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## **Pigments from microalgae**

*a new perspective with emphasis on phycocyanin*

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**Book of Abstracts and Proceedings**  
of the  
**7<sup>th</sup> International Congress on  
Pigments in Food**

***18-21 June 2013,  
Novara, Italy***



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**Book of Abstracts and Proceedings of the  
7<sup>th</sup> International Congress on Pigments in Food**

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## Preface

On behalf of the Organizers of the Seventh International Congress PIGMENTS IN FOOD, it is a pleasure to welcome all of you at the Dipartimento di Scienze del Farmaco, Novara (Università degli Studi del Piemonte Orientale "A. Avogadro").

After six successfully organized congresses, starting in Sevilla, Spain (1999) and passing through Lisbon, Portugal (2002), Quimper, France (2004), Stuttgart-Hohenheim, Germany (2006), Helsinki, Finland (2008), Budapest, Hungary (2010) the seventh event be held in Novara (2013), a beautiful town located in northern Italy, beside the Lake District of Piedmont.

The most important aim of "Pigments in Food" is to offer a possibility for meeting and discussion for scientists dealing with different aspects of food pigments, such as pigment chemists, food chemists, food technologists, agriculturists, nutritionists, but also industry people from all over the world. The "natural pigments" science is developing worldwide, particularly concerning technological novel solutions for foods and food supplements, and under the meaning of the "healthy functional properties".

A "comprehensive" scientific approach is particularly strategic, in order to discover, characterize and design new performing and functional pigments from natural food sources. Cool and charming topics like isolation of pigments from sustainable sources using sustainable "mild" techniques, novel technologies development for pigments stabilization, pigments stability bioactivity and functionality, regulatory affairs are the object of this edition of the Conference. The capacity to exploit new technological strategies and alternative food sources (also considering new promising microorganisms, like microalgae) increases more and more the interest towards this field of food science. Beside the scientific aspect of the Congress, hoping to share this Event with a significant number of Scientists from Academia and technicians from Industry, we really hope to host our guests in our beautiful Italian Region, offering nice coloured (and tasty) food ...

Pigments in Food VII 2013: a coloured vision on coloured food, quality and safety, for new functional foods with healthy profiles.

On behalf of the Organizing Committee  
**Marco Arlorio, Chair**

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# Session 1

## *Chemistry and Biochemistry*



## Plenary A

### NATURAL CAROTENOIDS: A STUDY IN OILS AND WATER COLOURS

#### Britton G.

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Colour has always been important in human life and culture. Brightly coloured foods are attractive to the eye and the bright colour is considered a sign of quality and freshness. This colour is provided by many different classes of chemical substances, pigments, among them the carotenoids, which are widespread and familiar in yellow, orange and red vegetables and fruits such as carrots, oranges and tomatoes, and in seafood.

We know the structures, we know the chemical and physical properties of the molecules and how these properties may be modified and the carotenoid protected and stabilised in natural foods *in vivo* by the molecular environment and interactions.

An important aspect of the large-scale production of manufactured foods now is to reproduce the colours of natural food by adding colouring materials during manufacture and processing, in the form of natural extracts, isolated compounds, synthetic dyes, or nature-identical colorants produced by chemical synthesis. The use of carotenoids for this poses particular challenges: they are, insoluble in water, not very soluble in vegetable oils, and susceptible to oxidative degradation, aggregation and crystallisation, leading to colour instability. Understanding the properties of the carotenoid molecules allows these difficulties to be overcome so that carotenoids are now used extensively as colorants, not only in oil-based applications but also in forms that allow dispersion in water so that they can be used for colouring drinks and other water-based products. The physical state or formulation also influences bioavailability of the carotenoids and their efficiency as health-promoting substances.

## Plenary B

### DIFFERENCES IN ANTHOCYANIN CONTENT OF FOOD AND NATURAL SOURCES CORRELATED WITH DIFFERENCES IN ANTHOCYANIN CHEMISTRY AND PROPERTIES

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*If* intake of anthocyanins has positive health effect(s) and *if* the various anthocyanins or their derivatives in the human body have different properties, then of course both the qualitative and quantitative anthocyanin content of our food as well as the *individual chemistry* of these compounds should be more closely correlated.

The major aim of this presentation is to show how the anthocyanins in fruits, vegetables and products thereof vary substantially with respect to structures and quantities, with serious impact on differences with respect to anthocyanin reactivity, stability and bioavailability, including formation of anthocyanin degradation products and phase II metabolites. We will show accurately that there exist a distinct difference between the anthocyanin content in vegetables and fruits of our diet, at least with respect to aromatic acylation and number of monosaccharide units. Thus, if anthocyanins or their derivatives have impact on our health, we have to design our diet with respect to choice of fruits and vegetables in a far more precise way than '5 A Day' to obtain optimum effects!

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## CAROTENOID ESTER PROFILES IN *SOLANUM TUBEROSUM* AND *SOLANUM PHUREJA* CULTIVARS

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Tubers of *Solanum* species are important staple foods and a continuous source of antioxidant pigments like carotenoids<sup>[1]</sup>.

We will present several profiles of free and esterified carotenoids found in the old *Solanum tuberosum* variety Shetland Black, the new breed Red Laura, and the *Solanum phureja* cultivars Mayan Gold and Mayan Twilight. Breithaupt and Bamedi <sup>[2]</sup> elucidated carotenoid ester profiles of potatoes available in Germany, showing the absence of carotenoid monesters. Our results, however, proof the occurrence of carotenoid monoesters and diesters in our cultivars. We identified lutein and zeaxanthin esters as well as their respective fatty acids in the all investigated varieties. Neoxanthin and violaxanthin predominantly occur in esterified forms as well. Lutein, however, is predominantly present in non-esterified form in our tubers.

The carotenoid ester patterns are different and typical for each *Solanum* species and variety investigated. Thus our results may also be used for authenticity considerations of raw and processed food and foodstuff based on these *Solanum* tubers.

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## INTRAMOLECULAR AND INTERMOLECULAR FACTORS AFFECTING THE DEGRADATION KINETICS OF XANTHOPHYLL ESTERS

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The kinetics of esterified xanthophylls degradation can depend on the structural features of the pigment, its intramolecular environment composed by fatty acids of different nature, and the intermolecular surrounding where the pigment is dissolved. In this study degradation of either free or esterified xanthophylls ( $\beta$ -cryptoxanthin, zeaxanthin, capsanthin and capsorubin) was monitored at four temperatures in two different environments (oil and oil-in-water emulsion) and considering that reaction can follow either zero or first order model to obtain kinetic and thermodynamic parameters. Results show that zero order model describes data from the oily environment while data from the oil-in-water emulsion fit to a first order kinetics. Free capsanthin and capsorubin are more stable than  $\beta$ -cryptoxanthin and zeaxanthin in oily media but when xanthophylls are emulsified in water differences in stability among pigments are vanished. This scenario changed for xanthophyll esters because there were not found significant differences in the stability of a pigment just changing the nature of the esterifying fatty acid. Thus, in the oily environment, capsanthin and capsorubin esters showed a degradation pattern not related to their esterification nature while kinetic constants of  $\beta$ -cryptoxanthin and zeaxanthin esters were different. However in the oil-in-water emulsion the intramolecular environment of any xanthophyll esters reached a higher significance in the kinetics, being responsible for an increased degradation rate. Considering the length of carbon chain of the fatty acid(s) esterifying the xanthophylls it was possible to establish it as an influencing factor, with negative consequences on the degradation profile of xanthophyll esters.

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## **ANALYTICAL AND TECHNOLOGICAL ASPECT OF CAROTENOIDS FROM RED-BELL PEPPERS**

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Ripe fruits of either vegetable or spice red-bell pepper are a good source of nutritionally important carotenoids. The attractive red colour of red-bell peppers is due to a diverse composition of several yellow-and red-coloured carotenoids, which occur esterified with fatty acids in form of mono- and di-esters. The content, composition and stability of carotenoids determine to a high extent, the quality of red pepper products and the acceptance of consumers towards them. Recent in-vivo or in-vitro epidemiological and chemotherapeutic studies confirmed cancer chemo-preventive activity of carotenoids from red-bell peppers.

Many chromatographic methods have been worked out and developed for the separation and determination of Carotenoids from red-bell peppers. In some of those methods the extract of fruits is simplified by alkaline hydrolysis of fatty acid esters and applied to a separation on reversed-phase adsorbent with gradient elution. In the other methods un-hydrolysed extracts were fractionated to their individual carotenoids by separation on reversed-phase column with analytical dimensions using gradient elution.

In the present work the recent advances in the analysis of carotenoids and carotenoid esters are described. Some methods were developed to ensure simultaneous, one-run analysis of naturally occurring and added carotenoids or synthetic dyes. In such methods analytical columns having adsorbents of 3 µm particle size were used with optimised gradient elution conditions that provided excellent separation of free xanthophylls, mono-esters, carotenes, di-esters and contaminating pigments or dyes.

The most recent development was in the application of reversed-phase material having cross-linked end-capping with high steric activity and stability to separate hydrolysed carotenoid extract of red-bell peppers under specific conditions similar to those of ultra-performance liquid chromatography. The run time of complete analysis was decreased from 40 min with conventional RP column to 16 min with cross-linked column.

Also included in this work is content of carotenoids in spice red pepper hybrids cultivated under plastic house conditions and their stability to different drying temperatures. In addition to many agronomic characteristics of some new hybrids, their carotenoid content and stability were significantly higher than those of the parent varieties.

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## ANTHOCYANIN-SYNTHESIZING TOMATO GENOTYPE 'SUN BLACK'<sup>TM</sup>, AS PRINCIPAL INGREDIENT FOR A NEW FUNCTIONAL TOMATO SAUCE

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'Sun Black'<sup>TM</sup> is a trademark protected tomato line characterized by a remarkable phenotype with deep purple pigmentation in the pericarp, due to an increased level of anthocyanins on the peel [1, 2]. Such line has been obtained by the combination of the *Anthocyanin fruit (Aft)* allele from *Solanum chilense* (a gene increasing the anthocyanin content of the fruit) with *atviolaceum (atv)* from *S. chesmaniae* (an allele enhancing the anthocyanin presence on stem and leaves). 'Sun Black' is therefore a breeding product, not a GMO product as in [3].

The anthocyanin pigments accumulate in the fruit epidermis, particularly the side much exposed to the sun. Anthocyanins have been extracted from the peel of ripe 'Sun black' tomatoes by ethanol acidified with 2% formic acid. At the HPLC analysis, while in the control tomato line there is no anthocyanin presence, in the 'Sun black' extract there are several peaks corresponding to delphinidin, petunidin and malvidin aglycones, differently glycosylated and acylated.

In order to increase the beneficial effect of consuming tomato sauce, we have planned to produce tomato sauce from 'Sun Black' tomatoes, obtaining thus a 'functional tomato sauce' with added nutraceutical value due to the anthocyanin presence.

The HPLC analysis of 'Sun Black' tomato sauce revealed the presence of anthocyanin molecules, even after pasteurization process.

The ORAC value of 'Sun Black' extract (peel or whole fruit) and ORAC value of 'Sun Black' tomato sauce extract is reported.

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## STUDIES ON COUPLING REACTIONS OF PROANTHOCYANIDINS AND MALVIDIN-3-O-GLUCOSIDE IN A WINE-LIKE MODEL SOLUTION SYSTEM

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Reactions involving wine polyphenols are seen as the key reactions changing the chromatic characteristics of red wines over storage time. Acetaldehyde, a reaction product occurring in course of wine oxidation, is in this context known to change color intensity and color hue, as it accelerates coupling reactions between anthocyanins and tannic structures to form polymeric pigments such as acetaldehyde-bridged anthocyanin-flavanol-adducts. Many of these pigments have been characterized by their absorption spectra. However, most studies focused solely on the products resulting from the reaction between one specific flavanol and one specific anthocyanin. Moreover, none of the studies hitherto have focused on the formation and decay of those products over time. In the presented study, a wine-like model solution containing different tannic structures extracted from grape skins or grape seeds and malvidin-3-O-glucoside was monitored over a time period of 45 days and analyzed by different chromatographic and spectrophotometric methods. The model wines were either spiked with acetaldehyde or not and were stored at different pH values. UV/Vis-spectroscopy was applied to measure the time-dependent changes in color intensity, color hue and color contribution of polymeric pigments. HPLC-DAD analysis was carried out to monitor the decreasing precursor compounds. LC-QToF-MS analysis was used to screen for the reaction products over time including potentially unknown anthocyanin-flavanol-adducts such as the 8-6-acetaldehyde-bridged malvidin-3-O-glucoside-catechin-dimer. Time elapsed regression fits were applied suggesting that the commonly known acetaldehyde-bridged anthocyanin-flavanol-adducts were not stable under wine conditions. However, subsequent derivatization reactions may finally lead to products with a higher stability, as seen for some hydroxyethyl derivatives.

## POST-HARVEST MODIFICATIONS ENHANCE THE ZEAXANTHIN CONTENT IN VEGETABLES

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Humans are subjected to oxidative stress and in order to counteract it, they are obliged to consume antioxidants and carotenoids of plant origin. Indeed, numerous studies show the need to include high amounts of the carotenoid zeaxanthin (Z) in the diet due to the fact that it has been negatively correlated to the development of age related macular degeneration [1] which leads to irreversible loss of vision. Enhancement of Z content would thus be a desirable trait to incorporate into crops in order to improve the nutrient intake. However, in green edible vegetables after harvest, Z is usually found in low amounts. In order to improve this, the aim of the present study was to increase the Z content in green vegetables by post harvest modifications. We found that light exposition before cooking and vinegar dressing in spinach and rocket respectively increased more than 3-fold the initial content of Z. On the other hand, dehydration speed could be critical for Z content in plants destined for storage as dry material such as parsley. Findings from this study revealed several post harvest treatments that can increase the nutritional value of food. Easy recommendations for improving food both to industry and to the common household quality can be derived from this finding.

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## DESCRIPTION OF A NEW CHLOROPHYLL CATABOLITE IN RIPENED FRUITS OF QUINCE (*Cydonia oblonga*, Mill.)

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Senescence means structural modifications on chlorophyll molecule producing terminal chlorophyll catabolites that accumulate in vegetal vacuoles. Since they were first described in 1991, only 13 different structures have been described to date. In this work we describe a new chlorophyll catabolite in ripened fruits of quince (*Cydonia oblonga*, Mill.) named *de-Zm-NCC1*. This was accomplished with an easy, rapid and reliable characterization of chlorophyll catabolites by a HPLC/ESI-TOF-MS method. High selectivity is achieved with the use of data post-processing software tools considering accurate mass, isotopic pattern and MS-MS fragmentation profile. We exploited the high selectivity of the instrument and software algorithm capabilities to show that a heterogeneous profile arose from the chlorophyll breakdown pathway, with different possible re-functionalizations reactions of a common structural precursor of chlorophyll catabolites. Screening was not the solely strategy for chlorophyll catabolites determination as we included in the target database elemental composition of either de-esterified or esterified chlorophyll catabolites with a methyl group at the C13<sup>2</sup> position for those structures in which such possibility has not been described to date. Consequently, our method proved to be a straightforward tool for screening of chlorophyll catabolites in vegetal tissues and for searching new structures expanding knowledge of chlorophyll catabolism routes.

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## RELATIONSHIPS AMONG FLAG LEAF CHLOROPHYLL CONTENT, AGRONOMICAL TRAITS, AND SOME PHYSIOLOGICAL TRAITS OF WINTER BREAD WHEAT GENOTYPES

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In this study, relationships among flag leaf chlorophyll contents of some winter wheat genotypes, agronomical traits, and some physiological characters such as canopy temperature, membrane thermostability, membrane injury, and relative water content of flag leaf were evaluated. The study was conducted in the Application and Research Area of Siran Vocational School of Gumushane University in the growth season of 2010-2011. Chlorophyll content of genotypes were measured by a portable chlorophyll meter at the start of anthesis (ZGS 60) and the early milky stage (ZGS 73). The mean chlorophyll content of the tested genotypes at ZGS 60 was 45.6 as SPAD unit, and ranged from 39.1 for line 51 to 54.0 for line 42. Chlorophyll content as the mean of all genotypes at ZGS 73 was 41.8 as SPAD unit. Mean chlorophyll content of the genotypes at this growth stage ranged between 35.2 for line 27 and 50.9 for line 44. The mean pigment loss was the percent of 8.3 as an average of all genotypes. The chlorophyll loss ranged between 1.7 % for line 75 and 19.2 % for line 32. The statistically significant correlations between chlorophyll contents and main yield components like grain number per spike and spike yield were obtained at both measuring stages. The significant correlation between chlorophyll loss and chlorophyll content was positive at ZGS 60, but negative at ZGS 73. These results show that determination of flag leaf chlorophyll content in winter wheat is important selection criteria for yield components in breeding programs.

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## OXIDATION ROUTES FOR BETACYANINS

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Betanin (5-O-glucosylated betanidin) is one of betacyanins, which are a group of natural, water soluble and non-toxic red-violet plant pigments. Because of the presence of a few functional groups in betanin structure, it is highly reactive and very sensitive to oxidation and factors such as increased temperature, presence of organic solvents and metal ions as well as low and high pH. Recent studies have shown importance of research on betacyanins oxidation paths, because of their high natural, antiradical and antioxidant activity and potential benefits to human health.

In this contribution, the identification of the products of betanin and its decarboxylated derivatives oxidation by ABTS cation radicals and horseradish peroxidase (HRP) in aqueous solutions at pH 3-8 is presented. The effects induced by these two different oxidizing agents were monitored by spectrophotometry and LC-DAD-MS/MS.

In general, the oxidation most probably results in a generation of quinonoid derivatives of the pigments at the first stage [1]. If oxidation of a betacyanin results in a formation of a semiquinone radical, it should undergo a subsequent oxidation resulting in a formation of its quinone methide intermediate and rearrangement to 2,3-dehydrogenated betacyanin as the most probable product since the formation of the aminochrome intermediate is impossible, because of the blocking hydroxyl at C-5 [1]. Therefore, one of the most frequently detected betanin and 2-decarboxy-betanin oxidation products is 2-decarboxy-2,3-dehydro-betanin.

