles are welcomed, including (but not limited to) identification of novel natural colorants, advances in sample treatment, improvements in analytical methods for quantitation and quality control purposes, new trends in colorants purification, stability studies, theoretical and experimental studies on the (bio)chemical properties of natural colorants, and potential industrial applications of new natural colorants. Deadline for manuscript submissions is 31 December 2020.

For more information, visit the following link: https://www.mdpi.com/journal/molecules/special_issues/Natural_Colorants

Special Issue "Recents Developments in Bioactive Molecules Evaluation" in Antioxidants (IF = 5.014)

EUROCAROTEN member Dr. Daniele Giuffrida will be guest editor of special issue of Antioxidants "Recents Developments in Bioactive Molecules Evaluation". Deadline for manuscript submissions is 31 December 2020.

For more information, visit the following link: https://www.mdpi.com/journal/antioxidants/special_issues/evaluation.

PUBLICATIONS BY EUROCAROTEN MEMBERS

Publication in Trends in Food Science and Technology

EUROCAROTEN members Paula Mapelli-Brahm, Francisco J. Barba, Fabienne Remize and Antonio J. Meléndez-Martínez are co-authors of review article "The impact of fermentation processes on the production, retention and bioavailability of carotenoids: An overview" published in Trends in Food Science & Technology. Article is available using the following link: https://doi.org/10.1016/j.tifs.2020.03.013.

Publication in Journal of Cleaner Production

EUROCAROTEN members Paula Mapelli-Brahm, Antonio J. Meléndez-Martínez, Isabel Vicario and Carla Stinco are co-authors of article "High-pressure homogenization as compared to pasteurization as a sustainable approach to obtain mandarin juices with improved bioaccessibility of carotenoids and flavonoids" published in Journal of Cleaner Production. Article is available using the following link: https://doi.org/10.1016/j.jclepro.2020.121325.

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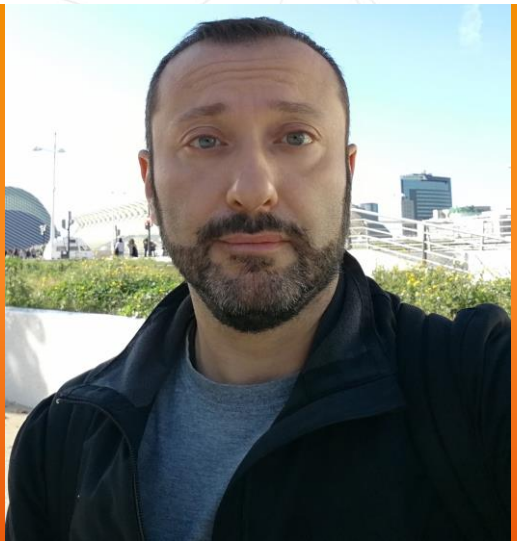
EUROCAROTEN
INTERVIEW

TALKING WITH:

Gianfranco Diretto



Affiliation	Italia Agency for New Technologies, Energy and Sustainable Development (ENEA)
Position	Research Scientist
Country	Italy
Area of Interest	Metabolic engineering, transcriptomics, metabolomics, bioinformatics

Please tell us a bit about your lab and what you work on?

We are a systems biology lab working on carotenoid and apocarotenoid metabolism in plants (solanaceae and iridaceae, mostly) by the use of mutants, transgenics and genome edited idiotypes, and the metabolomics characterization of a series of plant- and algal-based matrices. Overall, our objective is to identify novel cross-link, at transcript-metabolite level, in plant secondary metabolism.

In general terms, which area of the carotenoids do you find most interesting?

Regulation, physiology-related mechanisms, biotechnological application.

From your point of view, what are the greatest impacts that the study of the carotenoids has on society?

In terms of nutrition and of agronomical productivity and product attractiveness for the consumers.

As an STSM hosting lab, what type of collaborative projects would you envision?

