EUROCAROTEN

EUROCAROTEN CONFERENCE 2019

International Conference on Carotenoid research and applications in agro-food and health

BOOK *of* ABSTRACTS

COST ACTION CA15136

Lemesos (Cyprus), 26th-28th November 2019

Welcome

We are delighted to welcoming you to attend the Final meeting of Eurocaroten: European network to advance carotenoid research and applications in agro-food and health held under the auspices of the COST actions (European Cooperation in Science & Technology).

The Conference is a joint initiative of the Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology & Food Science and the Agricultural University of Athens, Greece.

The Scientific Program is comprised by 7 plenary talks and 56 oral/flash oral presentations, and is expected to cover the whole spectrum of research on carotenoids. Exceptionally, the Conference includes an inaugural pre-Conference talk about the employment of statistical tools and how to use data to problem solve in the Era of Analytics.

We aspire to offer a high-quality and interesting scientific program combined with an attractive social program.

The Convenors

George Manganaris
Cyprus University of Technology

Sekos Haroutounuian
Agricultural University of Athens

Conference Program

Monday, November 25, 2019	
16:00-19:00	**Attendee registration** (Royal Apollonia Hotel)
19:00-19:30	**A. Mavromoustakos** [Inaugural talk] How to educate and train yourself on using data to problem solve in the Era of Analytics

Tuesday, November 26, 2019	
07:30-08:30	**Attendee registration**
08:30- 08:45	Welcome Remarks
08:45-10:45	**Session I: Production & Development** Moderator: S. Haroutounian & J. Šic Žlabur
08:45-09:30	**P. Christou** [PLE-1] Third generation plant biotechnology products to address the Food-Feed-Nutrition-Health nexus
09:30-09:43	C. Zhu [OP-1] The subcellular localization of two isopentenyl diphosphate isomerases in rice suggests a role for the endoplasmic reticulum in isoprenoid biosynthesis
09:43-09:50	L. Schüler [OP-2] Improvement of lutein and β-carotene production by abiotic growth factors in a robust marine microalga *Tetraselmis* sp. CTP4
09:50-10:03	V. Křen [OP-3] Preparation of novel hybrid conjugates of carotenoids with flavonolignans and their antiradical properties
10:03-10:10	M. Macernis [OP-4] Modeling Raman and electronic states in carotenoids
10:10-10:23	S. Matsubara [OP-5] Toward understanding the regulation of carotenoid turnover in plants
10:23-10:30	T. Banh [OP-6] Whole plant $^{13}CO_2$-labelling for carotenoid turnover analysis in leaves
10:30-10:45	M. Čertík [OP-7] Production of carotenoid pigments by oleaginous fungi and yeasts
10:45-11:25	**Morning Tea/Coffee break**
11:25-11:30	**Group photo**

11:30-13:00	**Session II: Quality & Food Chain** Moderators: A. Mandic & T. Tsiaka
11:30-12:00	**N. O'Brien** [PLE-2] Pro-vitamin A carotenoids in foods: Nutritional significance in developing countries
12:00-12:13	R. Sturza [OP-8] Influence of thermal processing on carotenoid content and antioxidant activity in berry's pulp
12:13-12:20	M. Fikselova [OP-9] Carotenoid content in tomato products as affected by different processing methods
12:20-12:33	A. Ferrante [OP-10] The carotenoids as plant defense tools and bioactive compounds in food systems: the impact of postharvest treatments and storage conditions
12:33-12:40	A. Bockuviene [OP-11] Preparation and characterization of novel β-carotene loaded nanoparticles
12:40-12:53	M. Tsimidou [OP-12] The uncommon hydrophilic apocarotenoid picrocrocin and its importance in the quality control and applications of saffron
12:53-13:00	F. He [OP-13] Effects of root cooling on fruit quality of cocktail tomato
13:00-14:30	**Lunch**
14:30-16:00	**Session III: Nutrition & Health** Moderators: J. Dulinska & M. Iddir
14:30-15:00	**T. Bohn** [PLE-3] Carotenoids – are we ready for dietary intake recommendations?
15:00-15:13	R. Edge [OP-14] Are dietary carotenoids beneficial to health? The switch from anti- to pro-oxidant behaviour - the effect of oxygen
15:13-15:20	M. Cano [OP-15] Impact of high hydrostatic pressure and thermal treatment on the stability and bioaccessibility of carotenoid and carotenoid esters in astringent 'Rojo Brillante' persimmon during in vitro digestion
15:20-15:27	M. Cano [OP-16] Characterization of carotenoids, carotenoid esters and fatty acids in two lucuma (*Pouteria lucuma*) varieties experimentally grown in Spain
15:27-15:40	J. Ribot [OP-17] Fucoxanthin-rich ethanolic *Phaeodactylum tricornutum* extract ameliorates effects of diet-induced obesity in C57BL/6J mice

15:40-15:47	J. García-Alonso [OP-18] Post-harvest use of Light Emitting Diode (LED) to enhance carotenoid content in tomatoes
15:47-15:55	K. Kljak [OP-19] Carotenoid degradation during lactic fermentation of rehydrated maize grain from various hybrids
15:55-16:30	**Evening Tea /Coffee break**
16:30-18:00	**Session IV: Production & Development** Moderators: J. Hirschberg & L. Schüler
16:30-17:15	**G. Giuliano** [PLE-4] Convergent evolution of crocin biosynthesis in higher plants
17:15-17:23	I. Línzembold [OP-20] Covalent conjugation of carotenoids with flavonoids: efficient antioxidants showing supramolecular organization
17:23-17:30	J. Šic Žlabur [OP-21] Utilization of vacuum drying in retention of β-carotene content in winter squash (*Cucurbita moschata*)
17:30-17:40	E. Myrtsi [OP-22] Exploitation of Citrus juicing industrial by-products as a rich source of carotenoids
17:40-17:47	B. Rebelo [OP-23] In search of alternative plant-based systems for the production of astaxanthin and canthaxanthin
17:47-18:00	S. Koo [OP-24] Efficient synthesis of carotenoids for improved antioxidant activity
18:00-18:15	G. Diretto [OP-25] UGT709G1: a novel uridine diphosphate glycosyltransferase involved in the biosynthesis of picrocrocin, the precursor of safranal
18:30-20:00	**Working Group meetings**

Wednesday, November 27, 2019	
09:00-11:00	**Session IV: Nutrition & Health** Moderators: T. Bohn & K. Kljak
09:00-09:45	**A. Palou** [PLE-5] Health claims and nutrition information
09:45-09:58	S. Silva [OP-26] A single dose of marine Chlorella vulgaris increases plasma concentration of lutein, b-carotene and zeaxanthin in a 3 days intervention study with human healthy volunteers
09:58-10:05	A. Wawrzyniak [OP-27] Validation of Food Frequency Questionnaire methods for assessing lycopene consumption among young men
10:05-10:18	E. Georgiadou [OP-28] Fractionate analysis of lycopene metabolism gene expression levels and lycopene content in five tomato cultivars during ripening
10:18-10:25	Z. Vondráková [OP-29] Carotenoids and phenolic acids during ripening, harvest and storage in selected scab-resistant and mildew-tolerant apple cultivars
10:25-10:38	J. Keijer [OP-30] Altered homeostasis as a potential explanation for the adverse effects resulting from continuous high dose beta-carotene supplementation
10:38-10:50	D. Brown [OP-31] Astaxanthin supplementation improves endurance performance and fat oxidation during a 40 km cycling time-trial
10:50-11:30	**Morning Tea/Coffee break**
11:30-13:00	**Session V: Quality & Food Chain** Moderators: N. O'Brien & L. Van Meulebroek
11:30-11:45	M. Barros [OP-32] Scavenging and quenching activities of carotenoid extracts from citrus fruits and synergism with ascorbic acid in liposomes mimicking animal cell membranes
11:45-11:53	S. di Corcia [OP-33] Citrus juice concentrate obtained by coupling enzymatic liquefaction with microfiltration: carotenoid bioaccessibility
11:53-12:00	A. Focsan [OP-34] Enhancement of antioxidant activity of carotenoids in supramolecular complexes and the role of their redox potentials

12:00-12:07	M. Christofi [OP-35] Assessing the phenolic and carotenoid content of eight clingstone peach cultivars at harvest and after processing
12:07-12:15	V. Tumbas Saponjac [OP-36] Bioactive characteristics of sweet potato flesh and peel
12:15-12:23	A. Mišan [OP-37] Carotenoid profiling of selected strains of Cyanobacteria
12:23-12:30	K. Pumprova [OP-38] Fern fiddleheads as a potential source of lutein and antioxidants in the European diet
12:30-12:38	S. Häkkinen [OP-39] Autumn leaves – upgrading carotenoid rich park and garden waste to useful chemicals for industry
12:38-12:45	D. Lantzouraki [OP-40] Effect of natural antioxidants and citrus pulp supplementation to hen diet on pigmentation and carotenoid content of egg yolk
12:45-12:53	R. Sturza [OP-41] The impact of the carotenoid complex of rosehip and hawthorn powders on the accidental pathogenic microbiota in sausages
12:53-13:00	T. Tsiaka [OP-42] Natural deep eutectic solvents (NADES): Potentials and challenges of a state-of-the-art green technology for carotenoid extraction
13:00- 14:30	**Lunch**
14:30-16:20	**Session VI: Nutrition & Health** Moderators: J. Ribot & E. Georgiadou
14:30-15:15	**E. Johnson** [PLE-6] Lutein as a part of a nutrient pattern for brain health
15:15-15:28	L. Van Meulebroek [OP-43] Development of new carotenoid food additives from bacteria using gut metabolomics as innovative research tool
15:28-15:35	M. Dias [OP-44] Carotenoids intake by the Portuguese population based on a Total Diet Study approach
15:35-15:43	A. Gómez-Maqueo [OP-45] Carotenoid and carotenoid ester deposition in plastids of lucuma, avocado and persimmon and its effect on bioaccessibility

15:43-15:55	M. Iddir [OP-46] Influence of dietary proteins on the bioaccessibility of carotenoids from selected food matrices	
15:55-16:08	N. Gangadhar Katkam [OP-47] Design and development of a carotenoid enriched extract from microalgae using a liquid-liquid triphase system: its evaluation	
16:08-16:15	B. Olmedilla-Alonso [OP-48] Lutein and zeaxanthin concentrations: in serum, macular pigment optical density, feces and dietary intake of Spanish subjects aged 45 -65 years	
16:15-17:00	**Evening Tea /Coffee break**	
17:00-18:00	**Overview of COST action progress** Moderators: A. Melendez & C. Socaciu	
17:00-17:15	WG1: Production & Development Chair: J. Hirschberg	
17:15-17:30	WG2: Quality & Food Chain Chair: N. O'Brien & A. Mandić	
17:30-17:45	WG3: Nutrition & Health Chair: T. Bohn & J. Dulińska-Litewka	
17:45-18:00	WG4: Transfer & Exploitation Chair: A. Ritala	
19:30	**Departure to Limassol Marina**	
20:00-01:00	**Gala dinner (Marina Breeze)**	

Thursday, November 28, 2019

09:30	Departure from Apolonia Hotel
10:00-11:00	Guided Tour at Curium Palace
11:00-11:30	Departure from Curium to Pafos
11:30-12:30	Guided Tour at Pafos Mosaics
12:30-13:00	Free time in Pafos Old Harbor
13:00-13:30	Lunch basket
13:30-14:30	Departure from Pafos to Omodos
14:30-16:00	Guided Tour at Omodos Church and free time

16:00-17:45	**Session VII: Quality & Food Chain/ Nutrition & Health** **[Oenou Yi Conference Room]** Moderators: A. Mandic & E. Myrtsi
16:00-16:13	F. Mantzouridou [OP-49] Production of carotenoids from Blakeslea trispora: achievements and challenges
16:13-16:25	S. Hor [OP-50] Relation between fruit density and β-carotene content in ripe mango
16:25-16:32	V. Grujić [OP-51] The bioreactor system for the astaxanthin producing microalgae *Haematococcus pluvialis*: construction, quantification and determination of the astaxanthin
16:32-16:40	O. Demurtas [OP-52] Tonoplast transporters mediate vacuolar accumulation of crocins in saffron stigmas
16:40-16:47	S. Simões [OP-53] Lycopene extract incorporation in microemulsions for skin bioavailability enhancement
16:47-16:55	D. Segliņa [OP-54] Rapid determination of carotenoids in different pumpkin species by applying combined extraction and saponification procedure and separation via supercritical fluid chromatography
16:55-17:07	I. Petyaev [OP-55] Carotenoids reduces fat food digestibility-implications for health and nutrition
17:07-17:20	G. Dilena [OP-56] Carotenoids from traditional, new and sustainable sources
17:20-18:00	**A. Melendez** [PLE-7] Carotenoids and derivatives for the industry: Traditional, emerging and prospective applications
18:00-19:30	**Management Committee Meeting** Conference Wrap up and conclusions of COST Action Moderator: Antonio Melendez
19:30-21:00	**Wine tasting & Farewell dinner**
22:00	Arrival to Lemesos

Plenary Lectures, PLE

Code	Presenting Author- Title
PLE-1	**P. Christou** Third generation plant biotechnology products to address the Food-Feed-Nutrition-Health nexus
PLE-2	**N. O' Brien** Pro-vitamin A Carotenoids in Foods: Nutritional significance in developing World Countries
PLE-3	**T. Bohn** Carotenoids – are we ready for dietary intake recommendations?
PLE-4	**G. Giuliano** Convergent evolution of crocin biosynthesis in higher plants
PLE-5	**A. Palou** Health claims and nutrition information
PLE-6	**E. Johnson** Lutein as a part of a nutrient pattern for brain health
PLE-7	**A. Melendez** Carotenoids and derivatives for the industry: Traditional, emerging and prospective applications

PLE-1

Third generation plant biotechnology products to address the Food-Feed-Nutrition-Health nexus

P. Christou[1,2], C. Zhul[1], T. Capell[1]

[1]University of Lleida-Agrotecnio, Center, Lleida, Spain
[2]ICREA, Catalan Institute for Research and Advanced Studies, Passeig Lluís Companys 23, 08010 Barcelona, Spain

Enhanced nutrition is a major aim of contemporary research in agriculture. Future food and feed products need to supply more nutritious food/feed while minimizing the environmental footprint of human activities. The effectiveness of agriculture in addressing the nutritional needs of humans and animals on a global scale reflects game changing discoveries in biology and new developments in a multitude of other scientific and social disciplines. A key driver in this transition will be our ability to control plant metabolism with more precision and predictability. We will discuss a portfolio of engineered rice and maize lines designed to accumulate extraordinary levels of nutritionally important carotenoids and other nutritionally relevant molecules. Experiments demonstrating the beneficial effects of an engineered high carotenoid corn diet in human and animal health will be described. In moving forward one tantalizing strategy is to reduce plant cells to engineering components through abstraction, decoupling and standardization in the context of synthetic biology. We will discuss examples from our own laboratory focusing on the use of CRISPR genome editing to create a "plant shell" better able to serve as a starting point for complex genetic engineering interventions aiming to deliver highly nutritious food at source. We will also discuss the use of engineered plants as component molecules capable of neutralizing the HIV virus. Such combination microbicides hold great promise in halting the transmission of AIDS in developing countries.

PLE-2

Pro-vitamin A carotenoids in foods: Nutritional significance in developing world countries

N.M. O'Brien, T.P. O'Connor

School of Food and Nutritional Sciences, University College, Cork, Ireland

Pre-formed vitamin A (retinol/retinal) deficiency is a major nutritional issue worldwide particularly among children in developing countries. Vitamin A is crucial for growth and development, vision, immune function, gene expression. It is estimated that up to 30% of children under 5 worldwide may be deficient in vitamin A resulting in significant risk of associated morbidity and mortality. The vitamin A status of lactating mothers is also significant as this can influence transfer of vitamin A to breastfed infants. Supplementation programmes with high doses of pre-formed vitamin A are widely used interventions to help alleviate vitamin A deficiency. While these programmes are of very significant value, they do not address the fundamental nutritional reason for widespread vitamin A deficiency globally, i.e., poor dietary diversity. Very many people, in particular poorer people in developing countries, consume a non-diverse diet consisting principally of a few inexpensive starchy foods such as maize or sweet potato or cassava. This type of dietary pattern is typically low in micronutrient-dense foods including both foods rich in pre-formed vitamin A and, also foods rich in pro-vitamin A carotenoids which potentially can be bio-converted to vitamin A. While biofortification of starchy staple foods, for example sweet potato, is a valuable approach with potential to decrease the public health impact of vitamin A deficiency worldwide, most nutritionists emphasise the importance of public health education programmes to encourage people to increase dietary diversity by consuming micronutrient-rich foods, including foods rich in carotenoids. We will present findings from work conducted in two agro-ecological zones of rural Ethiopia among lactating mothers and their breastfed children. These findings clearly demonstrate the magnitude of the public health problem of poor dietary diversity and the need to encourage people in developing countries to increase consumption of nutrient-dense foods including foods rich in pro-vitamin A carotenoids.

PLE-3

Carotenoids – are we ready for dietary intake recommendations?

V. Böhm, G. Lietz, B. Olmedilla-Alonso, D. Phelan, E. Reboul, D. Bánati, P. Borel, J. Corte-Real, A.R. de Lera, C. Desmarchelier, J. Dulinska-Litewka, J.-F. Landrier, I. Milisav, J. Nolan, M. Porrini, P. Riso, J. M. Roob, E. Valanou, A. Wawrzyniak, B.M. Winklhofer-Roob, R. Rühl, T. Bohn

EU COST Action EUROCAROTEN & Luxembourg Institute of Health, Department of Population Health, L-1445 Strassen, Luxembourg

At present, dietary reference intakes for carotenoids are not defined, though suggestions exist for the vitamin A equivalence of pro-vitamin A carotenoids. There is uncertainty regarding carotenoid intake recommendations, as both positive and negative health effects have been found or correlated with carotenoid intake and tissue levels (including blood, adipose tissue, macula), depending on the type of study (epidemiological vs. intervention), the dose (physiological vs. supra-physiological) and the matrix (foods vs. supplements, isolated or used in combination). All these factors, combined with inter-individual response variations (e.g. depending on age, sex, disease state, genetic makeup), make the relationship between carotenoid intake and their blood/tissue concentrations often unclear and highly variable. Although blood total carotenoid concentrations <1000 nM have been related to increased chronic disease risk, no dietary reference intakes (DRIs) do exist. While high total plasma/serum carotenoid concentrations of up to 7500 nM are achievable following supplementation, a plateauing effect for higher doses and prolonged intake is apparent. As some carotenoids can be converted by unsaturated chain cleavage into bioactive retinoids and other apo-carotenoids by enzymes such as BCO1/BCO2, available information on the blood/tissue concentrations of these metabolites should be considered. In this presentation, it is strived to summarize the current knowledge on carotenoids and their main metabolites in serum/plasma and tissues and their relationship to dietary intake and health status, aiming to propose suggestions for a "normal", safe and desirable range of concentrations that presumably are beneficial for health. Existing recommendations are likewise evaluated and practical dietary suggestions are included.