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## **Session 2**

### ***Technology, Biotechnology and Processing***



## *Plenary C*

### **ARTIFICIAL INTELLIGENCE: IMPROVING THE COLOR MEASUREMENT**

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The overall appearance of any object is a combination of its chromatic and geometric attributes. The color is the first sensation that the consumer perceives and uses as a tool to accept or reject, because the color observation allows the detection of certain anomalies or defects of a product. Commercially available color-measuring devices are designed to contact the material for measurement. Since they measure a small area with a fixed geometry, repetitive measurements are necessary to increase accuracy for the mean color information of a heterogeneous food product. Computer vision based image analysis is a non-contact alternative technique taking the whole surface into account while measuring color. It does not only give mean information in any color space (Lab, RGB, XYZ), but also provide featured information such browning ratio for a product. In this presentation, basic principles of digital image analysis to obtain mean and featured color information from an object are discussed. Using custom-designed algorithms, potential applications of computer vision are exemplified for color measurements in various raw and processed foods.

**Keywords:** Color measurement, image analysis, artificial intelligence, mean color, featured color

## Plenary D

# MICROWAVE AND ULTRASOUND ASSISTED FOOD PIGMENTS EXTRACTION: HIGHLY EFFICIENT REACTORS FOR GREEN, SUSTAINABLE PROCESSES

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The design of efficient and sustainable extraction methods for vegetal matrices has been a hot research topic over the last decade. In spite of the scanty efficiency, maceration still remain the most common extraction technique also in industrialized countries. Conventional extraction processes are quite laborious, time consuming, involve large amounts of solvents and, ultimately, may cause some target molecule degradation. Great improvements can be achieved with the use of non-conventional techniques such as microwave [1] and ultrasound-assisted extraction [2]. The industrial production of natural colouring pigments, requires a technological innovation to improve extraction yield and minimize fading and degradation. With the aim to obtain extracts and pigments in high yield and outstanding quality, we developed several methods and equipments suitable for scaling up. These green extraction techniques applied to medium and large scale, possibly in flow-reactors, may lead to effective process intensification and also a carbon footprint reduction [3].

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## INFLUENCE OF SOME OAK WOOD COMPONENTS ON STABILITY OF MALVIDIN-3-GLUCOSIDE AND CHROMATIC CHARACTERISTICS IN MODEL WINE SOLUTIONS

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Many constituents can be extracted from staves during wine aging in barrels. The evolving environment inside oak barrels during the maturation of wines provides conditions for further reactions involving wood compounds and wine phenolic compounds.

The aim of the current study was to use model red wine solutions (12% ethanol and adjusted to pH 3.5) to evaluate the influence of furfural, eugenol, guaiacol, vanillin, ellagic acid and oak wood extracts on the changes in the levels of malvidin-3-glucoside and chromatic characteristics over a period of 64 days. The compounds were used in concentrations which are similar to the levels that occur in wine during aging in barrels. Malvidin-3-glucoside and ellagic acid were quantified by HPLC [1,2]. Furfural, eugenol, guaiacol and vanillin were quantified by GC [3]. Chromatic characteristics were calculated using CIELAB parameters.

The results showed that the decrease in malvidin-3-glucoside was more pronounced in the presence of ellagic acid and oak wood chip extracts. After 64 days, when incubated alone, the malvidin-3-glucoside content was 30.0 mg/L, and this fell to 21.0 mg/L in the presence of the oak extract and 19.0 mg/L when incubated with ellagic acid. Breakdown of malvidin-3-glucoside was also slightly more pronounced in the presence of guaiacol, furfural, vanillin and eugenol. Changes in the levels of furfural, guaiacol, eugenol and vanillin are characterized by a continuous steep decline throughout the storage period with no significant influence of malvidin-3-glucoside. For chromatic parameters,  $a^*$  values showed a more evident decrease in solutions containing malvidin-3-glucoside and oak wood extracts.

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## STABILIZATION OF ANTHOCYANIN–METAL CHELATES WITH HYDROCOLLOIDS FOR THEIR APPLICATION AS BLUE FOOD COLORANTS

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Recently, artificial dyes commonly applied in uncolored food and pharmaceutical products were suspected to be detrimental to human health. Consequently, replacement of these colorants by their natural counterparts is a major challenge for the industry. In contrast to red, orange and yellow hues, for blue tints only few and expensive natural colorants such as *Spirulina* and *Gardenia* Blue are commercially available, and cheaper ferric anthocyanin chelates revealing intense blue colors would be an interesting option [1].

For this purpose, optimal conditions for stabilizing these complexes with pectins were identified in a screening at a micro scale using juices and phenolic extracts of different pigment sources being frequently used as coloring foodstuffs [2]. Blue tints were limited to model systems consisting of amidated and high methoxylated pectins, and pH values  $\geq 4.0$ . Blue color hues and their thermal and storage stabilities markedly differed between the pigment sources. While model systems containing red cabbage extract and juice displayed appealing gentian blue hues, stabilities of these chelates were poor. Purple carrot extract proved to be the most promising pigment source, producing intense and stable cobalt blue colors during storage and heat treatment.

To elucidate the potential of ferric anthocyanin chelates in food matrices, such dyes were added to protein and polysaccharide based gels to evaluate the impact of storage conditions on color stability [3]. The trends regarding color hues and stability observed in the gels were consistent with previous findings, providing clear evidence that the basic knowledge gained in a small scale screening may easily be transferred to complex food matrices.

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## **STABILISATION OF BEETROOT DERIVED BETANIN THROUGH INTERACTION WITH AN EXTRACT FROM BARBADOS CHERRY**

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### Objective:

To develop a natural stabilisation system for betanin to increase its use as a food colour.

A concentrate of beetroot can be used to provide a red colour for a wide variety of food and beverages. The colouring pigment is betanin and a limiting factor to its increased use as a food colour is its heat stability.

Anti-oxidants have been used to slow the colour loss during processing and one of the most effective is ascorbic acid, often used in combination with citric acid.

A limiting factor with ascorbic acid is that at higher concentrations a pro-oxidant effect is observed and colour loss is promoted.

Barbados Cherry or Acerola extract is rich in Vitamin C and specifically a formulation was used from Diana Food Division containing a high level of naturally occurring Vitamin C.

Ascorbic acid, when delivered in the form of Barbados Cherry extract does not show the pro-oxidant effect and betanin colour loss exhibited by ascorbic acid in the pure form.

The use of ascorbic acid from Barbados Cherry allowed retention of 67% of betanin compared to an unstabilised beetroot which retained only 32% betanin under controlled heating conditions.

An additional advantage of this is that the final colour formulation has the current market requirement of a 'Clean label' ingredient.



## NATURAL HYDROXYANTHRAQUINOID PIGMENTS: CURRENT SITUATION AND FUTURE OPPORTUNITIES IN FOOD

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Natural pigments and colorants are widely used in the world in many industries such as textile dyeing, food processing or cosmetic manufacturing. Among the natural products of interest are various compounds belonging to carotenoids, anthocyanins, chlorophylls, melanins, betalains... This article emphasizes pigments with anthraquinoid skeleton and gives an overview on hydroxyanthraquinoids described in Nature. Main natural sources of such pigments are summarized, followed by discussion about toxicity and carcinogenicity observed in some cases. Current industrial applications of natural hydroxyanthraquinoids are described with two examples, carminic acid from an insect and Arpink red™ from a filamentous fungus. As a conclusion, it focuses on the description of the hydroxyanthraquinoid colouring compounds produced by filamentous fungi. The conclusions indicate that, even if the toxicological investigations of a new additive are not financially negligible, non-mycotoxigenic filamentous fungi such as strains of *Drechslera* spp., *Herpotrichia* spp., *Paecilomyces* spp. and *Isaria* spp. at least, could be used for the production of dyestuffs rich in hydroxyanthraquinoid pigments as potent natural food grade colorants, with different shades according to the biomass composition: such as red, reddish brown, bronze, maroon and orange-yellow.

**Keywords:** anthraquinone, hydroxyanthraquinone, natural colorant, food colorant, microbial pigment

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## DEGRADATION OF ANTHOCYANINS IN PROCESSED STRAWBERRY FRUIT

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The aim of the research work was to investigate selected enzymatic activities, including  $\beta$ -glucosidases (GOXD), polyphenol oxidases (PPO) and peroxidases (POXD), involved in the degradation and stability of anthocyanins in processed red fruits, as well as certain processing condition, using substrate models and *in vitro* strawberry fruit. Using the substrate models, catechin and guaiacol, the results showed the presence of PPO and POXD activities in the fruit; however, there was an absence of these activities when catechol and 4-methoxy- $\alpha$ -naphthol were used as substrates. The determination of glucose, resulted from the hydrolysis of endogenous anthocyanins by  $\beta$ -glucosidase activity, indicated its important role in the degradation of anthocyanins during the strawberry fruits processing. The use of selected carboxylic acids as inhibitors of the degradation of anthocyanins showed that 0.045% of  $\delta$ -gluconic acid lactone resulted in the highest rate of increase (26.1%) in total anthocyanins, followed by that of 17.9% by the combination (1:1, v/v) of 0.1% citric acid and 0.045%  $\delta$ -gluconic acid lactone, and then 14.6% by 0.1% citric acid. The results also indicated that the use of *collupulin HC* effectively inhibited the rate of oxidation of induced catechol and catechin in the fruit matrix. On the other hand, there was a gradual decrease in the anthocyanins content of 26.7 and 38.9%, when the fruit was incubated for 1 h at 60 and 80°C, respectively. The experimental findings showed that the increase in anthocyanins content of the thermal treated fruit (25°C, 3 h) decreased gradually as the pH value increased, with an optimum increase at pH 4.0.



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## **Session 3**

### ***Pigments from microalgae***



**Plenary E****PIGMENTS FROM MICROALGAE: A NEW PERSPECTIVE WITH EMPHASIS ON PHYCOCYANIN****Eriksen N.T.**

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C-phycoyanin (C-PC) is a blue light harvesting phycobiliprotein in cyanobacteria and microalgae, used as dye in cosmetics, in diagnostic applications, and in foods [1]. The chromophore phycocyanobilin, which structurally and chemically resembles biliverdin and shows similar physiological effects also makes C-PC a potential biopharmaceutical. C-PC is nowadays produced in the cyanobacterium *Arthrospira platensis* grown phototrophically in open ponds. The unicellular rhodophyte *Galdieria sulphuraria* is an alternative host for production of C-PC that offers a number of advantages because selected isolates of this alga maintain their pigments when grown heterotrophically. *G. sulphuraria* grows well in ordinary bioreactors where hygienic standards are higher than in open ponds. Light limitation is of no concern and biomass productivities have been up to 20-50 g L<sup>-1</sup> day<sup>-1</sup> in fed-batch and continuous flow cultures of *G. sulphuraria* [2]. Phototrophic cultures have biomass productivities around only 1 g L<sup>-1</sup> day<sup>-1</sup>. Therefore has also the productivity of C-PC been more than 10 times higher in *G. sulphuraria* than in *A. platensis* cultures; highest C-PC productivities have been 0.5-0.9 g L<sup>-1</sup> day<sup>-1</sup> [2]. C-PC from *G. sulphuraria* can be extracted and purified to similar standards as C-PC from *A. platensis* by combinations of ammonium sulphate precipitation, aqueous two-phase extraction, ultrafiltration, and anion exchange chromatography [3]. Although microalgae contain many different pigments, only a few are produced in significant amounts, largely because of low productivities. *G. sulphuraria* provides an excellent example of the larger productivity potential of heterotrophic compared to phototrophic microalgal cultures, even with respect to the production of a photosynthetic pigment.

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## ALGAL CAROTENOIDS AS NOVEL PIGMENTS IN NUTRITION

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Carotenoids are isoprenoid molecules which may be the first naturally occurring pigments. They are synthesized de novo by photosynthetic plants, fungi and algae and are responsible for the bright colors of various fruits and vegetables. They are lipid-soluble compounds which can be chemically classified into xanthophylls (oxygenated molecules) and carotenes (hydrocarbons lacking oxygen). Microalgae seem to be very promising sources of carotenoids and other novel functional ingredients. For example, *Spirulina* spp. is rich in  $\beta$ -carotene and *Hematococcus pluvialis* is rich in astaxanthin. Industrially, many carotenoids find application as food pigments in dairy products and beverages, as well as in salmonid and poultry feeds. Nowadays, this application has great importance due to the increased consumer demands for natural products. Besides, there has been considerable interest in dietary carotenoids with respect to their antioxidant properties and their ability to reduce the appearance of some chronic diseases involving free radicals, i.e. aging, atherosclerosis, cancer and neurodegenerative diseases. Consequently, carotenoid production appears to be one of the most successful cases of blue biotechnology and further increase is expected in the near future.

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## FUNCTIONAL FOOD DEVELOPMENT USING AQUEOUS EXTRACT OF ARTROSPIRA (SPIRULINA) MAXIMA RICH IN PHYCOBILIPROTEINS

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Phycobiliproteins are antenna pigments present in cyanobacteria and some algae (eg. rhodophytes, cryptomonads) that capture light energy and pass it to chlorophylls during photosynthesis. Phycobiliproteins are constituents of the phycobilisomes and are complex between proteins and covalently bound phycobilins that act as chromophores.

C-phycoerythrin (C-PC), A-phycoerythrin and phycoerythrin are the major phycobiliproteins and in *Spirulina* C-PC is the prevalent one and represent sometimes the main protein in terms of percentage. The aqueous extract of *A. maxima* is dominated by the blue colour of C-PC and can be used directly as dye in cosmetics and in foods.

Although phycobiliproteins, in particular C-PC, have demonstrated remarkable functional activities (eg. free radical-scavenging, anti-inflammatory) [1] they are very sensitive to thermal processing especially in presence of water [2,3]; the incorporation of this ingredient in food must be optimized to avoid loss of activities.

The present work shows the results of a new functional pasta enriched with aqueous extract of *Spirulina*. Stability of colour and biological activities (eg. antioxidant, ACE inhibitory activities) of the final product were assessed after different methodologies of incorporation of the functional ingredient and using the *Spirulina* extract in form of solution or spray dried in presence of some protective agents.

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## **Session 4**

### ***Health and Nutrition***



## ENHANCED BIOAVAILABILITY OF CAROTENOIDS: THE INFLUENCE OF CHROMOPLAST MORPHOLOGY, DIETARY LIPID, AND THERMAL PROCESSING

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A sufficient dietary supply of vitamin A is a prerequisite for human health, ensuring a functional immune system as well as normal growth and development. To date, a high prevalence of vitamin A deficiency is observed among children and pregnant women, especially in developing countries. Animal products rich in preformed vitamin A are often expensive or simply unavailable to poorer populations, thus highlighting the dietary importance of fruits and vegetables rich in provitamin A carotenoids. Although humans can convert these carotenoids (e.g.  $\beta$ -carotene) to vitamin A, their absorption and conversion is generally found to be low from plant foods [1]. In this presentation, three different strategies for enhancing carotenoid absorption were investigated in three clinical trials. First, the consumption of a liquid-crystalline form of  $\beta$ -carotene from papaya was shown to provide significantly higher levels of carotenoids than the consumption of solid-crystalline  $\beta$ -carotene from raw carrots and tomatoes. Secondly, the addition of dietary lipid resulted in an increase of the initially poor carotenoid absorption from carrots. Third, commercial processing of a novel tomato containing solid-crystalline  $\beta$ -carotene delivered a surprisingly high level of  $\beta$ -carotene, an effect which was further enhanced by dietary lipid. For diminishing vitamin A deficiencies worldwide, the education of affected populations to consume more dietary provitamin A was strongly recommended [1]. Co-consumption of dietary lipid should be heavily considered, particularly when carrots or other fruits and vegetables containing provitamin A carotenoids in solid-crystalline form are consumed. Furthermore, nutritional education programs should highlight local provitamin A carotenoid sources with highest bioavailability, like papaya fruits, in order to help diminishing this most prevalent but avoidable deficiency.

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## BIOACCESSIBILITY AND CHANGES IN THE CAROTENOID PROFILE FROM MURICI FRUIT AFTER *IN VITRO* GASTROINTESTINAL DIGESTION

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Murici (*Byrsonima crassifolia*) is a tropical tree native from North and Northeast regions of Brazil. Its spherical fruit (1-2 cm diameter) is appreciated by the local population due to its typical rancid cheese-like aroma and it is usually consumed *in natura*, and also as ice cream, juice, jelly and liquor. Considering that the bioactive compounds, such as carotenoids, must be bioaccessible to exert their biological functions, we determined the *in vitro* bioaccessibility<sup>1</sup> of the carotenoids from murici and the changes in the carotenoid profile<sup>2</sup> after *in vitro* digestion by HPLC-DAD-MS/MS. Moreover, the major free and esterified carotenoids were identified. The freeze-dried fruit was rehydrated to its original moisture content (75 g/100g) before analyses. In the freeze-dried fruit, the total carotenoid content was 101±10 µg/g (dry weight) and 9.0±1.8% of the carotenoids was bioaccessible after *in vitro* digestion. This value is in the range of the bioaccessibility of carotenoids from fruits and vegetables, which usually varies from 5 to 100% depending on the matrix.<sup>3</sup> Free lutein (38%), free zeaxanthin (14%), lutein-monoesters (10%) and lutein-diester (37%) were found in the freeze-dried murici. No qualitative changes were noticed in the carotenoid profile after *in vitro* digestion. In addition, xanthophyll ester hydrolysis was incomplete, and both free and ester forms were incorporated into the micelles. The relative proportion of free zeaxanthin (14%) after digestion did not change; however, the relative proportions of lutein (52%) and lutein-monoesters (23%) increased and of lutein-diester (13%) decreased.

Acknowledgments: CNPq and FAPESP.

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## A MINI REVIEW ON THE COLOURLESS CAROTENOIDS PHYTOENE AND PHYTOFLUENE. ARE THEY INVISIBLE BIOACTIVE COMPOUNDS?

