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ORAL PRESENTATIONS

USE OF SCANNING ELECTRON MICROSCOPY IN THE STUDY OF OLIVE OIL MICROBIOTA

G. Ciafardini, B.A. Zullo

Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy.

E-mail:ciafardi@unimol.it

Microorganisms are generally found in the production of many foods such as bread, cheese, cured meats, wine and many more foodstuffs. However, until a few years ago, olive oil was excluded from this extensive list as it was not considered a suitable habitat for microbial life. Moreover, during previous microbiological researches, where scanning electron microscopy (SEM) was used, we observed the presence and activity of microorganisms in olive oil, for the first time, which were represented mainly by yeasts engaged in some biochemical reactions, such as the debittering process of the newly produced olive oil during the sedimentation phase.[1-3] The SEM observations revealed the ultrastructural characteristics of the cells, as well as the damage showed by the yeasts which had been wall grown in oils rich in polar phenolic compounds or in flavoured olive oils containing lemon, garlic, chilli or oregano.[4] The use of electron microscopy has potentially great future perspectives in the study of microbiota of the extra virgin olive oil. Furthermore, promising results can be reached by SEM together with other techniques of molecular biology, in future studies on the non-cultivable microorganisms, including new species of yeasts, which are widespread in the various types of extra-virgin olive oils produced in the Mediterranean area.[5]

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X-RAY MICROSCOPY APPLIED TO FOOD SCIENCE

A. Gianoncelli

Elettra Sincrotrone Trieste, Italy

E-mail: alessandra.gianoncelli@elettra.eu

Investigating the biochemistry and the processes occurring at subcellular length scales in complex biological systems requires an interdisciplinary research approach. X-ray microscopy based on a synchrotron light has attracted the attention of life science scientists since it combines high resolution X-ray imaging with element sensitive spectroscopies, such as X-ray fluorescence (XRF) and X-ray absorption spectroscopy (XAS).[1,2] The major advantage of X-ray microscopes is their higher penetration depth and their easier sample preparation procedure, compared to electron-based microscopies. This presentation will emphasize the potential of soft X-ray microscopy, combined with X-ray imaging and low energy X-ray fluorescence (LEXRF) imaging in the food science field, presenting a short description of the methodology and selected results.[3-5]

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THERMOVALORIZATION OF PARMA HAM PRODUCTION WASTE: (E)SEM/EDX ANALYSES

M. Marmiroli, D. Imperiale, F. Mussi, N. Marmiroli

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy

E-mail: marta.marmiroli@unipr.it

Parma, at the heart of the Food Valley, has three main sectors in food production: dairy products, tomato sauce, and seasoned pork meat. Among the latter production chain, the Prosciutto Crudo di Parma (DOP) has the greater importance in terms of market (almost 3 billion Euros in 2016) and quantity of produce (2000 tons in 2016). However the production line for Parma ham generates a great amount of left overs in terms of pork meat, rind, gristle and bone, up to 20% of the starting material. An average processing house, producing 2200-2500 pieces per week, piles up 300-350 Kg of waste that have to be eliminated by expensive and rather polluting incineration.[1] The tens of tons of waste generated each year are not only adding to the market price of the goods, but should be disposed of in modern environmental friendly ways. One such strategy is to utilize the whole waste as feedstock for the production of syngas and biochar through pyrogassification. In fact, biochar, a carbon rich material, has a worldwide marked as fertilizer and soil conditioner thanks to its high microporosity.[2] The research and applicative collaboration between the University of Parma and the representative of the food industry within the Parma Province has among its main objectives to characterize the biochar obtained from ham production waste in order to optimize its qualities as soil amendment for the local agriculture. One of the most important techniques to study biochar is scanning electron microscopy (E)SEM, which, coupled with the EDX detector, allows to analyse both microstructure and elemental content of the biochar substrates[3]. Biochar is usually obtained from woody feedstock, rich in carbon from macromolecules such as lignin and cellulose, while the ham waste are abundant in nitrogen, from the meat, and carbon as fats and cartilage from gristle and rind. There are also micro and macronutrients elements, in particular Ca, Fe (from bone and meat) and Cl and Na from the salting process, that when in excess can lower the biochar quality. The raw material from Parma ham was analysed using Low-Vacuum SEM/EDX to identify significant structure's features that are conserved in the biochar in order to improve the product microporosity. This study will help to improve the quality of the outcome from the Parma ham waste disposal in order to decrease the cost of the operation.