The use of novel high-throughput technologies coupled to traditional molecular biology tools to unravel novel physiological processes.

What would you say to encourage a young student who is considering undertaking a career in carotenoid research?

Carotenoids play such high a number of functions and applications in base and industry-based research that just would be a pity not to work on them.

In your eyes, how can the EUROCAROTEN COST Action contribute to carotenoid research and how beneficiary was for you being part of this action?

The great extent of networking, allowing the establishment of new collaborations, the generation of novel ideas etc.

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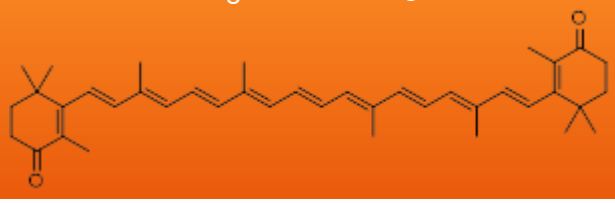
CAROTENOIDS IN OUR DAILY LIFE

CAROTENOID OF THE MONTH

Name: Canthaxanthin

Chemical Formula: $C_{40}H_{52}O_2$

Molecular Weight: 564.8 g/mol



Canthaxanthin, also known as β,β-carotene-4,4'-dione, is a di-ketocarotenoid with nine conjugated linear double-bonds and two oxo substituents in the β-ionone backbone. It has a characteristic red-orange colour due to its structure: the conjugated double-bond structure serves as a light-absorbing chromophore and the two keto groups shift the absorption maximum to higher wavelengths, in the same region as the acyclic lycopene. In nature, all-trans-canthaxanthin is mainly observed in carotenoid extracts, since all-trans configuration is more thermodynamically stable. Nonetheless, temperature, acidity or light can promote geometric isomerization of trans- to the cis-form, resulting in 9-cis- and 13-cis-canthaxanthin.
Canthaxanthin is biosynthesised from the precursor β-carotene, through the action of a single enzyme, known as β-carotene ketolase, bkt in algae, crtO in cyanobacteria and crtW in bacteria. The ketolation reaction necessary to produce ketocarotenoids, like canthaxanthin and astaxanthin, is restricted to a few species of bacteria, fungi and algae. Carotenoid denominations often give hints to the natural sources in which they could be found, as is the case of canthaxanthin. This pigment was first isolated from edible chanterelle mushroom, Cantharellus cinnabarinus, as a major component in a mixture of carotenoid extracts. Since then, canthaxanthin has been identified in algae, Chlorella zofingiensis, Dactylococcus dissociatus; in bacteria, Dietzia sp., Gordonia jacobaea, Bradyrhizobium sp.; and in fungi, Xanthophyllomyces dendrorhous, among others.

However, due to the high demand in the nutraceutical market, commercial canthaxanthin is supplied by chemical synthesis. It can be synthesised via Wittig olefination of suitable C10-trienedial and C15-Wittig salt, via C40-trisulfone intermediates or via Wittig–Horner condensation with C15-phosphonate and C10-trienedial.

Amongst hundreds of carotenoids, canthaxanthin belongs to the few that are marketed commercially with many applications, mainly in aquaculture and in the feed supplementation industry. Canthaxanthin causes a red-orange coloration after its uptake, as observed in the flamingo feathers, koi carp skin, salmon flesh and egg yolks. This pigment, being a ketocarotenoid, exhibits greater potential health benefits and antioxidant properties than other carotenoids. Studies have shown that canthaxanthin is capable of scavenging reactive oxygen species, such as superoxide and hydroxyl radicals, as well as of quenching singlet oxygen and preventing DNA damage.

In the past decade, the metabolic engineering of eukaryotes has progressed remarkably for the production of carotenoids towards its release into the market. In spite of this valuable research, the commercial production of canthaxanthin using biological systems remains challenging. Canthaxanthin market for personal care products and dietary supplements is growing rapidly, due to its medicinal properties. The scope to further extend the production of these molecules is high, with an increasingly solicited pipeline of natural products to compete with chemical synthesis.