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The colourless carotenoids phytoene (PT) and phytofluene (PTF) have not been studied thoroughly within the Food Science and Technology and Nutrition fields yet. This is surprising as they are the precursors of all the remaining carotenoids and they are readily bioaccessible and bioavailable in humans.

With the exception of tomato and tomato products, little quantitative information concerning food sources of PT and PTF have been reported, although their presence has also been described in pitanga, grapefruit, watermelon, orange and other citrus fruits, peach and apricot.

There are studies that report the presence of PT and PTF in animals and human plasma and tissues, above all, after the intake of tomato products. These studies indicate that they may have different metabolism and be more bioavailable and efficiently accumulated in some organs than LYC. However, almost all the studies dealing with the bioaccessibility (release from the food structure and processing into a potentially absorbable form) of carotenoids that have been carried out in the last years have not considered PT and PTF. A high-throughput digestion method was recently used to screen the release of carotenoids from their biological matrix in 69 introgression lines of tomato to single out those leading with the highest potential carotenoid bioavailabilities. As a result of this study it was observed that, on average, the release of PT was higher (ca. 46%) than that of LYC (ca. 40%), while that of PTF was ca. 33%.

Recent reviews indicate that some of the putative biological actions of LYC are actually observed when tomato carotenoid extracts (containing high levels of PT and PTF) are used. Taken into consideration these and other observations on the involvement of these colourless carotenoids in the promotion of health it appears necessary to expand our current knowledge on them. In this contribution we summarize important aspects of the current knowledge on PT and PTF related to their presence in foods, animals and possible involvement in biological actions.

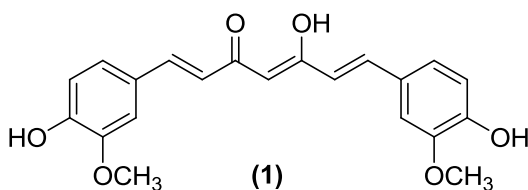
## DISSECTING THE PHARMACOPHORE OF CURCUMIN: TWO CASE STUDIES

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Natural pigments are widely used in the food and textile industry, and besides their chromatic features some of them, like flavonoids and carotenoids, have also important biological properties.

Since ancient times Curcumin (**1**), the yellow pigment (E 100) of the perennial herb *Curcuma Longa* (Turmeric), has been used in Indian cuisine as a spice, as yellow dye for textiles and it has been widely used as an Ayurvedic remedy with antibacterial and anti-inflammatory activity.



Nowadays Curcumin can be regarded as an universal platform of bioactivity, due to its capability of acting directly on more than 100 molecular targets. This pleiotropic pigment has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities. Considering all the different targets of Curcumin, a study of structure activity relationship (SAR) would be complex. For this reason, we have decided to focus our investigations on two of the most important targets of Curcumin: NF- $\kappa$ B and tubulin and two different approaches have been considered. In the study of NF- $\kappa$ B inhibitors we have synthesized different analogues by the introduction of carbon substituents on the aromatic moieties while for the anti-mitotic compounds we have focus our attention on the synthesis of shorter asymmetric analogues. The results have shown that the insertion of an *ortho*-prenyl group on the aromatic moieties has been detrimental for the activity against NF- $\kappa$ B, while the shorter asymmetric analogues were more potent of an order of magnitude than Curcumin as anti-tubulin agents.

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## Poster Session





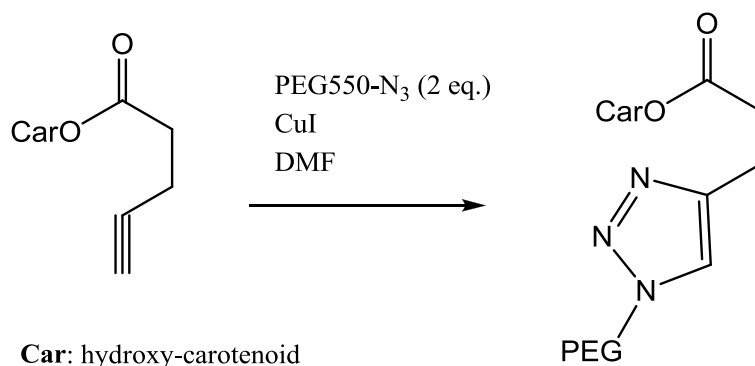
P 01  
SYNTHESIS OF WATER-SOLUBLE CAROTENOIDS  
VIA CLICK-REACTION

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Increasing the hydrophilicity of carotenoids would enhance their antioxidant effect, improve their pharmacokinetic properties and could result in their more widespread use as food additives and colorants. Previously, we synthesized carotenoid trimers and PEG-carotenoid derivatives via esterification. In this work our aim was the synthesis of similar compounds using the azide-alkyne click reaction.

The azide-alkyne click-reaction is a well-known, mild cycloaddition method that has nowadays a very widespread use among biomolecules. Our aim was to evaluate the synthesis of carotenoid derivatives with this method and optimize the conditions for these sensitive molecules. After finding the mildest conditions possible and the most suitable alkyne reactant for our purposes, we were able to use click-reaction for the synthesis of PEG-carotenoid conjugates from carotenoid pentynoates and PEG azides with acceptable yields. These derivatives have good water solubility as some previous examples have shown.



This study was supported by grant OTKA PD 77467 and K 83898 (Hungarian National Research Foundation) and SROP-4.2.2/B-10/1-2010-0029, Supporting Scientific Training of Talented Youth at the University of Pécs.

**P 02**  
**THERMAL AND LIGHT STABILITY OF**  
 **$\beta$ -CRYPTOXANTHIN ESTERS**

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$\beta$ -Cryptoxanthin esters are major components in orange fruits but also present in some vegetables [1]. Comparable bioavailability of free and esterified  $\beta$ -cryptoxanthin was found after supplementation in humans [2]. It was also showed that esterification with saturated fatty acids increases the thermal stability of  $\beta$ -cryptoxanthin [3]. The aim of the present study was the evaluation of thermal and light stability of  $\beta$ -cryptoxanthin esters with saturated and unsaturated fatty acids.

$\beta$ -Cryptoxanthin myristate, palmitate, oleate and linoleate were obtained by acylation with the corresponding acyl chlorides in dry pyridine and purified by HPLC. The compounds were dissolved in benzene and the initial concentration was adjusted at 3 +/- 0.2 micrograms/ml. Samples were kept at 40, 55 and 70°C for up to 130 hours. For the light stability test the samples were exposed to white light in the absence or in the presence of oxygen for up to 300 hours. Residual concentration was determined by HPLC-PDA on a C30 column. The rate constant of thermal and photooxidative degradation ( $k$ ) and the half-life periods ( $t_{1/2}$ ) were calculated by the integral method.

Esterification with both saturated and unsaturated fatty acids increased the thermal stability of  $\beta$ -cryptoxanthin compared to the free form. However, the thermal stability of unsaturated esters was lower than that of saturated ones. The light stability was not significantly influenced by esterification. Cis-trans isomerisation products significantly increased in samples exposed simultaneously to light and oxygen

Acknowledgement: This work was supported by PN-II-ID-PCE-2011-3-0721 research grant.

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**P 03**  
**EFFECT OF ESTERIFICATION ON THERMAL STABILITY AND  
ANTIOXIDANT ACTIVITY OF ZEAXANTHIN**

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Hydroxycarotenoids often occur in plants in the form of esters with various fatty acids. Carotenoid esters are major components in fruits and their thermal and photochemical stability, as well as the antioxidant capacity are important attributes. The studies pointing out the stability of xanthophylls esters are relatively scarce and the approaches used are very different [1, 2]. The aim of the present study was the evaluation of thermal stability and antioxidant capacity of different zeaxanthin esters compared to free zeaxanthin.

Zeaxanthin myristate, palmitate, oleate and linoleate were obtained by acylation with the corresponding acyl chlorides in dry pyridine and purified by HPLC [3]. The compounds were dissolved in benzene and the initial concentration was adjusted at 3 +/- 0.2 micrograms/ml. Samples were kept at 40, 55 and 70°C for up to 130 hours. Residual concentration was determined by HPLC-PDA on a C30 column. The rate constant of thermal degradation ( $k$ ) and the half-life periods ( $t_{1/2}$ ) were calculated by the integral method. The antioxidant activities were determined by TEAC and FRAP methods adapted for lipophilic compounds.

The zeaxanthin esters with saturated fatty acids had a better stability than free zeaxanthin. The ester with unsaturated fatty acids have a lower stability than those with saturated fatty acids but higher than free zeaxanthin.

Esterification with both saturated and unsaturated fatty acids did not significantly modify the antioxidant activity of zeaxanthin, similar values being obtained by both methods for free and esterified forms.

Acknowledgement: This work was supported by PN-II-ID-PCE-2011-3-0721 research grant.

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**P 04**

**MEASUREMENT OF ENZYMATIC HYDROLYSIS OF LUTEIN ESTERS FROM DAIRY PRODUCTS DURING *IN VITRO* DIGESTION**

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Xanthophylls are commonly present as fatty acid esters in fruits and vegetables so that dietary contribution of esterified xanthophylls is of marked value. As only free forms but not esters are found in human serum and peripheral tissues, bioaccessibility of esterified xanthophyll depends on the efficiency of the enzymatic hydrolysis during digestion<sup>1</sup>. In this study we evaluated the bioaccessibility of lutein esters added to dairy products (skimmed, semi-skimmed and whole milk or yogurt) using an *in vitro* digestion model. Lutein content in the micellar fraction was measured by HPLC to determine bioaccessibility, amount of lutein from the food matrix that is solubilised in mixed micelles, and the hydrolysis efficiency reached during digestion, ratio of free lutein to lutein esters in micelles. Milk and yogurt offer the same lutein bioaccessibility degree, but depending on the fat content the efficiency was different. Thus, skimmed milk and yogurt present a bioaccessibility efficiency lower than 15%, while the percentage transferred to micelles in semi-skimmed or whole products was higher than 35%. Although lutein esters were added to the products, lutein present in micelles was mainly in its free form either in milk and yogurt with any fat content, and hydrolysis efficiency reached a 2:1 ratio. Therefore, lutein esters are substrates for pancreatic lipase although previous work has shown a different result<sup>2</sup>, and hydrolysis efficiency is not influenced by food structure and amount of fat in the digesta.

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P 05

**OIL BODIES AS A POTENTIAL MICROENCAPSULATION CARRIER FOR ASTAXANTHIN STABILIZATION AND SAFE DELIVERY**

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Natural astaxanthin (AST) is a particularly valued molecule because of its high antioxidant properties. However, AST is extremely sensitive to oxidation and thus its bioactive properties decrease during processing and storage. Oleosomes isolated from plant seeds are oil bodies (OB) consisting of a triacylglycerol core bound by a phospholipid monolayer embedded with proteins known as oleosins [1,2,3]. The purposes of this study were to define conditions for microencapsulating astaxanthin in oil bodies (OB) from *Brassica napus* for the enhancement of its oxidative stability, and to test the bioactivity of the microencapsulated AST (AST-M) in CRL1730 endothelial cells. Conditions for maximizing microencapsulation efficiency (ME) [AST/OB, stirring speed and contact time] were determined using the Response Surface Methodology. The highest ME (> 99 %) was obtained with an AST/OB ratio of 0.14, 200 rpm, 5.3 h. OB loaded with AST showed a strong electrostatic repulsion in a wide range of pH (5.5 to 9.5) and ionic strength (0 – 150 mM NaCl). It was found that the half-life of AST-M exposed to air and light was twice as long as free AST, which indicates the protective role of OB. In addition, AST-M at a non-cytotoxic concentration (1 µg/mL) had a protective effect against reactive oxygen species. These results suggest that OB isolated from oil seeds offer a novel option for stabilizing and delivering AST with bioactive properties in industrial applications in the food and pharmaceutical fields.

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**P 06**  
**MICROENCAPSULATION OF ASTAXANTHIN OLEORESIN FROM**  
***Phaffia rhodozyma***

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Astaxanthin is a carotenoid used in functional foods for humans because of its antioxidant activity higher than Vitamin E, however its use in foods is limited due to their low solubility in water, in addition it has a low stability because is affected by the oxidation and could be easily isomerized due to heat, acids or light [1,2]. In this study, the astaxanthin was extracted from the yeast cells using the microwave as pretreatment at low power (105 W) for 1 minute, followed by extraction with ethyl acetate. The extraction was optimized using a design Box-Behnken, resulting as the best extraction conditions, a temperature of 65 °C, for 24 minutes, and a solid-solvent ratio of 1:19, obtaining an astaxanthin oleoresin. The astaxanthin oleoresin was dispersed using propylene glycol. Water-dispersible oleoresin encapsulated were obtained using a spray dryer, with inulin and maltodextrin as wall materials with inlet/outlet temperatures of 150/100 and 105/67 °C respectively. The encapsulated with inulin were more stable in solution ( $t_{1/2}=15.33\pm 0.686$  h) than maltodextrin ( $t_{1/2}=1.13\pm 0.141$  h). The shelf life of maltodextrin ( $t_{1/2}=34.48 \pm 1.57$  h) was higher than inulin ( $t_{1/2}=12.16\pm 0.622$ h) at 25 °C. The encapsulated presented a simple form and a diameter between 1 – 10  $\mu\text{m}$ . The antioxidant capacity of the oleoresin (mM Trolox / g sample) was of  $1.135 \pm 0.048$  and for microcapsules the values of  $10.105 \pm 0.502$  and  $10.509 \pm 0.281$  was obtained for inulin and maltodextrin respectively.

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**P 07**  
**EFFECT OF GENOTYPE AND GROWING CONDITIONS ON  
LUTEIN AND  $\beta$ -CAROTENE CONTENT OF GREEN LEAFY  
BRASSICA SPECIES**

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*Brassicaceae* vegetables are rich in nutritionally important constituents such as vitamins and minerals and they contain a broad spectrum of various secondary plant metabolites [1]. Their composition and content depends on genus and species, but also on genotype and agricultural factors. While *Brassicaceae* are particularly known for their high glucosinolate content, green leafy species are also rich in lutein and  $\beta$ -carotene. Moreover, their carotenoid content correlates with chlorophyll concentration.

The aim of the present study was to evaluate the impact of genotype and growing method on lutein and  $\beta$ -carotene contents of Japanese Greens and to establish correlations between carotenoid and chlorophyll contents. Twelve green leafy *Brassica* cultivars were produced in two different series. For spring production seeds were grown in the greenhouse either in beds or in pots, whereas summer cultivations took place either on open fields or under tunnels. Lutein,  $\beta$ -carotene as well as chlorophyll a and b contents of Japanese Greens were determined by HPLC-DAD [2].

Overall, lutein concentrations ranged from 3.8 to 10.5 mg/100 g FM and  $\beta$ -carotene contents from 2.1 to 6.8 mg/100 g FM. Botanical classification showed the strongest impact on carotenoid contents with *B. rapa* subsp. *chinensis* producing significantly more carotenoids than *B. rapa* subsp. *nipposinica* and *B. juncea*. The effect of growing conditions was less pronounced, but slightly higher  $\beta$ -carotene contents were observed in summer. In conclusion, choosing optimal genotypes and to some extent the manipulation of growing conditions offer strategies to increase lutein and  $\beta$ -carotene concentrations.

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**P 08**  
**EFFECT OF PROCESSING ON CONTENT OF VITAL  
CAROTENOIDS IN NEW VEGETABLE PUREE**

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Vital carotenoids such as lycopene,  $\beta$ -carotene and their biologically active derivatives have received an increasing attention during the last decades due to their association with the reduced risk of heart diseases and cancer. Recent investigations and researches suggested and recommended consumption of considerable amount of plant-derived products rich in bioactive nutrients such as carotenoids, poly-phenols and other bio-antioxidant compounds daily. The objective of the present work was to study the changes in content and composition of carotenoids as a function of processing of new vegetables puree (cream) prepared from red bell pepper, eggplant, tomato paste and onion under pilot plant conditions including washing, cutting, mixing in a double-jacket mixer, heating at 95°C for 2 min. The puree was packaged in plastic dishes, cooled and stored at 2-6°C.

Carotenoids were extracted by a conventional procedure using methanol first followed by 1,2-dichloroethane. The individual carotenoids were separated and determined by HPLC method using analytical RP-column and stepwise gradient elution starting with water-methanol and ending with methanol-acetonitrile-isopropanol. Identification was based on comparison of spectral characteristics and retention behaviour of sample carotenoids with those of standard materials or with literature data.

The red-bell pepper was the main source of vital zeaxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene and non-vital xanthiophylls, while sufficient amounts of bio-active lycopene were derived from tomato paste in the puree. It was found that processing caused substantial loss of carotenoids of red pepper, while lycopene was markedly protected against thermal degradation indicating that red pepper carotenoids (mainly xanthiophylls and their esters) were functioning as the first oxidation barrier under the chemical conditions of the new vegetable puree. These results are of special importance in the project since the aim is to produce vegetable puree rich in bio-active carotenoids beside other antioxidants and has accentuated sensory properties that increase consumer acceptance for the product.

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**P 09**  
**EFFECT OF ADDITION OF SODIUM ERYTHORBATE AND URUCUM ON THE LIPID OXIDATION IN PORK MEAT**

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Lipid oxidation is a major cause of food deterioration being responsible for the generation of undesirable flavors, decrease of shelf life, loss of nutritional value and formation of harmful compounds to human health, such as cholesterol oxides<sup>[1]</sup>. This study investigated the effect of sodium erythorbate (0.1%) and annatto powder (0.05%) on the inhibition of fatty acid and cholesterol oxidation in pork loin over 120 days of storage and subsequent heat treatment. Cholesterol and cholesterol oxides were identified and quantified simultaneously by HPLC-PDA-RID<sup>[2]</sup> and fatty acids by GC-FID<sup>[3]</sup>. TBARS values were determined by spectrophotometry. Five cholesterol oxides were identified: 7-ketocholesterol,  $\alpha$ -epoxycholesterol,  $\beta$ -epoxycholesterol, 7 $\alpha$ -hydroxycholesterol and 7 $\beta$ -hydroxycholesterol. The addition of annatto, sodium erythorbate and a mixture of sodium erythorbate with annatto inhibited the formation of cholesterol oxides since these compounds were found only in the control sample. The lowest TBARS values were observed in the samples containing annatto, sodium erythorbate and a mixture of sodium erythorbate and annatto during the first 75 days of storage. During storage the control sample showed a 18% reduction in PUFA's levels while the samples containing annatto, sodium erythorbate and a mixture of sodium erythorbate with annatto showed a reduction of 9%. On the other hand, heat treatment decreased MUFA and PUFA content despite the addition of annatto, sodium erythorbate and mixture of sodium erythorbate with annatto. The results demonstrated that the addition of mixture of erythorbate and annatto had higher antioxidant effect on lipid fraction of pork meat than the addition of each antioxidant alone.