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FOOD CHARACTERIZATION BY MULTIVARIATE IMAGE ANALYSIS

A. Ulrici

Department of Life Sciences, University of Modena and Reggio Emilia, Italy

E-mail: alessandro.ulrici@unimore.it

The basic tool for the evaluation of visual characteristics of a food product, often giving precious information about its quality, is eyesight. Unfortunately, human visual assessment is always affected by subjectivity, low sensitivity and scarce reliability. In this context, electronic eyes can be considered as valid substitutes of human eyes. Indeed, in the last decades, the use of digital images - including RGB, multispectral and hyperspectral images – has emerged as a powerful tool for the objective, reproducible and rapid assessment of food sample characteristics, which also widens the possibility to acquire information beyond the visible part of the electromagnetic spectrum. To reach these goals, Multivariate Image Analysis (MIA)[1] offers a wide range of effective tools to convert digital images in objective and transferable information. Essentially, MIA consists in the development and application of various statistical and computational strategies for the analysis of digital images, which contain a given number of picture elements (pixels), each one characterized by a series of color/spectral variables (channels). MIA can be applied to study in depth a single food sample by analyzing its image at the pixel-level, for example to clearly identify the areas where a specific feature (*e.g.* a defect) is present, or to map its chemical composition. Furthermore, high amounts of images taken from different food samples can be analyzed altogether at the image-level: this allows to quantify specific sample properties (*e.g.*, the content of a given pigment) directly from the relevant images,[2] or to automatically classify different samples based on their aspect or composition.[3] Thus, MIA can constitute a valid tool to extract useful information also from microscopic images. In this context, this communication is aimed at illustrating the basic principles of MIA and its potentials, through some representative applications for the characterization of various food types.

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OZONE SANITIZATION OF WITHERING GRAPES FOR AMARONE WINE PRODUCTION: A MULTI-MODAL IMAGING ANALYSIS

B. Cisterna[1], F. Boschi[2], A.C. Croce[3], R. Podda[1], M. Costanzo[1], P. Marzola[2], P. Bernardi[1], D. Degli Innocenti[1], G. Tabaracci[4], V. Covi[4], M. Malatesta[1]

[1]Dept. of Neuroscience, Biomedicine and Movement Sciences, University of Verona; [2]Dept. of Computer Science, University of Verona; [3]GM-CNR, University of Pavia; [4]San Rocco Clinic, Italy

E-mail: barbara.cisterna@univr.it

Amarone is a typical dry red wine characterized by peculiar colour, taste and perfume, and produced in Veneto wine region Valpolicella (Italy) from local grapes varieties among which Corvinone and Rondinella. After the harvest, the grapes slowly dehydrate in a large loft called *fruttaio* where the bunches remain until having lost about 40% of their original weight[1]. The berries are then crushed and the wine is left to age in barriques for at least three years before refining in bottles for six months. Although a phytosanitary checking guides the harvest of only healthy and ripe grapes, during the withering phase, mycotic infections develop especially due to *Botrytis cinerea.*[1] *B. cinerea* attacks the berry causing the gray mold which leads to a loss of product and a decreased yield for producers of Amarone wine. Ozone is a powerful oxidising gas able to inhibit the growth of the fungus, without however leading to phytotoxic injuries on berry tissue[2] or influencing wine aroma.[3] Thanks to a multimodal approach, we evaluated the effect of short and repeated treatments with low ozone concentrations during the withering phase of Corvinone and Rondinella grapes. We considered morpho-functional parameters taking into account the structure of the epicuticular layer of the berry skin and the *B. cinerea* morphology by scanning electron microscopy; the fluorescence properties of the berry by Optical Imaging; the berry dehydration grade by Magnetic Resonance Imaging and the colour of intact berry by a non-destructive optical analysis. Our results demonstrated that a mild ozonisation blocks the gray mold development without altering the withering process and skin colour, thus paving the way to long term studies on Amarone wine obtained with ozone treated grapes.

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MICROMORPHOLOGICAL ANALYSIS IN FLOWERS AND LEAVES OF GRAPEVINE (*Vitis vinifera* ssp. *sativa*)

E. Sgarbi[1,2], C. Bignami[1,2], L. Olmi[1,2]

[1]Department of Life Sciences; [2]Interdepartmental Center "Biogest-Siteia" University of Modena and Reggio Emilia, Italy