PLE-4

Convergent evolution of crocin biosynthesis in higher plants

G. Giuliano

Italian National Agency for New Technologies, Energy and Sustainable Development, 00123 Rome, Italy

Crocus sativus stigmas are the source of the most expensive spice on Earth, saffron, and owe their characteristic color to crocins, glycosylated apocarotenoids making up to 10% of the stigma dry weight. We identified and characterized candidate genes for the whole crocin biosynthetic pathway in *C. sativus* (1-3). The pathway starts in the plastid, with the cleavage of zeaxanthin by CCD2, continues in the ER and the cytoplasm, with the dehydrogenation and glycosylation by AlDH and UGT enzymes, and ends in the vacuoles, where crocins are transported by ABCC transporters. Crocins are synthesized by distantly related plants (*Crocus*, Iridaceae; *Gardenia*, Rubiaceae; and *Buddleja*, Scrophulariaceae). Expression of the CCD2 enzyme in *N. benthamiana* leaves results in the production of crocins, suggesting that the downstream steps in crocin biosynthesis and sequestration are ubiquitous and that their production just requires the evolution of a CCD enzyme with the appropriate cleavage activity. Based on these data, a model on the convergent evolution of crocin biosynthesis in different plant genera will be presented.

References:

1. Frusciante S et al (2014) Proc Natl Acad Sci USA, 111, 12246-12251.
2. Demurtas OC et al (2018) Plant Physiol, 177, 990-1006.
3. Demurtas OC et al (2019) Plant Cell, in press.

PLE-5
Health claims and nutrition information

A. Palou

Laboratori de Biologia Molecular, Nutrició i Biotecnologia (LBNB). Universitat de les Illes Balears (UIB; Centro de Investigación biomédica en red Fisiopatología de la Obesidad y Nutrición (CIBERobn); Fundació Institut d'Investigació Sanitaria Illes Balears (IDISBA); Alimentómica S.L. (Spin off UIB). Palma de Mallorca 07122, Spain

Regulation (EC) No 1924/2006 on nutrition and health claims (RNHC) is the legal framework in the EU enabling food operators to voluntarily highlight particular beneficial effects of food products, in relation to health and nutrition. It applies to commercial communications, whether in the labelling, presentation or advertising of foods to be delivered as such to the final consumer. It covers a very important part of the information to guarantee that Europeans are appropriately informed, as required by the EU general food law (Regulation 178/2002) to provide a basis for consumers to make informed choices in relation to food they consume and to prevent practices that may mislead the consumer. Consequently, food bearing claims that could mislead consumers are prohibited in the EU. However, foods promoted with claims, which are based on one or more of its components, may be perceived by consumers as having a health advantage over other products, while bearing excess of other components (such as sodium, trans fat, saturated fat or sugars), which may result in masking the overall nutritional status of a food product and encouraging consumers to make undesirable food choices. To address this potential problem a mandate to the European Commission was included in the RNHC (article 4) to establish (before 19 January 2009) the nutrition profiles (thresholds/levels content of different substances with a detrimental or beneficial nutritional or physiological effect) that foods must comply with in order to bear nutrition or health claims and the conditions for their use. However, after 10 years of delay, nutrient profiles have not been established due to difficulties to reconciliate the different superimposed conditionings in the Regulation, including the place and role of different foods in the overall diet, the variety of traditions, dietary habits and consumption patterns existing in the Member States, together with the objective of enhancement of product innovation. In the meantime, Regulation (EU) 1169/2011 on the provision of food information to consumers was adopted, including the on-packaging mandatory "nutrition declaration" that informs the energy value and the amounts of fat, saturates, carbohydrate, sugars, protein and salt. Also, the introduction of a legal limit of 2 g per 100 g of fat on industrially produced trans fats in food will come in force in 2021. Besides, an important controversy is emerging confronting two new proposals (*nutriscore* and *traffic lights*) to include front-of-pack nutritional labelling. Nutriscore (NS) is a composite label in which all nutritional information is traduced to one simple score (A through E, with A being the healthiest option). Traffic Light (TL) label provides a separate assessment for main ingredients (fat, saturated fat, sugar and salt) plus energy content. Both initiatives have important pros and cons and appears to relegate the discussion on nutrient profiles. However, this is unclear as in the essence of all this key legislation and initiatives the consensus is that all selected measures must be underpinned by strong science, which is the basis to authorize any health claim made on food. Given that NS and TL for each food can be considered specific health claims its scientific substantiation requires the scientific assessment by EFSA.

PLE-6

Lutein as a part of a nutrient pattern for brain health

E.J. Johnson

Tufts University, School of Medicine, USA

The earliest stage of AD is a critical time when nutrition may have a significant impact on disease onset and progression. While results from many clinical trials using supplementation with isolated nutrients have been mixed, interventions that incorporated foods or significant changes in dietary components have shown promising results on their protective effects against age-related neurodegeneration. This warrants an identification of what combination of nutrients, which is how they are consumed from the diet, is the most related to the absence or delayed progression of age-related cognitive impairment. The data set from the Georgia Centenarian Study provides a unique opportunity to examine the relationship between the status of fat-soluble nutrients in serum and brain of centenarians and cognitive function. In our study, measures of fat-soluble nutrients in serum and brain samples included carotenoids, retinol, α- and γ-tocopherols, phylloquinone, menaquinone-4, and fatty acids. Brain nutrient patterns were constructed with principal component analysis using concentrations averaged from the frontal and temporal cortices. Potential confounders were controlled for each analysis of association/correlation. Brain nutrient pattern high in carotenoids, saturated and n-3 polyunsaturated fatty acids, and low in monounsaturated FAs was significantly associated with better performance on global cognition, memory, language, activities of daily living, and lower depression in non-demented subjects. The findings from this research serve as a scientific evidence for establishing dietary recommendations, and support the emphasis on healthy dietary patterns as compared to individual nutrients.

PLE-7

Carotenoids and derivatives for the industry: Traditional, emerging and prospective applications

A.J. Meléndez-Martínez

Food Colour & Quality Laboratory, Area of Nutrition & Food Science, Universidad de Sevilla, 41012 Seville, Spain

Carotenoids are ancient, widespread and versatile isoprenoids that have been harnessed for different purposes by terrestrial plants, algae, fungi, bacteria and animals living in the most diverse conditions. Carotenoid-containing organisms can be found in the bottom of the seas, deserts or the highest mountains. Besides, carotenoids are thought to be key for the survival of organisms living in extreme conditions, including bacteria from the phylum Deinococcus–Thermus (that can endure extreme desiccation, radiactive and high temperature conditions) or the microalga *Dunaliella salina,* which thrives in hypersaline waters. Furthermore, carotenoids are precursors of a myriad of compounds. Thus, they can be oxidatively cleaved, either non-enzymatically (via singlet oxygen, lipoxygenase cooxidation or photooxidation) of enzymatically by means of carotenoid cleavage oxygenases. Carotenoid derivatives have different physico-chemical properties and actions and also play key roles in many processes. Examples of derivatives are retinoids (some of with exhibit vitamin A activity), a wide variety of odorant compounds, sapid compounds (for instance picrocrocin in saffron), fungal hormones (trisporic acid), insect repelents (grasshopper ketone, β-ionone) or phytohormones including abscisic acid or strigolactones, among many others. Due to their versatility and variety of actions, carotenoids and their derivatives can be used for different applications. As far as human foods and nutrition is concerned, they have been long used as natural pigments and as a source of vitamin A. In the last four decades, a large body of evidence has accumulated indicating that carotenoids may exert health-promoting biological actions, hence their interest for the development of functional foods and related products. Lately, they are attracting attention in the context of nutricosmetics. Apart from these and other classical examples of applications, there are emerging and possible future applications of carotenoids and derivatives that augur a brilliant future of applied research on these fascinating compounds.

Oral Presentations, OP

OP-1

The subcellular localization of two isopentenyl diphosphate isomerases in rice suggests a role for the endoplasmic reticulum in isoprenoid biosynthesis

X. Jin[1], C. Baysal[1], L. Gao[2], V. Medina[1], M. Drapa[3], X. Ni[2], Y. Sheng[2], L. Shi[4], T. Capell[1], P.D. Fraser[3], P. Christou[1,5], C. Zhu[1,2]

[1]*University of Lleida-Agrotecnio Center, Spain*
[2]*Changchun Normal University, China*
[3]*Royal Holloway University of London, UK*
[4]*Northeast Normal University, China*
[5]*ICREA, Catalan Institute for Research and Advanced Studies, Barcelona, Spain*

Isoprenoids are synthesized from the precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphosphate (DMAPP), which are interconverted by the enzyme isopentenyl diphosphate isomerase (IPPI). Many plants express multiple isoforms of IPPI, the only enzyme shared by the mevalonate (MVA) and non-mevalonate (MEP) pathways, but little is known about their specific roles. Rice (*Oryza sativa*) has two IPPI isoforms (OsIPPI1 and OsIPPI2). We therefore carried out a comprehensive comparison of IPPI gene expression, protein localization and isoprenoid biosynthesis in this species. We found that *OsIPPI1* mRNA was more abundant than *OsIPPI2* mRNA in all tissues, and its expression in de-etiolated leaves mirrored the accumulation of phytosterols, suggesting a key role in the synthesis of MVA pathway isoprenoids. We investigated the subcellular localization of both isoforms by constitutively expressing them as fusions with synthetic green fluorescent protein. Both proteins were localized to the endoplasmic reticulum (ER) as well as peroxisomes and mitochondria, whereas only OsIPPI2 was detected in plastids, due to an N-terminal transit peptide which is not present in OsIPPI1. Despite the plastidial location of OsIPPI2, the expression of *OsIPPI2* mRNA did not mirror the accumulation of chlorophylls or carotenoids, indicating that OsIPPI2 may be a dispensable component of the MEP pathway. The detection of both OsIPPI isoforms in the ER indicates that DMAPP can be synthesized *de novo* in this compartment. Our work shows that the ER plays an as yet unknown role in the synthesis of MVA-derived isoprenoids, with important implications for the metabolic engineering of isoprenoid biosynthesis in higher plants.

OP-2

Improvement of lutein and β-carotene production by abiotic growth factors in a robust marine microalga *Tetraselmis* sp. CTP4

L.M. Schüler, N. Gangadhar Katkam, T. Santos, P. Duarte, H. Pereira, L. Barreira, J. Varela

CCMAR – Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Because of the rising life expectancies and health trends, consumer interest in natural products and healthy food is growing. Microalgae are a rich source of nutritional and bioactive molecules such as carotenoids. This study aimed to investigate an industrial microalgal strain, *Tetraselmis* sp. CTP4 (*1*), under different physiological conditions for both optimal biomass growth and concomitant induction of carotenoid biosynthesis, particularly, of lutein and β-carotene. CTP4 was cultivated under a wide range of temperature (5-35 °C), light intensity (38-700 μmol/m*s), salinity (5-75‰) and nutrient availability for 2, 5 and 10 days. Carotenoids were extracted from wet biomass by repeated glass bead-assisted disruption with acetone. Quantification was done by RP-HPLC-DAD. CTP4 contained neoxanthin, violaxanthin, lutein and β-carotene, as expected for a *Tetraselmis* species (*2*). CTP4 produced more carotenoids in optimal growth conditions rather than under lipid-inducing nutritional stress. The obtained result might be explained by the fact that carotenoids in this species are located in the thylakoid membranes as components of the light harvesting complex rather than in lipid bodies. Additionally, cells at 30 °C contained the highest amounts of carotenoids (7.94±0.59 mg/g DW), probably due to heat-induced ROS formation resulting in the accumulation of scavenging pigments. Under these conditions, lutein content was 2.20±0.02 mg/g DW when cultures were exposed to light intensity of 700 μmol/m*s for 10 days. Conversely, β-carotene was induced at low light (38 μmol/m*s) reaching contents of 3.25±0.16 mg/g DW after 2 days. This study indicates that CTP4 is a promising strain for lutein and β-carotene production at locations where a robust, euryhaline, thermotolerant microalga is required.

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OP-3

Preparation of novel hybrid conjugates of carotenoids with flavonolignans and their antiradical properties

V. Křen, C.S. Chambers, D. Biedermann, K. Valentová

Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Biotransfor-mation, Vídeňská 1083, CZ 14220, Prague 4, Czech Republic

Antioxidants protect the structural and functional components in organisms against oxidative stress. Most of the antioxidants are of plant origin as the plants are permanently exposed to oxidative stress (UV radiation, photosynthetic reactions). Both carotenoids and flavonoids are prominent antioxidants occurring often together in the plant tissues acting both in lipophilic and hydrophilic milieu. They are complementary in their antioxidant activity. Combining antioxidants with different types of mechanism of action into one molecule often leads to a strong potentiation of the effect of both antioxidant partners in the molecule. This approach has already been used in hybrid molecules containing e.g. flavonolignans and fatty acids [1] or ascorbic acid [2] with improved antioxidant properties. In this study a series of hybrid ester conjugates of retinoic acid (Fig. 1) with various flavonolignans, such as silybin, 2,3-dehydrosilybin, and isosilybin was synthesized and structurally fully characterized (NMR, MS). Antioxidant/antiradical activities and bio-physical properties of novel synthesized carotenoid-flavonoid hybrids as well as various mixtures of the respective components were extensively investigated. This is probably the first example of such covalent supramolecular conjugate composed of carotenoid and flavonoid.

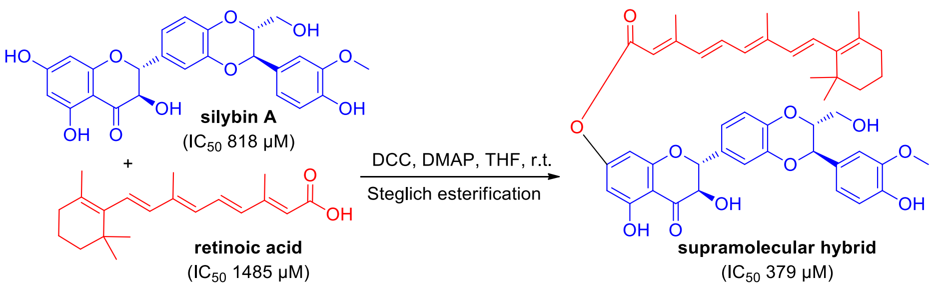

Figure 1: Carotenoid-flavonoid hybrid synthesis and DPPH scavenging properties; DCC (*N,N*-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine), THF (tetrahydrofuran).

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OP-4

Modeling Raman and electronic states in carotenoids

M. Macernis

Vilnius University, Faculty of Physics, Institute of Chemical physics, 3603 Saulėtekio av. 3, 10257 Vilnius, Lithuania

The color changes are related to variability of the internal electronic states of carotenoids (Cars) what also effect frequency related state properties due to structural changes. Thus, the example of key research questions: why tomatoes are red? Theoretical approach is the background for studies of various carotenoids (lycopene, β-carotene, peridinin, fucoxanthin, diadinoxanthin, alloxanthin and etc.) in order to understand possible reasons for the spectral changes are the main goal. Effects caused by various surroundings depending on their polarity as well as due to local protein environment were analyzed. All Cars have the lowest excited states, which are dark and, thus, not visible in the optical spectra. Quantum chemistry calculations of this dark excited state are complicated. There were performed using semi-empirical method which were continued with more accurate methods such as EOM-CCSD and CASSCI study. In parallel we investigated for charge transfer (CT) and intramolecular charge transfer (ICT) states which are predicted by explaining various experimental data. The Raman ν1 and optically allowed states (S2) were analyzed using various DFT approaches. The ensemble of alterations of the vibrational spectra of β-carotene upon introduction of conjugated phenyl groups at positions 13 and 13' are precisely predicted by the DFT method used according experimental data. We got there were the linear correlation between polarization and conjugation length of polyene and Cars: the longer the chain the higher polarizability according the global softness parameter. Raman ν1 band shift may be attributed to the increased polarizability in the Cars environments. However the Raman ν1 and ν2 shift are unclear in the diadinoxanthin, fucoxanthin (ν1 shift) and alloxanthin (ν1 and ν2 shift). The quantum molecular dynamics (QMD) was performed in order to study molecular properties. The present study showed there were the additional 3rd conformer according the ending groups of the studied Cars.

OP-5

Toward understanding the regulation of carotenoid turnover in plants

B. Thiele, T. M. A Banh, A. Chlubek, T. Hombach, A. Neuwohner, I. Janzik, S. Jahnke, E. Kleist, S. Matsubara

IBG-2: Plant Sciences, Forschungszentrum Jülich, 52428 Jülich, Germany

Progress in plant carotenoid research has brought the scientific community genetic and molecular tools to manipulate carotenoid composition and accumulation in model plants and crops. However, the outcome of such manipulation often remains unpredictable due to many "unknowns" in feedback regulation, protein modification and interaction, or compartmentalization. Unravelling the mechanisms that control flux, partitioning and sequestration in carotenoid biosynthesis and degradation pathways necessitates, among other things, turnover analysis of pathway intermediates and products. Isotopic labelling has been a powerful tool for investigation of metabolic pathways in cells and plants. Photosynthetic CO_2 fixation enables convenient incorporation of carbon isotopes in various metabolites under various conditions. While incorporation can be observed within seconds to minutes in and around primary metabolism, it can take hours to days for specialized metabolism, including that of carotenoids and apocarotenoids in photosynthetic and non-photosynthetic organs. Despite high sensitivity of detection, radioactive ^{14}C is difficult to quantify at the molecular level and experiments inevitably generate radioactive waste. Here, we present whole-plant $^{13}CO_2$ labelling systems combined with LC-MS and FTICR-MS analysis to study carotenoid turnover. Two experiments were conducted to test the applicability of the method. In the first experiment, a walk-in climate chamber was used to obtain ^{13}C-labelled peppermint plants. Subsequently, changes in carotenoid isotopologue composition were monitored in leaves of these plants in ambient air. In the second experiment, *Arabidopsis* plants grown in ambient air were transferred to a small $^{13}CO_2$ labelling chamber to analyze ^{13}C incorporation in leaf pigments for up to seven days. The mass spectra of carotenoids obtained from the two experiments showed similar patterns of isotopologue peaks, indicating that the method is compatible with pulse-chase labelling experiments in both directions ($^{13}CO_2/^{12}CO_2$ and $^{12}CO_2/^{13}CO_2$). This approach opens up new possibilities to elucidate the regulation of carotenoid metabolism in plants.