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CAROTENOIDS IN OUR DAILY LIFE

AUTUMN LEAVES – UPGRADING CAROTENOID RICH PARK AND GARDEN WASTE TO USEFUL CHEMICALS FOR INDUSTRY

Text by Häkkinen Suvi T, Ritala Anneli and Nohynek Liisa

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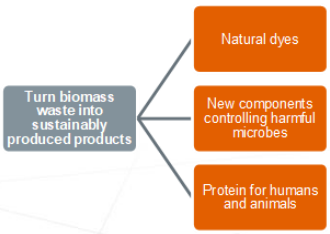
Every year waste treatment plants solely in Finland receive hundreds of tons of autumn leaves originating from parks and gardens in surrounding areas. So far, there has been only little use for the fallen autumn leaves, and currently this material is left on the ground, composted, incinerated or dumped into landfills. This is huge waste of valuable material containing carotenoids, anthocyanins and other components that should be further valorized.

At VTT Technical Research Centre of Finland, we wanted to find ways to take the valuable natural resource of autumn leaves into use. We identified several opportunities turning this waste biomass into sustainably produced products. Three main value chains were developed - i) natural dyes, ii) new components controlling unwanted microbes and iii) production of proteins for food and feed purposes.



i. **Natural dyes**: There is a fast-growing need for natural pigments in various industries around the world. One of the big challenges in the use of natural dyes is the lack of fastness. Many times application of inorganic mordants is required, which makes the dyeing environmentally risky and displays unwanted properties in final product with e.g. presence of harmful salts such as that of aluminium. Autumn leaves represent an interesting source for natural dyes as orange and yellow colours are derived from carotenoids and red ones from anthocyanins. We showed that extracts of autumn leaves, especially that of maple, was a promising dye for e.g. cotton, which is considered to be particularly difficult material to dye.

ii. **New components controlling unwanted microbes**: The second value chain was targeted in producing welfare by novel antimicrobial components which could be used as natural preservative in cosmetics or personal care products. Autumn leaves extracts and extract residues displayed significant inhibition of the growth of Staphylococcus aureus, which is a typical microbe e.g. in human skin.

iii. **Production of proteins for food and feed purposes**: Finally, the leaf residues were shown to have rather heterogeneous composition of carbohydrates and especially high contents of lignin. In this study, autumn leaf extraction residue was converted by microbial processes to single cell proteins with potential for e.g. animal feed, and was used as nutrient to cultivate edible mushrooms, replacing more expensive raw materials.

Here in North, with our wide forest areas and four seasons, we should take the autumn leaves to full use and exploit their valuable composition to benefit humans.

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CAROTENOIDS IN OUR DAILY LIFE

ESTABLISHMENT OF CAROTENOID ANALYSIS CAPACITY IN AFRICA TO SUPPORT BIOFORTIFICATION AND NUTRITION SENSITIVE AGRICULTURE

Text by Tawanda Muzhingi, PhD, Senior Scientist and flagship leader, International potato centre, ILRI Campus, Old Naivasha Road, Nairobi Kenya

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The Food and Nutritional Evaluation Laboratory (FANEL) was established in 2014 by the International Potato Center (CIP) in Nairobi, Kenya. CIP was founded in 1971 as a research-for-development organization with a focus on potato, sweetpotato and andean roots and tubers. It delivers innovative science-based solutions to enhance access to affordable nutritious food, foster inclusive sustainable business and employment growth, and drive the climate resilience of root and tuber agri-food systems. Headquartered in Lima, Peru, CIP has a research presence in more than 20 countries in Africa, Asia and Latin America. It is a CGIAR research center, a global research partnership for a food-secure future. CGIAR science is dedicated to reducing poverty, enhancing food and nutrition security, and improving natural resources and ecosystem services.