E-mail: elisabetta.sgarbi@unimore.it

SEM analysis represents an important tool to recognize some plant traits, morpho-physiological characters useful in studying reproductive fitness and plant-environmental relationships. Some cultivars of grapevine cultivated in Emilia, the Western part of Emilia-Romagna Region, have been studied to obtain a morpho-functional characterization of flowers. Malbo gentile, Trebbianina, Lambrusco di Sorbara, L. Montericco and L. Barghi showed hermaphrodite flowers with a normally developed gynoecium but back-reflexed stamens. Their flowers produced anomalous pollen, without furrows and pores-acolporated pollen unable to let pollen tube growth. In Termarina and Lambrusco salamino, differently, hermaphrodite flowers had erect stamens at the anthesis and produced normal tricolporate pollen. These results have been related with those reported from OIV and IPGRI grapevine descriptors,[1] relatively to the occurrence of morphologically hermaphrodite but functionally female flowers. Grapevine leaf traits are frequently related with susceptibility or tolerance to biotic and abiotic stress.[2-4] Moreover, seventeen *Vitis* genotypes, from different Italian Regions, have been analysed at SEM to characterize leaves traits. Results showed that the genotypes differ for stomatal density and size: mean stomata density ranged between 100 and 200 stomata/mm^2. Highest values of stomata density were recorded in the cultivar Nero d'Avola from Sicily and in *V. berlandieri,* an American species from which several currently-used rootstocks have been obtained, while the lowest value was in wild grapevine (*Vitis vinifera* ssp. *sylvestris*). Evaluating density, position and types of trichomes, a wide variation - mainly with respect of density - resulted within the observed genotypes. These characters are usually taken

in consideration as ampelographic descriptors[1] but they play also an important role in pathogen resistance and may be related with plant resistance to stress.

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COMPLEMENTARITY OF EM-BASED AND MS-BASED TECHNIQUES FOR THE PHYSICOCHEMICAL CHARACTERIZATION OF NANOMATERIALS

F. Cubadda

Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità - National Institute of Health, Rome, Italy

E-mail: francesco.cubadda@iss.it

Nanomaterials exhibit unique size- and structure-dependent properties distinct from those associated with larger sizes of the same material. Nanotechnologies, *i.e*. application of scientific knowledge to manipulate and control matter in the nanoscale, enable management of characteristics such as material size and morphology for the improvement or development of new process and product properties. In the food sector, three main categories of products/applications of nanotechnologies and nanomaterials can be identified, *i.e*. agricultural production (*e.g*. nano-formulated agrochemicals and animal feeds), food processing (nano-sized ingredients, nutritional supplements, additives), and food contact materials. Although making materials smaller can generate novel and useful properties, concerns exist on potential risks related to the interactions of nano-sized materials with cellular components, which may ultimately harm human health. The unique physicochemical properties of engineered nanomaterials may influence their toxicological properties, first of all the toxicokinetic behaviour, and pose the need to identify and characterise any potential hazard arising from such properties. In the present lecture it will be shown applications of Scanning and Transmission Electron Microscopy (EM), coupled with Energy Dispersive Spectroscopy, to the physicochemical characterization of nanomaterials intended for food use, either pristine or incorporated in food matrices, and to their detection in tissues from nanotoxicological studies, mainly focusing on oral exposure. It will be shown that EM is the technique of choice in such studies as it enables imaging of particles and the study of their morphology. On the other hand, quantitative data (size distribution and number concentration) can be difficult to be obtained when complex matrices are dealt with. It will be highlighted how, for inorganic nanoparticles, single particle-ICP-MS and ICP-MS in combination with a fractionation technique such as ultrafiltration or field flow fractionation (FFF) are ideal complementary techniques. FFF has the advantage of the possible combination with multiple detectors in addition to ICP-MS, such as UV, dynamic light scattering (DLS) and multiple angle light scattering (MALS), which can be used for organic nanoparticles as well. On the other hand, speed, sensitivity and determination of the number-based size distributions of the particles are the major advantages of single particle-ICP-MS.

POTENTIAL APPLICATIONS OF *Hemidesmus indicus* IN THE FOOD AND PHARMACEUTICAL SECTORS

C. Fimognari[1], E. Turrini[1], C. Calcabrini[1], E. Catanzaro[1], G. Greco[1], M. Tacchini[2], V. Pellicioni[1]

[1]Department for Life Quality Studies, University of Bologna; [2]Department of Life Sciences and Biotechnology, University of Ferrara, Italy

