EUROCAROTEN

European network to advance carotenoid research and applications in agro-food and health
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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

EUROCAROTEN 4th Management Committee & 3rd Working Groups meetings

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Workshop "Sustainable Production of Carotenoids: From Biosynthesis to Biotechnology"

October 16 -18 2017

Hotel Sveti Križ, Trogir, Croatia

UNDER AUSPICIES

Faculty of Food Technology and Biotechnology
University of Zagreb
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10 000 Zagreb
CROATIA

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Workshop Organizing Committees

Organizing Committee

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Program

Workshop program

Sunday October 15th 2017

14:00 – 19:00	***Registration***
19:00 – 21:00	*Dinner*
21:30 – 23:00	*Cocktail party*

Monday October 16th 2017

8:00 – 8:30	***Registration***
8:30 – 8:40	Opening of the EUROCAROTEN meeting – **Antonio J. Meléndez-Martínez** (Universidad de Sevilla, Spain), **Mladen Brnčić** (University of Zagreb, Croatia) and **Damir Jeličić** (Ministry of Science, Education and Sport, Croatia)
Session I	Chair: **Joseph Hirschberg** (The Hebrew University of Jerusalem, Israel)
8:40 – 9:05	**Gerhard Sandmann** (Goethe University Frankfurt, Germany) Diversity of reactions leading to the biosynthesis of common carotenoids: An introduction
9:05 – 9:40	**Enrique Cerdá Olmedo** (Universidad de Sevilla, Spain) Carotenoids and apocarotenoids from *Phycomyces blakesleeanus*
9:40 – 10:15	**Masha Sapojnik** (LycoRed Ltd., Israel) From petri dish to crystals: Natural β-carotene from *Blakeslea trispora*
10:15 – 10:45	***Coffee break***
Session II	Chair: **Mariá Jesús Rodrigo** (Institute of Agrochemistry and Food Technology IATA- CSIC, Spain)
10:45 – 11:20	**Ralf Welsch** (Albert-Ludwigs University Freiburg, Germany) Golden Rice – development, current state and future approaches for improvements

11:20 – 11:45	**Orly Dery** (The Hebrew University of Jerusalem, Israel) Operation of two competing pathways from phytoene to lycopene in transgenic plants expressing CRTI
11:45 – 12:10	**Xin Jin** (Universidad de Lleida, Spain) Regulation of β-carotene biosynthesis and accumulation in maize endosperm
12:10 – 12:35	Karolina Kaźmińska (Warsaw University of Life Sciences, Poland) Genetic mapping of QTLs related to high carotenoid content in winter squash fruit
12:35 – 13:30	***Lunch break***
Session III	Chair: **Gianfranco Diretto** (ENEA, Italy)
13:30 – 13:55	**Marilise Nogueira** (Royal Holloway University of London, United Kingdom) The development and evaluation of a new plant-based renewable source of ketocarotenoids
13:33 – 14:20	**Uri Karniel** (The Hebrew University of Jerusalem) Metabolic engineering of xanthophylls in tomato fruit
14:20 – 14:45	**Selma Ben Abdelaali** (IRESA-University of Sousse & IRESA-University of Carthage, Tunisia) Characterization of carotenoid diversity in traditional and emerging Tunisian sweet orange cultivars
14:45 – 15:00	***Coffee break***
Session IV	Chair: **Ludmila Bogacz-Radomska** (Wroclaw University of Economics, Poland)
15:00 – 15:25	**Hendrik Pollmann** (Goethe University Frankfurt, Germany) The red yeast Xanthophyllomyces dendrorhous as a production platform for the biosynthesis of phytoene and zeaxanthin
15:25 – 15:50	**José Sánchez del Pulgar J** (CREA – Food and Nutrition, Italy) Microalgae as novel and sustainable sources of natural carotenoids: a comparative study of four microalgae species
15:50 – 16:15	**Helena Melo Amaro** (University of Porto, Portugal) Microalgal Blue Biotechnology – an attempt to microalgal-based bioprocess optimization, towards bioactive compounds production
16:30 – 19:00	***Guided city tour of Split***
19:00 – 22:00	***Dinner***