As part of its nutrition-sensitive approach to reduce food insecurity globally, CIP developed biofortified orange fleshed sweetpotato (OFSP) varieties with high levels of beta-carotene, the precursor for Vitamin A in the human body. It is important to understand and therefore constructively manage the many factors that can affect pro-vitamin A concentrations: cultivar differences and the effects of production practices, growing location, harvest maturity and postharvest handling/processing. The required analysis is complex, needs careful sampling, controls, sample storage, for example and can be difficult to interpret.

Thus, a suitable nutrition laboratory, FANEL, with the necessary equipment was established at the Bioscience east and central Africa (BecA) facility, on the International Livestock Research Institute (ILRI) campus in Nairobi, Kenya.

FANEL has adapted carotenoid extraction protocols best suited for different matrices such as raw OFSP roots and processed products which include OFSP puree, OFSP flour, OFSP enriched baby complementary foods, OFSP baked products (bread, buns, biscuits/cookies), OFSP fried products (fries, doughnuts, chapati) and OFSP juice. The methods are also suitable for extraction of carotenoids from biofortified crops such as maize, cassava and plantain bananas. High-Performance Liquid Chromatography (HPLC) is used to identify and quantify carotenoids in different samples. Extraction methods have been optimized for the quantitative determination of retinoids, carotenoids and tocopherols and tocotrienols

The quantification of carotenoids in different crops has played a pivotal role in demand-led breeding programs for high Vitamin A crops in SSA, majorly contributing to the fight against Vitamin A deficiency (VAD) amongst underprivileged communities.

FANEL has become a regional center of excellence for food science and nutritional analysis. It has and continues to collaborate with Advanced Research Institutes in the UK, Europe and North America and with African based academic research institutes and universities. FANEL offers opportunism for students and visiting fellows to conduct high impact research in Africa and contributing to regional capacity strengthening of national laboratories in the region.

THINK TANK INFORMATION
PUBLICATIONS BY EUROCAROTEN ECIs

Isolation and characterization of novel *Chlorella vulgaris* mutants with low chlorophyll and improved protein contents for food applications

Lisa Schüler, Etiele Greque de Morais, Mafalda Trovão, Adriana Machado, Bernardo Carvalho, Mariana Carneiro, Inês Maia, Maria Soares, Paulo Duarte, Ana Barros, Hugo Pereira, Joana Silva and João Varela

https://www.frontiersin.org/articles/10.3389/fbioe.2020.00469/full

Microalgae are sustainable biological resources, widely used as food supplements due to their well-balanced biochemical profile, rich in protein, essential fatty acids as well as carotenoids. The addition of microalgal biomass to food products, however, is hampered by organoleptic factors such as a green color and a strong fishy taste and smell. Therefore, in this study two chlorophyll-deficient mutants of Chlorella vulgaris were obtained by random mutagenesis, presenting yellow and white color due to the presence of lutein and phytoene, respectively. Together with an increased protein content of the mutants compared to the WT makes them likely candidates to be feedstocks for the development of novel, innovative food supplements and foods.

Influence of soy and whey protein, gelatin and sodium caseinate on carotenoid bioaccessibility

Mohammed Iddir, Giulia Dingeo, Juan Felipe Porras Yaruro, Faiza Hammaz, Patrick Borel, Thomas Schleeh, Charles Desmarchelier, Yvan Larondelle and Torsten Bohn

https://pubs.rsc.org/en/content/articlelanding/2020/fo/d0fo00888e/unauth#!divAbstract

By means of a European consensus model for static *in vitro* digestion, we focused on the influence of WPI, soy protein isolate, sodium caseinate and gelatin on the bioaccessibility of individual carotenoids, i.e. β-carotene, lutein and lycopene. Insights obtained from such experiments can be valuable for fortifying food items with these health-associated pigments and to determine the optimal food matrix to support carotenoid bioavailability.