E-mail: carmela.fimognari@unibo.it

In traditional Indian medicine, the crude drug *Hemidesmus indicus* root -commonly known as Indian sarsaparilla- is used alone or in poly-herbal preparations for the treatment of a wide range of diseases.[1] The present study focuses on the cancer chemopreventive and therapeutic potential of *H. indicus* extracts on human cells. The possibility of modulating the cellular responses to mutagens by manipulating dietary factors has opened a new frontier for cancer control. DNA damage is frequently associated with chemical toxicity and carcinogenesis.[2] Preventing the manifestation of these events may prevent the induction of cancer. Through an integrated experimental approach, we explored the genoprotective potential of *H. indicus* extract against different mutagenic compounds. Moreover, we explored its pharmaceutical potential in the oncological field through the analysis of its cytotoxic, pro-apoptotic, and cytostatic properties on cancer cells. In our experimental conditions, the antigenotoxicity results showed that the extract was able to mitigate DNA damage, an essential mechanism for its applicability as a chemopreventive agent,[3] *via* either the modulation of extracellular and intracellular events involved in DNA damage. Moreover, *H. indicus* induced a cytotoxic effect on cancer cells involving the activation of both intrinsic and extrinsic apoptotic pathways and blocked the cell cycle in the S phase. These data add to the growing body of evidence that *H. indicus* can represent a

noteworthy dietetic strategy to prevent cancer and has an interesting therapeutic potential in the anticancer pharmacology.

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CREATINE PRESERVES THE MYOGENIC CAPACITY OF OXIDANT-STRESSED C2C12 MYOBLASTS THROUGH MITOCHONDRIALLY-TARGETED MECHANISMS

P. Sestili, C, Calcabrini, M. Battistelli, S. Salucci, M. Gervasi, E. Falcieri and E. Barbieri

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: piero.sestili@uniurb.it

Creatine (Cr) is a nutritional supplement promoting a number of health benefits, whose use is spreading in the prevention of muscle aging and treatment of neuromuscular maladies[1]. Indeed Cr has been shown to be beneficial in disease-induced muscle atrophy, improve rehabilitation and afford mild antioxidant activity[2]. The beneficial effects of its supplementation are likely to derive from pleiotropic interactions. In accord with this notion, we previously demonstrated that multiple, pleiotropic effects account for the capacity of Cr to prevent the differentiation arrest caused by oxidative stress in C2C12 myoblasts, namely: increased expression of muscle regulatory factors mRNA, antioxidant activity, amelioration of energy status and preservation of mitochondrial damage[3]. Given the importance of mitochondria in supporting the myogenic process, here we further explored morphologically and biochemically the protective effects of Cr on the structure, function and networking of these organelles in C2C12 cells differentiating under oxidative stressing conditions (acute exposure to 0.3 mM H_2O_2); the effects on the energy sensor AMPK, on PGC-1α, which is involved in mitochondrial biogenesis and its downstream effector Tfam were also investigated. Our results indicate that damage to mitochondria is crucial in the differentation imbalance caused by oxidative stress and that the Cr-prevention of these injuries is invariably associated with the recovery of the normal myogenic capacity. We also found that Cr-activates AMPK and induces an up-regulation of PGC-1α expression, two events which are likely to contribute to the protection of mitochondrial quality and function.

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FOOD IMAGING THROUGH MICRO-TOMOGRAPHY: WHICH POSSIBILITIES?

V. Zambelli[1], P. Delvecchio[1], A. Panariti[1,2], G. Bellani[1,2]

[1]Department of Medicine and Surgery, University of Milano Bicocca, Monza; [2]Department of Emergency and Intensive Care, San Gerardo Hospital, Monza, Italy

E-mail:giacomo.bellani1@unimib.it

X-ray computed tomography (CT) was introduced into clinical practice in 1972 and it is considered the first of the modern imaging techniques. It uses X-rays that have short wavelength and high energy, thus penetrating objects better than light. It records 2D X-ray images from various angles around an object and reconstructs them in a 3D-rendered volume, allowing dimensional and volumetric analysis. In the relative recent years, micro-CT have been developed by microfocusing X-ray tubes, leading to major improvements in the resolution, in order to determine microstructure of biological samples. Besides the large use of CT in the study of the anatomy of the human body, micro-CT finds application in biological research, material sciences, geosciences, since it allows the investigation of the morphology of the samples in a non-destructive manner. Micro-CT scanners are specifically designed for non-medical applications, thus involving high resolutions (in the micrometer range). Micro-CT is a versatile technique that permits precise measurements and not limited to the external anatomy. The micro-CT management is quite low cost and the sample preparation is unnecessary or minimal. In the food field, the nondestructive properties of X-ray CT allow to assess the quality of raw ingredients and final products. Moreover, it permits to evaluate component volume fractions, their size and shape distribution or to detect unknown material to ensure product safety. It can help to detect internal food defects or to assess new product formulations.