Tuesday October 17th 2017

Session V	Chair: **Paul Fraser** (Royal Holloway University of London, United Kingdom)
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9:05 – 9:40	**Giovanni Giuliano** (ENEA, Italy) Italian Saffron crocin biosynthesis: A highly compartmented pathway
9:40 – 10:15	**Meike S. Andersson** (HarvestPlus, Colombia) Tackling hidden hunger: Boosting Vita-A through enhancement and reducing degradation
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Session VI	Chair: **Carmen Socaciu** (University of Agricultural Sciences and Veterinary Medicine, Romania)
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11:10 – 11:35	**Sofia G. Papadaki** (National Technical University of Athens, Greece) Encapsulation of microalgal origin carotenoids in ulvan nanomatrices using electrohydrodynamic process
11:35 – 12:00	**Vesna Tumbas Šaponjac** (University of Novi Sad, Serbia) Modelling the accelerated solvent extraction of carotenoids from carrot
12:00 – 12:25	**Dan Lewitus** (Shenkar – Engineering. Design. Art., Israel) Astaxanthin-based polymers as new antimicrobial compounds
12:25 – 13:00	**General Discussion** **Paul Fraser** (Royal Holloway University of London, UK)
13:00 – 14:00	***Lunch break***

EUROCAROTEN Working Group meetings

Tuesday October 17th 2017

WG1: Production: developing resources and biosynthetic pathways
WG2: Quality along the food chain
WG3: Nutrition and health
WG4: Transfer, dissemination and exploitation

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14:30 – 15:15	WG1, WG2 & WG3 parallel meetings
15:15 – 15:30	***Coffee break***
15:30 – 17:30	WG1, WG2 & WG3 parallel meetings
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EUROCAROTEN Management Committee meeting

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8:30 – 9:30	Management Committee meeting
9:30 – 9:45	***Coffee break***
9:45 – 11:00	Management Committee meeting
12:30 – 13:30	***Lunch***

Abstracts

Invited Speakers

1. Gerhard Sandmann

Biosynthesis Group, Institute for Molecular Biosciences, Faculty of Biological Sciences Goethe University Frankfurt, Germany

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Diversity of reactions leading to the biosynthesis of common carotenoids: An introduction

Economic chemical carotenoid synthesis is restricted to a few compounds, whereas natural sources provide a broad range of different carotenoid structures. These carotenogenic plants and microorganism may be biotechnologically exploited. This introduction to carotenogenesis will contribute to the understanding of how the carotenoid pathways are organized and operate. Knowledge on the biochemistry and metabolic reactions behind is helpful not only for a general understanding of carotenoid biosynthesis but also for the optimization of carotenoid production and for genetic pathway engineering. An overview on carotenoid structure diversity will be presented together with a focus on the basic and common reactions in carotenoid biosynthesis. This includes phytoene synthesis, desatuations, cylization and oxygenation reactions together with the diversity of the genes involved. Finally, metabolic engineering strategies are outlined and examples of successful approaches shown.

2. Enrique Cerdá Olmedo

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Carotenoids and apocarotenoids from Phycomyces blakesleeanus

The mucorales *Phycomyces blakesleeanus* and *Blakeslea trispora* (Zygomycotina) have been noted research subjects for the biosynthesis of β-carotene and apocarotenoids since the discovery of these chemicals. These fungi offer convenient and sometimes unique research methods. A major deficiency is the lack of stable transformation. Successes include broad views on terpenoid pathways, unravelling of complex regulations, ascertainment of functions, and procedures for the industrial production of β-carotene and lycopene.

3. Masha Sapojnik

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From petri dish to crystals: Natural β-carotene from Blakeslea trispora

Natural β-carotene, which it is converted to vitamin A in our body, is an important contributor to our eye health, immune system, skin health and cardiovascular system. An all-natural and sustainable source of β-carotene is our *Blakeslea trispora* fungi biomass produced by a fermentation process.

A fascinating, challenging and ever evolving fermentation process is performed in a 37 years old plant called "Vitan". Ukraine based "Vitan" is owned by Lycored Ltd and is one of the largest producers of *Blakeslea trispora* fungal biomass in the world. From the "Vitan" facility the dry biomass is shipped to an extraction facility in Israel. The highly concentrated crystals are used for preparation of β-carotene-based food colouring formulations and dietary supplements.

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4. Ralf Welsch

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Golden Rice – development, current state and future approaches for improvements

Vitamin A deficiency is a public health problem affecting about 250 million preschool children and 19 million pregnant women worldwide and causes blindness in 500 000 and mortality in 250 000 cases every year. It is estimated that sufficient vitamin A supply could reduce child mortality by 23-34%. Rice is the staple food of more than half of the world's population, but among the 40 000 varieties of rice worldwide, there is not a single one which accumulates even trace amounts of provitamin A carotenoids in its endosperm. During a Rockefeller Foundation Workshop in 1993, a group of carotenoid biochemists, geneticists and plant molecular biologists evaluated possible strategies for increasing provitamin A content in rice endosperm. This was the initiation of the development of *Golden Rice*, a rice variety produced through genetic engineering which uses biofortification, the plants own biosynthetic capacity, to synthesize β-carotene in the endosperm. In this presentation, I'll give an overview on the developmental steps of *Golden Rice*, which began with initial approaches to assess the usability of isoprenoid precursors in rice endosperm for carotenoid biosynthesis, continues with the first generation of *Golden Rice* with low levels of β-carotene of 1 µg g^{-1} dry mass and the second generation with strongly increased levels of up to 37 µg g^{-1} dry mass. A special focus will be on pathway-specific challenges which needed to be met, on rather unexpected findings which strongly fostered the development of *Golden Rice* and on the roles of supporting organizations enabling its continuous development. Novel approaches aiming at increasing the stability of provitamin A carotenoids during seed storage will also be presented.