Sea buckthorn oil as a valuable source of bioaccessible xanthophylls

Cristina Tudor, Torsten Bohn, Mohammed Iddir, Francisc Vasile Dulf, Monica Focşan, Dumitriţa Olivia Rugină and Adela Pintea

https://www.mdpi.com/2072-6643/12/1/76/htm

In this study, a physiologically standardized in vitro digestion protocol was applied in order to investigate the bioaccessibility of carotenoids from cold-pressed sea buckthorn oil as compared to the bioaccessibility of carotenoids from an oil-in-water emulsion prepared with the use of the above-mentioned oil.

Strengthening the immune system and reducing inflammation and oxidative stress through diet and nutrition: Considerations during the COVID-19 crisis

Mohammed Iddir, Alex Brito, Giulia Dingeo, Sofia Sosa Fernandez Del Campo, Hanen Samouda, Michael R. La Frano and Torsten Bohn

https://www.mdpi.com/2072-6643/12/6/1562

Challenges arise concerning how to optimally support the immune system in the general population, especially under self-confinement. In this review, we highlight the importance of an optimal status of relevant nutrients to strengthen the immune system during the COVID-19 crisis, focusing on the most relevant constituents that reduce inflammation and oxidative stress.

THINK TANK
OF EARLY CAREER INVESTIGATORS AND OTHER YOUNG RESEARCHERS

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WORKING GROUP NEWS

At the final meeting of COST Action EUROCAROTEN held in November 2019, in Lemesos , Cyprus, Torsten Bohn, presented an overview of WG3 progress so far.

Within the WG3 – Nutrition and Health – we have been continuing to work toward our main goals, including:

a) the targeted reviews,
b) providing training opportunities,
c) the carotenoid questionnaire,
d) the carotenoid tissue database, and
e) setting goals toward the SOPs.

As further discussed in Lemesos, the 3 scientific reviews include one on Carotenoid Intake Recommendations, which has meanwhile been accepted in Nutrition Reviews, a second one on Carotenoid Mechanisms Implicated in Diseases which by now is in its final draft, and a third one on Carotenoids and Prostate Cancer, for which a draft exists. A final EUROCAROTEN training school was organized in Palma de Mallorca by Joan Ribot and Jaap Keijer/Evert van Schothorst, where students were able to learn about functional and omics analysis of carotenoid interventions, including cell culture experiments, animal modelling and nutrigenomics.

This workshop was visited by ca. 20 young researchers from 10 countries. More data on carotenoids obtained by the questionnaire was meanwhile collected by Joan Ribot, and collection is ongoing in additional countries such as Poland, and an online version of the 10 questions is targeted, together with WG2. At the University of Graz, Brigitte Winklhofer-Roob and Daniela Nedelkovska continued their work on the carotenoid database by means of a STSM; at present data from about 250 articles has been collected comprising tissue concentrations in healthy and diseased subjects. Finally, SOPs on carotenoid extractions and determination from different specimens will be collected and made available via a Google Doc document, coordinated by Mohammed Iddir, Ng'Andwe Kalungwana and Torsten Bohn, and aided by Manuele Rodriguez.

WG leader: Torsten Bohn (torsten.bohn@lih.lu)

WG vice leader: Joanna Dulinska Litewka (joanna.dulinskalitewka@uj.edu.pl)

ACKNOWLEDGEMENTS

We would like to thank everyone who has so kindly contributed with the content present in this newsletter:

Antonio J. Meléndez Martínez and Cristina L.M. Silva for their guidance and supervision during the making of the EUROCAROTEN Newsletter.

Gianfranco Diretto for his contribution to our EUROCAROTEN Interview.

Bárbara A. Rebelo, M. Rita Ventura, Rita Abranches, Häkkinen Suvi T, Ritala Anneli, Nohynek Liisa and Tawanda Muzhingi, for their contribution to our "Carotenoids In Our Daily Life" rubric.

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