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MICROSTRUCTURE AND QUALITY OF PARMIGIANO REGGIANO CHEESE: MULTI-SCALE VIRTUALIZATION AND THERMO-RHEOLOGICAL INVESTIGATION

P.M. Falcone[1], E. Sabatinelli[1], L. Valentini[2], P. Gobbi[2], M. Moscatti[3], A. Bianchi[4], M. Nocetti[4]

[1]Department of Agricultural, Food and Environmental Science, Polytechnic University of Marche, Ancona; [2]Department of Biomolecular Sciences, University of Urbino Carlo Bo; [3]TEC Eurolab, industrial tomographic facility, Modena; [4]Consortium of Parmigiano Reggiano, Reggio Emilia, Italy

E-mail: pm.falcone@univpm.it

The Parmigiano Reggiano cheese (PR) is an Italian artisanal cheese for which the European Commission deserved the protection of designation of the origin and imposed quality requirements about making technology, ripening, grainy structure and fracture properties. Unfortunately, the latter two distinguishable traits are specified without a clear definition and analytical method for quantitative measurement. Aiming to individuate the relationships between microstructure and mechanical properties that behind such two physical requirements, PR cheeses having different ripening (from 12 to 72 months) have been investigated coupling data from two non-destructive imaging techniques, fracture resistance and thermo-rheological behavior under isothermal creep and temperature-sweep experiments. EDAX-ESEM was used to evaluate the elemental composition of the fracture surface in relation to their morphology over a large range of scale. X-Ray Micro-Tomography was used to virtualize three-dimensional volumes aiming to evaluate size, number and distribution of the main structure-active components. At sub-microscopic scale, PR cheese can be considered a multiphasic and hierarchical matrix mainly consisting of a dense casein network entrapping low-density regions consisting of globular and free fat pockets. From microscopic to macroscopic scale, PR cheese appear as a non-continuum matrix with randomly dispersed macro-discontinuities that include star-shaped cracks separating curd junctions, salt crystals aggregates and widely spread spongy microstructure. Fracture mechanism of more ripened cheeses followed a "fragile" mode with the two new fracture surfaces showing high complementary morphology. An opposite role of thermal-induced softening of lipids and hardening of caseins network at the higher temperatures affected fracture resistance and thermo-rheological behavior, decreasing with ripening. Opening a new exciting perspective for the comprehensive investigation of evolving microstructure during cheese making, imaging and rheological markers can be considered as key quality drivers of granular aspect and fracture requirements of Parmigiano Reggiano cheese.

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HISTOLOGICAL AND NUTRITIONAL ASPECTS OF PHENOLIC COMPOUNDS AND POLYSACCHARIDES IN DIFFERENTS CEREAL SEEDS

A. Panato[1], F. Bortolotti[1], P. Rodegher[1], E. Antonini[2], P. Ninfali[2]

[1]Dept. of Diagnostic and Public Health, University of Verona; [2]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: anna.panato@univr.it

The first part of the present study was focused on the optical microscopy analysis of histological sections from different cereals such as wheat, barley, emmer wheat, and oat. The use of Azan trichrome and Periodic Acid-Schiff staining allowed for the identification of the aleuronic layer and of the starchy endosperm. Taking into consideration the native fluorescence of the phenolic compounds contained in the fibrous structures of the aleuronic layer, the same sections analyzed by the optical microscopy were observed by the fluorescent microscopy. In this case the polyphenols looked green colored. Moreover, the use of fluorescence microscopy allowed for the identification of the beta-glucans, which, after staining with Calcofluor Blue, appeared blue-colored. An interesting aspect to be investigated by the morphological analysis is the changes caused by the transformation of the cereal seeds in flour for the production of pasta and bread. Indeed, the way of grinding affects the percentage of fibers, polyphenols and other nutrients, which can be saved only in the production of whole flour. For the European Food Safety Authority in the case of whole flour "*small losses of components, that is less 2% of the grain or 10% of the bran that occurs through processing methods consistent with safety and quality are allowed* (HEALTH GRAIN 2016)". This definition is a guarantee for the citizens who prefer whole flour because of its capacity to control the cholesterol level and then to prevent the cardiovascular diseases. On this basis, the second part of the present work has been dedicated to investigate the possibilities offered by the optical microscopy to analyze sections of pasta and bread in order to verify the loss of nutrients caused by grinding. The sections from whole flour products have been compared with sections from products made with refined flour and with legume flour. The comparative analysis showed significant differences among the different products, then encouraging the use of morphological analysis for

studying the nutritional quality of different types of pasta and bread.