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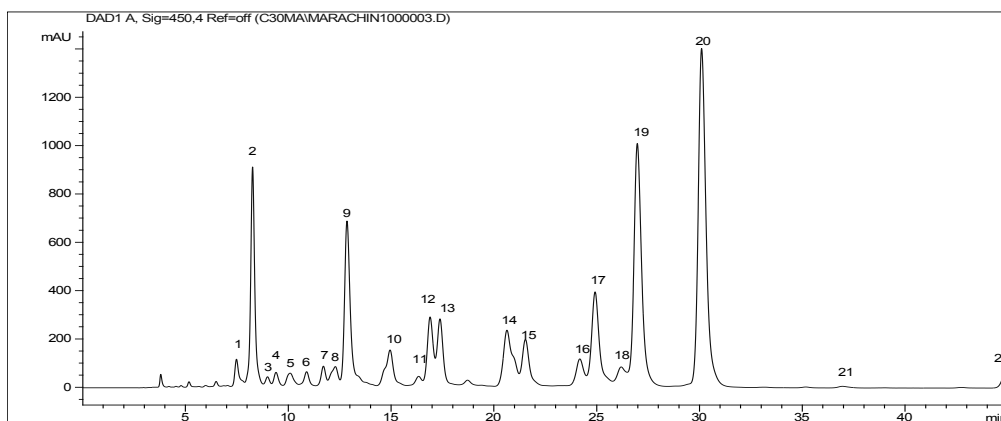
## P 10

IDENTIFICATION OF *Cionosicyos macranthus* CAROTENOIDS

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*Cionosicyos macranthus* (Chinese passion fruit) is a cucurbit fruit, native of Central America and Mexico, which grows as the passion fruit. The pulp of this fruit is red by the high content of carotenoids with termination keto- $\kappa$ . We recently found that *Pouteria sapota*, other fruit native to this region, is also characterized by the high content of carotenoids with termination keto- $\kappa$ . This is the first report on the separation and identification of all *Cionosicyos macranthus* carotenoids. The samples used were obtained directly from the tree and extracted fresh. Carotenoids were identified by their spectra UV / Vis and molar mass, obtained by HPLC-DAD and HPLC-MS, respectively. To minimize the interference caused by the overlapping of peaks, the saponified extract was separated into four fractions by column chromatography packed with  $Al_2O_3$  (ether: hexane 10%, ether:hexane 50%, ether 100% and methanol:ether 5%) . The figure shows the HPLC chromatogram of the original extract saponified, with the numbers corresponding to carotenoids identified.



The cryptoxanthin (peak 20) is the main carotenoid of *Cionosicyos macranthus*, followed cryptocapsin (peak 19). The composition of the extract indicates most carotenoids are epoxides or derivatives thereof. It is known that  $\kappa$  ring carotenoids are formed by pinacol arrangement of 5,6-epoxides. When comparing *Cionosicyos macranthus* with *Pouteria sapota* carotenoids, we found that both are the same but in different proportions.

**P 11**  
**BIOACTIVE COMPOUNDS IN SUPERCRITICAL CO<sub>2</sub>-EXTRACTED PUMPKIN OIL**

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Pumpkin (*Cucurbita moscata* Duch.) flesh has an intense yellow/orange color owing to its high levels of carotenoids, mainly  $\alpha$ - and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein and zeaxanthin [1]. These pigments are largely used in food preparation to make color appealing to consumers and improve food healthiness and stability because of their antioxidant and pro-vitamin A activities. Supercritical carbon dioxide (SC-CO<sub>2</sub>) is a “green” technology suitable for carotenoid extraction. It gives extracts totally free of toxic organic solvents.

Few reports describe SC-CO<sub>2</sub> carotenoid extraction from pumpkin [2,3]. The authors used freeze-dried and milled flesh to feed the extractor, getting a maximum carotenoid yield of ~10.9 mg/100 g dry weight (d.w.). In this work a process for obtaining a considerably higher carotenoid yield by SC-CO<sub>2</sub> extraction from dehydrated pumpkin flesh is described. Our results show that dehydration method is a key factor to improve carotenoid extraction from pumpkin. Oven-drying increased SC-CO<sub>2</sub> extraction yield of total carotenoids of ~8.5 folds with respect to freeze-drying (49.2 mg/100 g d.w. vs 5.8 mg/100 g d.w., respectively). Moreover, mixing the oven-dried flesh with milled pumpkin seeds (1:1 by weight) further increased carotenoid yield of ~1.6 fold (79.2 mg/100 g d.w. of fed pumpkin flesh). At the same time, it led to an enrichment of the extract with tocopherols, tocotrienols and PUFA from seed oil.

These findings encourage further studies in order to scale up the process for possible industrial production of high quality bioactive ingredients from pumpkin for functional food or cosmeceutical preparations.

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**P 12**  
**EVALUATION OF CAROTENOIDS AND CAPSAICINOIDS  
CONTENT IN POWDER OF CHILLI PEPPERS DURING ONE YEAR  
OF SHELF-LIFE**

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Red peppers are often employed as a source of pigment with the purpose of conferring colour to food preparations. The intense red colour is conferred to the fruits by the presence of almost 50 carotenoids, both free or esterified with fatty acids. The use of powdered dried fruits has largely increased during the last years all over the world. During storage of the powdered berries, a progressive loss of colour intensity is observed, causing the product to change from brilliant red to dull brown. The oxidative degradation of carotenoids seems to be the main reason for colour degradation. Factors as high temperature, humidity, water activity, exposure to light and contact with oxygen have been found to play an important role in causing deterioration.

Piquancy is the main tasty feature of red chilli peppers, and it is assured by the presence of a group of molecules belonging to the family of capsaicinoids. In the present study, the profiles of carotenoids and capsaicinoids in paprika samples stored along one year under different conditions have been recorded. Liquid chromatography coupled to mass spectrometry has been employed for carotenoid analysis, while DAD detector was used for capsaicinoids detection. Samples of powdered dried berries were stored at room temperature and in the dark in envelopes made of paper/PET. One more aliquot was stored at refrigerating conditions (4°C). Aliquots of each sample were analysed along a 12 months period. Data obtained showed that carotenoids higher retention occurred in the samples stored under refrigerating condition with a relative small percentage loss going from 6 to 12 months of storage; whereas the carotenoid retention in sample stored at R.T., significantly decreased going from 6 to 12 months of storage.

As for capsaicinoids, no differences were found during the first six months-period in all aliquots stored at the different conditions, while from nine months onwards a slight decrease in capsaicinoids content was registered in the samples stored at room temperature. On the contrary, the piquancy of the refrigerated samples was found unaltered during the entire period.

**P 13**  
**CAROTENOIDS IN RED FLESHED SWEET ORANGES**

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The orange carotenoids are responsible for the color of the peel and juice that are important quality factor, influencing its acceptance for the consumers and have also important functions for the human health. Orange is the fruit more consumed worldwide both as fresh and juice. In Brazil are being cultivated varieties of red fleshed sweet oranges, which have distinct red pulp due to the presence of the lycopene in their carotenoid composition. The aim of this study was to compare three varieties of red fleshed sweet oranges (Sanguínea de Mombuca, Baia Cara-Cara and Valência Puka with a variety Valencia in relation to the major carotenoids composition and color parameters. The carotenoids were separated and quantified by liquid chromatography and with C<sub>18</sub> column. The mobile phase was composed of acetonitrile:methanol:ethyl acetate, in gradient elution: 0 min (99:1:0), 30 min (64:1:35), 35 min (64:1:35) and 58 min (99:1:0)<sup>1</sup>. Flow rate was 0.6ml/min and the peaks were monitored to 450 nm. The results showed that red fleshed sweet oranges when compared with Valencia had juices less acid and a darker color with greater saturation of red coloration and higher vivacity. The content of carotenoids showed no significant differences ( $p < 0.05$ ) between the varieties of red pulp oranges, but these proved to have 20-76% more total carotenoid that Valencia oranges<sup>2</sup>, and the same behavior was observed for concentrations of  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and lutein which respectively showed an increase 230-375%, 36-65% and up to 37%.

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**P 14**  
**COLOUR CHANGES OF HEAT-TREATED ORANGE JUICE  
DURING AMBIENT STORAGE**

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Orange juice is one of the most consumed fruit juices and appreciated for its high content of vitamin C. The attractive orange colour is known as an important sensory attribute to evaluate juice quality. Changes of colour during processing and storage indicate the occurrence of several chemical reactions, one of them is non-enzymatic browning. Ascorbic acid degradation is considered as one of the main contributors to browning in orange juice<sup>1</sup>. Meanwhile, Maillard reaction may also occur but is considered to be of minor importance<sup>2</sup>. Several components such as ascorbic acid, sugars, citric acid, and oxygen were identified as major factors affecting browning in orange based juices<sup>3</sup>.

Studies have been undertaken to understand the cause of browning and identify the main factors involved in colour changes in orange juice. Pasteurised single-strength orange juice (11.2 °Brix) in PET bottles were stored at 20, 28, 35 and 42 °C. Change in colour was assessed using the CIE-L\*a\*b\* colour system. The L\* value decreased during storage which indicates a darker appearance of the juice. Higher losses of total vitamin C and oxygen were found at higher storage temperature. After 8 weeks of storage, the vitamin C retention was 25% at 42 °C meanwhile at 20 °C the retention was 68%. A sharp decrease from the first to the second week of storage may imply oxidative changes in the orange juice. Furthermore, kinetic modelling has been applied to describe the evolution of several quality attributes of orange juice during storage.

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P 15

## **CAROTENOID DEPOSITION AND PROFILES IN PEACH PALM (*BACTRIS GASIPAES* KUNTH) FRUITS, AND THEIR IMPLICATION ON ITS NUTRITIONAL POTENTIAL**

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The tropical peach palm develops 3-6 cm broad, edible drupes with a yellow, orange, or red exocarp. The yellow to light orange fruit pulp has been reported to be rich in starch, lipids and provitamin A carotenoids<sup>1</sup>.

In this study, major nutrients and the genuine carotenoid profile of highly diverse peach palm fruits, purchased at local Costa Rican markets, were investigated. Selected accessions showed widely ranging levels of (all-*E*)- $\beta$ -carotene (0.4-5.4 mg/100 g on fresh weight (FW)) and (Z)- $\gamma$ -carotene isomers (total 0.2-5.3 mg/100 g on FW), representing predominant carotenoids of the raw mesocarp. Total carotenoid levels in the edible portion of the fruit ranged from 0.7 mg to 14.6 mg/100 g on FW. Orange-fleshed fruits were shown to contain 9- and 6-fold higher amounts of total carotenoids and (all-*E*)- $\beta$ -carotene, respectively, than yellow-fleshed ones. Provitamin A equivalents, i.e. retinol activity equivalents (RAE), of uncooked fruits ranged from 38  $\mu$ g to 642  $\mu$ g RAE/100 g on FW.

Light microscopic studies of mesocarp tissue revealed the occurrence of globular chromoplasts, where carotenoids are assumed to be lipid-dissolved. Solubility estimations based on lipid contents of the fruit supported carotenoids to be deposited in a lipid-dissolved state, since fruit-own lipid contents would suffice to dissolve total carotenoids contained. Consequently, improved bioaccessibility of  $\beta$ -carotene and further lipid-dissolved carotenoids from peach palm fruit is expected due to their deposition in globular-tubular chromoplasts<sup>2</sup>. In summary, peach palm fruits represent a most promising source for dietary provitamin A, potentially being highly bioavailable due to their concomitant high lipid contents.

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**P 16**  
**DEPOSITION OF LYCOPENE,  $\beta$ -CAROTENE, AND**  
 **$\beta$ -CRYPTOXANTHIN IN DIFFERENT CHROMOPLAST**  
**SUBSTRUCTURES IN PAPAYA FRUITS**

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Papaya fruits commonly are a rich source of  $\beta$ -carotene and esterified  $\beta$ -cryptoxanthin, whereas the additional accumulation of lycopene was only observed in red-fleshed papaya fruits<sup>[1]</sup>. In this study, the role of different chromoplast substructures should be elucidated, considering the deposition form of the respective carotenoids. For this purpose, carotenoid profiles of one red- and one yellow-fleshed genotype were compared using HPLC-PDA-MS<sup>n</sup> in order to confirm similarity of patterns regarding the occurrence of  $\beta$ -carotene and  $\beta$ -cryptoxanthin esters. Both (*all-E*)-lycopene and some minor (*Z*)-lycopene isomers were demonstrated to exclusively occur in the red-fleshed genotype. Green-ripe as well as fully ripe yellow- and red-fleshed fruits were subsequently investigated by light and electron microscopy, identifying the carotenoid-bearing chromoplast substructures. Undifferentiated proplastids and plastids with plastoglobules were observed in the uncoloured mesocarp of green-ripe fruit. In both ripe fruits, tubular chromoplast structures were found being associated with the deposition of  $\beta$ -carotene and  $\beta$ -cryptoxanthin. Crystalloid elements were exclusively identified in red-fleshed genotypes and, therefore, hypothesized to be the deposition site of lycopene. Electron micrographs from lycopene-rich tomato mesocarp revealed striking similarities to the “lycopenic” substructures in red-fleshed papaya chromoplasts<sup>[2]</sup>. While carotenoids in chromoplast tubules were previously described to be deposited in a liquid-crystalline physical state, crystalloid substructures were related to the solid crystalline accumulation of the respective carotenoid<sup>[3]</sup>.

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**P 17**  
**EVALUATION OF QUALITY PARAMETERS AND CAROTENOID  
CONTENT OF THREE CULTIVARS OF MANGO  
(*MANGIFERA INDICA L.*) FROM REUNION ISLAND**

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Mango (*Mangifera indica L.*) is a well-accepted fruit worldwide thanks to its qualities, such as taste, aroma, color and health promoting content, making it one of the first tropical fruit produced <sup>[1]</sup>. Most imported mangoes in Europe are "American-type" characterized by a blush on the fruit's sun exposed side (ex: Kent). In this study, we compared three cultivars produced in Reunion Island: Kent (American-type, well known in international market), Cogshall (American-type) and José (Indian-type, imported by the first Reunion Island inhabitants). Fruit mass, Total Titrable Acidity (TTA), Total Soluble Solids (TSS) and carotenoid content of flesh were measured on ripe fruits harvested at a commercial maturity stage and ripened in relative humidity and temperature controlled chamber <sup>[2]</sup>. Mango taste was estimated using TSS/TTA ratio indicating that José was sweeter than the two other cultivars, with a ratio about 10. Cogshall and Kent had a lower ratio, about 4 and 3, respectively. Total carotenoid content suggested that Kent mangoes had the highest level followed by Cogshall and José, equal to 44.9, 35.9 and 34.3µg eq. β-carotene/g Fresh Matter, respectively. A correlation was found between the hue angle (h°) value and carotenoid content for American-type mangoes Kent and Cogshall showing a direct link between the two cultivars. Subsequently quantification of Cogshall's carotenoid content was experimented using High Performance Liquid Chromatography. Identification was obtained by comparison of retention times versus pure commercial standards <sup>[3]</sup>. This study allowed us to get a first identification of Cogshall's carotenoids and quantify the β-carotene.

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P 18  
**GENUINE PROFILES AND BIOACCESSIBILITIES OF  
CAROTENOIDS FROM RED- AND YELLOW-FLESHED MAMEY  
SAPOTE (*Pouteria sapota*) FRUITS**

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Mamey sapote (*Pouteria sapota* (Jacq.) H.E. Moore & Stearn) is an underutilized carotenoid-rich fruit containing rare  $\kappa$ -ring carotenoids such as the newly identified sapotexanthin [1]. Although different genotypes showing distinct pulp colors are available, knowledge on differences in their carotenoid composition is still scarce. Therefore, the goal of the present research work was to qualitatively and quantitatively describe the carotenoid profiles of different genotypes using HPLC-DAD coupled to mass spectrometry. Validation of the extraction method applied was performed for violaxanthin,  $\beta$ -cryptoxanthin, and  $\beta$ -carotene. While high amounts of sapotexanthin and cryptocapsin were observed in red-fleshed genotypes, high concentrations of free  $\beta$ -cryptoxanthin and violaxanthin esters were found, for the first time in yellow-fleshed fruits. However,  $\beta$ -carotene contents were comparatively low, resulting in low retinol equivalent values for both red and yellow-fleshed sapote. An *in vitro* digestion model was used to assess the liberation of sapote carotenoids during digestion, indicating low carotenoid bioaccessibility from both the raw and cooked fruit matrix, possibly due to the high contents of acid-labile violaxanthin esters.

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**P 19**  
**TRANSGENIC TOMATOES AND THEIR CAROTENOID AND  
FLAVOUR PROFILES**

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Transgenic tomatoes (*Solanum lycopersicum*) containing genes of the *Ccd1* and *Ccd4* type genes from *Cucumis melo*, *Osmanthus fragrans*, *Malus domestica* and *Rosa damascena* were evaluated for differences in their carotenoid and flavour profiles.

We will present the carotenoid and respective norisoprenoid profiles of these transgenic tomatoes. These results will provide insight into the flavour and carotenoid metabolism with a special focus on the action of the transgenic *CcdX* genes used for our tomato varieties.

All carotenoids and flavour compounds were extracted simultaneously from the same tomatoes and subsequently analyzed by GC-MS (flavour compounds, after concentration on SPME fibers) and by LC-MS-MS (carotenoids). The simultaneous extraction and analysis of our samples provided excellent comparability between all transgenic tomato strains while ensuring the direct correlation of carotenoids and their related flavour compounds within the respective strains.