5. Salim Al-Babili

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Biosynthesis and novel functions of apocarotenoids in plants

Carotenoids are precursors of several plant signaling molecules and hormones, including abscisic acid and strigolactones (SLs), which arise by oxidative cleavage of double bonds in carotenoid backbone. Enzymatic cleavage of carotenoids is generally catalyzed by carotenoid cleavage dioxygenases (CCDs). According to their biological function, these enzymes differ in their substrate, regio- and stereo-specificity. SLs are a class of novel plant hormones with different developmental functions, which also act as rhizospheric signal released by roots to attract symbiotic mycorrhizal fungi. However, SLs have been co-opted by root parasitic weeds of the Genus *Striga* for host-recognition. *Striga* and related parasitic weeds infest many crops, including cereals, tomato and sunflower, devastating yields and representing a severe agricultural problem in sub-Saharan Africa, South Europe, the Middle East and Asia. The biosynthesis of SLs is an exciting example for the diversity of reactions catalyzed by CCDs. In this talk, we will describe the elucidation of SL biosynthesis and how knowledge about the pathway can be used to combat root parasitic weeds.

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6. Giovanni Giuliano

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Saffron crocin biosynthesis: A highly compartmented pathway

Crocus sativus stigmas are the source of the most expensive spice on Earth, saffron, and owe their characteristic color, taste and aroma to the apocarotenoids crocin, picrocrocin and safranal. The identification of genes catalyzing apocarotenoid biosynthesis and sequestration is a necessary prerequisite for the engineering of the pathway in heterologous systems. We identified and characterized candidate genes for the whole biosynthetic pathway, from the initial cleavage of zeaxanthin, to the dehydrogenation and glycosylation steps, to the vacuolar sequestration of glycosylated crocins. CCD2, a member of a novel CCD clade, has a plastidial localization and, when expressed in *E. coli* or in *Z. mays*, is able to cleave zeaxanthin, yielding the crocin precursor crocetin dialdehyde. In vitro assays defined the stereospecificity of CCD2 and the two-step conversion of zeaxanthin to crocetin dialdehyde via 3-OH-β-apo-8'-carotenal. The two downstream steps, dehydrogenation of crocetin dialdehyde and glycosylation of crocetin, take place in the cytoplasm, and are mediated by enzymes localized on insoluble complexes. The final step of the pathway, vacuolar sequestration, is mediated by tonoplast transporters that display stereospecificity and cooperative transport. The compartmentation of crocin biosynthesis in saffron stigmas displays amazing similarities to that of other plant secondary metabolites, such as steviosides or ABA-GE.

7. Meike S. Andersson, Joe Tohme, Wolfgang Pfeiffer

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Tackling hidden hunger: Boosting Vita-A through enhancement and reducing degradation

HarvestPlus leads a global interdisciplinary alliance of research institutions and implementing agencies with the vision of a world free of hidden hunger. The initiative is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). Our approach is to actively add micronutrients to the diets of those who are deficient, by developing and delivering new, more nutritious varieties of staple food crops that provide higher amounts of vitamin A, iron, or zinc – the three micronutrients identified by the World Health Organization as most lacking in diets globally. This innovative approach – called biofortification – complements other nutrition interventions, and is evidence-based, cost-effective, and sustainable. HarvestPlus' applied and strategic research is driven by an impact/product pathway that integrates crop development, nutrition, seed production, capacity building, marketing, advocacy and monitoring/impact research in country-specific crop delivery plans which span the entire value chain. By now, HarvestPlus and its partners have successfully developed >150 varieties of agronomically competitive, profitable micronutrient dense crops through conventional breeding. As a result, >5 million farming households —25 million people— have already been reached with biofortified crops in >30 countries in Asia, Africa and Latin America. Our goal is to reach 20 million farming households—100 million people— with biofortified nutritious foods by 2020. By strengthening our partnerships and delivery efforts, we are confident that we can reach 1 billion people with biofortified foods by 2030.