ALEURONE AND STARCHY ENDOSPERM OF THREE DIFFERENT CEREAL CARYOPSES GAINED USING ESEM-EDS AND AFM

E. Antonini, C. Zara, L. Valentini, P. Gobbi, P. Ninfali, M. Menotta

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: elena.antonini@uniurb.it

The anatomical structure of the cereal caryopsis is constituted by the germ, the outer layers and the endosperm, composed of the aleurone and the starchy endosperm.[1] Each layer has its own structural organization and chemical composition, in order to store nutrients in a compartmentalized fashion. The endosperm contains starch, accounting for 80% of weight, in the form of granules. The content and organization of the starch granules depend on the cereal type.[2] The aleurone is composed of one or more cell layers, depending on the cereal type, containing proteins, ferulic acid and electron-dense globoids, which constitute the main storage site of phosphorous and metal ions.[3] In this study, we applied Environmental Scanning Electron Microscopy-Energy Dispersive Spectroscopy (ESEM-EDS) and Atomic Force Microscopy (AFM) analysis in order to determine the chemical elemental composition of protein body globoids of the aleurone and starchy granules in three different cereal caryopses: barley, oats and einkorn wheat. Regarding the morphology, barley showed the thickest outer layers. The aleurone of each cereal type contained globoids within its cells. Large type-A and small type-B starch granules were revealed in the endosperm of barley and einkorn wheat, whereas irregular starch granules were found in oats. The starch granule elemental composition, detected by ESEM-EDS, was rather homogenous in the three cereals, whereas the outer layers and globoids showed heterogeneity. In the globoids, oats showed higher P and K concentrations than barley and einkorn wheat. Regarding the topographic profiles, detected by AFM, einkorn wheat starch granules showed a surface profile that differed significantly from that of oats and barley, which were quite similar to one another. In conclusion, this study provides information on both nutritional and technological potential of different cereal caryopses. In particular, we underlied the highest nutrient bioaccessibility of einkorn wheat, in comparison with oats and barley, due to its thinner and smaller starchy granules.

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MICROSCOPY TECHNIQUES ARE ESSENTIAL TOOLS FOR UNDERSTANDING PHENOMENA, OPTIMIZING PROCESSES AND SOLVING PROBLEMS IN DAIRY TECHNOLOGY

P. D'Incecco, S. Limbo, L. Pellegrino

Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy

E-mail: paolo.dincecco@unimi.it

Finished food products are constantly analysed by manufacturers to ensure the highest quality standard. However, the way food components organize themselves and interact is strategic to achieve the target stability of the product during both processing and storage. Confocal Laser Scanning Microscopy (CLSM) and Transmission Electron Microscopy (TEM) have been successfully used to support the dairy sector like in the following case studies. To get access to emerging markets, stability of UHT milk currently needs to be prolonged up to 12 months. A double high-pressure homogenization of milk avoided fat globule rising up to 18 months. CLSM images showed a narrower fat globule distribution compared to a single homogenization process. Furthermore, TEM showed broken fat-protein aggregates.[1] Hard cheeses undergo long ripening during which, besides the typical taste and structure, characteristic macroparticles (white spots) and microparticles (crystals) appear.[2] Differently from the surrounding cheese, spots showed an irregular protein matrix (TEM) and a significant accumulation of hydrophobic free amino acids. Microcrystals showed variable shapes and a complex ultrastructure outlined as: a central star-shaped crystal, an intermediate zone and a peripheral shell at the interface with the cheese. Confocal Raman microscopy showed the star-shaped crystal to be calcium phosphate. Overall, these structures in hard cheeses represent signs of their unique ripening process.

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POSTERS

ULTRASTRUCTURAL CHARACTERIZATION OF LIPOSOMAL IRON CARRIERS

M. Battistelli[1], S. Salucci[1], T. De Paoli[2], B. Riccardi[2], E. Falcieri[1]

[1]Department of Biomolecular Sciences (DiSB), University of Urbino Carlo Bo, Italy; [2]Lipotech S.A., Buenos Aires, Argentina.

E-mail: michela.battistelli@uniurb.it

Iron deficiency is one of the most frequent health problems, affecting approximately one third of the entire population. Its request may not be satisfied with a regular diet, due to a low iron bioavailability and to dietary factors that limit its absorption. Iron supplementation is considered a global economic and effective strategy for prevention and control of anemia, in particular, during pregnancy.[1] Therefore, there is the need to develop new experimental protocols to improve its bioavailability. Liposomes[2] are vesicular structures with double-layer lipid membrane, which are proved to be particularly interesting in the biomedical field as a delivery system of pharmaceutically active substances.[3] In this study, ultrastructural analyses have been carried out to verify if liposome dehydration process induces structural alterations of lipid membranes, which could compromise carriers integrity and function. Morphological integrity of Biofer and Lifervit (two new commercial iron carriers), both in liquid form and in dried powder, has been investigated by means of transmission electron microscopy.[4] Moreover, liposome interaction with U937 cell line, a well-known phagocytosis human model, has been studied. Both compounds revealed a good stability and the ability to penetrate into cells, interacting with cytoplasmic organelles without inducing, at least apparently, any ultrastructural damage and toxicity. Therefore, Biofer and Lifervit do not cause cellular damage, and for that they can be considered potential candidates in iron vehiculation, in agreement with the current literature. Further studies are in progress to evaluate their interaction with intestinal cell models.