OP-6

Whole plant $^{13}CO_2$-labelling for carotenoid turnover analysis in leaves

T.M.A. Banh, B. Thiele, A. Chlubek, T. Hombach, E. Kleist, S. Matsubara

IBG-2: Plant Sciences, Forschungszentrum Jülich, 52428 Jülich, Germany

Understanding the regulation of carotenoid metabolism (synthesis, conversion and degradation) in plants requires turnover measurement of individual carotenoids, apocarotenoids and precursors. In our previous study, we demonstrated continuous turnover of carotenes together with chlorophyll *a* in illuminated leaves of *Arabidopsis thaliana* by using $^{14}CO_2$ pulse-chase labeling. In contrast to carotenes and chlorophyll *a* that are bound in photosystem reaction center complexes, xanthophylls and chlorophyll *b*, which are bound in light-harvesting antenna complexes, were hardly labeled by ^{14}C within a day, even when the total amount of xanthophyll-cycle pigments (zeaxanthin, antheraxanthin, and violaxanthin) was increasing, presumably by *de novo* synthesis, under strong light. In order to obtain quantitative information of carotenoid turnover in leaves of intact plants, we constructed a labelling chamber in which 15 small plants, such as *Arabidopsis*, can be synchronously labelled by $^{13}CO_2$ over days. First we tested the chamber by operating with $^{12}CO_2$ while continuously monitoring the conditions inside the chamber (light intensity, air temperature and humidity, CO_2 concentration, pressure). Then a protocol was established to grow 15 *Arabidopsis* plants (wildtype Columbia-0) in the chamber under the light intensity of ~200 μmol photons m^{-2} s^{-1}. Switching to $^{13}CO_2$, plants were grown in the chamber under the same conditions for up to seven days. LC-MS analysis of pigments extracted from rosette leaves of the ^{13}C-labelled *Arabidopsis* plants showed a substantial incorporation of photosynthetically fixed ^{13}C into β-carotene and lutein along with chlorophyll *a* and chlorophyll *b*. The proposed system can be used for pulse-chase experiments (from $^{12}CO_2$ to $^{13}CO_2$ and vice versa) to estimate the turnover rate of carotenoids and chlorophylls as well as other plant metabolites. Results from such experiments could provide missing pieces of information in the current picture of metabolic pathway regulation in plants.

OP-7

Production of carotenoid pigments by oleaginous fungi and yeasts

M. Čertík, T. Klempová, J. Tkáčová, O. Slaný,

Institute of Biotechnology, Faculty of Chemical and Food Technology, Slovak, University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

One of the main targets of pharmaceutical, medical, cosmetic, veterinary and food/feed industries are carotenoids, a group of structurally diverse and biologically active metabolites. Because of their scarcity from conventional agricultural and animal sources, commercial demand has led for searching of alternative natural sources of these compounds. Microbial biotechnology represents another option and promising method for the production of natural pigments. Oleaginous microorganisms including some fungi (Zygomycetes) and yeasts (Ascomycetes and Basidiomycetes) that accumulate substantial amounts of intracellular carotenoids (β-carotene, γ-carotene, torulene, torularhodin, astaxanthin) are suitable candidates to fulfill this task. Pigment production varies among species and it is affected by different fermentation conditions and physiological factors (e.g. various C/N sources and their ratio, environmental stress, light source and its wavelength, etc.). In our laboratory, two basic biotechnological processes have been developed for microbial carotenoid production: submerged and solid-state fermentations. Both fermentation processes provide specific advantages in each industrial application that in turn depends on the product form, metabolite yield and cost of production process. Submerged cultivation of red yeasts maximally yielded up to 56 mg β-carotene/L (*Rhodotorula glutins*) or 110 mg astaxanthin/L (*Phaffia rhodozyma*). On the other hand, *Umbelopsis isabellina* was considered as the most valuable fungus for production of beta-carotene (40.7 mg/L). Zygomycetous fungi also effectively utilized cereals during solid-state fermentation and enriched them with 260 mg beta-carotene/kg fermented cereals.

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OP-8

Influence of thermal processing on carotenoid content and antioxidant activity in berry's pulp

A. Ghendov-Moșanu[1], C. Socaciu[2], A. Pintea[2], C. Bele[2], C. Tudor[2], R. Sturza[1]

[1]Technical University of Moldova, 168, Ștefan cel Mare Blv., MD-2004, Chisinau, Republic of Moldova
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Mănăștur 3-5, 400372, Cluj-Napoca, Romania

The capacity to improve the health of the population depends greatly on the quality of the consumed food, which is in turn influenced by their thermal processing. Berries can be eaten fresh, frozen or dried. For research, 3 types of berries from the Republic of Moldova were selected: sea buckthorn (*Hippophae rhamnoides*), rosehip (*Rosa canina*) and mountain ash (*Sorbus aucuparia*), which can be recommended for producing functional food as a source of biologically active compounds as natural dyes and antioxidants. The aim of the study was to investigate the influence of thermal processing on carotenoid content and antioxidant activity in berry's pulp. Berries of native indigenous population were selected for the needs of the current study. Harvest took place from September to November 2018. Fruit was frozen at temperature of -18±1°C and dried at room temperature and at temperature of 65±1°C. The carotenoid content and antioxidant activity in berry's pulp were comparatively determined by spectrophotometric and high performance liquid chromatography (HPLC) methods. Antioxidant activity was determined using the method based on stabilized silver nanoparticles. In the case of frozen berries, the highest carotenoid content was recorded in rosehip, followed by sea buckthorn and mountain ash. Thus, the carotenoid content varied, for frozen berries, between 37.16 ± 1.2 mg/100g dry weight and 68.73 ± 5.02 mg/100g dry weight. The carotenoid content decreased by approximately 2-fold in the dried sea buckthorn, of 1.3 times in the dried rosehip and of 1.6 times in the dried mountain ash. The temperature of the drying agent did not essentially influence the carotenoid content of the dried berries. The antioxidant activity of the thermal processing berries was also investigated. In the case of frozen samples, the highest antioxidant activity was observed in mountain ash berry (with an average of 440.66 ± 3.15 mg GAE/100 g dry weight) followed by rose hip berry (430.47 ± 6.13 mg GAE/100 g dry weight) and the lowest in case of sea buckthorn (148.82 ± 0.17 mg GAE/100 g dry weight). Antioxidant activity of dried berries at 65°C was found to be higher than that of frozen samples. Thus, thermal processing has been found to have a different influence on the carotenoid content and antioxidant activity in the investigated berries.

OP-9

Carotenoid content in tomato products as affected by different processing methods

A. Mendelová[1], M. Fikselová[2], L. Mendel[3]

[1]Slovak University of Agriculture, Department of Technology and Quality of Plant Products, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, Nitra, 949 76 Slovakia
[2]Slovak University of Agriculture, Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, Nitra, 949 76 Slovakia
[3]The National Agricultural and Food Centre, Research Institute of Plant Production, Bratislavská cesta 122, 921 68 Piešťany, Slovakia

The aim of this work was to monitor how different processing affects the total carotenoid content of the final product made of tomatoes. Five tomato cultivars (*Lycopersicon esculentum* Mill.) such as Uno Rosso F1, Pavlína, Mobil, Zőmők, Denár were used. Dry tomato slices were prepared (3 mm) and dried under three temperature regimes: 40 °C, 55 °C and 70 °C. Drying process was performed until the water content of the final product was lower than 18 %.The average length of drying at 40 °C was 24 h., at 55 °C for 20 h., at 70 °C for 17 h. Tomato juice using thermal treatment (93 °C), then tomato puree by classic way at 95 °C with oxygen access and tomato puree by vacuum technology, without oxygen access were prepared as well. The content of carotenoids was determined spectrophotometrically and expressed in % of dry matter. The results were processed statistically by ANOVA test and Tukey HSD test. Compared to different temperatures used, the highest content of carotenoids was found to be in 70 °C slices (127.8 mg.100g^{-1} DM compared to 40 °C, 111.3 mg.100g^{-1} DM), more than 75 % of carotenoids retained. It was found that cultivar and temperature have statistically significant effect. Zőmők cultivar can be recommended for processing with the highest retained content of carotenoids. By decreasing the temperature and increasing the time of drying the content of carotenoids decreased. Treatment of tomato into juice showed the decrease in carotenoid content by 8.3 %. Production of the puree by classic way with oxygen showed another decrease in carotenoid content by 28.9 %. Vacuum technology without oxygen access showed the highest mean content of total carotenoids comparable to carotenoid content in fruits, so we can recommend it for processing of these kind of products.

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OP-10

The carotenoids as plant defense tools and bioactive compounds in food systems: the impact of postharvest treatments and storage conditions

A. Ferrante
Department of Agricultural and Environmental Sciences, University of Milano, via Celoria 2, 20133, Milano, Italy

Carotenoids are important leaf molecules that are involved in the photo-protection of chlorophylls. They act as shields for avoiding the photo-oxidation of leaf pigments with loss of leaf functionality. There is a tight correlation between carotenoids concentration and chlorophylls content. The increase of carotenoids is associated to the increase of chlorophylls. The carotenoids through their catabolism provide the substrate for the indirect biosynthesis of abscisic acid (ABA) that is an important plant hormone involved in several plant abiotic stress responses. ABA is mainly involved in the stomata closure and its concentration increase especially under drought stress. The carotenoids are also antioxidant compounds with a long carbon chain and have scavenger activities for the double bonds in their molecules. Leafy vegetables and vegetable fruits can have high carotenoids concentration with positive effect on the quality retain during storage and shelf life. The carotenoids have also important role in the human diet for their beneficial effect on the health. The aim of the study was to evaluate how light conditions and antioxidant postharvest treatments can be used for the preserving the carotenoids in leafy vegetables during storage. On tomato as fruit vegetables, instead, the study was performed on transgenic tomato over-expressing the 9-cis-epoxycarotenoid dioxygenase (NCED) gene aiming to evaluate how the plant hormones can enhance ripening and the role of carotenoids on plant hormones biosynthesis especially on the abscisic acid (ABA). The effect of NCED over-expression on fruit quality and carotenoids during storage was evaluated. Postharvest treatments such as ascorbic acid can preserve the carotenoids more than 10% of carotenoids in leafy vegetables. Storage under light conditions can preserve the carotenoids with values about 10-20% higher than dark storage. The effect of lighting on the carotenoid concentrations was tightly correlated with light intensity and duration. Tomato fruits, harvested from plants over-expressing the NCED gene which encodes for the key enzyme of the ABA biosynthesis, showed during storage a reduction of β-carotene, lutein, and phytoene compared the control. The lycopene, instead, did not significantly change, while the ABA increased. There is a clear connection between ABA and carotenoids content. This relationship can be exploited for selection tomatoes that have lower ABA concentration and higher carotenoids which may show longer shelf life. Recent studies demonstrated the positive effect of carotenoids in the removing the reactive oxygen species. Carotenoids should be preserved during postharvest for providing to the consumers the highest content of these functional compounds. Postharvest treatments and storage conditions are studied for preserving the concentration of carotenoids through the food chain. The scavenger ability of carotenoids has a primary importance in the human diet and health.

OP-11

Preparation and characterization of novel β-carotene loaded nanoparticles

A. Bockuviene[1,2], E. Rodríquez[3], J. Sereikaite[1], B. Olmedilla-Alonso[4]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
[2]Department of Polymer Chemistry, Institute of Chemistry, Vilnius University, Vilnius, Lithuania
[3]Facultad de Farmacia, Universidad Complutense de Madrid, Spain
[4]Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid, Spain

Carotenoids (CAR) are a class of natural pigments found in fruits and vegetables. CAR are widely used as food colorants and are very important in human diet. However, CAR are very sensitive to light, temperature, acids and are poorly water soluble. In this study, we report a successful synthesis of two component β-carotene- chitooligosaccharides (IIC-CHIOS-βCAR) nanoparticles by mechanochemical complexation method and three-component β-carotene-chitooligosaccharides-poly-γ-glutamic acid (IIIC-βCAR-CHIOS-γPGA) nanoparticles by encapsulation method. The interaction of components and physicochemical characteristics, i.e. morphology, structure, size, polydispersity index (PDI) and zeta potential, of both nanoparticles were investigated by ^{1}H-NMR, FTIR, DSC, SEM and DLS methods. Stability against light (UVC and day light), temperature (25°C, 40, °C 60°C, 80°C, 100°C) and pH (3-8) of β-carotene encapsulated in novel nanoparticles were evaluated by UV and HPLC methods. Nanoparticles were investigated by an *in vitro* digestion method. The encapsulation efficiency and the hydrodynamic size in the aqueous solution of both nanoparticles was between 60-80 % and 50-200 nm, respectively. Analysis of ^{1}H-NMR, FTIR, RAMAN, XRD and DSC provided the evidence of nanoparticle formation and βCAR interaction with CHIOS and γPGA. The SEM experiments revealed visual changes in surface morphology during mechanochemical and encapsulation method. The relative stability of β-carotene under various conditions in IIC-CHIOS-βCAR and IIIC-CHIOS-βCAR-γPGA complexes were in the range from 30 % to 60 % and from 60 % to 80 %, respectively. Therefore, the coating of IIC-CHIOS-βCAR nanoparticles with γPGA strongly increases β-carotene stability towards light irradiation, temperature and pH conditions. *In vitro* digestion test revealed that recovered (bioaccessible) βCAR content (μg/100 mg) in IIIC-CHIOS-βCAR-γPGA nanoparticles after *in vitro* digestion were 25.76 μg/100 mg. Novel three component IIIC-CHIOS-βCAR-γPGA nanoparticles are better than IIC-CHIOS-βCAR due to higher stability. Novel nanoparticles could serve for β-carotene delivery and food fortification.

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OP-12

The uncommon hydrophilic apocarotenoid picrocrocin and its importance in the quality control and applications of saffron

A. Kyriakoudi and M.Z. Tsimidou

Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Food Chemistry and Technology, 54124 Thessaloniki, Greece

The unique sensorial properties of saffron, the dried red stigmas of the plant *Crocus sativus* L., which is the most expensive spice worldwide, are closely related to the presence of various apocarotenoids [1]. Among them, picrocrocin, (4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde), is the colorless, hydrophilic compound that co-exists with crocins, the most well-known apocarotenoids of saffron, in the vacuoles. Picrocrocin is transferred to saffron aqueous extracts, which are characterized by a unique bitter taste [2,3]. Lack of a suitable analytical approach delayed the investigation on the actual amounts of this apocarotenoid in saffron, as well as on its sensorial attributes, stability and bioavailability. In the present work, evidence is presented on its occurrence and levels (~ 12% of dry stigmas weight) in a great number (*ca.* 80) of saffron samples from different geographical origin (e.g. Greece, Spain, Italy, Iran) of various years of production, stability upon storage [4] or *in vitro* digestion [5], bioavailability [6] and sensory properties [2]. The importance of this evidence for saffron quality control and future food and pharmaceutical applications is discussed.

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OP-13

Effects of root cooling on fruit quality of cocktail tomato

F. He[1], B. Thiele[1], M. Watt[1,2], T. Kraska[2], A. Ulbrich[3], A. Kuhn[1]

[1] Forschungszentrum Jülich, IBG-2: Plant Science, Wilhelm-Johnen-Straße, 52428 Jülich, Germany
[2] Bonn University, Faculty for Agriculture, Nußallee 21, 53115 Bonn, Germany
[3] Osnabrück University of Applied Sciences, Faculty of Agricultural Sciences and Landscape Architecture, Albrechtstr. 30, 49076 Osnabrück, Germany

Modern horticulture systems rely on the ability to alter microclimate components surrounding crops, such as temperature and light, to improve yield and quality of products. Root temperature has also long been considered as an important ecological factor that determines a variety of structural and functional characteristics. However, little is known about the effect of root temperature on food quality of horticultural crops. Cocktail tomato, as the hybrid of cherry tomato with normal sized tomato, is getting increasingly popular among the consumers, due to its suitable size. Additionally, cocktail tomatoes contain higher level of carotenoids and other antioxidants than normal sized ones. The effects of root temperature on fruit quality (sensorial attributes and nutritional components) were examined in two cocktail tomato varieties "Amoroso" and "Delioso" during the winter of 2017-2018. Plants were grown hydroponically on rockwool with varied root temperature (16-24°C and 10°C) from inflorescence to harvest inside the greenhouse. Root temperature 10°C was manipulated independently from air temperature (16-29°C) by circulating cooling water. Reductions of total and marketable yield per plant in both varieties were observed in response to root cooling by 7.7-17.9% and 7.9-20.9%, respectively. Glucose and fructose concentration increased by 7.8-16.1% and 7.4-14.0% with the application of root cooling. Lycopene concentration increased 15.9% in "Delioso", β-Carotene increased 10% in "Amoroso" and ascorbic acid concentration increased 21 % in "Delioso". Besides, N, K, S, Fe concentration reduced by 6.8-18.1% in "Delioso", whereas no statistical changes in elements concentration were observed in "Amoroso". Thus, manipulation of root temperature could be a feasible method to improve overall fruit quality of cocktail tomato.