Early Career Investigators (ECI)

1. Operation of two competing pathways from phytoene to lycopene in transgenic plants expressing CRTI

Dery O.[1]*, Filler S.[1], Welsch R.[2], Enfissi E. M.[3], Fraser P. D.[3], Hirschberg J.[1]

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Metabolic engineering of carotenoids has been successfully achieved in a number of crop plants, mainly to enhance β-carotene. In most cases, for example "Golden Rice", a transgenic bacterial phytoene desaturase (CRTI) was employed. CRTI converts 15-cis-phytoene to all-trans lycopene and thus bypasses a four-enzyme plant pathway consisting of phytoene desaturase (PDS), ζ-carotene isomerase (Z-ISO), ζ-carotene desaturase (ZDS) and carotene isomerase (CRTISO). To study the mode of action of the two parallel phytoene desaturases in the same plant we investigated carotenoid biosynthesis in transgenic tomato over-expressing CRTI (35S::CRTI). Our data revealed that in fruits, phytoene produced by phytoene synthase 1 (PSY1) is preferentially metabolized by PDS and proceeds through ZISO, ZDS and CRTISO even in the presence of a functional CRTI. CRTI was able to produce lycopene when PDS was inhibited by norflurazon in both leaves and fruits. However, when PDS was eliminated via gene silencing, CRTI was able to produce lycopene in fruits but not in leaves. Our results support the hypothesis of a multiprotein carotenoid biosynthesis complex that exists in plastids in which phytoene produced by PSY is preferentially metabolized by PDS and the carotenoids are flux proceeds through Z-ISO, ZDS and CRTISO. The results suggest that in addition to desaturation of phytoene, the PDS polypeptide also plays another indispensable function possibly by stabilization of the complex in chloroplasts but not in chromoplasts.

2. Regulation of β-carotene biosynthesis and accumulation in maize endosperm

Jin X.[1]*, Bai C.[1], Berman J.[1], Farre G.[1], Capell T.[1], Christou P.[1,2], Sandmann G.[3], Zhu C.[1]

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Comparatively little is known about the regulation of endogenous carotenogenic gene expression in higher plants, and this limits our ability to predict how engineered plants will perform in terms of carotenoid content and composition. Maize β-carotene mhydroxylase 2 (BCH2, also termed crtRB1, or HYD3) controls the critical steps in the conversion of β-carotene into zeaxanthin via β-cryptoxanthin in the endosperm. Here we report that *ZmBCH2* is preferentially transcribed in maize endosperm and ism regulated developmentally. Moreover silencing of *ZmBCH2* by RNAi resulted in increased β-carotene content in the endosperm in different maize genetic backgrounds. In order to understand the regulatory mechanisms controlling *ZmBCH2* expression, the 5´-flanking region of *ZmBCH2* containing 2036 bp upstream from the translation start site was isolated from yellow maize inbred B73, and subjected to functional testing in transgenic rice plants. A 774 bp basal promoter region from the translation start site of *ZmBCH2* contains all the necessary cis-regulatory elements responsible for higher GUS expression in transgenic rice endosperm. Furthermore, the 5´ non-translated region containing the first intron in *ZmBCH2* plays a critical role in terms of conferring high levels of expression in the endosperm. This basal promoter contains a single prolamin box and one AACA motif, known to be specifically recognized and bound by endosperm-specific prolamin-box binding factor (PBF) and GA-regulated MYB (GAMYB) transcription factors, respectively, in cereals. Transgenic maize is now being used to characterize whether ZmPBF and ZmGAMYB regulate individually or synergistically endosperm-specific *ZmBCH2* expression.

3. *Genetic mapping of QTLs related to high carotenoid content in winter squash fruit*

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High content of carotenoids, carbohydrates and dry matter makes winter squash (*Cucurbita maxima* Duchesne) fruit valuable material for food industry. Due to their nutritional value, long shelf-life and health protective properties, winter squash fruits have gained increased interest in recent years and are used in the development of novel types of food products. Moreover, no need for chemical crop protection and high level of stress tolerance makes *C.maxima* an interesting vegetable crop for the ecological production. Despite these advantages, genetic and genomic resources available for *C.maxima* are still limited. The aim of this study was to use quantitative trait loci (QTLs) genetic mapping approach to identify QTLs related to carotenoid content in winter squash fruit - such QTLs have not yet been identified. Unique *C.maxima* population of recombinant inbred lines (92 F6 RILs) was developed from a cross between two lines that differ in terms of carotenoid content in fruit. RILs population was used for genetic map construction and carotenoid content evaluation (HPLC, two years experiments). Constructed genetic map consisted of 37 SSRs, 1004 SNPs and 666 SilicoDArTs markers distributed across 20 linkage groups. An average genetic distance between markers was 1,67 cM. For α-carotene, β-carotene, lutein, zeaxanthin, violaxanthin and anteraxanthin content in the fruit several QTLs, including major QTLs, which could explain greater than 20% of the phenotypic variation, were identified.