Whereas we could proof that carotenoids and directly derived flavour compounds are closely linked to each other, the influence of transgenic *Ccdx* genes on flavour formation and carotenoid composition in *Solanum lycopersicum* fruits was not evident in our data.

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## STUDY OF THE TIME-COURSE *CIS*/*TRANS* ISOMERISATION OF LYCOPENE, PHYTOENE AND PHYTOFLUENE FROM TOMATO

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Carotenoids are susceptible to geometrical isomerization, so they can exist as all-*trans* (all-*E*) or *cis* (*Z*) isomers. The formation of the latter as analytical artifacts is well-known although they can also be naturally found in many sources. The study of the geometrical isomerism of carotenoids is interesting as there can be differences among isomers in aspects so important to explain the levels and actions or functions of these compounds like reactivity, specificity for cleavage enzymes, release from the matrix, bioavailability, vitamin A activity, etc. The presence of certain geometrical isomers in foods can also provide information about industrial processing and storage or cooking conditions. In this study we have stereomutated lycopene (LYC), phytoene (PT) and phytofluene (PTF) from a natural extract of lycopene-rich tomatoes following two different methodologies. The main objectives were to compare the different isomeric sets at the equilibrium and to obtain information about kinetically- and thermodynamically-favoured compounds by evaluating their formation over time. Our results indicated that regardless of the compound and the stereomutation procedure *cis* carotenoids clearly predominated over their all-*trans* counterparts at the equilibrium. Except in the case of LYC, important differences were observed in the isomeric sets at the equilibrium as a function of the stereomutation method followed. The data on the relative stability of geometrical isomers obtained from the isomerization reactions are being compared to those obtained from *in silico* approaches.



**P 21**  
**CAROTENOID COMPOSITION OF THREE**  
**HUNGARIAN ALGAE SPECIES**

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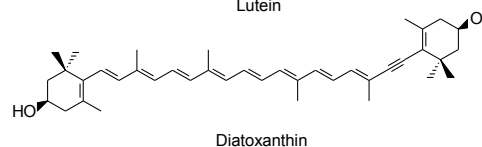
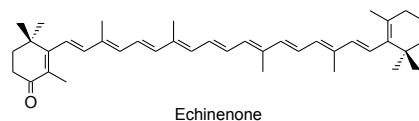
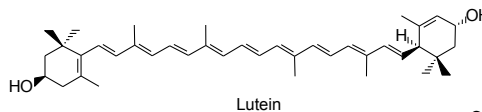
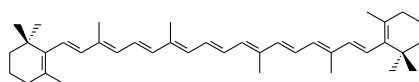
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To survive in a competitive environment, algae have developed defense strategies that result in a significant level of structural-chemical diversity. The current application of metabolites isolated from diverse classes of algae is increasing. Over 15,000 novel compounds have been chemically assigned. It suggests that algae are a promising group to furnish novel biochemically active substances. The exploration of these metabolites and organisms for pharmaceutical purposes for human nutrition and for other utilities is justified. The natural mass of algae, called algal-bloom, which a common phenomenon in fresh and marine waters a cheap and adequate natural resources for exploring bioactive metabolites. In this study, the patterns of carotenoids were investigated by HPLC-DAAD-MS from algal blooms occurring in Hungary.

The three investigated algae species collected in eastern Hungary showed three different carotenoid compositions. The main carotenoid of *Dunaliella* specie was lutein (~50%). The *Nostoc* contained echinenone (35%) and  $\beta$ -carotene (36%) as major compounds, while the *Euglena saugvinea* lutein (23%) and diatoxanthin (39%). Based on their UV-VIS and mass spectrum as well as co-chromatography with authentic samples neoxanthin, (9Z)-neoxanthin, antheraxanthin, zeaxanthin,  $\beta$ -carotene (*Dunaliella*), canthaxanthin,  $\beta$ -carotene 5,6-epoxide,  $\beta$ -carotene 5,8-epoxide, (9Z)- and (13Z)- $\beta$ -carotene (*Nostoc*), and (9Z)-neoxanthin (*Euglena*) were identified as minor carotenoids.



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**P 22**  
**HPLC METHOD VALIDATION FOR THE  
DETERMINATION OF FUCOXANTHIN**

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The Fucoxanthin is a carotenoid present primarily in brown seaweeds (Feoficee) of the genus *Undaria pinnatifida* (commonly called Wakame), *Sargassum fulvellum*, *Laminaria japonica* (better known as Kombu) and *Hizikia fusiformis*, which gives them the peculiar dark color, overlapping the typical greenish color of the common algae.

Fucoxanthin is a characteristic xanthophyll present in brown seaweed and the most abundant amongst aquatic carotenoids, accounting for more than 10% of estimated total natural production of carotenoids (Prabhasankar et al., 2009).

The fucoxanthinol, primary active metabolite of this molecule, has been recently found to have anti-obesity effects through the mobilization of adipose deposits for energy purposes, as well in the production of DHA (docosahexaenoic acid) which is claimed to provide a variety of different health benefits (Maeda et al., 2006).

In this work a HPLC-Vis validation method for determination and quantification of *all-trans*-fucoxanthin (CAS number 3351-86-8) in dry extracts of Wakame, used as raw material for the formulation of dietary supplements and in several commercial samples (fucoxanthin-based), was reported.

The first step of this research was the optimization of the chromatographic conditions and its validation in accordance with the international guidelines ICH (*International Conference on Harmonization*), also applying common statistical tests suitable for validation of a chromatographic method.

The second step included the optimization of a protocol for extraction of fucoxanthin and its quantification in dry extracts of Wakame seaweed and several commercial products.

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**P 23**  
**CAROTENOIDS STABILISATION FOR USE IN BEVERAGES:  
TWO DIFFERENT APPROACHES**

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Over the last few years, Naturex has developed several techniques to extend the stability of natural colours for applications in the beverage industry. The two following examples illustrate some of the technical improvements achieved.

Oil soluble paprika is commonly incorporated in water based beverages as an emulsion stabilised by non-ionic surfactants. The incorporation of Polysorbate 80 for example leads to the production of clear acid stable products. However, high content of Polysorbate 80 can cause foaming issues and impact the taste of beverages. Naturex has developed a series of polysorbate 80 free emulsions presenting a similar acid stability, transparency and shelf life. The droplet size distributions of both formulations are presented together with the light stability results.

Carotenoid-based colours are generally prone to oxidation. There are several combinations of oil soluble antioxidants that can be used to optimise the stability of the colour through various food & beverages processes. Oil soluble Rosemary extracts standardised in carnosic acid and carnosol added in the colour formulation significantly improve the stability of carotenoids based colours. In addition to this oil soluble form of Rosemary extracts, Naturex has developed a range of water soluble Rosemary extracts, StabilEnhance<sup>®</sup> WSR that can prolong beverages stability when incorporated as an antioxidant directly in the final application.

**P 24**  
**EFFECT OF HEAT PROCESSING ON THE PROFILE OF  
PIGMENTS AND ANTIOXIDANT CAPACITY OF JALAPEÑO  
PEPPERS AT INTERMEDIATE RIPENING STAGES**

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Recent studies have demonstrated that household heat-treatments alter differentially the content (qualitative and quantitative) and antioxidant capacity (AOC) of pigments in green and red Jalapeño peppers.<sup>1,2</sup> However, these attributes have not been evaluated in Jalapeño peppers at intermediate ripening stages. In this study, profiles of carotenoids (free and esterified), chlorophylls, and chlorophyll derivatives were evaluated by HPLC-APCI-TOF-MS analysis in raw and heat-processed (boiled or grilled) Jalapeño peppers at intermediate ripening stages (brown, 50% red, and 75% red). The pigment extracts were also evaluated for AOC (DPPH and FRAP methods). The AOC values were higher for 75% red peppers than for peppers at other ripening stages. The AOC was differentially affected by heat-processing style. Sixty-four compounds were observed in the pigment extracts, being the all-*trans*-capsanthin and the chlorophyll *a* the most abundant. Chlorophyll *b* was not observed in 75% red peppers. Nine compounds were generated by heat-processing, highlighting several pheophytins (*a*, *a'*, *b* and *b'*) and two  $\beta$ -cryptoxanthin isomers. Finally, the concentration of pigments was decreased by boiling and grilling. Our results reveal the importance of Jalapeño peppers at intermediate ripening stages, which might provide a greater health effects than green or red peppers since they contain a more complex mixture of pigments.

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**P 25**  
**MICELLARIZATION AND DIGESTIVE STABILITY OF PIGMENTS  
FROM JALAPEÑO PEPPERS AT INTERMEDIATE RIPENING  
STAGES**

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The effect of the interaction between different factors on the micellarization of carotenoids has been scarcely studied.<sup>[1,2]</sup> We evaluated the effect of different fat sources (soybean oil and beef tallow) and heat treatments (raw, boiling, and grilling) on the *in vitro* digestive stability and micellarization and carotenoids (free and esterified), chlorophylls, and chlorophyll derivatives from Jalapeño peppers at intermediate ripening stages (brown, 50% red, and 75% red). Around of 76 pigments were identified by HPLC-MS analysis in tested fruits. Fifty four pigments were micellarized, including free and esterified pigments. The micellarization of free carotenoids was higher with brown and 50% red than with 75% red peppers. Chlorophyll derivatives were micellarized only with brown and 50% red peppers. The interaction effect between dietary fat and heat treatments determined the digestive stability and micellarization of pigments. Dietary fat, mainly soybean oil, increased the micellarization of esterified carotenoids, pheophytins, and the less polar free carotenoids. The micellarization of free and monoesterified carotenoids was higher with raw than with processed peppers. Grilling increased the micellarization of carotenoid diesters. Micellarization values of esterified carotenoids were influenced by their fatty acid moiety. The results showed that the interaction between the tested factors determined the micellarization of the pigments from Jalapeño peppers. Peppers at intermediate ripening could show an advantage on the green or red peppers, providing a higher variety of pigments.

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**P 26**  
**CHANGES IN LUTEIN, CHLOROPHYLLS AND CHLOROPHYLL  
DEGRADATION PRODUCTS IN PISTACHIO KERNELS  
(*PISTACIA VERA* L.) DURING ROASTING**

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Bright green pistachio kernels are desirable in the food industry and a loss of color can be associated with a change in quality. Chlorophylls *a* and *b*, as well as lutein, are the predominant pigments in pistachios. The heat treatment of chlorophyll containing foods results in color degradation due to the conversion of chlorophylls (bright green color) to pheophytins and pyropheophytins (yellow-brown olive color). In this work changes in lutein, chlorophylls and chlorophyll degradation products in pistachios were monitored during oven roasting for the first time. A high performance liquid chromatography method with photodiode-array detection (HPLC-PDA) was developed to separate and quantitate the pigments in pistachios. Interestingly, chlorophylls *a* and *b* were higher in pistachio kernels roasted for 5 and 10 minutes than in the raw kernels. The most drastic losses were observed with pheophytins *a* and *b*, which both decreased by approximately 85% after 60 minutes of roasting. On the other hand, pyropheophytins *a* and *b* increased significantly during roasting and were 10-12x higher in the pistachios roasted for 60 minutes than in the raw pistachios. Lutein concentration increased by 37% after 5 minutes, but did not change significantly after that. Color measurements of the pistachios supported changes in chlorophyll pigments observed at the later roasting times. Initial increases in chlorophylls *a* and *b* and lutein were likely due to enhanced extractability with roasting.

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**DECOLOURATION PROCESSES UNDER NON-OXYGEN  
THERMAL AUTO-OXIDATION OF CHLOROPHYLL AND  
CAROTENOID FRACTIONS IN VIRGIN OLIVE OILS****Aparicio-Ruiz R., Gandul-Rojas B\***

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Kinetic models are capable of predicting shelf life in keeping with the different variables that can affect the degradation of the food item. Numerous experimental works describe virgin olive oils (VOOs) degradation, but until recently the kinetic performance in oxidation parameters<sup>1</sup> and individual pigment thermodegradation products have not been reported<sup>2-3</sup>. In this work, VOOs collected at three ripening stages with high, medium and low pigment content respectively, were thermodegraded to characterize the kinetic and thermodynamic parameters for the global degradation of chlorophyll (CHL) and carotenoid (CAR) fractions. A first-order kinetic mechanism was appropriate for describing the decolouration processes under non-oxygen thermal auto-oxidation. A marked effect of temperature has been pointed out, with the CARs being the most affected by heat. The kinetic constants for the CARs degradation were 3.6 times higher than the respective for CHLs that showed a more stable structure to decolouration by heat. As well, higher activation energy of CHLs ( $16.03 \pm 0.26$  kcal/mol) as compared to CARs ( $15.45 \pm 0.17$  kcal/mol) implies that a smaller temperature change is needed to increase the kinetic constant of CHLs. Neither isokinetic ratio and nor compensation existed between the three VOO matrices and further, for each pigment fraction (CHLs or CARs) all kinetic constants were explained by a single Arrhenius line. Consequently, the oily medium did not significantly affect the decolouration mechanisms, and moreover, the kinetic parameters obtained as temperature functions according to Arrhenius model, can be used to develop a prediction mathematical model for CHL and CAR decolouration in VOO over time and depending on temperature.

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**P 28**  
**PIGMENT CHANGES DURING PROCESSING OF GREEN TABLE  
OLIVE SPECIALITIES TREATED WITH ALKALI AND  
WITHOUT FERMENTATION**

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Table olives are among the most important fermented vegetable foods produced in the world. The main green table olive commercial preparation is the so-called Spanish or Seville-style which comprises an alkaline treatment of the olives and subsequent natural lactic fermentation in brine. Recently, other green table olive preparations, which include an alkaline treatment but not preservation by natural fermentation, are gaining importance in international trade, being included in the trade standard applying to table olives as "Specialities"<sup>1</sup>. One of the main highly valued characteristic for this product is a bright green colour, as similar as possible to the fresh fruit. Generally, colour is one of the most important quality attributes for table olives and can be considered as a quality index. The pigments of green table olives treated with alkali but not fermented (such as Castelvetro and Campo Real-styles) have been scarcely studied for different commercial products and no detailed study about the pigment transformation during processing has been carried out. Colour of green table olives is due to the presence of two main families of natural pigments, chlorophylls and carotenoids<sup>2</sup>. In the present work, changes in the chlorophyll and carotenoid composition of olive fruits during Castelvetro-style olive processing (lasting two weeks), and their involvement in the colour of the final product, were investigated. During processing, the main transformations of pigments were due to allomerization reactions of chlorophylls *a* and *b*, what do not imply colour change of the green fruit. Certain degradation to uncoloured compounds was also detected. With respect to the carotenoid fraction, any noticeable change took place.

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**P 29**  
**POLYPHENOLS AND VOLATILE COMPOUNDS IN OGLIAROLA  
AND CELLINA OLIVE**

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The Italian region Apulia is characterized by a large virgin oil production; in Italy, about 80% of olive oil production is located in southern regions, where Apulia represents the most important area. Owing to the difficult in modernizing the traditional olive growing designs, from one side super high density models are proposed [1] and from the other monovarietal oils from particular geographic areas were characterized [2]. In this study, monocultivar olive (Ogliarola and Cellina di Nardò) at different ripening degree from Apulia were analysed. The spectrophotometric and HPLC/DAD/MS quali-quantitative analyses of CMP were performed. CMP play an important role in diet, because they seem to prevent many diseases; several pharmacological studies demonstrated that the phenolic fraction extracted from virgin olive oil, and from waste waters collected within the production of olive oil, are able to prevent the LDL oxidation and can cause a decrease in the platelet aggregation induced by collagen in conditions of oxidative stress. Other aim of this study was the in vivo analyses of volatile compounds without a previous pre-treatment of the sample with PTR-TOF-MS instrumentation, a non-invasive technique which allows the achievement of whole mass spectra with a time of resolution lesser than 1 second and the detection of high molecular weight molecules with a high resolution power and to provide unambiguous determination of chemical formula leading a better interpretation of mass spectra [3].

Further analyses are in progress to validate a non-destructive optical method (as Multiplex sensor) to monitoring anthocyanins accumulation directly on olive fruits.

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**P 30**  
**CHLOROPHYLLIAN PIGMENTS IN EXTRA VIRGIN OLIVE OILS**

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The chemical composition of the olive oils pigments (*Olea europaea* L.) varies on the base of parameters such as variety, degree of ripeness, environmental conditions, growing region, processing and storage.

The pigments, in particular the chlorophylls and pheophytins affects considerably the preservation of this important diet mediterranean product as pro-oxidants in synergy with metals trace eventually present enhancing the autooxidation process.

In virgin olive oils the principal compounds present in decreasing content order are pheophytins A and A' followed from pheophytins B and B', pyropheophytin A and at last chlorophyll A.

This type of analysis is conducted as quality parameter to point out high oxidized lipidic status or detect eventually deodorization process or thermal treatment.

The official method to quantify these compounds is ISO 29841:2009 method, but on the base of our big experience on a lot of samples of this lipidic matrix we have evidenced that applying the over method the % content of pyropheophytin A respect to pheophytins A+A+pyropheophytin A is increased until to 40 %, respect to the data obtained after direct injection of a sample solution in acetone in a HPLC-UV system: the passage through the silica column cause a partial modification of pigments profile, in particular chlorophyll A, pheophytins B+B', pheophytins A+A' are partially detained on column in particular these last.