OP-14

Are dietary carotenoids beneficial to health? The switch from anti- to pro-oxidant behaviour - the effect of oxygen

F. Boehm[1], R. Edge[2], T.G. Truscott[3]

[1]*Photobiology Research, IHZ, Berlin, Germany*
[2]*Dalton Cumbrian Facility, The University of Manchester, Westlakes Science Park, Moor Row, Cumbria, UK*
[3]*School of Physical Science (Chemistry), Keele University, Staffordshire, UK*

Carotenoids are known to act as antioxidants, quenching both singlet oxygen and a range of free radicals, meaning they are of wide interest due to their potential health benefits. This study shows that a range of dietary carotenoids can protect human lymphoid cells from membrane damage caused by free radicals produced by ionising γ-radiation, and also by nitrogen dioxide radical, generated photolytically. Blood was taken from volunteers (n=3) who had supplemented their diet with large doses [typically, 70-90 mg/day] of a specific carotenoid for 2 weeks or had minimized carotenoid-rich fruit and vegetables in their diet. Radical-induced cell membrane destruction was shown by cell staining with eosin. All carotenoids studied imparted protective effects, however, protection was reduced as the oxygen concentration increased, particularly for damage due to γ-radiolysis ($p<0.01$, Mann–Whitney U-test). The oxygen effect was observed to be most pronounced for lycopene, where there was almost no protection under 100% oxygen, down from 5-fold protection at 21% oxygen and, an extremely high, 50-fold, protection in the absence of oxygen. Studies using β-carotene and the xanthophylls, astaxanthin, zeaxanthin and lutein, have shown a reduced, but still significant, oxygen effect. A range of non-cellular gamma radiation studies of carotenoids in simple solutions have also been undertaken to help understand the molecular mechanisms for the observed oxygen effect. The remarkable reduction in the protection by carotenoids at high oxygen concentrations may, perhaps, be one reason why in lung cancer epidemiological trials, β-carotene supplementation was shown to increase the number of tumours with statistical significance. However, the effect could possibly be exploited to enhance radiation procedures for therapy and the variation observed between carotenoids indicates that supplementation with certain carotenoids may be more suitable than others, for protection in environments with different oxygen partial pressures.

OP-15

Impact of high hydrostatic pressure and thermal treatment on the stability and bioaccessibility of carotenoid and carotenoid esters in astringent 'Rojo Brillante' persimmon during *in vitro* digestion

M.P. Cano[1,2], A. Gómez-Maqueo[1,2], J. Welti-Chanes[2], T. García-Cayuela[3]

[1] *Institute of Food Science Research (CIAL; CSIC-UAM), Nicolás Cabrera 9, 28049 Madrid, Spain*
[2] *FEMSA Biotechnology Center, School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Mexico*
[3] *Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Zapopan, Jalisco, Mexico*

Persimmon is a rich source of carotenoids, carotenoid esters, ascorbic acid, fiber and condensed tannins with potential health benefits. To determine the potential health properties of this widely consumed fruit, the stability and bioaccessibility of its carotenoids and carotenoid esters should be studied. The aim of this study was the characterization of their profile in astringent persimmon fruits, cv. 'Rojo Brillante', and study of their stability and bioaccessibility in an *in vitro* digestion assay following the INFOGEST recommendations and an adapted methodology to efficiently extract and analyze the carotenoid compounds from each phase of the in vitro digestion. Carotenoid and carotenoid ester profile showed a total of 38 carotenoids, corresponding to 21 free carotenoids (13 xanthophylls and 8 hydrocarbon carotenes) and 17 carotenoid esters . The stability of persimmon carotenoids ranged 61-74%, depending on the digestion phase, being (all-*E*)-β-cryptoxanthin and (all-*E*)-antheraxanthin 3-O-palmitate the most stable carotenoids. At the final step of the digestion (oral+gastric+duodenal phase), only traces of (all-*E*)-antheraxanthin, (all-*E*)-lutein and (all-*E*)-β-cryptoxanthin were found in control samples (no processed ones) due to the low efficiency of carotenoid micellarization, which was affected by the high pectin content present in persimmon tissues. Processing affects positively the carotenoid bioaccessibility, showing and increase up to 54% for pressurized samples or up to 25% for pasteurized ones. This effect depends on the chemical structure of the carotenoids, being (all-*E*)-β-cryptoxanthin and (all-*E*)-β-cryptoxanthin laurate the most bioaccessible carotenoids in pressurized samples and (all-*E*)-β-cryptoxanthin laurate and (all-*E*)-antheraxanthin in pasteurized ones. Collectively, high hydrostatic pressure and pasteurization are promising technologies for obtaining most bioaccessible carotenoid persimmon products without the inclusion of other dietary fats.

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OP-16

Characterization of carotenoids, carotenoid esters and fatty acids in two lucuma (*Pouteria lucuma*) varieties experimentally grown in Spain

M.P. Cano[1], A. Gómez-Maqueo[1,2], E. Bandino[1]

[1]Institute of Food Science Research (CIAL; CSIC-UAM), Nicolás Cabrera 9, 28049 Madrid, Spain
[2]FEMSA Biotechnology Center, School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Mexico

Lucuma is a tropical fruit native to the Andean valleys found in Peru, Ecuador and Chile. It has been successfully grown in experimental parcels belonging to the Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM-UMA-CSIC) in Malaga (Spain). This is the furthest zone from the Equator capable of its production and the only zone of commercial lucuma production in continental Europe. Although it is widely consumed in South America, it has not been studied regarding its carotenoid, carotenoid ester and fatty acid composition. The aim of this study was the characterization of the profile of each chemical family of these constituents in three ripening stages of two lucuma cultivars ('Molina' and 'Beltran') grown in Málaga, Spain. Carotenoids were analyzed in saponified and direct extracts by liquid chromatography (HPLC-DAD-MS) with a reversed-phase C_{30} column. Triglycerides were derivatized into fatty acid methyl esters (FAMEs) and analyzed by HPLC-FID. A total of 32 carotenoids were identified composed of 11 xanthophylls, 4 carotenes and 17 xanthophyll esters. The most abundant carotenoids in both varieties were (all-*E*)-lutein, (all-*E*)-cryptoxanthin and violaxanthin esters as well as (all-*E*)-ß-carotene and (all-*E*)-α-carotene. Molina presented a higher overall carotenoid content, however Beltran was the only variety to contain lycopene. 'Beltran' and 'Molina' lucuma contained 4,6 and 4,1 mg of fatty acid methyl esters/ g dry weight, respectively. Fully ripe, unripe and unripe cool room-stored lucuma did not present significant differences in triglyceride content. Fatty acid profile was 34% α-linoleic, 25% palmitic, 20% oleic, 6% linoleic, 5% stearic, 3% myristic and other minor fatty acids. Collectively, lucuma fruits cultivated in Spain are rich sources of carotenoids with interesting fatty acid composition which might benefit their bioaccessibility. To our knowledge, this is the first time the carotenoid profile and lipid composition in lucuma fruits has been reported.

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OP-17

Fucoxanthin-rich ethanolic *Phaeodactylum tricornutum* extract ameliorates effects of diet-induced obesity in C57BL/6J mice

J. Ribot[1], A. Gille[2], B. Stojnic[1], K. Briviba[2], A. Palou[1], M.L. Bonet[1]

[1]Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears-CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN)-Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma de Mallorca, Spain
[2]Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Physiology and Biochemistry of Nutrition, Karlsruhe, Germany

The marine diatom microalga *Phaeodactylum tricornutum* is enriched in the ω-3 PUFA eicosapentanoic acid and the carotenoid fucoxanthin, two lipophilic constituents with proposed anti-obesity and anti-diabetic properties. We investigated the effect of an ethanolic *P. tricornutum* extract (PTE) on energy metabolism in obesity-prone mice fed a high fat diet (HFD). 6-8 week-old male C57BL/6J mice were switched to an HFD and, at the same time, received orally the vehicle or PTE (100 mg or 300 mg/kg body weight/day, providing ~2.4 mg fucoxanthin or ~7.1 mg fucoxanthin/kg body weight/day respectively). Body weight, body composition and food intake were monitored. After 26 days, blood and tissue samples were collected for biochemical, morphological and gene expression analyses. PTE-supplemented mice with the higher dose accumulated fucoxanthin metabolites in white adipose tissue (WAT) depots (1.48±0.76 μg fucoxanthinol/g *vs.* below detection in inguinal depot) and attained a significant 35% lower body weight gain, independent of decreased food intake. At the end of treatment they showed a significant 25% lower body fat content and reduced inguinal WAT adipocyte size compared to controls (adipocyte diameter 48.4±0.4 *vs.* 54.5±0.6 μm, $p<0.05$). PTE supplementation was associated with: lower expression of *Mest* (a marker of fat tissue expandability) in WAT depots; decreased expression of genes related to lipid uptake and turnover in visceral WAT; increased expression of genes key to fatty acid oxidation and thermogenesis (*Cpt1*, *Ucp1*) in subcutaneous WAT; and signs of thermogenic activation including higher UCP1 protein in interscapular brown adipose tissue. In conclusion, these data show the potential of PTE to ameliorate HFD-induced obesity *in vivo* through mechanisms that include changes in lipid and energy metabolism in adipose tissues. Knowledge on the anti-obesity action of *P. tricornutum* and its mechanisms may pave the way for novel uses of this microalga in the functional food and nutraceutical arena.

OP-18

Post-harvest use of Light Emitting Diode (LED) to enhance carotenoid content in tomatoes

J. García-Alonso, C. Iniesta, R. González-Barrio, N. Baena, M.J. Periago

Department of Food Science and Nutrition, Veterinary Faculty, n° 17. Campus de Espinardo 30100, University of Murcia, Spain

Tomato is rich in different bioactive compounds, especially carotenoids (lycopene and β-carotene) and phenolic compounds, which are associated with various health benefits. Therefore, different strategies have been developed to increase the concentration of bioactive compounds during growing and post-harvest. Among them, treatments with ultraviolet light (UV) and light emitting diode (LED) –alone or in combination– have been shown to be of interest to enhance bioactivity. The aim of this study was to evaluate the effect of ultraviolet (A and C) and LED light on the concentration of carotenoids, especially lycopene and antioxidant capacity, during the refrigeration storage in green tomatoes (*Lycopersicon esculentum*) variety "asurcado-Raff type". In addition, special attention has been paid to other parameters such as weight loss, colour, acids, soluble solids, flavour index, antioxidant capacity (FRAP) and total phenolic compounds. The tomatoes were pre-treated with ultraviolet light A or C and stored for 7 days in a cooling chamber at 6°C, under different lighting conditions (dark or LED). Exposure to post-harvest LED light in refrigerated conditions increased up to 3 times the total carotenoid content compared to tomatoes refrigerated in the dark, while UV treatments alone did not affect the carotenoid content. Also, exposure to LED increased the total antioxidant capacity of tomatoes by 60%, which, together with the increase in carotenoids, can be considered an improvement in the nutritional value of tomatoes. LED treatments fostered ripening, without causing significant weight losses, and slightly increased of the flavour index associated with an increase of the soluble solids. Further studies must be carried out to evaluate the impact on sensory attributes and consumer acceptance.

OP-19

Carotenoid degradation during lactic fermentation of rehydrated maize grain from various hybrids

K. Kljak[1], M. Duvnjak[1], J. Šic Žlabur[2], D. Grbeša[1]

[1]University of Zagreb Faculty of Agriculture, Department of Animal Nutrition, 10000 Zagreb, Croatia
[2]University of Zagreb Faculty of Agriculture, Department of Agricultural Technology, Storage and Transport, 10000 Zagreb, Croatia

Lactic fermentation is commonly used in preservation of high-moisture feeds making them available for animal feeding throughout a year. Maize is often ensiled and even dry grain could be ensiled after rehydration. Maize grain is the only cereal with significant carotenoid content and this study aimed to examine the effect of ensiling on changes in carotenoid content in rehydrated grain compared to dry grain during prolonged storage. Grain of seven maize hybrids (Bc 344, Bc 418b, Bc 424, Bc 525, Bc 572, Kekec and Pajdaš) was harvested after physiological maturity. One part of grain was rehydrated to 32% of moisture and ensiled with the addition of commercial inoculant containing lactic acid bacteria in vacuum bags at room temperature (22-25 °C). The other part was stored in conventional silo where temperature was monitored daily. The ensiled rehydrated and dry grain were sampled on days 0, 21, 63, 100, 185, 310 and 420 of the experiment. Lutein, zeaxanthin, β-cryptoxanthin and β-carotene in all samples were quantified using reverse-phase HPLC method. Contents of determined carotenoids varied across tested hybrids ($P<0.001$), and their ranges (μg/g DM) at the beginning were 25.83-9.47 for lutein, 19.35-11.18 for zeaxanthin, 4.71-1.70 for β-cryptoxanthin and 2.06-0.56 for β-carotene. Carotenoid contents in all hybrids decreased both in dry and ensiled rehydrated grain ($P<0.001$); in comparison to the beginning, total carotenoid content was lower 30.10-43.61% in dry and 16.37-47.34% in ensiled rehydrated grain on 420th day of the experiment. However, the changes in dry and ensiled rehydrated grain throughout the experimental period differed. While carotenoid contents decreased linearly in dry grain (R^2=0.98 for total carotenoids), in ensiled rehydrated grain, polynomial dependence was observed. Contents of all determined carotenoids in ensiled rehydrated grain of tested hybrids sharply decreased to 63rd day (up to 50%), then increased to 185th day (up to 39% compared to 63rd day) and then linearly decreased to the end of the experimental period. Although carotenoids degrade in acidic conditions during ensiling, results suggest that some carotenoids were captured in maize microstructure and released upon changes in microstructure during prolonged ensiling.

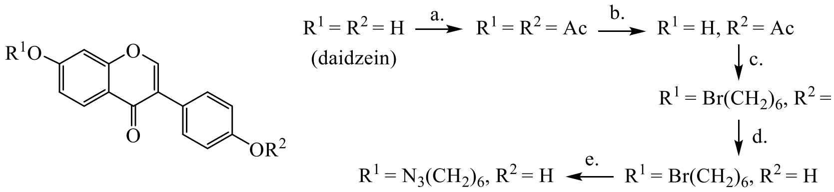
OP-20

Covalent conjugation of carotenoids with flavonoids: efficient antioxidants showing supramolecular organization

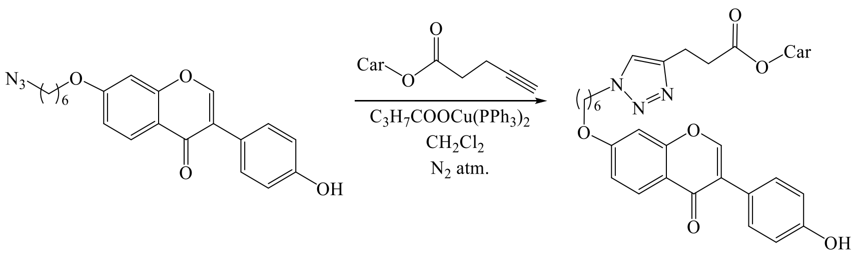
I. Línzembold[1], D. Czett[1], A. Agócs[1], G. Gulyás-Fekete[1], A. Takátsy[1], J. Deli[1,2], T. Kurtán[3], V. Nagy[1]

[1]University of Pécs, Department of Biochemistry and Medical Chemistry, Medical School, 7624 Pécs, Hungary
[2]University of Pécs, Department of Pharmacognosy, Faculty of Pharmacy, 7624 Pécs, Hungary
[3]University of Debrecen, Department of Organic Chemistry, Faculty of Sciences, 4032 Debrecen, Hungary

Flavonoids and carotenoids are crucial in preventing diseases and staying healthy. Due to their structure, these natural bioactive molecules possess high antioxidant capacity as well as a wide range of other advantegeous pharmacological properties. In the present research, different hydrophobic carotenoids were covalently coupled with hydrophilic flavonoids in order to increase their stability, bioavailability and antioxidant effect, even synergisticly. Azide-alkyne [4+2] cycloaddition reaction was proven to be a mild and appropriate method for the synthesis of carotenoid-flavonoid conjugates. The natural flavonoids (daidzein, chrysin, quercetin) were transformed to their 7-azidohexyl ether derivatives and coupled with carotenoid pentynoates giving the corresponding triazols, in the presence of bis-triphenylphosphano-copper(I)-butyrate complex as a catalyst.



a) Ac_2O, Py 60 °C; b) imidazole, -15 °C; c) 6-bromohexanol, DIAD, PPh_3, THF; d) NaOMe/MeOH; e) NaN_3, 60 °C



Car: 8'-apo-β-carotenol, zeaxanthin, capsanthin

The synthetized triazols may have increased antioxidant effect, as well as, having amphipathic structure, they showed self-assembly in aqueous medium.

OP-21

Utilization of vacuum drying in retention of β-carotene content in winter squash (*Cucurbita moschata*)

M. Marelja[1], K. Kljak[2], M. Brnčić[1], F. Dujmić[1], S. Rimac Brnčić[1], J. Šic Žlabur[2*]

[1]*University of Zagreb Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia*
[2]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

Among unit operations, conventional convection drying process is one of the most energy-intensive, stimulating degradation of heat-sensitive food nutrients. To overcome such constraints, vacuum drying can be a potential alternative. The low pressure applied in vacuum drying allows water evaporation at lower temperatures allowing preservation of heat-sensitive bioactive compounds. The aim of this study was to determine the effects of vacuum drying on β-carotene content in winter squash. Fresh winter squash fruits (cv. 'Piena di Napoli') were prepared for drying, peel and seeds were removed while pulp was cut into thick slices (3 mm). The conduction drying was performed at atmospheric pressure (1013.25 mbar) at 70 °C, while for vacuum drying, pressures (50 and 150 mbar) and temperatures (60 and 70 °C) were varied. Winter squash slices were dried to a final water content of 14%. The β-carotene content in all samples was determined spectrophotometrically at 450 nm. Drying by conduction had the most adverse effect on β-carotene content (70.53 mg/100 g DW). The highest β-carotene content (153.69 mg/100 g DW) was determined in sample dried by vacuum at 50 mbar and 60 °C. Regardless of temperature, β-carotene content was lower in samples dried at 150 mbar compared to 50 mbar. Additionally, regardless of applied pressure, lower β-carotene content was determined at higher temperatures. Based on the obtained results, it can be concluded how proper combination of pressure and temperature provide an effective way for the retention of β-carotene content in plants processed by vacuum drying. Also, it should be emphasized that vacuum drying is an effective drying process in preservation of unstable food nutrients, such as carotenoids.

OP-22

Exploitation of Citrus juicing industrial by-products as a rich source of carotenoids

E.D. Myrtsi, E. Evergetis, S.D. Koulocheri and S.A. Haroutounian

Department of Nutritional Physiology and Feeding, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

Citrus juicing process generates considerably large amounts of by-products, since only the 50% of the fresh fruit's mass is transformed into juice [1]. The remaining residues, consisted of peel, pulp and seeds are of limited economic interest, although their contentin bioactive natural products is indicative of their potential for utilization as biorefinery raw materials. Among the numerous by-products obtained during the juice-making process,only the Cold Pressed Essential Oils (CPEOs) have currently found wide applications by food, beverage, cosmetic and pharmaceutical industries, mainly as flavouring and fragrance agentsdue to their characteristic aroma profile [2].The citrus juicing CPEOs consist of two fractions,containing a sum of approximately 200 compounds [3]: a thoroughly studied major (volatile) fraction, containing mainly D-limonene as prevailing component, and a minor, non-volatile residue, which is composed of hydrocarbons, sterols, fatty acids, waxes, carotenoids, coumarins, psoralens and flavonoids [4]. Endeavour herein refers to the exploitation of the less studied, non-volatile fraction as a rich source of carotenoids and the assessment of their applicability as pigments and/or bioactive extracts. In this context, the CPEOsoffour different Citrus species –namely orange (*C. sinensis*), lemon (*C. limon*), grapefruit (*C. paradisii*) and mandarin (*C. reticulate*)–were investigated in order to determine their efficacy to act as carotenoids source under various production conditions (technique, temperature, pressure). In particular, the most abundant carotenoids detected in CPEOs (using UPLC/MS-MS) were the molecules of β-cryptoxantin, β-carotene, zeaxathin and lutein. The detailed results of CPEOs yields, carotenoids quantitation and bioactivities evaluation will be presented.