4. The development and evaluation of a new plant-based renewable source of ketocarotenoids

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In aquaculture, ketocarotenoids are essential feed components necessary to obtain the desired red coloration of trout and salmon flesh, an important consumer trait. At present, ketocarotenoid supplements are mainly petrochemical derived and represent 20% of the feed costs. The present study reports the development of an environmentally friendly technology platform for the effective delivery of plant-based ketocarotenoid feed supplements. Firstly, genetic intervention has been used to create high ketocarotenoid tomato fruits (3.0 mg/g DW). These prototypes have been characterized and production scaled-up for agronomic evaluation. The potential of feed formulations using tomato powder and tomato ketocarotenoid extracts were assessed over two trout trials performed in fresh and brackish water. The plant-based feeds were more efficient than the synthetic feed to color the flesh of trout. This study demonstrates the production and economic feasibility of a new technology platform for the generation of aquaculture feed supplements with improved environmental credentials.

5. Metabolic engineering of xanthophylls in tomato fruit

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In recent years it has been recognized that the β-xanthophylls β-cryptoxanthin and zeaxanthin provide significant health benefits as dietary anti-oxidants and preventive agents against degenerative diseases. In particular, β-cryptoxanthin has been implicated in reduction of lung cancer in humans. These xanthophylls are not readily available in our diet. Zeaxanthin occurs in small amounts in green plant tissues, and in higher concentration in some yellow fruits and vegetables. Tomatoes, an important crop worldwide, constitute a major dietary source of carotenoids in the human diet. Processed tomato varieties provide a cheap source of raw materials for industrial purposes. These attributes make tomato a preferred crop for metabolic engineering to produce xanthophylls. During ripening, the tomato fruit accumulates lycopene in micro-crystalline structures, but not xanthophylls. Our goal is to create tomato varieties that produce and accumulate β-cryptoxanthin and zeaxanthin in fruits. β-cryptoxanthin is synthesized from β-carotene by hydroxylation of a single β-ionone ring. As a first step we developed a tomato line that accumulates β-carotene in fruits based on a newly identified allele of *BETA.* Taking a non-GMO strategy, this line was crossed with a tomato mutant *hp3*, which is impaired in zeaxanthin epoxidase (ZEP). The double mutant *BETA/zep* accumulated significant levels of zeaxanthin but only negligible amount of β-cryptoxanthin. In a second approach, a transgenic expression of β-carotene hydroxylase (*CHYb*) from *Citrus clementina* was established to divert the flux from β-carotene to the downstream products of β-cryptoxanthin and zeaxanthin. Over-expression of *C. clementina CHYb* in tomato nearly doubled the level of free zeaxanthin in fruits. Experiments to elevate xanthophylls in tomato are in progress. Results of this study will generate a sustainable source of unique carotenoids with high nutritional and commercial value.

6. Characterization of carotenoid diversity in traditional and emerging Tunisian sweet orange cultivars

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Citrus are among the most cultivated fruit crops worldwide and occupy a paramount importance in the Tunisian agricultural sector being one of the strategic products. Despite of that, little is known about the quality parameters of Tunisian sweet oranges and some cultivars have not been characterized yet. This work aims to study the coloration diversity of twenty-five Tunisian grown oranges by analyzing their total and individual carotenoids content. Experimental results proved large diversity in the carotenoid composition of the studied orange juices. Maltaise demi-sanguine cultivar was distinguished from the rest due to its relative high content of ζ-carotene, lutein and total carotenoids while in the juice of Meski Ahmer. The presence of the unusual carotene lycopene was detected. The Tunisian grown Washington Navel cultivar ranked within the rich-carotenoids juices. Regarding color parameters and carotenoid composition, (all-*E*)-violaxanthin and ζ-carotene were both significantly correlated to yellow color intensity index (b*) while zeaxanthin and lutein were positively related to Chroma (C*) and (b*) indices. These findings are of interest since the carotenoids content and composition are directly related to color and citrus juice quality. The results obtained could be part in a selection program-guide of Tunisian cultivars and help to understand the physiological and biochemical bases of sweet orange coloration.