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**P 31**  
**SUBCELLULAR DISTRIBUTION IN OLIVE FRUIT OF  
PEROXIDASE ACTIVITY ON CHLOROPHYLL SUBSTRATE**

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The catabolism of chlorophylls has been mainly studied on leaf senescence, assuming that the degradation of these pigments should be similar in fruits. However, recent research has revealed important differences on chlorophyll metabolism in leaves and fruits. Even, it has been postulated the coexistence, only in certain fruits, of an exclusively oxidative metabolism concomitant with the general chlorophyll degradation pathway proposed by Kräutler and Hörtensteiner<sup>1</sup> (2011). Type III peroxidase (EC 1.11.1.7) is the enzyme most clearly involved on chlorophyll oxidative metabolism. In this work we studied the distribution of chlorophyll oxidative peroxidase activity (POD) in different subcellular fractions from mesocarp cells of olive fruit (*Olea europea* L.): Arbequina and Hojiblanca varieties. The specific methodology used for olive fruit it is based on Salas *et al.*<sup>2</sup>, which implies differential centrifugation techniques. The POD activity in the cell fractions enriched in the thylakoid membranes was monitored throughout the whole life cycle of the olive fruit and a positive correlation between the levels of the maximum activity of POD and the presence of oxidized derivatives was obtained in the fruits of the Arbequina variety. The study also showed varietal differences in the distribution of POD activity present in different subcellular fractions that explain the greater accumulation of oxidised derivatives of chlorophyll in fruits of the Arbequina than in Hojiblanca variety<sup>3</sup>. This work supposes one step further on deciphering chlorophyll degradation pathway on fruits.

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**P 32**  
**CHLOROPHYLL AND CAROTENOID PIGMENTS IN A SURVEY OF  
MARKETED APPLE VARIETIES**

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Domesticated apple (*Malus x domestica* Borkh) varieties have been traditionally consumed due to their sensory quality attributes and in relation to their health benefits which are associated with their high content in phenolic compounds, vitamin C, antioxidants and dietary fibre. Apart from the anthocyanins, responsible for the reddish colour of the skin of many apple fruit, chloroplastic pigments (chlorophylls and carotenoids) contribute also to the external (skin) and internal (flesh) fruit colouration. Apple has always been considered a fruit with low chloroplastic pigment content, especially carotenoids, when compared with other fruit species. In the last decade increasing efforts are being dedicated to the selection of fruits with enhanced levels of phytonutrients, including the carotenoids, involving multidisciplinary breeding programmes. In the present study we have characterised the chlorophyll and carotenoid composition of 13 marketed apple varieties, distinguishing between skin and flesh. All the varieties were characterised by common pigment profile, composed by chlorophyll *a* and *b*, neoxanthin (all-*trans* and 9-*cis* isomers), violaxanthin (all-*trans* and 9-*cis*), lutein and  $\beta$ -carotene as major compounds. Several 5,8-epoxide derivatives (neochrome, luteoxanthin and auroxanthin) were also found in certain varieties, whereas  $\beta$ -cryptoxanthin and antheraxanthin were mostly detected as minor compounds. Especial attention has been paid to determine the presence of carotenoid esters, being their detailed identification under study. Total chlorophyll and carotenoid contents were always higher in the skin, where lutein was the main carotenoid followed by violaxanthin, neoxanthin and  $\beta$ -carotene, a characteristic chloroplastic profile. On the contrary, neoxanthin and/or violaxanthin dominated the carotenoid composition in pulp, presenting also a higher proportion of esterified xanthophylls (mainly violaxanthin and neoxanthin).

**P 33**  
**QUANTITATION OF POLYPHENOLS IN DIFFERENT APPLE  
VARIETIES CULTIVATED IN AOSTA VALLEY**

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The aim of this study was to compare polyphenols content in different apple varieties (Renetta Canada, Golden Delicious, Gala, Jonagold, Red Delicious, Fuji, Pinova, Falstaff, Mairac, Topaz, Goldrush) cultivated in orchards situated in different areas of Aosta Valley, at harvest time and after controlled atmosphere (CA) storage, over four consecutive years. Harvest time was decided according to commonly used ripening indices: flesh firmness, sugar content, titrable acidity and starch. Samples were extracted from fresh fruit with a mixture of acetone/water to achieve a good extraction of polyphenols, following the method of Mattivi et al. [1]. The total amount of polyphenols was measured with an optimized Folin-Ciocalteu assay [2], according to which interfering compounds were removed by cleanup on a C18 cartridge. Quantitation of ascorbic acid was also performed in all the varieties in this study. The polyphenol content depends on the year and on the apple variety; Renetta Canada has a much higher content of total polyphenols than any other variety studied, according to other authors. Over four years, the average content of total polyphenols detected in the apple at harvest time was between 65.40 and 156.63 mg/100g of fresh fruit according to the variety in the following increasing order: Fuji, Gala, Goldrush, Pinova, Golden Delicious, Mairac, Falstaff, Topaz, Red Delicious, Jonagold and Renetta Canada, with some differences depending on the harvest year. After storage no highly significant change in polyphenol content was observed in all the years considered and in all the apple varieties collected.

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**P 34**  
**ANTHOCYANINS, PHENOLIC ACIDS AND ANTIOXIDANT  
ACTIVITY IN YELLOW, RED AND PURPLE-FLESHED POTATOES  
AFTER STEAM COOKING**

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Potatoes, belonging to the species *Solanum tuberosum* L., constitute a food consumed worldwide prepared and served in a variety of ways. Coloured potatoes with high levels of anthocyanins, total phenols and antioxidant activity, represent healthy food choice for consumers and a potential source for natural food colorants. This study aimed to determine anthocyanin and phenolic acid content in potato flesh with different colours, and to evaluate the antioxidant activity of these cultivars.

Potatoes, after boiled steam cooking, were extracted with 70% EtOH adjusted to pH 2.0 by HCOOH, as already described in a previous study [1] and the compounds were identified by HPLC/DAD/MS according to our previous work [2]. The ABTS method described by Lachman *et al.* (2011) was used for the antioxidant activity determination.

Six yellow-, two purple- and three red-fleshed varieties were studied. For purple- and red-fleshed cultivars, average total anthocyanin content values ranged from 64,1 to 437,2 µg/g of fresh material and a correlation with antioxidant activity was found. The lowest antioxidant activity was achieved by the group of varieties with yellow flesh, averaging 0,36 µg ascorbic acid equivalent/mL extract, instead in the group of red- and purple-fleshed cultivars it was higher 3,25 times considering the variety with the highest total anthocyanin content.

For these coloured potatoes, differences in antioxidant activity were found and the analysis showed a strong correlation between antioxidant activity and total anthocyanin content. Consequently, red and purple-fleshed cultivars could be a promising source of important antioxidants in human nutrition.

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**P 35**  
**CHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY**  
**OF SIX RICE CULTIVARS GROWN IN PIEDMONT**  
**(PIGMENTED AND NON-PIGMENTED)**

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Different extracts from pigmented rice cultivars grown in Piedmont (Artemide, Venere, Nerone (black); Ermes, Russ (red); Selenio (white)) were analyzed by high-performance liquid chromatography coupled with diode array detection and electrospray ionization - mass spectrometry (HPLC-DAD-ESI-MS/MS). The aim of this work was to identify the anthocyanins, flavonols, and flavan-3-ols contents so characterizing the different typologies of rice [1]. The stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) has been used to compare the antioxidant activities of the different rice cultivars and moreover total phenolic content has been examined. Black rice cultivars contained eight anthocyanins and all the three black varieties Artemide, Venere, and Nerone presented the same qualitative composition of anthocyanin pigments [2, 3]. The flavonols that occur in rice samples made up the series of 3-glc, and 3-rut of quercetin and isorhamnetin. Derivatives of dihydroquercetin and dihydroisorhamnetin were detected only in the 3-glc form. The total sum of flavan-3-ol monomers and dimers was substantially different considering the six rice varieties; Artemide, Ermes, and Russ presented significantly higher values (193.1, 101.8, and 63.7 mg/kg rice respectively) if compared to the others. The free phenolic content of 6 rice varieties ranged from 1404 ± 322 (Selenio) to 11872 ± 1161 (Artemide) mg of gallic acid equiv/kg of dry weight. Considering the antioxidant activity, the black rice cultivars showed the highest antiradical potential (DPPH<sup>•</sup> scavenging of 88.4%, 53.6% and 51.2% for Artemide, Nerone and Venere, respectively), while the lowest activity was observed for the non-pigmented rice sample (Selenio: 5.36%).

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## P 36

**EFFECT OF THE USE OF ENZYMATIC PREPARATIONS ON EXTRACTION OF PHENOLIC COMPOUNDS FROM BLUE MAIZE (*ZEA MAYS* L.), FROM THE REGION OF TLAXCALA, MEXICO**

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The presence of anthocyanins on blue maize (*Zea mays* L.), converts it on a potential supply of natural colorants and antioxidants<sup>1</sup>. Mexico has a diversity of maize germplasm<sup>2</sup>, but with a very low exploitation, despite its nutritional and nutraceutical properties, so its use has potential for the development of new products. While, enzymatic degradation of vegetables cell walls is considered essential on the extraction of phenolic compounds from fruits and vegetables<sup>3</sup>. In this work, it was evaluated the use of commercial enzymatic preparations on the extraction of total phenolic compounds (TPC), total monomeric anthocyanins (TMA), antioxidant capacity (AC) and color intensity (CI) from two blue maize varieties. Anthocyanin extracts from pericarp and aleurone from grains (1, 2, and 3 g) were obtained using phosphate buffer at four pH intervals (2.5, 3.0, 3.5 y 6.5), adding three enzymatic preparations and a combination between them (Pectinase®, Viscozyme® y Protease®). A MANOVA statistical analysis was performed, and the data obtained shows differences ( $P \leq 0.05$ ) between the studied factors and variables. Treatments using Pectinase®, buffer pH 3.0 and 1 g of blue maize from variety 1, and Viscozyme®, buffer pH 3.5, and 1 g of blue maize from variety 2, showed the best interaction conditions with values of  $17.11 \pm 0.77$  and  $35.77 \pm 0.81$  of TPC mg gallic acid/100g maize,  $2.49 \pm 0.13$  and  $23.75 \pm 0.69$  of TMA mg cyanidine-3-glucoside/100g maize,  $0.1053 \pm 0.0002$  and  $0.1773 \pm 0.0015$  of AC mM Trolox/100 g maize, and  $0.2198 \pm 0.0035$  and  $2.4773 \pm 0.0022$  of CI (Abs), respectively; evidencing a favorable effect of enzymatic preparations on extraction.

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**P 37**  
**TECHNO-FUNCTIONAL PROPERTIES OF TOMATO SAUCE  
FORTIFIED WITH ANTHOCYANIN PIGMENTS**

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Tomato sauce can be considered a 'staple food' not only in Italy but all over the world, because of its usage in many recipes. It can be also considered a 'functional food', due to epidemiological evidence of reduced risk of certain types of cancers and CVD related to its high content of antioxidant molecules, first of all lycopene, and also vitamins C and E [1, 2].

The nutraceutical value of tomato sauce can be increased by the presence of added antioxidant compound, obtaining thus a 'fortified tomato sauce' or a 'functional tomato sauce'.

With this aim, we added to control tomato sauce (Von Felten, Parma) (10-12° Brix) different % of anthocyanin pigments, obtained from natural source (*Prunus mahaleb* L. fruits, *Daucus carota* L. var. *atrorubens*, *Vitis vinifera* L. fruit skins). The addition of the anthocyanin extracts at maximum % (1% w/w) has decreased the pH values (- 0.1 points) to acceptable values for processed products, whereas the soluble solids have increased (+ 3° Brix). Since the addition of anthocyanin extracts is early in the production flowchart, the evaluation of colour (made by colorimeter with CIELAB parameter) has revealed a good colour retention even after pasteurization.

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**P 38**  
**EFFECT OF POST-HARVEST TREATMENT ON ANTHOCYANIN  
CONTENT AND TOTAL PHENOLICS IN MANGO  
(MANGIFERA INDICA L.) PEELS**

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Mango (*Mangifera indica* L.) is one of the most important tropical fruit crops. Its worldwide popularity and extensive processing led to a serious disposal problem of perishable by-products. Peels and kernels were shown to be rich in health promoting bioactive phenolic compounds such as quercetin, mangiferin, gallotannins, and anthocyanins<sup>[1, 2]</sup>. The effect of post-harvest treatment on mango peels was assessed comparing drying of peels without and after previous steam blanching to prevent phenolic compounds from enzymatic oxidation.

Peels of the mango cultivars Palmer, Nam Doc Mai, Tommy Atkins, and Kent were analyzed for their total monomeric anthocyanin content. Individual anthocyanins were characterized using HPLC-MS<sup>n</sup> and quantified by HPLC-DAD. Furthermore, total phenolic contents and antioxidative capacities were determined using Folin Ciocalteu- and FRAP-assay, respectively. Additionally, fruit ripeness was determined according to previous work<sup>[3]</sup>.

In accordance with literature, cyanidin 3-O-galactoside and 7-O-methylcyanidin 3-O-β-D-galactopyranoside were identified, showing the latter as predominant anthocyanin in the heterogeneously pigmented cultivars<sup>[1]</sup>. Cv. Palmer displayed the highest anthocyanin contents as compared to the three other varieties. While cv. Nam Doc Mai was devoid of anthocyanins, it was the richest source of total phenolics (107 mg gallic acid equivalents/g DM) exerting the highest antioxidant capacity (78 mg trolox equivalents/g DM). Blanching was shown to reduce anthocyanin contents, total phenolics, and antioxidative capacities as compared to peels dried without previous blanching. Peel preservation by blanching lowered the contents of bioactive compounds, supposedly due to thermal degradation. Additionally, spectrophotometrical measurement was shown to underestimate anthocyanin values as compared to HPLC-DAD determination.

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## P 39

**MAQUI (*ARISTOTELIA CHILENSIS* (MOL.) STUNTZ) – DETAILED ANALYSIS OF THE HIGHLY PIGMENTED “SUPERFRUIT”****Brauch J., Buchweitz M., Carle R.**

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Maqui (*Aristotelia chilensis* (Mol.) Stuntz), a South American evergreen shrub belongs to the Elaeocarpaceae family [1]. Due to its high phenolic levels maqui fruits have been suggested to exhibit health-promoting properties. In particular its high anthocyanin content, being responsible for the intense blackish color, has aroused increasing interest in maqui fruits as a healthy coloring food additive. Although maqui has previously been classified as novel food, its consumption in Europe is well documented since the end of the 19th century [2].

Detailed analyses of maqui fruit constituents are still scarce. Hence, lipids of dried and ground maqui berries were characterized by GC-MSn after accelerated solvent extraction (ASE). Further fruit constituents were analyzed by HPLC-MSn. Antioxidant activities and total phenolics were determined using FRAP-, TEAC- and the Folin-Ciocalteu-assay, respectively. In addition, total monomeric pigment content and the browning index were assessed spectrophotometrically. Sugar contents and edible acids were quantitated using enzymatic assays.

The lipid content of maqui was higher than usually observed in berries, predominantly consisting of linoleic and oleic acids contained in the seeds. High amounts of flavonols, ellagic acid and hydroxycinnamic acid derivatives and anthocyanins were found in the pulp. In accordance with literature, eight pigments were characterized, representing mono- and di-glycosides of cyanidin and delphinidin [3]. Total phenolic contents and exceptionally high antioxidant activities confirmed the high phenolic content as determined by HPLC-DAD. The berries contained moderate amounts of D-glucose and D-fructose, and citric acid was found to be the major acid.

The results confirm the potential of maqui as a so far under-estimated pigment-rich “superfruit” revealing promising properties as functional food additive.

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**PRUNUS MAHALEB, L. FRUIT EXTRACTS: A NOVEL SOURCE FOR NATURAL FOOD PIGMENTS****Gerardi C.<sup>1</sup>, Albano C.<sup>1</sup>, Blando F.<sup>1</sup>, Pinthus E.<sup>2</sup>, Rescio L.<sup>3</sup>, Mita G.<sup>1</sup>.**<sup>1</sup>Institute of Food Production, CNR, Territorial Unit Lecce, Italy.<sup>2</sup>FRUTAROM Ltd, Haifa, Israel.<sup>3</sup>Pierre s.r.l., Galatina, Lecce, Italye-mail: [carmela.gerardi@ispa.cnr.it](mailto:carmela.gerardi@ispa.cnr.it)

*Prunus mahaleb* is a deciduous tree native in Mediterranean region, Iran and central Asia. The tree prefers warm and dry climate, well-drained soil and is robust and resistant to diseases. For these characteristics, Mahaleb cherry trees are used as rootstock in grafting cherries, especially in Italy and above all in Apulia region. The plant is used also for a spice obtained from the seeds in Turkey, Armenia and Greece. *P. mahaleb* fruits are small thin-fleshed cherry-like drupe, green at first, turning red than purple to black when mature. They have no importance for crop production due to their astringent and sour taste. These fruits had been preliminary characterized [1], revealing a high content in anthocyanins, whose main qualities are an attractive vivid pink-red colour and numerous bioactive effects like inhibition of growth of human colon cancer cells [2] and high antioxidant and anti-inflammatory activity [3]. The aim of this study is the identification of the better extraction conditions to obtain from *P.mahaleb* fruits an extract rich in bio-functional compounds that can be used as a natural pigment for food industry. With this aim we extracted chemically untreated fruits and used food grade chemicals. The better total extract and anthocyanin yield were obtained using ethanol + 1% citric acid 1M as extraction solvent. Successively the ethanol was removed and we obtained a concentrate fruit juice with high anthocyanin content ( $9,852 \pm 0,49$  mg/ml) and antioxidant activity. A more detailed chemical characterization of the concentrated *P.mahaleb* fruit juice is in progress.

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**RED ORANGE ANTHOCYANINS IN COLORED JUICES AND DRINKS: ANALYTICAL METHOD VALIDATION**

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An increasing diffusion of drinks including red orange juice as ingredient is spreading on the market. Because of the red orange juice high cost, the red color is often achieved by the addition of natural and synthetic dyes (black carrot, red berries, carminic acid, allura red, etc.), reducing the amount of red orange juice in the final product even up to not use it as ingredient.