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OP-23

In search of alternative plant-based systems for the production of astaxanthin and canthaxanthin

B. Rebelo, R. Abranches

Plant Cell Biology Laboratory, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

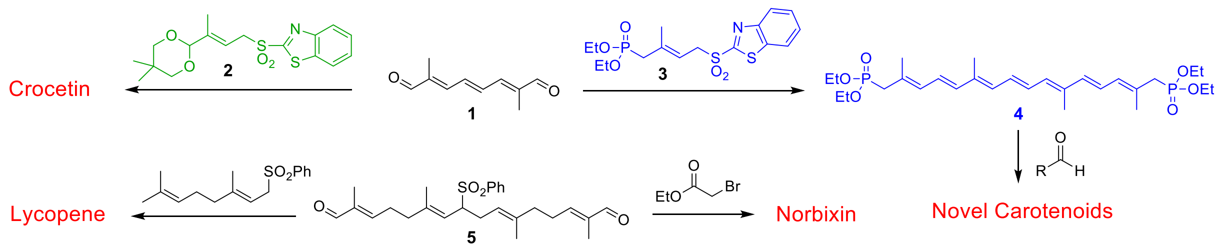
Molecular farming is a promising alternative technology for the production of high added value products in a cost-effective way. These plant-based systems are maintained in a controlled, contained and sustainable environment, and can be used to produce recombinant proteins or secondary metabolites with high yields. In this work, we are exploring an alternative system for the production of ketocarotenoids, namely astaxanthin and canthaxanthin, which are highly valuable for application in nutraceutical, cosmetic and feed industries. In nature, a ketolation reaction is necessary to obtain these ketocarotenoids, which is restricted to a few bacteria, fungi and unicellular green algae. Our goal is to produce these high value carotenoids in an alternative sustainable system, namely in plant cell suspension cultures such as tobacco BY-2 cells. Using genetic engineering tools we will manipulate the carotenoid pathway by introducing or removing enzymes responsible for ketolation or divergence in the carotenoid pathway. To this end, we are carrying out a systems biology approach to analyse the enzymes involved in carotenogenesis, in both tobacco and other ketocarotenoid producing organisms such as microalgae and fungi. Ketocarotenoids may have higher capacity of anti-oxidant and anti-inflammatory activity than other carotenoids and, for that, have a high market demand. With this project, we aim to establish a new production system that could became a commercially sustainable source for these high value ketocarotenoids.

OP-24
Efficient synthesis of carotenoids for improved antioxidant activity

D. Kim, G. Shi, MS. Alam, S. Koo

Department of Chemistry; Department of Energy Science and Technology Myongji University, Yongin, Korea

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 the use of organo-sulfur chemistry.[1] Various building blocks containing a sulfone group were devised for efficient construction of the polyene chains of carotenoids. Benzothiazolyl (BT) sulfone **2** containing an acetal moiety was devised for iterative chain extension of apocarotenoids from C_{10} dial **1**, and crocetin was efficiently prepared.[2] BT-sulfone **3** with a phosphonate moiety reacted with C_{10} dial **1** to produce C_{20} diphosphonate **4**, which provided novel carotenoids upon olefination with various aldehydes (R-CHO). 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] Lycopene synthesis had been demonstrated by the sulfone olefination protocol from geranyl sulfone and C_{20} dial **5**,[4] which was applied to norbixin synthesis by olefination with readily available ethyl bromoacetate.[5] Our recent syntheses of carotenoids for improved antioxidant activity will be presented.



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OP-25

UGT709G1: a novel uridine diphosphate glycosyltransferase involved in the biosynthesis of picrocrocin, the precursor of safranal

G. Diretto[1], O. Ahrazem[2], A. Rubio-Moraga[2], A. Fiore[1], F. Sevi[1], J. Argandoña[2], L. Gómez-Gómez[2]

[1]Italian National Agency for New Technologies, Energy, and Sustainable Development, Casaccia Research Centre, 00123, Rome, Italy
[2]Instituto Botánico, Departamento de Ciencia y Tecnología Agroforestal y Genética, Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071, Albacete, Spain

Saffron, constituted by the dried red stigmas of *C. sativus*, is one of the oldest natural food additives used as a colouring and flavouring agent. *C. sativus* is characterized by lilac flowers with long red stigmas, which produce and store significant quantities of the glucosylated apocarotenoids crocins and picrocrocin, greatly contributing to its colour and pungent taste, respectively. Contrary to crocins, which are ubiquitous in almost all Crocus spp., picrocrocin accumulation is exclusive of few members as *C. sativus* and *C. cartwrightianus*. The saffron apocarotenoid pathway starts with the oxidative cleavage of zeaxanthin in a reaction catalysed by CsCCD2L; crocins are subsequently produced by the sequential action of an aldehyde dehydrogenase and a glucosyltransferase (UGT74AD2), which is unable to synthesize picrocrocin. Finally, in the processed stigma, picrocrocin is converted to safranal, giving saffron its typical aroma, in a heat-induced spontaneous reaction. Here, we describe the molecular cloning and functional characterization of a novel apocarotenoid glucosyltransfease (UGT709G1) from saffron, involved in picrocrocin formation, by using a targeted search for differentially expressed UGTs in different *Crocus* transcriptomes. Biochemical analyses reveal that UGT709G1 possessed a high catalytic efficiency toward the apocarotenoid substrate, 2,6,6-trimethyl-4-hydroxy-1-carboxaldehyde-1-cyclohexene (HTCC), making it suited for the biosynthesis of picrocrocin, the safranal precursor, in saffron stigmas. The role of UGT709G1 in the second step in picrocrocin/safranal biosynthesis was confirmed by the absence or presence of gene expression in a screening for HTCC and picrocrocin production in different *Crocus* species, and by a combined transient expression assay with *CsCCD2L* in *N. benthamiana* leaves. Overall, our data allow identifying a novel UGT responsible for the saffron pungent taste and aroma, and shed light on its evolutionary appearance in the saffron germplasm.

OP-26

A single dose of marine *Chlorella vulgaris* increases plasma concentration of lutein, β-carotene and zeaxanthin in a 3 days intervention study with human healthy volunteers

A.T. Serra[1,2], S. Silva[1], L. Gouveia[3], N. Fernandez[1], A. A. Matias[1], C. Marques[3], N. Bandarra[4], J. Perdigão[5], A. Álvarez-Bautista, G. Real[5], P. Escudero, M. R. Bronze[1,2,3]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal, [2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), 2780-157 Oeiras, Portugal, [3] Faculdade de Farmácia da Universidade de Lisboa, Av das Forças Armadas, 1649-019 Lisboa, Portugal, [4] Portuguese Institute for the Sea and Atmosphere (IPMA), Department of Sea and Marine Resources, Lisbon, Portugal, [5] Buggypower (Portugal), Lda., Alameda dos Oceanos,Parque das Nações, 1990-203 Lisboa, Portugal

Chlorella species have been reported to potentially prevent lifestyle related diseases, including cardiovascular diseases and neurological disorders, and these effects are mainly attributed to their high content in vitamins (B12, B9), iron and carotenoids, namely lutein and β-carotene. Despite there are several published human intervention studies accessing the health promoting effect of *Chlorella* products, there are few information regarding the bioavailability of the main bioactive compounds, such as carotenoids. This study aims to evaluate the bioavailability of carotenoids during 3 days after intake of a single dose (6 g) of a dried marine microalgae, *Chlorella vulgaris*, providing about 7.08 mg of lutein, 1.88 mg of β-carotene and 1.47 of mg zeaxanthin. The bioavailabilty of other compounds namely PUFAs (α-linolenic acid, linoleic acid, EPA and DHA) and mineral salts (Se and I) was also evaluated. Eleven healthy males (age: 30.5± 4.2 years old; BMI: 24.6 ± 2.6 kg/m^2) were included in this study and were instructed to eat a low carotenoid diet one week before the study and during experimental phase. On the day of the experiment, volunteers ingested the microalgae encapsulated in gelatin capsules with water, bread (40 g) and red fruit jam (10 g). Blood samples were collected just before eating and at 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after ingestion. Plasma samples were separated and analyzed by HPLC UV-DAD for carotenoid concentration. Results showed that for all carotenoids the values of $AUC_{0h\text{-}72h}$ and C_{max} are significantly different from zero, indicating that a single dose of marine *Chlorella vulgaris* (6g) increased plasma concentrations of lutein, β-carotene and zeaxanthin in healthy men. The highest $AUC_{0h\text{-}72h}$ and Cmax values were obtained for β-carotene (1302.4 μg.h/L, 34.9 μg/L), followed by lutein (1001.6 μg.h/L, 20.4 μg/L) and zeaxanthin (122.2 μg.h/L, 3.4 μg/L). Notably, results obtained in this human intervention study are in accordance with the *in vitro* bioaccessibility assays performed previously, using the INFOGEST standardized *in vitro* gastrointestinal digestion protocol. This study suggests that the intake of a single dose of marine *Chlorella vulgaris* may lead to a significant increase in plasma concentration of carotenoids in the short term.

OP-27

Validation of food frequency questionnaire methods for assessing lycopene consumption among young men

A. Wawrzyniak[1], W. Pakosz[1], D. Głąbska[2]

[1]Department of Human Nutrition, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C, 02-787 Warsaw, Poland
[2]Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C, 02-787 Warsaw, Poland

Due to the proposed health-promoting effects of lycopene, it is important to assess its consumption in every day food rations, using validated methods. The group sensitive to the shortage of antioxidants, including lycopene, could constitute men who are more exposed to the development of cardiovascular diseases. The aim of the study was to validate the food frequency questionnaire method (FFQ) for the assessment of lycopene consumption in the diet of young men and compare to the results obtained by the alternative method of 3-day food records. Men, aged 20-30, after initial recruitment (n = 110), were asked to complete an original questionnaire on the frequency of consumption of selected lycopene products (FFQ) (16 items) and a 3-day dietary questionnaire on current intake. The food frequency questionnaire was filled twice, with an interval of 6 weeks denoted as FFQ1 and FFQ2, while the questionnaire of the 3-day food records was filled out once, together with FFQ1. Data for the assessment of lycopene consumption came from the USDA database (2017). Ultimately, 84 respondents completed the whole study. Reproducibility of results obtained by FFQ method with their validity in relation to the 3-day food records method were assessed. Lycopene sources in the diet were identified. Statistical analysis showed statistically significant correlations between the results of lycopene consumption obtained by the FFQ method repeated at FFQ1, FFQ2 ($r=0.637$; $p \le 0.05$) and with the 3-day food record method ($r=0.283$ for FFQ1; 0.269 for FFQ2; $p \le 0.05$). The mean intake of lycopene among the respondents was 6 mg/d in studies using FFQ and 12.4 mg using the 3-day food records method. The sources of lycopene in the respondents' diet were fresh tomatoes (20%) and processed tomato products such as ketchup (20%) and tomato paste (22%). Other products included in the FFQ containing lycopene were chosen less often by the respondents. The developed FFQ method for the assessment of lycopene consumption is characterized by acceptable reproducibility, which allows its use in studies conducted in the same group of respondents at various intervals.

OP-28

Fractionate analysis of lycopene metabolism gene expression levels and lycopene content in five tomato cultivars during ripening

E.C. Georgiadou, C. Antoniou, V. Goulas, P. Filippou, P. Challouma, M. Keveze, V. Fotopoulos

Cyprus University of Technology, Department of Agricultural Science, Biotechnology and Food Science, 3603 Limassol, Cyprus

Lycopene is a naturally occurring chemical compound that gives fruits and vegetables a red/orange color. As an intermediate metabolite of β-carotene biosynthesis, lycopene represents one of the major carotenoids. Interest in lycopene is growing rapidly following the implication of lycopene in the prevention of cancer and cardiovascular diseases. The present work studies the gene expression pattern of lycopene biosynthesis/catabolism genes and the accumulation pattern of lycopene at three different developmental stages and tissues in five tomato cultivars commonly consumed in Cyprus. Four of the cultivars (cvs. 'Cherry Ninolino F1', 'Elpida F1', 'Daphne F1' and 'Eliseo Plum F1') studied are red in colour, while cv. 'Oxheart' is pink. Furthermore, lycopene content was correlated with *in vitro* antioxidant activity. Higher levels of lycopene were observed in the skin than in the other tissues, with 'Oxheart' displaying the highest content. Lycopene showed maximal content at the fully ripe stage, thus choosing this stage for all subsequent analyses. Differential regulation of lycopene biosynthetic genes (*SlZDS* and *SlCRTISO*) was demonstrated in skin and flesh of all tomato cultivars compared with the seeds of each corresponding cultivar. Interestingly, a gene implicated in lycopene catabolism (*SlbLCY*) demonstrated a general induction in the tested tissues of the different cultivars. Lycopene content correlates with significant induction of both *SlZDS* and *SlCRTISO* in 'Oxheart'; contrarily, *SlbLCY* was significantly induced in 'Eliseo Plum'. Significantly increased antioxidant activity (FRAP and TEAC) was observed in the skin of 'Cherry Ninolino' and 'Elpida' cultivars compared with other tested tissues (flesh, seeds) across all cultivars, whereas significantly lower antioxidant activity was observed in the tested tissues of 'Daphne', 'Eliseo Plum' and 'Oxheart' cultivars compared with the other cultivars. The exploration of lycopene activity in different cultivars and the consumption of ripe tomatoes including the skin could be useful for the protection of human health.

OP-29

Carotenoids and phenolic acids during ripening, harvest and storage in selected scab-resistant and mildew-tolerant apple cultivars

Z. Vondráková, A. Trávníčková, J. Malbeck, D. Haisel, R. Černý, M. Cvikrová

Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Lysolaje, Czech Republic

The aim of this study was to characterise the changes in concentration and composition of antioxidants during ripening, harvest and after 5 months of storage in three commercially successful scab-resistant and powdery mildew-tolerant apple cultivars originating from the Institute of Experimental Botany. The detailed description of free and glycosylated phenolic acid profiles and content of 6 selected carotenoids - neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and ß-carotene will serve for outcomes of major characteristics of these apple cultivars. The standard spectrometric methods (incl. LC-MS) were optimized for determinations of carotenoids and phenolic compounds in apples. The relative proportions of measured carotenoids were similar in peels and fleshes; however higher levels of them were detected in peels. Extremely low content of carotenoids was found in all apples at the beginning of growth (in June) and during maturation and ripening (July, August and September) - less than 5 µg/g DW, followed by an increase (50-80 µg/g DW) toward harvest (October). The total content of carotenoids was reduced during storage (to 30–50 µg/g DW). Broad spectra of free and glycosylated phenolic acids were found in peel and flesh of all apple cultivars. Chlorogenic acid was the major free phenolic acid in all of them, and protocatechuic acid the most abundant glycosylated form. Levels of phenolic acids were highest at the beginning of apple fruit growth (c. 2000 µg/g DW) and markedly decreased during ripening and then increased towards harvest (800–1000 µg/g DW) and during the storage (1200-1500 µg/g DW). The information dealt with the concentration and composition of antioxidants in selected apple cultivars and is aimed for consumers in view of health benefits of phenolic compounds and carotenoids.

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OP-30

Altered homeostasis as a potential explanation for the adverse effects resulting from continuous high dose beta-carotene supplementation

J.Keijer and Y.G.J. van Helden

Human & Animal Physiology, Wageningen University, Wageningen, The Netherlands

Beta-carotene (BC) is dietary component that naturally present in a wide variety of foods. It is a pro-vitamin A and has anti-oxidant properties. It was thought that BC could contribute to protection against lung cancer via it anti-oxidant properties. However, two intervention trials with continuous high dose BC supplementation disproved this. The ATBC and CARET trials resulted in an increased, rather than a decreased lung cancer risk. A sound mechanistic explanation for the adverse effects is still lacking. Here, it is argued that the continuity of the supplementation may have played a role, since every-other-day high dose supplementation did not show the adverse effects. The continuous supplementation may have altered homeostatic setpoints leading to dysregulation, with retinoic acid resistance and prolonged activation of developmental frizzled signaling as potentially affected molecular pathways. This still needs to be verified experimentally. In the meantime it seems wise take the intake regimen of BC, and any other supplement for that matter, into account when considering its safety.

OP-31

Astaxanthin supplementation improves endurance performance and fat oxidation during a 40 km cycling time-trial

D.R. Brown[1], A.R. Warner[2], S.K. Deb[3], L.A. Gough[4], S.A. Sparks[1], L.R. McNaughton[1]

[1]Edge Hill University, Department of Sport and Physical Activity, Ormskirk, L39 4QP, UK
[2]University of Hull, Department of Sport, Health and Exercise Science, Hull, HU6 7RX, UK
[3]University of Westminster, Department of Life Sciences, Westminster, W1W 6UW, UK
[4]Birmingham City University, School of Health Sciences, Birmingham, B15 3TN, UK

Improvements in exercise metabolism and endurance performance are reported in mice models following supplementation with astaxanthin, a liposoluble carotenoid. In contrast, current evidence surrounding astaxanthin supplementation in exercising humans is somewhat equivocal. The current study aimed to investigate whether 7 days of astaxanthin supplementation is able to improve exercise performance and metabolism during the completion of a 40 km cycling time-trial. Twelve recreationally trained cyclists (age: 28 ± 6 years, height: 178 ± 7 cm, body mass: 78.3 ± 7.6 kg, body fat: 13.7 ± 2.6%) were recruited. Prior to each experimental trial, participants were supplemented with either 12 $mg \cdot day^{-1}$ astaxanthin or an appearance-matched placebo for 7 days (after 14 days of washout), using a randomized, double-blind counterbalanced design. On day 7 of supplementation, participants completed a best effort indoor 40 km cycling time-trial on a cycle ergometer, with indices of exercise metabolism measured throughout. Time to complete the 40 km cycling time-trial was significantly improved by 1.2 ± 1.7% following astaxanthin supplementation, from 70.76 ± 3.93 min in the placebo condition to 69.90 ± 3.78 min in the astaxanthin condition (mean improvement= 51 ± 71 s, 95% CI= 6 to 96 s, p= 0.029, Hedges' *g*= 0.21). Whole-body fat oxidation rates were also significantly greater (mean difference= +0.09 ± 0.13 $g \cdot min^{-1}$, 95% CI= 0.00 to 0.17, p= 0.044, Hedges' *g*= 0.52), and the respiratory exchange ratio significantly lower (mean difference= -0.03 ± 0.04, 95% CI= -0.01 to -0.06, p= 0.024, Hedges' *g*= 0.60) between 39-40 km following astaxanthin supplementation compared to placebo. In conclusion, supplementation with 12 mg of astaxanthin for 7 days provided an ergogenic benefit to 40 km cycling time-trial performance when compared to placebo and enhanced whole-body fat oxidation in the final stages of this endurance-type performance event.