Keywords: Citrus, diversity, oranges, carotenoid content

7. The red yeast Xanthophyllomyces dendrorhous as a production platform for the biosynthesis of phytoene and zeaxanthin

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The colorless phytoene and the yellow zeaxanthin are health-promoting carotenoids in humans with applications for skin care and for prevention of macular degeneration, respectively. However, their availability is limited. To meet the economic demand the red yeast *Xanthophyllomyces dendrorhous* was used as a production platform. By genetic modifications of a high-yield astaxanthin mutant the phytoene desaturase gene *crtI* was inactivated and precursor supply for phytoene synthesis was enhanced by over-expression of the genes *HMGR*, *crtE* and *crtYB* which encode limiting enzymes of the pathway. The combination of this engineering approaches resulted in a phytoene producing strain which accumulated 7.5 mg/g dw in shaking cultures and 10.4 mg/g dw in a three-step bioreactor process. The generation of a zeaxanthin producing strain involved the inactivation of the astaxanthin synthase gene *asy* and the heterologous expression of a codon-optimized bacterial β-carotene hydroxylase CrtZoXd and led to a concentration of 3.5 mg/g dw zeaxanthin. Subsequently, the carotenoid content of the phytoene and zeaxanthin producers were increased up to 60 and 92 %, respectively, by the use of xylose and a xylose-rich wheat straw hydrolysate as the carbon source. These results show the strong potential of herbal waste for the development of a sustainable and cost-effective biotechnological carotenoid production. Moreover, novel multioxygenated zeaxanthin derivatives with a high beneficial potential for human health were obtained by combinatorial biosynthesis. This was achieved by the integration of three microbial genes into the genome of a β-carotene mutant leading to the accumulation of rare 4-keto-nostoxanthin and 4,4'-diketo-nostoxanthin and finally by the chemical reduction to the novel 4-hydroxy-nostoxanthin and 4,4'-dihydroxy-nostoxanthin.

8. Microalgae as novel and sustainable sources of natural carotenoids: a comparative study of four microalgae species

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Microalgae, photosynthetic organisms at the base of the aquatic food chain, are rich sources of nutrients and bioactive molecules. They can thus be used for direct animal and human consumption (feed and food sectors) or in the nutraceutic, pharmaceutic and cosmetic sectors as sources of high value products. In particular microalgae are one of the richest and most varied sources of carotenoids.

Microalgae withstand extreme environmental conditions, require no land to grow, absorb excess CO_2 from the atmosphere and have higher productivity than traditional crops. For these reasons microalgae could represent a novel and sustainable source of natural carotenoids. Therefore, the aim of this study was to assess the carotenoid profile of four marine microalgae species (*Isochrysis galbana, Tetraselmis suecica, Nannochloropsis gaditana, Porphyridium cruentum*).

Carotenoid extraction was carried out with a mixture of acetone: methanol (70:30 v/v) with a previous sonication. The extracts were analyzed by HPLC-PDA. Fucoxanthin, Violaxanthin, Lutein, Zeaxanthin, α-carotene and β-carotene were the most represented carotenoids. All xanthophylls and carotenes were found to be present in their free form.

β-carotene was present in all four species at a concentration ranging from 48 mg to 108.7 mg/100 g alga d.w. depending on the species, while α-carotene was detected only in *T. suecica* at a concentration of 26.5 mg/100 g. *I. galbana* confirmed to be a very high source of fucoxanthin (1.3 g/100 g). High levels of violaxanthin were detected in *N. gaditana* (336.7 mg/100 g) and *T. suecica* (81.8 mg/100g). This latter species contained also a high amount of lutein (85.4 mg/100g). Zeaxanthin was detected at a level of 94.2 mg/100 g in the Rhodophyta *P. cruentum*.

9. Microalgal Blue Biotechnology – an attempt to microalgal-based bioprocess optimization, towards bioactive compounds production

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Biotechnology of microalgae has been gaining great importance in recent decades, particularly in production of bioactive compounds with nutra- and pharmaceutical uses – such as carotenoids, due it capacity to act, between other, as antioxidant.

A usual bioprocesses entail 5 stages: 1) selection of microalgal specie; 2) set of ideal of growth conditions; 3) biomass harvesting; 4) compounds extraction and 5) compounds purification.

However, high extraction costs of microalgal metabolites limit its commercial exploitation. Therefore, the work developed attempted overcomes these bottlenecks in microalga-based processes –acting in stage 2, 4 and indirectly in 5. Besides, it was selected a prokaryotic microalga poorly studied so far, *Gloeothece* sp. Hence, efforts were focused in I) optimization of the carotenoids production using light spectrum as a tool; and II) optimization of extraction conditions of bioactive components.

Thus, different LED wavelengths – blue (B), red (R) and two combinations thereof (BR), were tested in terms of its effects on carotenoids and fatty acids (FA) and biomass (PX) productivity, and antioxidant capacity compounds (AC). Also, infrared (IR) LEDs were tested in addition to the best performing visible LEDS.