In order to assess the occurrence of red orange juice in colored drinks, a qualitative HPLC/PDA/ESI-MS/MS analytical method was developed to detect red orange typical anthocyanins (cyanidin 3-glucoside and cyanidin 3-(6"-malonyl) glucoside) [1-2]. The method was statistically validated in terms of accordance (100%, n=10), specificity/selectivity, repeatability of retention times (<0.5%), instrumental limit of detection (0.05 mg/L), method limit of decision (CC $\alpha$ , 0.36 mg/L) and limit of detection (CC $\beta$ , 0.40 mg/L). The chromatograms were evaluated by photo diode array and by tandem mass spectra of the quasi-molecular ions [M+H]<sup>+</sup> at *m/z* 449u and *m/z* 536u, limiting the UV-Vis interference due to other dyes eventually added. Because the cyanidin 3-(6"-malonyl) glucoside in processed and stored juices easily hydrolyses to cyanidin 3-glucoside, the judgment of conformity is expressed according to the following criteria:

- the presence of red orange juice in the analyzed sample is confirmed if the amount of cyanidin 3-glucoside > CC $\alpha$ ;
- the absence of red orange juice in the analyzed sample is established if the amount of cyanidin 3-glucoside < CC $\alpha$ .

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**ANTHOCYANINS EXTRACTION FROM MULBERRY BY A COMBINATION OF HIGH HYDROSTATIC PRESSURE AND ENZYMATIC HYDROLYSIS AS EMERGING TECHNOLOGY****Kim C.-T.<sup>1</sup>, Maeng J.-S.<sup>1</sup>, Kim C.-J.<sup>1</sup>, Cho Y.-J.<sup>1</sup>, Kim N.<sup>1</sup>, Oh H.-J.<sup>1</sup>, Kwon S.-J.<sup>1</sup>, Sung G. B.<sup>2</sup>**<sup>1</sup> Functional Materials Research Group, Korea Food Research Institute, Seongnam, Korea.<sup>2</sup> National Academic of Agricultural Science, Rural Development Administration, Suwon, Korea.e-mail: [ctkim@kfri.re.kr](mailto:ctkim@kfri.re.kr)

Natural colours such as anthocyanins, carotenoids, chlorophylls and betalains have used in as alternative source in food industry. Most natural colours commonly extracted by aqueous mixtures of organic solvent such as ethanol, methanol or acetone which contain a small amount of hydrochloric acids or others. Traditional extraction methods for natural colours have several disadvantages, such as involving high energy consumption, much time, low selectivity and low extraction efficiency. A combination of high hydrostatic pressure and enzymatic hydrolysis (HHP-EH) is a relatively new extraction technique that is programmable and thus is very reproducible with respect to product development. The aim of this research was to develop the practical application of HHP technology to extract anthocyanins from fresh mulberry in combination with enzymes Termamyl, Celluclast and Viscozyme L. This HHP-EH technique may provide a safe, simple, highly efficient and cost-effective method of extraction without using solvent or excessive heat. Fresh mulberry was chopped, homogenized and liquefied by a colloid mill. The suspension was mixed with enzymes Termamyl, Celluclast and Viscozyme L. The mixture was poured into a plastic bag, excess air carefully removed and then transferred to the programmable high pressure treatment apparatus set at pressures of 0.1 and 100 MPa for 12 h at 50 °C. Under the HHP-EH condition, there are significantly higher yields of anthocyanins, total phenolics and total flavonoids than those obtained with no enzymatic or high pressure treatment.

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**ANTHOCYANINS AND BIOACTIVES CONTENT IN HEALTHY  
RED FRUIT DRINKS**

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Nowadays one of the interesting topics for the juice industry is the investigation in new healthy red beverages. Anthocyanins are natural red pigments present especially in fruits as black grapes, strawberries and raspberries. These kinds of polyphenols are of interest because of its potential effect in reducing the incidence of cardiovascular disease, cancer and other chronic diseases through the intake of anthocyanin-rich foods (1). Evaluation of anthocyanins content and other bioactive polyphenols as flavonoids (anthocyanins, flavonols, flavanols, flavones, flavanones, isoflavones), phenolic acids (hydroxybenzoics and hydroxycinnamics), stilbenes and lignans, may be related to a beneficial effect on consumers' health.

Codex Stand 247-2005 includes the analysis methods for verification of composition, quality and authenticity of fruit juices and nectars. IFU (International Federation of Fruit-Juice Producers) method nº 71 (1998) determine the anthocyanins profile by HPLC with visible detection. This method has been validated, type I, by the IFU analytical commission (2).

The present work has studied anthocyanins and bioactives content of four commercial antiox red fruit juices and three smoothies. A modification of IFU method nº 71 was used for analyses (3). This method allow in a single analysis, without any sample pre-treatment, to identify and quantify not only anthocyanins but also other bioactive polyphenols. Polyphenols from vegetal residues were extracted with methanol and water-HCl for an accurate total individual polyphenol determination. From 25 to 30 different polyphenols such as flavonoids, phenolic acids and stilbenes were identified in every studied sample. Total polyphenol values were around 90 mgGAE/100 ml in antiox juices and around 130 mgGAE/100 ml in smoothies.

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**BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN FRUITS FROM ATLANTIC RAINFOREST, SOUTHEAST BRAZIL.****Azevedo-Silva N.<sup>1</sup>, Rodrigues E.<sup>2</sup>, Mercadante A.Z.<sup>2</sup>, Oyama L.M.<sup>3</sup>, De Rosso V.V.<sup>1</sup>**

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This study aimed to quantify the bioactive compounds, identify anthocyanins and carotenoids and determine antioxidant capacity by chemical and cellular methods in fruits from Atlantic Rainforest. The identification was performed using HPLC-PDA-MS/MS. To determine total phenolic compounds was used Follin-Ciocalteu method and the antioxidant activity was established by radical ABTS<sup>o+</sup> and peroxyl (ORAC and cellular methods). In relation to total carotenoids the fruits showed values varying between 76.82±21.82µg/100g in araçá and 1331.96±21.40µg/100g in uvaia. The grumixama and juçara had concentrations of 483.25±170.46 and 737.59±75.80µg/100g, respectively. The majority carotenoids in all fruits were all-*trans*-lutein, all-*trans*-β-cryptoxanthin and all-*trans*-β-carotene. Anthocyanins were identified and quantified only in grumixama and juçara. The first presented monomeric anthocyanins level of 2.90±0.1mg/100g and the majority was identified as cyanidin-3-glucoside. In juçara majority anthocyanin was cyanidin 3-rutinoside. The monomeric anthocyanin value was found to be 201.4±6.4mg/100g. In the analysis of total phenolics compounds, the araçá, grumixama and uvaia showed similar values (138.1 to 156.9mgGAE/100g). Juçara showed 553.7±3.6mgGAE/100g, the higher content of phenolic compounds of the all fruits. In determining the chemical antioxidant activity by ABTS method, the juçara (67.5±1.4µmoltrolox/g) showed the highest activity followed by grumixama, araçá and uvaia. The same order was maintained for testing peroxy radical deactivation in hydrophilic extracts. In the cellular antioxidant activity assay showed the highest activity juçara (108.9µmolQE/100g), followed by grumixama (99.3µmolQE/100g), araçá (66.9µmolQE/100g) and uvaia (19.1µmolQE/100g). Taking into account the levels of bioactive and antioxidant activity analyzes already conducted, it is recommended the insertion of juçara and grumixama in the Brazilian diet.



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**P 45**  
**PHENOLIC COMPOSITION OF NEBBIOLO GRAPES FROM  
PIEDMONT: CHANGES DURING RIPENING AND IDENTIFICATION  
OF GEOGRAPHIC ORIGIN**

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Polyphenols are widely distributed in the plant kingdom and involve a very heterogeneous group of molecules. They are responsible of several properties of vegetables (fruits, flowers, leaves), such as colour, bitterness and astringency and are directly involved in the defence mechanisms of the plants. Moreover, polyphenols possess a variety of recognized beneficial effects on human health, such as anti-inflammatory, antimicrobial and antiaging effects, and are also considered as key compounds for their antioxidant potential.

Grapes (*Vitis vinifera* L.) are rich in polyphenols; the phenolic composition of grape is very complex, including both monomeric and/or polymeric molecules as anthocyanins, flavan-3-ols, flavonols, dihydroflavonols, various phenolic acids and hydroxystilbenes.

Grape polyphenols are also important from the taxonomical point of view; in fact, the patterns of some classes of flavonoids are under strict genetic control and their distribution varies considerably among different grape cultivars, even if their absolute concentrations can vary widely depending on both environmental and agronomical factors.

Since polyphenols are important compounds determining the grape characteristics (and consequently the wine quality), and could be also potentially used to discriminate different cultivars, the principal aim of this work was to characterize the phenolic composition of Nebbiolo grapes. The amount of total polyphenols, anthocyanins and tannins, the antioxidant activity (DPPH method) and the chromatic characteristics of phenolic extracts obtained from Nebbiolo grapes (from Piedmont, four different geographic origin) were determined. Three different ripening stages were considered: before veraison, at the veraison and at the physiological ripeness (harvest), in order to follow the changes of phenolic fraction during berry development. Liquid chromatography (RP-HPLC/DAD) has been also applied for the characterization of individual polyphenols. Finally, the different Nebbiolo samples at maturity stage were compared with other Piedmont grape cultivars (Uva rara, Vespolina) and analysed in order to recognize their geographic origin.

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**ANTIOXIDANT PIGMENTS IN RED GRAPE JUICES (*VITIS VINIFERA* L. CV. AGLIANICO N.): *IN VITRO* BIOACCESSIBILITY, BIOAVAILABILITY AND PLASMA PROTEIN INTERACTION****Tenore G.C.<sup>1</sup>, Ritieni A.<sup>1</sup>, Campiglia P.<sup>2</sup>, Manfra M.<sup>3</sup>, Coppola L.<sup>4</sup>, Novellino E.<sup>1</sup>**<sup>1</sup>Department of Pharmacy, University of Naples "Federico II", Naples, Italy<sup>2</sup>Department of Pharmaceutical Sciences, University of Salerno, Salerno, Italy<sup>3</sup>Department of Science, University of Basilicata, Potenza, Italy<sup>4</sup>Coppola S.p.a., Scafati (Salerno), Italy

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Positive health benefits related to the consumption of red grape juice and the *in vitro* pharmaceutical potential of its polyphenols have been previously reported.<sup>1-3</sup> To achieve any effect in a specific tissue or organ, polyphenols must be available, which refers to the compound tendency to be extracted from the food matrix and then be absorbed from the gut via the intestinal cells. The aim of the present work was to evaluate the *in vitro* bioaccessibility and bioavailability of red grape juice polyphenols, by simulating an *in vitro* gastrointestinal (GI) digestion model that could reproduce the parameters at the base of the bioaccessibility and bioavailability of bioactive compounds in the human organism. GI digestion was distinguished into salivary, gastric and duodenal digestive steps; Caco2 cell lines were adopted to mimic the intestinal transit; the interaction of polyphenolic compounds with plasma albumins and lipoproteins after intestinal absorption was evaluated. Red grape juice provided a good intestinal bioaccessibility and bioavailability of oligomeric procyanidins. Since 49.4% of native procyanidins were not absorbed, they are expected to accumulate in the intestinal lumen where an antioxidative protection and a potential inhibition capacity of cellular cholesterol uptake could be assumed. The permeated procyanidins (6.7% of their native pattern, 12.0% of intestinal procyanidins) significantly bound (58.7%) to plasma HDLs, suggesting a major role in cholesterol metabolism. Our results would indicate red grape juice and its potential nutraceuticals as potent systemic antioxidants and effective tools in the regulation of plasma cholesterol levels.

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**P 47**  
**STABILITY OF NATURALLY**  
**COLOURED FOOD PLANT EXTRACTS**

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The use of food colorants as additives in the food industry is an important factor for both food companies and consumers in determining the acceptability of processed food. In recent years, due to the potential harmful effects (i.e. allergies and intolerance) of synthetic pigments, the use of natural pigments in foodstuff has some marketing advantages and therefore it has been increasing. The use of natural pigments (main groups are anthocyanins, chlorophylls, carotenoids, curcuminoids, and betacyanins) can cause problem due to their stability, so detailed knowledge about their behaviour at different pH, temperature, and light conditions are needed in order to optimize the industrial production and storage of the final products.

In the present investigation the stability of different aqueous and methanolic colored plant extracts to 1) thermal treatment at three different temperatures for different time, 2) different pH conditions, 3) sunlight and 4) UV radiation, were detected. Furthermore the antioxidant and antiradical activity of the plant extracts were evaluated.

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**COLOR DIVERSITY AND ANTIOXIDANT ACTIVITY IN CACTUS PEAR FRUITS FROM SOUTHERN ITALY GENOTYPES****Albano C.<sup>1</sup>, Aprile A.<sup>2</sup>, Negro C.<sup>2</sup>, Gerardi C.<sup>1</sup>, Mita G.<sup>1</sup>, Miceli A.<sup>2</sup>, De Bellis L.<sup>2</sup>, Blando F.<sup>1</sup>**

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Betalains are a water-soluble nitrogen-containing pigments that are responsible for the bright red or yellow coloration of fruits, flowers, roots and leaves of plant belonging to the order of *Caryophyllales*. One of this plant, *Opuntia ficus-indica* (L.) Mill. (cactus pear) contains in fact betalains in the fruits, particularly betacyanin in the red variety and betaxanthin in the yellow variety. Cactus pear has been considered as good source for red and yellow food colorants for use at neutral pH [1]. Recently, cactus pear has attracted attention due to the nutritional and health-promoting benefits [2].

With the aim to characterize the betalain composition and antiradical activity of *Opuntia ficus-indica* grown in South Apulia, we extracted with 10 ml ethanol:formic acid:water (50:5:45  $V/V$ ) 1g of lyophilized fruits of different colours: the purple, the yellow and the green one. The lack of commercially available standards make HPLC betalain analysis and quantification much difficult [3]. For this purpose we identified the unique betacyanin of *Amaranthus retroflexus* L. (betanin) and used it as standard retention time. We developed different HPLC analytical method in order to separate betacyanin (DAD at 535 nm) and betaxanthin peaks (DAD at 484 nm). As an average for all the methods, in purple variety the betacyanin peaks account for nearly 65% of the total, instead betaxanthin nearly 35% of the total. In the yellow variety, betacyanin account for 4% of the total, instead the betaxanthin for the 96% of the total. We report here also the TEAC (Trolox Equivalent Antioxidant Capacity) of fruit extract of different varieties.

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**P 49**  
**BETANIN STABILITY IN SELECTED AQUEOUS-ORGANIC SOLUTIONS INFLUENCED BY HEAVY METALS**

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An influence of several heavy metal cations on betanin stability was investigated at different physico-chemical conditions in selected aqueous-organic solutions. In general, heavy metal ions lead to betalainic pigments discoloration and decomposition [1] which is accelerated by high or very low pH. A possibility of a formation of complexes with metal cations is also considered, because of a presence of new maxima of absorption in betanin Vis spectra in tested solutions.

The study proved very low betanin stability under the presence of heavy metal ions with high organic solvent concentration. The decomposition of betanin catalyzed by heavy metal ions most probably proceeds by oxidation of the pigment. The highest influence on betanin was noticed for Cu(II), Fe(II), Fe(III) and Ni(II) ions.

The fastest changes in betanin spectra effected by Cu(II) are observed at pH 6-8 within first minutes of reaction. The presence of new absorption maxima could be an effect of a complex formation or betanin oxidative degradation. For the alcoholic and acetonitrilic solutions containing Fe(II) ions, there is no shift of the absorption band observed even during a few hours of reaction. The fastest degradation proceeds mainly at pH 3, especially in ethanolic solutions. In the case of Fe(III), the pigment degradation proceeds much faster than for Fe(II). Moreover, during the reaction, a bulk precipitation is observed. A possibility of a complex formation between betanin and Ni(II) is stated for pH 7-8 when its relatively high stability in aqueous samples is observed.

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**P 50**  
**TEACHING FOOD BIOTECHNOLOGY IN SECONDARY SCHOOLS**  
**USING RIBOFLAVIN AS EXAMPLE**

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Food Biotechnology accompanies mankind since thousands of years by using micro-organisms to produce food. Typical biotechnological products such as beer, bread, wine and vinegar are established topics in chemistry classes of upper secondary level. A lot of publications can be found dealing with the usage of food in chemistry classes. But here, food is used to teach different analytical methods, to analyse different food additives or to talk about good nutrition. Modern ways of food production and processing are usually not taught in German Secondary Schools.

Modern Food Biotechnology, however, includes more than just the production of food itself. Optimizing production technologies is also a vital part of this field, but this is not well known. Another example is that basic knowledge on the biotechnological production of vitamins is not widespread in public. Using the example of riboflavin [1] [2], which is also an important food colour, it is possible to teach the basic biotechnological manufacturing processes used for food production as well as consequences of using food biotechnology for the environment. We will present some ideas about how to include this topic into the chemistry curriculum of upper secondary level.

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**APPLICATION AND STABILITY OF THE NATURAL PIGMENT**  
**NEOCANDENATONE IN CANDY PRODUCTS IN COMPARISON**  
**WITH A COMMERCIAL ANTHOCYANIN**

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Neocandenate is a natural pigment from *Dalbergia congestiflora* heartwood [1]. This pigment displays red-purple colours similar to anthocyanin and it has shown excellent solution stability [2], therefore its addition to food products could meet the needs of these compounds, due to the growing trend in the market for the use of natural products.

In this study application tests in model systems of candy products were performed, various doses of pigment and citric acid were used in a candy with protein (gummies) and without protein (hard candy). To match commercial products 0.007, 0.014, 0.028 and 0.031% of neocandenate and 0.025, 0.050, 0.062 0.1% of anthocyanin at 3% was used. Additionally, colour changes were obtained when percentage of citric acid was modified from 0.3 to 1.0 % in the formulation. L, a, b \* CIELAB parameters and Chroma and H angle values were determinate for products pigmented with neocandenate and compared with those obtained with the commercial anthocyanin. The H values obtained for Neocandenate were from  $1.78 \pm 0.92$  to  $79.42 \pm 0.06$  and for anthocyanin from  $7.19 \pm 1.36$  to  $45.54 \pm 1.0$ , indicating that the neocandenate obtained with a wider range of colours in the final product. Accelerated shelf life assays were performed for all products at 40 °C for 192 h showing not colour changes in the pigmented products with both pigments in that conditions. These results showed that both pigments could be used for colouring candy products and they are stable in these matrices.