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OP-32

Scavenging and quenching activities of carotenoid extracts from citrus fruits and synergism with ascorbic acid in liposomes mimicking animal cell membranes

M.P. Barros[1,2], J. Zacarías[1], F. Rey[1], L. Zacarías[1], M.J. Rodrigo[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Departamento de Biotecnología de Alimentos, Calle Catedrático Agustín Escardino 7, 46980 Paterna, Spain
[2]Institute of Physical Activity and Sports Science (ICAFE), Cruzeiro do Sul University, Rua Galvao Bueno 868, B-Tower, 13th floor, 01506000, Sao Paulo, SP, Brazil

Regular consumption of citrus fruits in humans has been associated with lower incidence of chronic-degenerative diseases, especially those mediated by reactive oxygen/nitrogen species. Most of the health-promoting benefits provided by the regular uptake of citrus fruits derives from their carotenoid (antioxidants and pro-vitamin A compounds) and ascorbic acid (ASC; antioxidant and enzyme cofactor) content. We have investigated the scavenging (against hydroxyl radical, HO•) and quenching activities (against singlet oxygen, $[O_2(^1x_g)]$) of carotenoid extracts from citrus fruits in liposomes that mimic the lipid composition of animal cell membranes (48% egg-yolk phosphatidylcholine, 20% cholesterol, 16% sphingolipid (ceramide), and 16% egg-yolk phosphatidylethanolamine) in presence and absence of micromolar ASC. Accurate controls characterized the oxidizing behavior of our innovative liposomal system, in comparison to usual phosphatidylcholine liposomes. We strategically selected citrus fruits with singular carotenoid composition in the pulp (quantified by HPLC-PDA): (i) 'Pinalate' sweet oranges, rich in colorless phytoene and phytofluene carotenes; (ii) 'Nadorcott' mandarins, rich in the xanthophyll x-cryptoxanthin; (iii) 'Ruby Valencia' sweet orange, a new lycopene-containing mutant, and (iv) the sweet orange 'Valencia' with typical violaxanthin-rich content. The fluorescent probe C_{11}-BODIPY$^{581/591}$ was incorporated into liposomes to detect membrane oxidation promoted by HO• (Fenton reaction) and results were compared with thiobarbituric acid-reactive assays (TBARS). The singlet oxygen antioxidant capacity assay (SOAC) was performed with carotenoid extracts against $[O_2(^1x_g)]$ in organic solvent, and also in micellar systems to investigate the putative synergistic effect of water-soluble ASC. The carotenoid extracts were also assayed against $[O_2(^1x_g)]$ produced in UV-photoexcited systems (O_2-bubbled solutions of rose Bengal, methylene blue, or hydrogen peroxide/hypochlorite) and monitored by the fluorescence probe Sensor Green®. Minor synergistic effects were observed between carotenoids and ASC, except for their quenching activities in lycopene-rich extracts of 'Ruby Valencia' sweet oranges. Possible synergistic mechanisms between carotenoid extracts and ASC are discussed.

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OP-33

Citrus juice concentrate obtained by coupling enzymatic liquefaction with microfiltration: carotenoid bioaccessibility

S. di Corcia[1], C. Dhuique-Mayer[1,2], M. Dornier[1]

[1]QualiSud, Univ. Montpellier, Cirad, Montpellier SupAgro, Univ. Avignon, Univ. La Réunion, Montpellier, France
[2]CIRAD, UMR QualiSud, F-34398 Montpellier, France

Citrus juices represent a significant dietary source of carotenoids. These micronutrients contribute to their health effects in combination with vitamins and polyphenols. Often coupled with enzymatic treatment to improve filterability, crossflow microfiltration (CMF) is an innovative process that allows carotenoid content to increase without heating[1]. However, this process modifies juice characteristics that could probably alter the bioaccessibility of carotenoids. In this study, the effect of enzymatic liquefaction coupled to CMF on carotenoid bioaccessibility was investigated. A formulation consisting of a mixture of 60/40 *Citrus clementina* and *C. paradisi* juices was chosen to obtain a balanced β-carotene/β-cryptoxanthin/lycopene profile. Carotenoids in the juice and the concentrate obtained were analyzed by HPLC. The individual bioaccessibility was assessed using an *in vitro* digestion model[2]. Granulometry, viscosity, and pectin content were chosen as structural indicators to better understand the process impact on bioaccessibility. The concentrate was obtained coupling an enzyme liquefaction using Ultrazym AFP Novo (300 mg L^{-1}, 45 min, 30°C) and a CMF step with a 0.2 ×m tubular ceramic membrane at 30°C. Bioaccessibility of the 3 carotenoids in the juice was improved by the enzymatic liquefaction. This positive effect can be explained by particle size, viscosity, and pectin content reduction due to enzymatic degradation of cell walls. The CMF step also decreased the average diameter of insoluble particles but simultaneously increased viscosity and pectin content of the concentrate (9-fold). Because of these combined effects, carotenoid bioaccessibility was not significantly enhanced by CMF (in range of 25% for β-cryptoxanthin, 11% for β-carotene and 0.7% for lycopene). However, CMF was particularly relevant because carotenoid content in the concentrate increased by a factor of 9 for the 3 carotenoids. These results showed the interest to combine enzymatic liquefaction with CMF not only to increase the product filterability but also to improve its nutritional quality, combining carotenoid content with bioaccessibility. So, granulometry, viscosity and pectin content measured could be reliable indicators to predict carotenoid bioaccessibility.

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OP-34

Enhancement of antioxidant activity of carotenoids in supramolecular complexes and the role of their redox potentials

A.L. Focsan[1], N.E. Polyakov[2,3], L.D. Kispert[4]

[1]Valdosta State University, Department of Chemistry, Valdosta, GA 31698
[2]Institute of Chemical Kinetics and Combustion, 630090, Novosibirsk, Russia
[3]Institute of Solid State Chemistry and Mechanochemistry, 630128, Novosibirsk, Russia
[4]The University of Alabama, Department of Chemistry, Box 870336, Tuscaloosa, AL 35487

Electron transfer plays an important role in many biological processes with participation of carotenoids, including their antioxidant activity. We have found using Electron Paramagnetic Resonance (EPR) spin trapping technique that the ability of carotenoids to scavenge the reactive oxygen species (ROS) is extremely sensitive to their redox potentials, and increases sharply with increasing oxidation potentials of carotenoids [1]. Carotenoid bixin exhibits the highest measured oxidation potential (0.94 V) to date and its scavenging ability towards ROS was estimated to be 17 times higher than that of astaxanthin [2]. Another advantageous property of bixin is that it does not form H-aggregate in aqueous solutions like zeaxanthin, lutein and astaxanthin do [3]. H-aggregation significantly reduces the scavenging ability of carotenoids towards ROS. Encapsulation of carotenoids in different delivery systems is an innovative approach shown to increase their solubility, stability and bioavailability features important for practical food and health applications. We have demonstrated that incorporation of highly hydrophobic carotenoids into water-soluble delivery systems significantly affects the properties of carotenoids, including their water solubility, stability, optical properties, self-association ability, oxidation potentials and even stability of radical paramagnetic forms [4]. For example, inclusion of some carotenoids into glycyrrhizin complex affected their oxidation potentials resulting in increased antioxidant activity and prevented aggregation of xanthophyll carotenoids in aqueous solutions. This work was supported by the U.S. Department of Energy and National Science Foundation (L.D.K), FRSG Program at Valdosta State University (A.L.F.) and the Russian Science Foundation (N.E.P.).

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OP-35

Assessing the phenolic and carotenoid content of eight clingstone peach cultivars at harvest and after processing

M. Christofi[1], T. Tsiaka[2], D.Z. Lantzouraki[2], P. Drogoudi[3], P. Zoumpoulakis[2], S.A. Haroutounian[4], G.A. Manganaris[1]

[1]Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology & Food Science, 3603 Lemesos, Cyprus
[2]Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, 48, Vas. Constantinou Ave., 11635 Athens, Greece
[3]Pomology Institute, Hellenic Agricultural Organization 'Demeter', Directorate General of Agricultural Research, 38 R.R. Station, 59035 Naoussa, Greece
[4]Nutritional Physiology and Feeding Laboratory, Department of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece

Peach (*Prunus persica*) is considered as a classic fruit for the consumer either as fresh or as processed. Having significant impact on human nutrition, peach is the second most important temperate fruit crop worldwide in terms of production volumes. Peach canning is one of the most popular of all processed fruit, with Greece being the leading country in clingstone peach production and exports of canned peaches at international level. Notably and despite its economic importance, information on bioactive profile analysis of widely grown cultivars and changes induced after canning process is very limited. Thus, in the present study, bioactive compounds including carotenoids and phenolic compounds were determined in fresh and canned tissue; aiming at the evaluation of the impact level of processing on the composition of bioactive compounds in peach fruit, with special reference to carotenoid content. Towards this aim, peach fruit of eight commercially important clingstone cultivars were harvested according to their maturity stage and size with scalar ripening (spanning from beginning of July to mid of September), and were subsequently processed. Initially, the extractions of carotenoids and phenolic compounds from lyophilised peach powder were performed using methanol solvent and methanol: water (7:3, v/v) respectively. Thereafter, total carotenoid (TC) and total phenolic composition (TP) of methanolic extracts were determined by spectrophotometric assays, followed by LC-MS/MS analysis of individual identification and quantification of TC and TP content. Data were subjected to statistical analysis and differences among the examined cultivars before and after canning process were monitored. Notably, results indicated great differences among cultivars, highlighting the genotype effect. Additionally, the effect of processing on individual carotenoid and phenolic compounds content is also discussed.

OP-36

Bioactive characteristics of sweet potato flesh and peel

V. Tumbas Šaponjac, V. Šeregelj, J. Vulić, G. Ćetković, J. Čanadanović-Brunet, S. Stajčić

University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Agro by-products represent an important source of phytochemicals possessing a wide range of functional activities. Sweet potato (*Ipomea batatas* L.) peels are a major waste generated during root processing and currently have little commercial value although it contains, as the flesh, various types of bioactive compounds such as carotenoids and phenolics. Recycling of agro wastes into high valuable products with potential health benefits could be achieved through the extraction of their bioactive compounds. The present study was aimed to evaluate the extraction efficiency of carotenoids and phenolics of flesh and peel sweet potato using two different extraction solvents (100% acetone and acetone:ethanol mixture 36:64 v/v) and the antioxidant activity of the extracts, as well as application possibilities of peel extracts in the food industry. The total carotenoid (TCar) and phenolic (TPh) contents, as well as DPPH radical scavenging activity (SA) and reducing power (RP) were determined spectrophotometrically. TCar content of the flesh extract did not differ significantly in relation to the applied extraction solvent (19.50 and 19.67 mg β-carotene/100 g for acetone and acetone:ethanol, respectively), as well as for the peel extract (12.01 and 12.71 mg β-carotene/100 g for acetone and acetone:ethanol, respectively). However, the flesh and peel extracts prepared with acetone and ethanol mixture had significantly higher levels of TPh (109.77 and 152.32 mg gallic acid/100 g, respectively), SA (300.02 and 315.16 μmol trolox /100 g, respectively), and RP (236.21 and 306.70 μmol trolox /100 g, respectively), compared with acetone extracts (TPh (29.65 and 31.14 mg gallic acid/100 g, respectively), SA (139.00 and 131.32 μmol trolox /100 g, respectively), and RP (18.79 and 40.57 μmol trolox /100 g, respectively)). These results show that acetone:ethanol extraction method was more effective, comparing to aceton solvent and, that there is a big potential for sweet potato peel as an antioxidant in food systems due to its high content of carotenoids, phenolics and antioxidant activity.

OP-37

Carotenoid profiling of selected strains of Cyanobacteria

I. Milovanović[1], D. Kovač[2], J. Simeunović[2], M. Pojić[1], A. Mandić[1], A. Mišan[1]

[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

Cyanobacteria represent some of the oldest living organisms which show great biological diversity. Their mass cultivation is considered to be of a high sustainability, as they absorb CO_2 from the atmosphere, withstand extreme environmental conditions, have a high productivity and do not compete with terrestrial crops for agricultural land to produce a wide range of biomolecules. In this work, the carotenoid profiles of biomass from selected cyanobacterial strains of *Spirulina*, *Anabaena*, and *Nostoc* genera, originating from Serbia were investigated. Additionally, the influence of nitrogen level during growth on the production of carotenoids was estimated for *Nostoc* and *Anabaena* strains. Accelerated solvent extraction (Dionex ASE 350 Thermo Scientific, USA) with n-hexane was applied for pigment extraction. Extracted pigments were separated and analyzed by HPLC-DAD using a reversed-phase C18 column. Investigated cyanobacteria showed strain-specific carotenoid profiles. Beta-carotene was the only carotenoid common to all strains and it was present in the content range from 2.25 to 7.58 mg/g dry mass. The strains of *Spirulina* genera were characterized by the presence of zeaxanthin, traces of different xanthophylls, as well as very low content of lutein, all of which were not present in other samples. In addition to zeaxanthin, the dominant carotenoids in *Spirulina* strains were auroxanthin (8.86 mg / g in S1 and 1.61 mg / g in S2) and β-carotene (5.82 mg/g in S1 and 7.58 mg/g in S2). In *Nostoc* and *Anabaena* strains, the dominant carotenoids were β-carotene, canthaxanthin and echinenone. The addition of nitrogen into the growth medium decreased the content of total carotenoids in both *Anabaena* and *Nostoc* strains. Regarding the relatively high carotenoid content, which can be further tailored by changing the conditions of growth, investigated cyanobacterial strains of *Spirulina, Anabaena*, and *Nostoc* present a valuable and sustainable source.

OP-38

Fern fiddleheads as a potential source of lutein and antioxidants in the European diet

L. Langhansova[1], K. Pumprova[1], D. Haisel[1], Z. Antoninova[1], A. Pavicic[1], L. Skalova[2]

[1]The Czech Academy of Sciences, Institute of Experimental Botany, 165 02 Prague, Czech Republic
[2]Charles University, Faculty of Pharmacy, Department of Biochemical Sciences, 500 05 Hradec Králové, Czech Republic

Fern fiddleheads are part of the diet in East Asia, North America or Oceania. However, the importance of ferns in diet as well as in traditional medicine had been forgotten in Europe. In our study, we analysed nutritional and antioxidant potential of fiddleheads and leaves of 26 fern species. Regarding the carotenoid content, we found most of the examined species as a good source of carotenoids, mainly lutein (in average 162 μg/g dry weight in fiddleheads and 191 μg/g dry weight in leaves), comparing to common leaf vegetable such as spinach (69 μg/g DW) or rocket (55 μg/g DW). Generally, we found slightly higher content of carotenoids in leaves (in average 20 % higher compare to fiddleheads) but lutein content was higher in few fiddleheads mostly of the *Dryopteridaceae* family. The cytotoxicity test showed no toxic effect of ferns (the LDH assay was applied to sheep hepatocytes), therefore we suggest further research leading to production of fern fiddleheads for food industry. The antioxidant capacity was evaluated via total phenol content and ORAC assay. We found stronger antioxidant capacity in fiddleheads comparing to leaves and most of the ferns species distinguished by antioxidant capacity that exceed the standard antioxidant Trolox. Fifteen out of 26 tested fiddlehead extracts exceed 1 g Trolox equivalent per gram of extract dry weight. Fern genera have a few unique secondary metabolites, which have not been discovered in higher plants (Cao H et al, 2017 Phytochem Rev 16:379-440). Therefore, discovering specific compounds in ferns and their use in medicine might bring valuable knowledge for improving the human health. Based on the results in this study we selected species from families *Athyriaceae*, *Dryopteridaceae*, and *Polypodiaceae* for further research on biological activity.

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OP-39

Autumn leaves – upgrading carotenoid rich park and garden waste to useful chemicals for industry

S.T. Häkkinen, S. Alakurtti, A.-S. Jääskeläinen, S. Ellilä, T. Seppänen-Laakso, R. Puupponen-Pimiä, A. Ritala and L. Nohynek

VTT Technical Research Centre of Finland Ltd, Tietotie 2, P.O.Box 1000, 02044 VTT, Finland

Every year waste treatment plants at 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. Therefore, the aim of the study was to take this valuable natural resource into use. For example, there is a fast-growing need for natural pigments in various industries around the world, and autumn leaves represent an interesting source for natural dyes for they derive their colours from orange and yellow carotenoids and red anthocyanins. In addition, plant leaves contain a range of other compounds with commercial value, such as polyphenols, lignin, carbohydrates and protein, potentially available in autumn leaves. One of the big challenges in the use of natural dyeing ingredients 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, besides possessing anthocyanins and carotenoids, contain large amounts of tannins, which have shown to act as natural mordants. We used extracts of fallen autumn leaves as natural colouring solutions in textile dyeing and very good fastness without additional mordant was proven. In addition, feeding of pigment fractions into plant cell cultures was initiated in this project, showing response in intracellular carotenoid content, which originally was at modest level (0.1 - 0.8 mg/g DW). In this study, extraction residue was converted by microbial processes to single cell proteins and was used as nutrient to cultivate edible mushrooms. Finally, when we modified extraction residue, antimicrobial activity against harmful microbes were proven in growth inhibition assays.