In an attempt to optimize extraction of lipid components, a laboratory-made continuous pressurized solvent extraction system (CPSE) was built, aimed to optimizing extraction of referred compounds. Biomass amount in the extraction column, solvent flow rate, pressure, temperature and overall solvent volume – including extract fractioning and degree of solvent recirculation, were sequentially optimized as operation parameters in an attempt to meet the Green Chemical Principles.

10. Enhanced bioaccesibility of β-carotene through microencapsulation by emulsion-electrospraying

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The development of carotenoid-enriched functional foods is limited by the low bioaccessibility of these bioactive compounds. The aim of this work was to improve the bioaccessibility of β-carotene after *in-vitro* digestion through its encapsulation within electrosprayed protein microparticles. Electrospraying has recently emerged as an alternative for the production of dry, edible polymeric particles incorporating bioactive agents in a one-step process and mild, food-grade conditions. This technology consists of subjecting a polymer solution or dispersion, which is pumped through a conductive capillary, to a high voltage electric field in such a way that a charged polymer jet is ejected towards a grounded collector. During the flight this jet is broken down into fine droplets due to electrostatic interactions, generating a dry powder upon solvent evaporation. For the incorporation of hydrophobic compounds within the capsules, a plausible approach is to prepare emulsions prior to electrospraying. In this work, two different protein matrices, namely zein and a whey protein concentrate (WPC), and two emulsification procedures, i.e. high-speed homogenization and ultrasonication, were used to prepare the microcapsules through emulsion-electrospraying. A soy bean oil was used as a carrier oil for β-carotene. The impact of the emulsion properties on the microencapsulation efficiency (MEE) and the bioaccessibility of β-carotene was then studied. Results showed that the stability of the prepared emulsions was the main factor affecting the microencapsulation efficiency. The highest MEE was achieved for the capsules obtained from zein emulsions. Finally, the impact of microencapsulation on the bioaccessibility of β-carotene was studied. The results showed that all the encapsulation structures, except those obtained from WPC emulsions prepared by high-speed homogenization, increased the bioaccessibility of β-carotene after *in-vitro* digestion.

11. *Encapsulation of microalgal origin carotenoids in ulvan nanomatrices using electrohydrodynamic process*

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Diet rich in carotenoids is positively correlated with a decreased risk of developing several chronic diseases. However, their bioavailability is often compromised due to incomplete release from the food matrix, poor solubility and potential degradation during digestion. Carotenoid's encapsulation in nano-systems develops an effective thermodynamical and physical barrier against deteriorative environmental conditions, such as water vapor, oxygen, light, enzymes or pH, enhancing their bioavailability, solubility and interfacial properties. Polysaccharides are widely used as encapsulation matrix for bioactive components. Polysaccharides of high density, such as marine origin sulfated polysaccharide Ulvan, exhibit particular interest because of their satisfactory solubility in aqueous systems. The sulfated polysaccharides are used in the formation of nanostructures because of their ionic nature which permits the formation of complexes with oppositely charged polyelectrolytes, useful in designing drug carriers since the polyelectrolyte complexes permit binding of drugs in the polymer matrix at a molecular level. An innovative and promising encapsulation technique of bioactive compounds for food applications is the electrohydrodynamic process. During this procedure a viscous solution of bioactive compounds and matrix material charged with a high voltage that creates an electric field, forces the polymer to be spun out in thin threads. The comparative advantages of this method, is the absence of toxic solvents- high interest in food and nutraceutical applications, the operation in room temperature preserving the thermosensitive ingredients, the process stability that permits tunable fiber diameter or particle size with controlled size distribution and high encapsulation efficiency. In this study, the recovery of carotenoids from the microalgae species *Haematococcus pluvialis* (astaxanthin), *Phaeodactylum tricornutum* (fucoxanthin), and their encapsulation in an emulsion form through the electrohydrodynamic process using ulvan polysaccharide as matrix was studied.