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**P 52**  
**CHARACTERIZATION AND GENETIC FINGERPRINT**  
**OF SAFFRON**

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The dried stigmas of *Crocus sativus* L. are a very expensive spice known as saffron used as food flavouring and colouring agent and as traditional herbal medicine. Crocus is cultivated in India, Iran, Spain, Greece and Italy. The production process involves a big manual work and cannot be completely mechanized. In Italy from a 1000 m<sup>2</sup> area about 120000-150000 flowers can be obtained (4000-5000 kg) which give rise to 5-7 kg of fresh stigma, i.e. 1.0-1.3 kg of dried product.

Many papers deal with analytical aspects to set up methods for the separation and determination of the biological active components [1, 2], and aroma components [3].

The purpose of this paper is the analysis of stigmas from *Crocus sativus* cultivated in Italy, Iran and Marocco to characterize secondary metabolites and the quality of commercial saffron.

The major biologically active components of saffron are crocin analogues which are all glycosides of *trans*-crocetin, a carotenoid derivative which are responsible for colour. Safranal which is responsible for the characteristic aroma of saffron, is formed during the storage by dehydration of picrocrocine which is responsible for its bitter taste.

Stigmas of *Crocus sativus* L. samples were analysed for their crocins and flavonols content.

Identification of crocins, safranal, picrocrocine, and flavonols (kaempferol derivatives) was carried out by HPLC/DAD/MS analysis.

Other aim of this study will be the definition of a genetic fingerprint useful for the characterization of Italian germplasm.

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**P 53**  
**EXTRACTION METHODS OF NATURAL PIGMENTS FROM  
STAMEN OF SAFFRON FLOWER**

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In Iran annually produce high amounts of petal and stamen of saffron flower as by product. Stamen contains many kinds of valuable components such as antioxidants, phenolic compounds, carotenoids and anthocyanins. The possibility of extraction and applying of natural pigments from stamen is the purpose of this paper. The extraction of pigments was done by different solvents in an ultrasound apparatus at different times (5-15 minutes) and intensities (20-100%). Results showed the stamen contain three components of natural pigment: soluble and insoluble water carotenoids and anthocyanin. The best time and intensity of ultrasound with the highest yield and antioxidant activity were introduced.

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## P 54

**EFFECT OF SALT-STRESS ON THE PRODUCTION OF PIGMENTS BY *CHLORELLA VULGARIS* UNDER HETEROTROPHIC CULTURE****Benavente-Valdés J.R.<sup>1</sup>, Montañez J.C.<sup>2</sup>, Aguilar C.N.<sup>1</sup>, Méndez-Zavala A.<sup>2</sup>**<sup>1</sup> Department of Food Research, Universidad Autónoma de Coahuila, Saltillo, México.<sup>2</sup> Department of Chemical Engineering, Universidad Autónoma de Coahuila, Saltillo, México.

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Nowadays microalgae represent a potential, economic and ecological option for the development of new products in the alimentary industries [1]. These photosynthetic microorganisms are an exclusive biological source of pigments such as carotenoids and chlorophylls [2]. Nitrogen and phosphate deprivation, high light intensity and salinity are factors than can improve the pigments and lipid content [3]. The effect of salt concentrations (0, 1, 2 and 3% NaCl w/v) was evaluated under two light regimens (constant illumination and dark). Cultivation was carried out in heterotrophic conditions using glucose (2 g L<sup>-1</sup>) as a carbon source during 216 h at 30 °C and 145 rpm. Growth was determined gravimetrically and pigments (total chlorophyll and carotenoids) were quantified spectrophotometrically. The microalga *Chlorella vulgaris* was able to grow in all the NaCl concentrations tested under both conditions. The biomass yields was higher using 1% of NaCl in comparison with basal medium (without NaCl), however, at higher concentrations of NaCl (2 and 3%) the growth decreased for both light conditions. Microscopic observation revealed an increment in cell volume with green-orange content under NaCl concentrations of 2 and 3% in dark and light conditions. The salt-stress treatment under darkness increased the accumulation of the total carotenoids per dry biomass to 1.45 mg g<sup>-1</sup> in comparison with non-stressed cells (0.77 mg g<sup>-1</sup>). These findings indicate that salt-stress may have a potential commercial importance; simplifying the algae culture in open system in which light availability is low and the saline environment reduce the risk of contamination.

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**P 55**  
**CAROTENOIDS PROFILE OF ULTRASOUND-ASSISTED  
EXTRACT *PHORMIDIUM SP.***

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Microalgae are recognized as a source of natural colorants such as carotenoids. Ultrasound-assisted extraction (UAE) is an environmentally friendly technique that cut down working times and a solvent consumption. In this study, the carotenoid profile from microalgae *Phormidium sp.* used in agroindustrial wastewater treatment, extracted by conventional and UAE techniques, was investigated. The experiments were carried out in a bubble column bioreactor, initial cell concentration of 0.1 g.L<sup>-1</sup>, and absence of light. The biomass was separated from the culture medium by centrifugation. Carotenoids were extracted exhaustively with acetone in a homogenizer, in conventional method. For the UAE extract obtaining, the instrument was operated with an amplitude wave of 20% for 20min. The carotenoids were determined by high performance liquid chromatography coupled to photodiode array detector (HPLC-PDA, Shimadzu) on a C30 column. The identification was performed according to the information: elution order, co-chromatography with authentic standards, UV-visible spectrum, compared with literature (Britton, 2004; Zepka & Mercadante, 2009). A total of 19 different carotenoids were separated in both extracts. Although the same carotenoids have been found in the different extracts analyzed, the proportion among they wasn't equivalent. While all-*trans*- $\beta$ -carotene was the major carotenoid found in the extract from conventional method (31%), all-*trans*-zeaxanthin was the major in UAE extract (32%). Echinenone, all-*trans*-lutein, and 9-*cis*- $\beta$ -carotene also was present in representative amounts in both extracts. In summary, compared with conventional extraction, the time used in UAE is shorter, with higher extraction yield. UAE, therefore, is a rapid, competitive method for extraction of carotenoid from microalgae.

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**P 56**  
**MICROORGANISMS USED AS PIGMENT SOURCE**

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Production food coloring agent from microbial source is an important issue. The color is very important for the acceptance of foods. Pigment producing microorganisms exist commonly in nature and various pigments are produced from these microorganisms. One of these microorganisms is microalgae. Microalgae are microbial sources that they show saving feature on some valuable commercial metabolite.  $\beta$ -carotene, lutein, phycoerythrin, xanthophyll and phycocyanin examples of pigments that are produced by microalgae. Five different pigment producing microorganism are Monascus, Penicillium, Dunaliella, Haematococcus and Parohyridium. Carotenoid, melanine, flavine, quinone, monascin, violacein, phycocyanin and indigo are examples of pigments that produced by these microorganisms. These pigments are used in various field including foods. Food coloring agents producing by fungus take researchers attention. Pigments producing by microorganism and microalgae are explained with examples in this review.

**Keywords;** microorganism, microalgae, fungus, pigment

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**PIGMENTED FILAMENTOUS FUNGI ISOLATED FROM TROPICAL MARINE ENVIRONMENTS AROUND REUNION ISLAND****Fouillaud M., Boyer E., Fel A., Caro Y., Dufossé L.**LCSNSA, University of La Réunion, Reunion Island  
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Filamentous fungi are ubiquitous organisms, whose role is often overlooked in marine ecosystems [1]. They are otherwise potential producers of natural substances of interest to many industries [2, 3]. This study initiated the search for filamentous fungi in some marine biotopes of La Reunion island's reef flat. The focus was on fungal populations with low culture requirements which would facilitate the expression of potentially workable strains in an industrial context, especially with regard to biomass or pigments production. Samples were collected in the back reef-flat and on the external slope of the coral reef, on the west coast. Sediments, open water, hard substrate and coral's fragments from different sites were analysed for fungal contents. The fungi were recovered through cultivation under oxygenic conditions on synthetic media prepared with sea water. About 40 different species were identified. Amongst the more frequent species recovered, the *Aspergillus* and *Penicillium* genera are very represented. Twenty five isolates at least produced pigments during their growth. The shades observed range from pale yellow to dark brown, through red colors, according to the strains. Some species such as *Monascus ruber*, *Penicillium citrinum*, *Penicillium rubrum* or *Penicillium purpurogenum* are already known as pigments producers. The study confirms that La Reunion island's back reef flat and coral reef shelter many revivable fungi. The isolated species, usually considered as terrestrial could just survive or could have been accustomed to the marine environment. Whatever, the strains which could be new sources of pigments for food or dyeing industries shall be further investigated.

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**P 58**  
**VALORISATION OF VINASSE, A RUM DISTILLERY EFFLUENT,  
BY THE PRODUCTION OF CAROTENOID PIGMENTS USING  
FILAMENTOUS FUNGI**

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Among natural pigments, carotenoids are lipophilic molecules that are commonly found in fruits and vegetables<sup>1</sup>. These molecules are known for their antioxidant properties and provitamin A activities. Filamentous fungi constitute an alternative source for the production of carotenoids. The originality of our work lies in the use of vinasse as a growth substrate for those fungi. Vinasse is a dark brown effluent obtained by the distillation of rum. It is poorly valorised and pollutant for the environment (high Biological Oxygen Demand and Chemical Oxygen Demand). Having an acidic pH, vinasse contains organic molecules and can be a potential good source of carbon and nitrogen for the growth of filamentous fungi<sup>2</sup>.

We worked with 10 strains from 6 different species which were chosen for their ability to produce carotenoids. Once the fungus was cultivated, the biomass was lyophilized then crushed and carotenoids were extracted with methanol and methyl tertiary butyl ether. The absorbance of samples was measured by a spectrophotometer (450nm) and the total carotenoids concentration was calculated by the Beer-Lambert law. HPLC was used to evaluate the qualitative production of carotenoids.

Among the 10 strains only *Mucor circinelloides* and *Phycomyces blakesleanus* were able to grow on vinasse and to produce significant amounts of pigments. HPLC experiments showed that  $\beta$ -carotene was the major product for all strains but chromatographic profiles were different depending on the culture medium and the lighting conditions. Those results showed that carotenoid production using fungi could be an interesting and alternative way to valorize vinasse.

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## PIGMENTS PRODUCED BY THE BACTERIA BELONGING TO THE GENUS *ARTHROBACTER*

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Since several decades, pigments have been used as a taxonomic tool for the identification and classification of bacteria. Nowadays, pigment producing microorganisms have been also widely interested in scientific disciplines because of their biotechnological potential. With the growing interest in microbial pigments because of factors such as production regardless of season and geographical conditions, novel microorganisms which their pigments can be extracted are being evaluated. In the nature, a numerous number of microorganisms e.g. yeast, fungi, algae and bacteria produce pigments. The genus *Arthrobacter* is one among diverse microorganisms which has been found to produce pigments. Most of bacteria in this genus produce a range of pigments. Several previous studies show that pigments produced by bacteria belonging to the genus *Arthrobacter* have various hues depending on the chromophore which is present, e.g. yellow by carotenoid and riboflavin, green and blue by indigoidine and indochrome, and red by porphyrins and carotenoids. Since long time numerous strains in this genus have been reported that their colonies are colored; however, the purification and characterization of their pigments were not frequently conducted until well know chemical structures and role in these strains. Consequently, a study of pigments produced by the genus *Arthrobacter* may be worthy to play attention for discovering a novel source of natural colourants.

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**CHARACTERIZATION OF *ARTHROBACTER ARILAITENSIS***  
**PIGMENTATION USING SPECTROCOLORIMETRY**

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Spectrocolorimetry was used for the evaluation of *Arthrobacter arilaitensis* pigmentation. A total of 14 strains of *Arthrobacter arilaitensis* isolated from smeared cheeses were cultivated on milk ingredients-based agar. After 7 days, the bacterial biofilms were measured and expressed by the CIE  $L^*a^*b^*$  colorimetric system. Alignments of hue value from each experimental ( $a^*b^*$ ) pair ranged from 72.39 to 240.83°. The effect of light exposure against storage in the dark was also investigated using this approach. The color intensity (function of chroma,  $C^*$ ) of 8 strains have been significantly decreased under darkness. Three groups with distinct behaviors by hue angle were demonstrated for the 14 *A. arilaitensis* strains.

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**P 61**  
**MODELING THERMAL STABILITY OF RED PIGMENTS  
PRODUCED BY *PENICILLIUM PURPUGENUM* GH2**

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In recent years studies focused on the production of natural pigments have received much attention due to their potential applications as an alternative to synthetic food colorants. It has been reported that some microorganisms have the ability to produce pigments in high quantities [1]. Microorganism are more feasible sources of pigments in comparison to pigments extracted from plants and animals because they do not have seasonal impediments and could be produced in high yields [2]. The knowledge of how the process conditions affect the stability of natural pigments is important to allow their promising use as food additives in the food industry [3]. Optimization of food processes relies on adequate degradation kinetic models to reduce nutrients damage and to increase product quality. This study aimed to evaluate the influence of temperature on the degradation kinetics of a red pigment extract (RPE) produced by *Penicillium purpurogenum* GH2 in order to predict its impact on thermal processing. RPE was treated at different time-temperature combinations in the range of 0-270 min and 30-80 °C. Results showed that a fractional conversion model was the best describing the time-dependent pigment degradation ( $R^2=0.95-0.98$ ) with k-values ranged between  $0.210 \text{ min}^{-1}$  and  $0.235 \text{ min}^{-1}$  and D values between 182.7 h and 18.83 h. Results suggested that RPE is a relatively thermostable pigment with a z-value of 50.66 °C and  $E_a$  of 43.67 kJ mol<sup>-1</sup>.

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**pH STABILITY OF RED PIGMENTS PRODUCED BY *PENICILLIUM PURPUROGENUM* GH2**

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Stability of natural pigments and bioactive content in thermal and non-thermal processing technologies has been a major challenge in food processing [1]. To date, the application of heat is the most common method for processing food. However, pigments stability is not only a function of the processing temperature, it is also influenced by other properties such as pH, chemical structure, enzymes, proteins and metallic ions and other storage conditions like light, and oxygen [2]. There are many studies in degradation of food compounds such as anthocyanins, carotenes, enzymes [3], but there is scarce information in literature about stability of natural pigments produced by microorganisms. The aim of this study was to evaluate the influence of pH on the stability of red pigments produced by *Penicillium purpurogenum* GH2. Red pigment solutions were adjusted to different pH values (4.0, 5.0, 6.0, 7.0 and 8.0) and incubated at 80 °C from 0 to 270 min. Results showed that red pigments were less stable at acidic pH values (4 and 5), whilst at pH values rinsing to neutrality (6 and 7) and alkaline (8) the pigments were more stable. The red pigments were capable to maintain 48%, 50%, 61%, 63% and 65% of the initial colour at pH values of 4, 5, 6, 7 and 8 after 270 min, respectively. Despite the low pH stability determined in this study, *Penicillium purpurogenum* GH2 pigments compares well with other natural pigments, so that these pigments are still a promising colour food additive.

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**PREPARATION OF BROWN-COLOURED SUBMICRON-SIZED  
HAZELNUT SKIN FIBER WITH HIGH ANTIOXIDANT CAPACITY  
USING HIGH SHEAR HOMOGENIZATION**

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With its natural brown color, hazelnut skin that is a by-product of roasted hazelnuts offer potential for utilization as a coloring agent in foods. However, its use for this purpose needs further modification of this fiber-rich material. Ground hazelnut skin contains approximately 15% of lipids that should be removed prior to use. Large particles of ground hazelnut skin is not useful for direct utilization in food formulation.

In this study, sub-micron sized particles from hazelnut skins defatted by hexane were obtained by means of high shear homogenizer. Half grams of defatted ground skin material was suspended in 100 ml of water. The suspension was pre-homogenized using a low shear mixer (Heidolph, Silent M Crusher) at 25000 rpm for 8 minutes. Then, pre-homogenized suspension was passed through a high shear microfluidizier (M110P, Microfluidics, Newton, MA, USA). High shear homogenization treatment was performed under various processing conditions applying different pressures (10000 or 30000 psi) and cycle times (1, 3, 5 and 10). Homogenized suspensions were then lyophilized to obtain sub-micron sized solid particles. Obtained materials were characterized by measuring particle size distribution, color, phenolic compounds profile and total phenolic compounds , and total antioxidant capacity. The results indicated that high shear homogenization process significantly improves physical and chemical properties of hazelnut skin as a potential coloring agent and bioactive material. Brown color intensity of hazelnut skin could be improved by high shear homogenization process. Decreasing the particle size significantly increased availabe concentration of bioactive phenolic compounds.

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## **SURVEY ON OCCURRENCE OF AMINOCARMINIC ACID IN E120 (CARMINE)-LABELED FOOD ADDITIVES AND BEVERAGES**

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With the aim of gaining a greater understanding on the conformity and legal compliance of additives used for the preparation of commercial red juice-based beverages, our research focused on an in-depth study of the chemical structure of an unknown colouring stated on the label as E120 (Carminic acid). This unknown red-purple colorant presented spectroscopic, chromatographic and mass spectrometric properties different from carminic acid [1]:

- retention time of the unknown molecule, under the chromatographic analytical conditions, eluted about 7 minutes later from carminic acid;
- UV-Vis spectrum at pH 1 showed maximum of absorptions at 530 and 564 nm, differently, carminic acid showed only a maximum at 490 nm;
- ESI/MS<sup>n</sup> studies, NMR and elemental composition confirmed that the unknown molecule is a semi-synthetic amino derivate of carminic acid, namely the 4-aminocarminic acid [2, 3].

Analyses of about 30 samples of commercial E120-labeled red-colored-beverages and E120 additives, collected in the Italy during Ministry quality control investigation, demonstrated that more than 50% of the investigated samples contained aminocarminic acid, evidencing the alarming illicit employ of this semi-synthetic carmine acid derivate instead of carminic acid [1].

Aminocarminic acid is not present in the lists of authorized additives (EC Reg. 1333/2008) and there are no current studies on its toxicity.

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