OP-40

Effect of natural antioxidants and Citrus pulp supplementation to hen diet on pigmentation and carotenoid content of egg yolk

D.Z. Lantzouraki[1,2,3], V.J. Sinanoglou[3], M. Goliomytis[4], S.G. Deligeorgis[4], A.C. Calokerinos[1], P. Zoumpoulakis[2]

[1]*Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece*
[2]*Institute of Biology, Medicinal Chemistry and Biothechnology, National Hellenic Research Foundation, 11635 Athens, Greece*
[3]*Laboratory of Chemistry, Analysis & Design of Food Processes, Department of Food Science and Technology, University of West Attica, 12210 Egaleo, Greece*
[4]*Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 11855 Athens, Greece*

The nutritional profile of egg yolk, including carotenoids as lutein and zeaxanthin, can be manipulated through the hen's diet, leading to "designer eggs" with specific attributes in order to meet the demands of health-conscious consumers. Within this framework, the present study aims to examine the effect of feed supplementation with citrus pulp and natural antioxidants on the carotenoid composition of egg yolks. A total of 189 laying hens (*Gallus gallus domesticus* L.) were randomly distributed into three dietary groups and the experiment was conducted for one month. The groups were parted to the control that was offered a basal ration, the second group that was offered a diet supplemented with 9% w/w dried pulp of *Citrus sinensis*, and the phenolic antioxidant group that was offered the basal diet supplemented with 0.767 g hesperidin and 0.002 g naringin per kg of feed (concentration levels equivalent to the citrus pulp as determined with LC-MS). The yolks were separated from the albumen and lyophilized, and the colour parameters were measured with a chromameter. Total carotenoids were extracted and they were determined spectrophotometrically. Moreover, an LC-MS analysis using a triple-quadrupole linear ion trap mass spectrometer was conducted for the quantitation of main carotenoids in the yolk extracts. According to our results, hens fed with citrus pulp produced eggs with significantly ($P<0.05$) lower concentration of carotenoids in relation to the control group, while yolk colour shifted from orange to greenish. This could be attributed to the low palatability of the pulp that resulted to reduced feed intake. On the contrary, the supplementation with hesperidin and naringin was shown to increase ($P<0.05$) total yolk carotenoids concentration. Taking into consideration that phenolic compounds possess antioxidant activity, it seems that naringin and hesperidin enhance the antioxidant potential of the yolk, thus preventing the oxidation of lipids, including carotenoids.

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OP-41

The impact of the carotenoid complex of rosehip and hawthorn powders on the accidental pathogenic microbiota in sausages

D. Cojocari[1], R. Sturza[2], V. Rudic[1], E. Sandulachi[2], A. Macari[2], G. Balan[1], A. Ghendov-Moșanu[2]

[1]State University of Medicine and Pharmacy "Nicolae Testemitanu", 165, Stefan cel Mare Bd., MD-2004, Chisinau, Republic of Moldova
[2]Technical University of Moldova, 168, Stefan cel Mare Bd., MD-2004, Chisinau, Republic of Moldova

One of the problems in the food industry is the food contamination with pathogenic microorganisms of human origin as a result of inadequate handling and processing. Microbial contamination reduces the shelf life and quality of foods that can cause food infections and food poisoning. Because of the bacterial resistance increase to antibiotics, a particular interest has been shown to investigate the antimicrobial effects of natural bioactive compounds against pathogenic bacteria in order to produce harmless foods for consumers. Recent studies have demonstrated that rosehip and hawthorn powders manifested antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*. The purpose of this research was to investigate the antimicrobial properties of rosehip and hawthorn powders against pathogenic microorganisms, *S. aureus* ATCC 25923, *Salmonella* Abony ATCC 6017, *Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922 in sausages. In this research we used rosehip (*Rosa canina)* and howthorn (*Crataegus monogyna*) berry powder that was dried at room temperature. The carotenoid content in powders was determined by spectrophotometric and high performance liquid chromatography (HPLC) methods. The carotenoid content in the hip powder was 18.3 times higher than in the hawthorn powder. Samples of sausages were contaminate with strains of *S. aureus* ATCC 25923, *Salmonella* Abony ATCC 6017, *Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922. The growth of tested cultures in situ (sausages), in control samples (with no addition of berries extract), and in the samples with rosehip and hawthorn were determined. Incubation was performed at 37 °C. The rates of growth of pathogenic microorganisms were monitored after 24, 48, 72 and 96 hours. The results of this study showed that the use of rosehip and hawthorn powders in sausage processing had diminished the rate of growth of pathogenic microorganisms on purposely contaminated samples. By studying the Lag phase and the Logarithmic growth phase of the strains, it was found that hawthorn had a greater bacteriostatic effect on *S. aureus* strains ATCC 25923 and *E. coli* ATCC 25922, than on the other microorganisms, while the rosehip had important bacteriostatic effect on *Salmonella* Abony strains ATCC 6017 and *Klebsiella pneumoniae* ATCC 13883.

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OP-42

Natural deep eutectic solvents (NADES): Potentials and challenges of a state-of-the-art green technology for carotenoid extraction

T. Tsiaka[1], S. Koutsoukos[2], A. Tzani[2], A. Detsi[2], P. Zoumpoulakis[1]

[1]*Institute of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece*
[2]*Laboratory of Organic Chemistry, Department of Chemical Sciences, School of Chemical Engineering, National Technical University of Athens, 15780 Zografou, Greece*

Currently, nutraceutical and food industries are steering their research towards the re-valorization and commercialization of putative bioactive compounds from their natural agro- and seafood byproducts. Since the promotion of sustainable and greener sample preparation strategies is of major importance, there is an urgent need for a) the implementation of faster, cost-saving nonconventional extraction methods (i.e. high energy extraction techniques) and b) the utilization of eco-compatible innovative extraction solvents. Thus, the present work was focused on increasing the efficiency of carotenoid extraction from apricot and shrimp byproducts through the introduction of a combinatorial methodology that includes i) the use of ultrasound- (UAE) and microwave-assisted extraction (MAE) and ii) a natural deep eutectic solvent (NADES) derived from a 2:1 choline chloride (CC) and tartaric acid (TA) mixture. To this extent, a hydrophilic CC/TA NADES was used for the first time to recover non polar compounds, like carotenoids. In particular, a 80:20 v/v NADES:MeOH mixture was utilized for extracting b-carotene from apricot pulp and astaxanthin from shrimp head by applying US or MW irradiation. The comparison of carotenoid extraction yields provided by NADES with those obtained when conventional organic solvents (methanol, ethanol, *n*-hexane, chloroform, acetone and their mixtures) were used, revealed the great potential of NADES as extraction medium which can reach up to 4-times higher carotenoids yields when longer extraction time occurs. For example, b-carotene yields of NADES-UAE was 41.3(±1.9) mg/100 g dry sample, while conventional UAE extracted 11.51(±0.29) mg of b-carotene/100 g dry sample (*p*-value≥0.05). However, at short extraction periods (≤10 minutes) where astaxanthin extraction was performed, NADES-based extraction was favored only in the case of MAE (26.7(±2.5) mg of astaxanthin/100 g dry sample) due to the higher extraction temperatures applied (45-50 °C) compared to UAE (~30 °C), where astaxanthin extraction yield was 7.85(±0.47) mg/100 g dry sample. To conclude, the integration of high energy techniques and novel NADES systems emerge as a promising alternative to the traditional extraction practices. Nonetheless, the future large-scale application of carotenoid-NADES high energy extraction merit further investigation.

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OP-43

Development of new carotenoid food additives from bacteria using gut metabolomics as innovative research tool

L. Van Meulebroek[1], S. Possemiers[2], C. Duysburgh[2], L. Vanhaecke[1,3]

[1]Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Laboratory of Chemical Analysis, 9820 Merelbeke, Belgium
[2]ProDigest, 9052 Zwijnaarde, Belgium
[3]Queen's University, School of Biological Sciences, Institute for Global Food Security, 1NN Belfast, Northern Ireland, United Kingdom

Due to the chemical instability of carotenoids, food manufacturers have difficulties to incorporate these health-promoting compounds into their products and achieve acceptable bioavailability. Therefore, the EU-funded COLORSPORE project was established to assess certain bacterial species of the *Bacillus* genus that are able to form endospores, which are able to survive pasteurization and short-term exposure to high baking temperatures as well as transit through the GI-tract. In the gut itself, the spores may produce highly bioavailable carotenoids, including amongst others lycopene, α- and β-carotene, astaxanthin, zeaxanthin, lutein, 4-etozeaxanthin, echinenone, phenicoaxanthin, and canthaxanthin. As such, these spores would be an interesting carotenoid-delivery vehicle. Two strains of the Bacillus genus (i.e. *Bacillus indicus* HU36® and *firmus* GB1®) were identified to hold most promise for industrial purposes, as they were evaluated as safe and the bacteria-derived carotenoids enclosed higher antioxidant activity (factor ten, by *in vitro* digestion) and bioavailability (factor four, by *in vitro* and *in vivo* digestion by rats) than common dietary carotenoids. To valorize these findings and to confirm the bacterial strains as probiotic food ingredients, the CaroDel project was founded. Within this new project, we performed metabolomics analyses to monitor meaningful alterations in metabolism based on *in vitro* gut models and *in vivo* animal studies. Hereby, a strategy of generic extraction and ultra-high performance liquid-chromatography and Orbitrap-mass spectrometry was implemented to map the polar metabolome and assess metabolic alterations that were induced by carotenoids, and this for administration of i) vegetative *Bacillus* cells, ii) *Bacillus* spores, and iii) extracted carotenoids. Significant alterations in metabolism were revealed by multivariate statistics (PCA-X and OPLS-DA modelling with p-value < 0.05 and $Q^2 > 0.5$), whereby the spore-based delivery vehicle showed high potential to make administered carotenoids bioavailable to humans, and may therefore be used in human trials to verify any health-promoting effects.

OP-44

Carotenoids intake by the Portuguese population based on a Total Diet Study approach

M.G. Dias, E. Vasco, L. Oliveira

Monitoring and Surveillance Unit, Food and Nutrition Department, National Institute of Health Doutor Ricardo Jorge, IP, Av. Padre Cruz, 1649-016 Lisboa, Portugal

Carotenoids are a family of compounds, usually with colours near yellow, orange and red occurring widely in nature. Human beings cannot synthetize them, however they are crucial for the prevention of illnesses related to vision, cognitive function, certain cancers. Total Diet Studies (TDSs) are a tool to evaluate the ingestion and exposition of the populations to substances of interest, taking into account the whole diet and food as consumed. The main dietary primary sources of carotenoids are fruits and vegetables. The objective of this study was to evaluate the intake of carotenoids by the Portuguese population based on a TDS approach. Based on the national food consumption survey (excluding supplements) "Portuguese Population's Food Habits and Lifestyles", 164 composite samples were arranged from the collection of 1968 food items and prepared accordingly to the consumption habits. These samples were analysed and carotenoids determined by HPLC. The ingestion of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin by the Portuguese population was evaluated. MCRA software was used to estimate the dietary ingestion of carotenoids by a probabilistic approach. Results indicated that mean ingestion of β-carotene and lutein were 2.0 and 1.5 mg/day, respectively, for adults and elderly, without difference between females and males. The elderly population seems to ingest more β-carotene and lutein, 2.4 and 1.6 mg/day, respectively, than adults. Fifty percent of the population ingested less than 0.84 and 0.85 mg/day, of β-carotene and lutein, respectively. Regarding lutein, only 1.5% of the population ingests more than 10 mg/day. The main contributors for the intake of β-carotene were mixed vegetable soup, carrot, lettuce, legumes soup and cabbage, and for lutein, raab, mixed vegetable soup, lettuce, cabbage and carrot. Based on the TDS approach, the Portuguese population will benefit from an intensification of the consumption of fruits and vegetables and consequent increase of carotenoid intake.

OP-45

Carotenoid and carotenoid ester deposition in plastids of lucuma, avocado and persimmon and its effect on bioaccessibility

A. Gómez-Maqueo[1,2], E. Bandino[1], M.P. Cano[1,2]

[1]Institute of Food Science Research (CIAL; CSIC-UAM), Nicolás Cabrera 9, 28049 Madrid, Spain
[2]FEMSA Biotechnology Center, School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Mexico

Carotenoids play critical roles in human nutrition and health as essential components of the human diet. However, their bioaccessibility is affected by components in fruits such as fiber, starch and lipids as well as their physical deposition state. *Pouteria lucuma, Persea americana* and *Diospyros kaki* are rich sources of carotenoids and carotenoid esters with diverse profiles and macromolecular composition. The aim of this study was the assessment of the effect of plastid deposition on the stability and bioaccessibility of carotenoid and carotenoid esters in the afore mentioned fruits following the adapted INFOGEST recommendations to extract and analyze carotenoids from each in-vitro digestive phase. Pulp sections were analyzed by confocal laser scanning and optical microscopy to examine their carotenoid deposition in fruit tissue and in each digestive phase. Carotenoid identification and quantification was analyzed by HPLC-DAD-MS using a reversed-phase C_{30} column. Carotenoids in ripe lucuma (var. Beltran and Molina) were deposited in amyloplasts in the membrane enclosing the starch granules. During digestion, carotenoids were concentrated in the amyloplasts by a significant reduction size due to the dissolving of starch in the digestive medium. Carotenoids in ripe avocado (var. Hass) were found in elaioplasts along with a large content of lipid vesicles. Fatty acid content (637,4 mg FAME/ g dry weight) and profile (61% oleic, 17% palmitic, 11% linoleic, 5% cis-vaccenic, 4% palmitoleic and 2% other fatty acids) in avocado contributed to high carotenoid bioaccessibility. Carotenoids in ripe astringent persimmon (var. Rojo Brillante) were found in globular chromoplasts in the cells surrounding vascular bundles. They were the least bioaccessble because of (i) low fatty acid content and (ii) because they were trapped in pectin polysaccharide chains during digestion. The further study of carotenoid deposition in fruits is fundamental for understanding their potential bioaccessibility which contributes to the design of processed fruit products oriented to improve the intestinal absorption of carotenoids.

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OP-46

Influence of dietary proteins on the bioaccessibility of carotenoids from selected food matrices

M. Iddir[1,4], G. Dingeo[1], E. Cocco[2], C. Desmarchelier[3], T. Schleeh[2], C. Guignard[2], P. Borel[3], Y. Larondelle[4], T. Bohn[1]

[1]Luxembourg Institute of Health, Department of Population Health, Strassen, Luxembourg
[2]Luxembourg Institute of Science and Technology, Belvaux, Luxembourg
[3]C2VN, INRA, INSERM, Aix-Marseille Univ, Marseille, France
[4]Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium

Carotenoids are lipophilic phytochemicals and their intake has been associated with the prevention of chronic diseases. Their bioaccessibility, depends on dietary as well as host-related factors (1, 2). Among the lesser studied dietary aspects are influences of proteins on carotenoid bioaccessibility, since proteins may foster micellization due to their potential emulsifying properties. The aim was to scrutinize the effect of proteins on carotenoid bioaccessibility. Therefore, we employed the European consensus model for static *in vitro* digestion to investigate the influence of: a) digestive conditions (concentrations of pepsin, pancreatin and bile, kinetic energy, digestion time, and the amount of co-digested lipids) on beta-carotene bioaccessibility digested with whey-protein isolate at different concentrations (equivalent to 0/10/25/50% of the RDA), b) frequently consumed proteins (whey protein isolate, soy protein isolate, sodium caseinate and gelatin), at various concentrations, on the bioaccessibility of pure carotenoids (beta-carotene, lutein, lycopene), and carotenoid-rich food matrices (tomato juice, carrot juice, spinach). Under the standardized digestion parameters recommended by the European consensus model: a) Proteins tended to enhance bioaccessibility of beta-carotene by up to 20% ($p<0.001$), especially under higher kinetic energy or reduced amount of dietary lipids. Conversely, they impaired bioaccessibility to one third ($p < 0.001$) under incomplete digestive conditions. b) Different proteins showed a generally positive trend on the bioaccessibility of carotenes, while showing an inhibitory effect on xanthophylls causing a decrease in their bioaccessibility by approx. 50% ($p<0.001$). Experiments including carotenoid-rich foods are ongoing; preliminary results indicate matrix-depending effects. Results suggest that proteins may modulate carotenoid bioaccessibility depending on a) digestive conditions, b) type and concentration of proteins, type of carotenoid (i.e. carotene vs. xanthophylls), and the co-digested matrices, possibly via their interaction during the processing of lipid droplets into mixed micelles. Results may be important under specific circumstances, such as intake of carotenoid-rich food items low in lipids, or under maldigestion conditions.

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OP-47

Design and development of a carotenoid enriched extract from microalgae using a liquid-liquid triphase system: its evaluation

N. Gangadhar Katkam, L.M. Schüler, T. Santos, H. Pereira, L. Barreira, J. Varela

University / CCMAR – Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

In recent years, microalgae-based ventures worldwide are looking for appropriate biorefinery technologies to produce value-added products from microalgal biomass, carotenoids in particular. Microalgae are a rich source of carotenoids (carotenes and xanthophylls) which can play important roles in prevention (and even treatment) of human diseases and health conditions. Indeed, microalgal crude extracts are comprised of a complex mixture of biochemicals being particularly rich in carotenoids, polyunsaturated fatty acids (PUFA) containing triacylglycerols (TAGs) and polar lipids, among others. Unfortunately, the fractionation/or refining of microalgae crude extracts into different market-end products is a less developed field in the context of a microalgae biorefinery. Therefore, the main objective was to design and develop an effective downstream processing method for enriching specific fractions with carotenoids with known antioxidant properties (e.g., lutein) from *Tetraselmis* sp. CTP4 using a centrifugation-assisted liquid-liquid triphase system (LTPS). *Tetraselmis* sp. CTP4 biomass was grown using a modified ALGAL medium in 200-L plastic airlifts at controlled temperature and light for 15 days at Necton, Portugal. Wet biomass was used for extraction using ethanol followed by drying. The dried crude alcoholic extract was dissolved in a mixture of hexane and water to generate a triphasic system upon centrifugation. These fractions were analysed, identified and quantified by HPLC-DAD. Three streams/phases, organic (OP; upper), colloidal (CP; interfacial) and aqueous (AP; bottom), were characterized for total carotenoids. CP showed a high proportion of lutein (54.3%), neoxanthin (22.5%), violoxanthin (20.8%) and beta-carotene (1.0%), whereas the OP contained mostly beta-carotene (71.0%) and lutein (23.0%). The high distribution of xanthophylls in the middle layer might be related to the occurrence of polar hydroxyl groups in these carotenoids, imparting more amphiphilic characteristics to them. Colloidal phase exhibited a dose-dependent effect, presenting IC_{50} values of, in terms of radical scavenging activity against DPPH (12.3 ± 2.0 mg/mL) and ABTS (1.52 ± 0.19 mg/mL) radicals as well as redox metal chelating activities namely iron (5.20 ± 0.59 mg/mL) and copper (7.80 ± 1.15 mg/mL). The high amount of xanthophylls (e.g., lutein, neoxanthin and violoxanthin) as well as phospholipids present in the CP suggest that this fraction can display a strong potential for future nutraceutical and cosmeceutical applications, which will be critical for upgrading the value of the microalgal biomass produced.