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ULTRASTRUCTURE OF EXPERIMENTAL INFANT BISCUITS AND INTERACTION OF THEIR DIGESTATES WITH AN *IN VITRO* MODEL OF HUMAN SMALL INTESTINE

M. Bottani[1], S. Cattaneo[2], A. Ferraretto[1], I. De Noni[2]

[1]Department of Biomedical Health Sciences, University of Milan; [2]Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy

E-mail: michela.bottani@unimi.it

Three experimental infant biscuits (IBs) were prepared by oven baking doughs containing different dairy ingredients: milk protein concentrate (IB 1), whey protein isolate (IB 2) or skim milk powder (IB 3). Additional samples were prepared without dairy ingredients (IB 0) or without gluten protein (IB 4). Ultrastructure of IBs was investigated by SEM and IBs were *in vitro* digested using a static protocol mimicking the gastrointestinal conditions of infants. Four different doses of the obtained digestates were administered to a co-culture of Caco2/HT-29 70/30, which showed morpho-functional features similar to the human small intestine,[1] to evaluate a possible effect on the paracellular permeability and the production of the anorectic hormones Peptide-YY (PYY) and cholecystokinin (CCK). The SEM showed similar protein matrices in IBs 0 to 3, with starch granules distributed inside. Differently, SEM of IB 4 showed a less regular structure likely due to the absence of gluten protein. Overall, the SEM images of IBs 0 to 3 showed the baking process to induce minimal and similar changes in the characteristics of starch granules, which were not easily recognizable only in the SEM image of IB 4. Results from *in vitro* experiments showed that digestates of IBs did not affect the permeability of the Caco2/HT-29 70/30 coculture. Only IBs 2, 3 and 4 induced CCK secretion (19.72±0.01, 14.67±0.11 and 16.24±0.76 pg/mL, respectively) and significantly (P<0.01) increased the PYY production (144±12, 130±5 and 124±10, as percentage *vs* control, respectively). Overall, the presence of dairy ingredients seemed to increase PYY and CCK production depending on the dose of administered IB digestates. Nonetheless, the likely diverse digestibility of IB matrices could account for these findings.

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TYROSOL PREVENTS SKELETAL MUSCLE DEATH

S. Burattini, D. Burini, G. Zappia, E. Falcieri, S. Salucci

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: sabrina.burattini@uniurb.it

Tyrosol (Tyr) is a phenolic compound present in extra-virgin olive oil with antioxidant, antimicrobial and anti-inflammatory properties. This compound is a known scavenger of peroxynitrite and superoxide anion.[1] In this study, Tyr effect has been investigated in C2C12 muscle cell line exposed to known oxidative stress inducers.[2,3] In particular, Tyr protection against oxidative stress and cell death has been investigated through morpho-functional analyses, focusing our attention on the mitochondrial behaviour. Data obtained evidence Tyr ability to prevent skeletal muscle damage and to preserve the mitochondrial membrane integrity and functionality. These findings demonstrate Tyr antioxidant properties in skeletal muscle model and suggest, for this molecule, a potential protective role against muscle diseases related to reactive oxygen species production and accumulation.

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ALBEDO FROM "*LIMONCELLA*": A PRELIMINARY MORPHOLOGICAL, PHYLOGENETIC AND CHEMICAL CHARACTERIZATION FOR ITS BIODIVERSITY AND ECO-SUSTAINABILITY VALORIZATION

L. Di Patria, L. Potenza, R. Saltarelli, S. Burattini, D. Burini, E. Falcieri, P. Gobbi, L. Valentini, D. Sisti, V. Stocchi, G. Annibalini, E. Barbieri

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy University of Milan, Italy