12. Modelling the accelerated solvent extraction of carotenoids from carrot

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The aim of this work was to use accelerated solvent extraction (ASE) for the extraction of carotenoids from carrot (*Daucus carota*). The traditional solvent extraction methods often used for extraction of different bioactive compounds have certain drawbacks such as low selectivity or low extraction yields, consuming more time, labour and toxic organic solvents. Therefore, contemporary extraction techniques have been focused on the use of sub- and supercritical fluids and GRAS (Generally Recognized as Safe) solvents. One of them is ASE that uses elevated temperatures and pressures to achieve efficient and reproducible extraction in a short period of time. This study is focused on studying the effects of extraction parameters, i.e. temperature, extraction time, and number of extraction cycles, on efficiency of carotenoids isolation from carrot with ethanol using ASE. The optimal parameters were defined by response surface methodology (RSM), adopting Box-Behnken experimental design for three variables at three levels. The yields of carotenoids, screened spectrophotometrically, were used as a response for the optimization. According to the results obtained in the experiments the significant ($p=0.0002$) quadratic model was obtained, presenting significant ($p<0.05$) linear effects of all three variables, as well as quadratic effect of temperature on carotenoid extraction yield. The total determination coefficient ($R2=0.9902$) indicate high agreement between experimental and predicted values of the carotenoid content. The optimal conditions for carotenoid maximum yield (30.91 mg/100 g) were determined to be: 42.84 minutes, 96 °C and 3 extraction cycles. Due to experimental validation of this optimum, the experiment was performed under these conditions. The experimental values were found to be in high agreement ($p<0.05$) with the predicted ones.

13. Astaxanthin-based polymers as new antimicrobial compounds

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Astaxanthin (ATX), a xanthophyll bearing a symmetric chemical structure with two hydroxyl groups, has been used a building block for the synthesis of a library of novel high molecular weight polymer, polyastaxanthin (pATX). ATX is a powerful antioxidant, with known therapeutic properties, thus, its copolymerization with various diacids resulted in therapeutic polyesters with varying chemico-physio-mechanico properties (e.g., storage moduli range: 125-1300 MPa and wetting angles of 40-110°). The potential of pATX as a therapeutic material for medical devices was demonstrated in vitro against clinically relevant bacteria (*Staphylococcus aureus* MRSA252 and MSSA476; *S. epidermidis* 1457) showing significant reduction of both bacterial growth and biofilm formation. Additionally, we establish that using pATX films in vivo had no adverse effect in direct contact with open wounds, or on the complete physiological process of whole animal wound healing. Thus, we have successfully demonstrated our capability to turn ATX into a viable plastic material with great potential in various therapeutic applications.

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Late Abstracts

1. *Microalgae as β-carotene source*

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Today, researches on algal biotechnologies are carried out to utilize natural bioactive compounds which have various pharmacological effects. Despite of being used in Asian countries for years as a food, algae have come into prominence as a novel source for bioactive compounds such as β-carotene over the last decade. Consumption of microalgae oil ensures intake of carotenoids. The intake of carotenoids gives the microalgae oils a nutritional added value compared to fish oil. According to their structural properties, amount of β-carotene accumulated in algal cells can be changed in various environments. In this study, effects of light cycle, temperature and nitrogen amount on β-carotene content of *B. braunii* and *M. aeruginosa* were determined and commented by Box-Behnken statistical model to find out the optimum conditions for the usage in further pharmaceutical applications. In comparison between these two species, *B. braunii* showed the highest β-carotene production. There is not any study investigated the effect of parameters on β-carotene production from algae. Obtained product is encapsulated via electrospraying as novel encapsulation method to protect bioactives from degradation and improve adsorption property for pharmaceutical applications. 120 nm average particle size has been achieved with this technique. Data from this study will provide original contributions to the literature.

2. The extraction of fucoxanthin from Nitzschia thermalis using response surface methodology

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Today microalgal biotechnology has gained importance. Microalgae are considered as new sources of carotenoids due to their biodiversity. Fucoxanthin is a type of carotenoid and has powerful antioxidant activity mostly produced by brown algae that also gives their color.

This study aimed to determine the fucoxanthin (FX) that is the major carotenoid in brown microalga, *Nitzschia thermalis* obtained from Ege University Microalgae Culture Collection (Ege-MACC).

The experiments regarding the extraction of carotenoids were carried out using response surface methodology (RSM) to optimize the extraction step. The effect of the extraction time (T), the extraction temperature (C) and the biomass ratio (B) on the amount of FX (mg g^{-1}) were chosen as the design variables and the calculated amount of FX was assessed as the response variable when a Box-Behnken design was used. The three levels of the mentioned design variables, namely T (10, 20 and 30 min), C (25, 35 and 45 °C) and B (0.005, 0.001 and 0.015 g ml^{-1}) were calculated to investigate their impact on the above-stated response variable.

The separation of FX was achieved by C30 column and it was determined at 450 nm by HPLC-DAD using internal standard method.

In the present study, optimum extraction conditions providing the maximum amount of FX were selected by applying the "desirability" function approach in RSM. Finally, the temperature was found to be 28 °C, the extraction time was 10 minutes and the biomass ratio was 0.005 g ml^{-1}. Under these conditions, the maximum fucoxanthin level was determined as 7.89 mg g^{-1}. In conclusion, this study can serve as a reference for the analysis of FX from other brown microalgae.

Keywords: fucoxanthin, carotenoids, brown microalgae, RSM, HPLC-DAD

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