OP-48

Lutein and zeaxanthin concentrations: in serum, macular pigment optical density, feces and dietary intake of Spanish subjects aged 45-65 years

E. Rodríguez-Rodríguez[1], R. Estévez-Santiago[2], B. Beltrán-de-Miguel[3], M. Sánchez-Prieto[2], B. Olmedilla-Alonso[2]

[1]*Faculty of Pharmacy, Department of Chemistry in Pharmaceutical Sciences, 28040 Madrid, Spain*

[2]*Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Department of Metabolism and Nutrition, 28040 Madrid, Spain*

[3]*Faculty of Pharmacy, Department of Nutrition and Bromatology I, 28040 Madrid, Spain*

Lutein and zeaxanthin accumulate in retina (macular pigment) and, an adequate nutritional status (serum concentrations, macular pigment optical density [MPOD]) is associated with a lower risk of age-related macular degeneration. In a recent study, the MPOD showed age-specific correlations and is influenced by the presence of lutein+zeaxanthin in relation to circulating lipids (in subjects aged 45-65 y but not in those aged 25-35y) (Olmedilla-Alonso et al., Nutr. J. 2014). Here we present preliminary data of a project to assess the lutein and zeaxanthin dietary intake effect on the MPOD and the relations between intake, status and visual function. Lutein and zeaxanthin concentrations were analyzed in serum (n=104), feces (n=18), dietary intake (n=50) and MPOD (n=104) in subjects aged 45-65 y (150 included in the cross-sectional study). Inclusion criteria: cholesterolemia (<250mg/dl), BMI (<30kg/m^2), varied diet, no dietary supplements intake, no drugs/foods to lower cholesterol, no chronic diseases. Lutein and zeaxanthin in fasting blood and faeces were analyzed by HPLC, MPOD by heterochromic flicker photometry (MPS 9000 desktop device). Dietary intake by three 24 h recalls and a carotenoid database and software (Beltrán et al., Nutr. Hosp.2012; Estévez-Santiago et al., Nutr. Hosp.2013). The concentrations, expressed as mean±SD (median), were: in serum 16.8±7.2 (15.8) lutein and 3.8±2.3 (3.1) zeaxanthin µg/dl; in feces: 131 ±207 lutein and 258 ±252 zeaxanthin ug/100 g dry weight. Dietary intake (µg/day): lutein+zeaxanthin: 1634±1591 (1065). MPOD (density units, n=208): 0.33 ±0.15 (0.34). Significant correlations (Spearman's ρ, *p* value): MPOD–serum: Lutein (0.183, *p*=0.008), zeaxanthin (0.223, *p*=0.001), lutein+zeaxanthin (0.202, *p*=0.004), lutein+zeaxanthin/cholesterol (0.212, *p*=0.002). MPOD-dietary intake: no significant correlations were found. Data from the present study will contribute to the establishment of normal /reference serum lutein and zeaxanthin concentration and MPOD ranges for subjects aged 45-65y, and, with data on feces and with dietary intake, to the assessment of their apparent bioavailability.

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OP-49

Production of carotenoids from *Blakeslea trispora*: achievements and challenges

F. Mantzouridou

Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

The world market for carotenoids reached 1.24 billion dollars in 2016 and is projected to reach 1.53 billion dollars in 2021, highlighting them as an emerging market with very good growth prospects and many business opportunities. 95% of the world market for carotenoids is achieved through synthetic routes using petrochemical sources. The growing consumer demand for food products using natural colorants (natural carotenoids) has led to a significant growth in this market (an average annual rate of ~ 4% for the period 2016-2023), while natural carotenoids are mainly produced from plants and only 1% by microorganisms. As compared to the recovery of natural carotenoids from plant sources, their microbial production has been increasing due to factors such as the possibility of using low or even no-cost substrates, designation of natural substances, small area required for production and controlled operation, and decoupling from environmental conditions. For instance, lycopene was priced at over $6,000 per kg in 2007 making its market expansion intrinsically difficult. The high cost is due to the low efficiency of conventional extraction processes with food-grade organic solvents and the need of pesticide-free fresh tomatoes grown with organic farming. The production of microbial lycopene is expected to be economically more favorable compared to that derived from tomatoes. *Blakeslea trispora* is one of the most promising and economically attractive sources of natural products. Key products are β-carotene and lycopene. In this work, the factors inducing carotenoid accumulation in *Blakeslea trispora* and the knowledge acquired on the metabolic pathways responsible for fungal biosynthesis achievements are outlined. The challenge of the development of a fungal micro-factory for sustainable production of economically competitive carotenoids is also discussed.

OP-50

Relation between fruit density and β-carotene content in ripe mango

S.Hor[1,2], S.Avallone[1], H.Mith[2], M.Lechaudel[1,3], C.Bugaud[1,4]

[1]Qualisud, Montpellier SupAgro, CIRAD, Univ d'Avignon, Univ Montpellier, Univ de la Réunion, Montpellier, France
[2]Faculty of Chemical Engineering and Food Engineering, Institute of Technology of Cambodia, Phnom Penh, Cambodia
[3] CIRAD, UMR Qualisud, F-97130 Capesterre-Belle-Eau, Guadeloupe, France
[4]CIRAD, UMR Qualisud, F-34398 Montpellier, France

β-carotene content is one of the main factors that determines the nutritional quality and orange color of ripe mango fruit. It is known as the pro vitamin A, and it is generally the predominant carotenoid in ripe mango. Currently, fresh fruit sector demands a reliable non-destructive indicator to better predict the nutritional quality of ripe mango, and especially in terms of β-carotene content. Fruit density has been applied in horticulture sector to predict dry matter or maturation stage of mango. It would interesting to know if it can be a relevant non-destructive indicator of the carotenoid content in fruits Therefore, the aim of this study was to identify the relationship between mango density and β-carotene content at the ripe stage. Mangoes (cv. 'Kent'), from Ivory Coast, Peru, and Brazil were selected from a mango importer in France at a green mature stage (day 0). Then, all mangoes were ripened at 18 °C and 80% of relative humidity. Color of ripe mango pulp and β-carotene contents were assessed 11 and 15 days after (day 0) corresponding to a ripe stage of consumption. Fruit density was significantly correlated to the pulp color and β-carotene contents of ripe mangoes, whatever the origin (p-value < 0.05). Mango fruits with a high density were characterized by high value of Chroma, lower value of Hue angle, and high contents in β-carotene. β-carotene content was found in range of [92-307 $\mu g.100g.FM^{-1}$] for low density mango, and in range of [365-924 $\mu g.100g.FM^{-1}$] for high density mango. So, fruit density, measured at the green-mature stage, could be a reliable indicator to predict the nutritional quality of mango fruit at the ripening stage, which is extremely useful for fresh fruit sector for grading or sorting mango fruit early in the supply chain.

OP-51

The bioreactor system for the astaxanthin producing microalgae *Haematococcus pluvialis:* construction, quantification and determination of the astaxanthin

V.J. Grujić[1], Z. Molnar[3], T. Todorović[4], J. Ambrožič-Dolinšek [1,2]

[1]*University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Koroška cesta 160, SI-2000 Maribor, Slovenia*
[2] *University of Maribor, Faculty of Education, Department of Elementary Teacher Education, Koroška cesta 160, SI-2000 Maribor, Slovenia*
[3]*Szechenyi Istvan University, Faculty of Agricultural and Food Sciences, Department of Plant Sciences, H-9200 Mosonmagyarovar, Hungary*
[4]*University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia*

The purpose of the research was to establish the astaxanthin producing bioreactor system for the cultivation of the growth demanding microalgae *Haematococcus pluvialis.* We designed a flexible laboratory bioreactor system with the ability of automatic regulation of the environmental factors such as CO_2 flow, pH, temperature, the intensity and wavelength of LED light sources. To obtain as possible as high producing line for further studies, we screened biomass and astaxanthin production of different microalgal strains, obtained from CCALA (Culture collection of Autotrophic Organisms, http://ccala.butbn.cas.cz/en). We observed statistically significant differences in biomass production among different strains and not statistically significant differences in the astaxanthin production. The highest yielding strain S4 produced 1462 mg L^{-1} of dry weight and the highest (41 mg L^{-1}) astaxanthin content. The strain S4 was determined as the most appropriate for further research of the influence of environmental factors on the biomass production and for the research of influence of stress or elicitation on astaxanthin production. Currently we are testing different cell disruption methods, stabilization and extraction methods and trying to evaluate the diversity and content of the carotenoid compounds in alga *H. pluvialis* using Q-TOF Mass Spectrometer analysis.

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OP-52

Tonoplast transporters mediate vacuolar accumulation of crocins in saffron stigmas

O.C. Demurtas[1], R. de Brito Francisco[2], G. Diretto[1], P. Ferrante[1], S. Frusciante[1], M. Pietrella[1,3], G. Aprea[1], L. Borghi[2], M. Feeney[4], L. Frigerio[4], A. Coricello[5], G. Costa[5], S. Alcaro[5], E. Martinoia[2*], G. Giuliano[1*]

[1]ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, C.R. Casaccia, 00123, Rome, Italy
[2]Department of Plant and Microbial Biology, University of Zurich, 8008 Zurich, Switzerland
[3]Council for Agricultural Research and Economics (CREA), Research Center for Olive, Citrus and Tree Fruit, 47121 Forlì, Italy
[4]School of Life Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom
[5]Department of Health Sciences, Magna Græcia University of Catanzaro, 88100 Catanzaro, Italy

The saffron spice is composed of dried stigmas of the plant *Crocus sativus*. It owes its red color to crocins, apocarotenoid glycosides that accumulate in the intracellular vacuoles and reach up to 10% of the spice dry weight. We have previously shown (Frusciante et al., 2014; Demurtas et al., 2018) that their synthesis starts in the chromoplast through the cleavage of zeaxanthin by CCD2, and proceeds in the ER through dehydrogenation by ALDH3I1 and in the cytosol through glycosylation by UGT74AD1. In this work, we elucidate the last step of crocin biosynthesis: transport into vacuoles.

OP-53

Lycopene extract incorporation in microemulsions for skin bioavailability enhancement

S.Simões[1], A. Costa[1], A. Ascenso[1], J. Marto[1], M. Carvalheiro[1], L.M. Gonçalves[1], M. Marques[2], A. Paiva[2], M. Bento[3], P. Simões[2], H.M. Ribeiro[1]

[1]iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[2]LAQV-REQUIMTE, Chemistry Department, FCT/Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[3]Italagro SA, Lezíria das Cortes, 2600-634 Castanheira do Ribatejo, Portugal

Lycopene is a natural carotenoid present in red-colored fruits and vegetables. Due to its well-known antioxidant properties, this compound is being used in skin care formulations. However, lycopene topical formulations are quite challenging as this lipophilic molecule tends to accumulate on the outermost layer of the skin without reaching the viable epidermis. In this work, lycopene-enriched extracts (LEE) from tomato processing industry residues were obtained by CO2 supercritical extraction. To assess the biosafety of the LEE, a cell bioavailability assay (MTT) was performed in human keratinocytes (HaCaT cells) and the capacity of LEE to reduce the ROS production was evaluated using HaCaT cells exposed to H2O2 or irradiated with a UV-B single dose. LEE showing no toxicity and presenting antioxidant activity were formulated in microemulsions comprising a blend of monoacylglycerol and diacylglycerol as the oily phase, with the aim of developing a topical dosage form able to supplement the skin with lycopene. Polyoxylglyceride was selected as the surfactant agent at different concentrations (2.5-40% total weight), and combined with a co-surfactant and purified water. After adding the oily phase to aqueous phase and stirring for 15 mins at room temperature, the formation of a spontaneous transparent and isotropic microemulsion was observed at surfactant concentration higher than 30%. The formed microemulsions showed <50 nm droplet size, monodisperse population, and a pH approximately 6.5. These microemulsions presented physical stability. Incorporation up to 2% (w/w) LEE was obtained by dissolving it in the oily phase. Lycopene microemulsions were tested for skin delivery using a Franz cell permeation assay and tested formulations remarkably increased lycopene penetration in the viable epidermis using new born pig skin as the biological membrane. These results demonstrate the skin lycopene delivery using LEE incorporated in microemulsions.

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OP-54

Rapid determination of carotenoids in different pumpkin species by applying combined extraction and saponification procedure and separation via supercritical fluid chromatography

D. Segliņa[1], I. Mišina[1], K. Majore[1], I. Tauvēna[2], P. Górnaś[1]

[1]Institute of Horticulture, Graudu Street 1, Ceriņi, Krimūnu distr., Dobele reg., LV-3701, Latvia
[2]Riga Stradinš University, Dzirciema Street 16, LV-1007, Latvia

In the current time, rapid, simplified and environmentally friendly techniques are required for the determination of bioactive compounds, such as carotenoids, in the food and plant material. To meet these demands, the extraction and saponification procedure were combined, while carotenoids separation was performed via supercritical fluid chromatography (SFC). Carotenoids in the edible parts of twenty-one genotype samples of three pumpkin species (*Cucurbita moschata*, *Cucurbita pepo*, and *Cucurbita maxima*) were studied. Different variants of carotenoids extraction and saponification, including a combination of these two procedures, and ultrasounds treatment, were studied. The separation was done via SFC system due to the environmental and speed reasons. The combination of extraction and saponification procedures led to a significant reduction in the consumption of solvents and time required. Sthe obtained results of the developed method were similar to the recommended assays for carotenoid determination. The application of the SFC system with UV diode array detection (DAD) resulted in the rapid separation of all carotenoids detected in the pumpkin samples with a total analysis time below 5 min. The SFC-DAD approach was precise, accurate, and most importantly environmentally friendly compared to the liquid chromatographic methods. The principal component analysis, allowed to differentiate the analyzed pumpkin genotypes in terms of the predominant type of carotenoid. The optimized method consisting simultaneously an extraction and saponification along with SFC separation were successfully applied for the carotenoids determination in all pumpkin samples.

OP-55

Carotenoids reduces fat food digestibility-implications for health and nutrition

I.M. Petyaev

Lycotec Ltd., Cambridge, United Kingdom

Fifty percent of the world popluation are overweight or obese, conditions which are one of the main factors responsible for the continuing spread of cardiovascular diseases and diabetes. Consumption of refined oils is the one of the main sources of excessive calorie intake. The refining process of pressed plant oils, essential to remove proteins and carbohydrates, is important to improve their particular cooking properties. However, this process also removes carotenoids, which are always present in plant lipids. As a result of this, lipid droplets of refined oils become very small and easy to digest. Reintroduction of carotenoids back to the oils leads to a significant increase in the size of these droplets and consequently to a reduction in their rate of digestion. When carotenoids are absent, or reduced, in the diet of dairy animals, their concentration in the body and in their milk is very low. As a result of this, the size of the lipid droplets of the butter is significantly smaller than the butter from green-grass fed milk. Introduction of carotenoids to the former results in an increase in the size of these droplets and hence reduction in their digestibility. In clinic we demonstrated that ingestion of plant or animal fat products with increased lipid droplet size, restoring them to their unrefined or green-grass fed level, resulted in reduced postprandial lipidaemia. Moreover, this positive effect, repeated on a daily basis for 4 or 8 weeks replacing conventional fat food with low digestibility fat products, translated into a significant reduction in the blood triglyceride and cholesterol in volunteers who had an elevated level before the studies. To conclude, reintroduction of carotenoids to restore health beneficial properties of unrefined oils or green-grass fed dairy products would help to develop new functional foods with lower lipid digestibility, hence contributing to a reduction of overall calorie intake.

OP-56

Carotenoids from traditional, new and sustainable sources

G. Di Lena, M. Lucarini, J. Sanchez del Pulgar, G. Lombardi-Boccia

CREA - Research Center for Food and Nutrition, Via Ardeatina 546, Rome, Italy,

Among the carotenoids found in nature, only β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin represent more than 95% of the blood carotenoids. Fruits and vegetables are the main sources of carotenoids in the human diet. Nowadays great attention is focussed on the recovery from agri-food waste of nutrients and molecules of interest for new food applications and various industrial purposes. In this study some traditional, new or sustainable sources of carotenoids are compared. The example of pistachios (*Pistacia vera,* L.) as a traditional source of carotenoids and of six commercial microalgae (*Porphyridium cruentum, Isochrysis galbana, Phaeodactylum tricornutum, Tetraselmis suecica, Nannochloropsis gaditana and Arthrospira platensis*) as sustainable sources are reported. Furthermore, in the framework of the H2020 BBI-JU EXCORNSEED Project, the potential of sidestreams from biofuel biorefineries, i.e. corn oil and thin stillage from corn bio-ethanol production plants, as new sources of carotenoids, are presented. Carotenoids were analyzed by HPLC-PDA using reverse-phase C-18 and C-30 columns after solvent extraction. Pistachio was rich mainly in lutein (1.33 mg/100 g d.w.), followed by β-carotene (0.19 mg/100 g d.w.). Neolutein b was the main isoform in pistachio, while fucoxanthin, violaxanthin, lutein, zeaxanthin, α-carotene and β-carotene were the most represented carotenoids in microalgae. Levels of α-carotene and lutein in *T. suecica* (41.8 and 85.4 mg/100 g d.w., respectively), β-carotene and violaxanthin in *N. gaditana* (100.1 and 336.7 mg/100 g d.w.), zeaxanthin in *P. cruentum* and *A. platensis* (94 and 108 mg/100 g d.w.) compared favourably to that of traditional vegetable sources, expressed on a dry weight basis. Lutein and zeaxanthin were the main carotenoids present in corn-bioethanol co-products, confirming the advantage to recover them at industrial level through an integrated process of green technologies. Carotenoids were present in large amounts in new and sustainable sources, comparable to that of traditional dietary sources, highlighting their potential for the functional food and nutraceutic markets. The high concentration and variety of carotenoids in microalgae make them a valuable source to be exploited with a biorefinery approach, where several valuable compounds are valorized. Furthermore, the recovery of lutein and zeaxanthin from corn bio-ethanol co-products for specific market applications represents an example of a new bio-based value-chain from sustainable feedstocks.

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