E-mail: l.dipatria@campus.uniurb.it

Limoncella is the common name for a fruit tree of the Rutaceae family considered as a rare and ancient Mediterranean variety belonged to the *Citrus* genus. The fruit is composed of flavedo (10-20%) and pulp (35-40%). The albedo is the spongy white layer below the flavedo, a source of flavanones[1] and usually food waste for the industries. The albedo together with the septum and the core, constitutes 30-40% of the fruit weight. The percentage composition depends on varieties. In this study, the albedo from *Limoncella* of Mattinata in Puglia - Italy, particularly thick and sweet, was morphologically and chemically characterized. Molecular phylogeny of this fruit was also inferred through Internal Transcribed Sequence (ITS) analysis. Preliminary morphological observations of fresh, dry and 2.5% glutaraldehyde fixed albedo were carried out by different microscopy approaches: light, fluorescence, transmission, scanning and environmental scanning electron microscopy (TEM, SEM and ESEM). In particular, the ESEM was equipped with an Energy Dispersive Spectrometer for the semi-quantitative chemical analyses. The ITS fragments including ITS1, ITS2 and 5.8S were amplified as described in Potenza *et al*.[2] All morphological approaches allowed us to observe parenchymal cells with a considerable central vacuole. Cells are slightly turgid and few plastids appear, most of which are leucoplasts. Furthermore, we can observe apoplasts composed of intracellular empty spaces and plasmodesmata, that are able to enhance cell communication through the secondary wall. Elements of secondary wall lignified characteristic of the xylem were observed such us spiral tracheids, with regular pitting walls. Chemical analyses showed that the albedo contains carbon (73.79%), oxygen (25.47%), chloride (0.16%), potassium (0.27%) and calcium (0.31%). Phylogenetic identification inferred through ITS (tree length = 71, consistency index = 0.930, retention index = 0.982) showed that *Citrus medica* is the closest relative species. Further studies are necessary for a better biodiversity and eco-sustainability valorization of the *Limoncella* biovar, and its albedo, a product useful for recycling of organic waste in agriculture and food industries.

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COMPARATIVE INTERNAL MORPHOLOGY OF EDIBLE SEED'S PROTOPLASTS FROM DICOTYLEDON ANGIOSPERMS

D. Fraternale, P. Gobbi, L. Valentini, A. Bruno, P. Ninfali

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: daniele.fraternale@uniurb.it

The internal morphology of seeds of Flax (*Linum usitatissimum L;* Linaceae) and Buckwheat (*Fagopyrum esculentum;* Poligonaceae) has been investigated by means of an Environmental Scanning Electron Microscope, equipped with an Energy Dispersive Spectrometer (ESEM–EDS) in order to compare aleuronic granules, oleosomes and amyloplasts, with the aim to provide further information related to nutrition and industrial processing. The comparative analy-

sis of the internal morphology of buckwheat and flax seeds reveals that both seeds are characterized by two cotyledons, which contain cells, rich of aleurone granules. The granules of buckwheat seed are numerous and smaller in size with respect to those found in the cotyledons of flax seed. In the buckwheat, the amyloplasts appear simple and small in size (about 7 µm), grouped into large structures of round-oval shape. In the flax seed, the amyloplasts show composed granules, due to aggregation of several small polyedric starch granules of irregular form. In the flax seed, the walls of the endosperm cells, containing starch, are very thick a made of compact structures of cellulose and lignin. In the flax seed, there are several oleosomes of 1-2 µm in size, full of TAG, utilized, as energy source by the embryo. The oleosomes were not observed in buckwheat endosperm. In the flax seed, the pericarp is thin, whereas in the buckwheat seed the perycarp, appears thick due to a strong network of cellulose and lignin. The ultrastructural analysis allows the definition of the nutritional aspects of the both seeds, in terms of structure and contents. Buckwheat, represents a source of bio accessible starch due to an abundant amount of small size granules of simple starch. The thick pericarp requires an accurate dehulling and pearling processes, to avoid an excess of indigestible bran. The Flax is a remarkable source of TAG, particularly rich in omega 3 fatty acids. Nevertheless, the morphology reveals that the oleosomes are enclosed in cells with a thick wall, that can be responsible for the cumbersome industrial extraction process of the TAG. At nutritional level, the seeds have to be reduced in fine particles during the milling, to make TAG more bioaccessible to the digestive enzymes.

MICROSCOPIC METHODS AND COMPUTER IMAGE ANALYSIS ARE USEFUL TO DISTINGUISH AND CLASSIFY MARINE/FISH MEALS CONTAINING PELAGIC AND FARMED FISH *VS* SEA MAMMALS (NO TARGET SPECIES)

M. Ottoboni, L. Pinotti

Department of Health, Animal Science and Food Safety, University of Milan, Italy

E-mail: matteo.ottoboni@unimi.it

The aim of this study was to investigate whether microscopic methods and computer image analysis are useful to distinguish and classify marine/fish meals containing pelagic and farmed fish (*e.*g. salmon) *vs* sea mammals (no target species). Accordingly, eight samples of controlled origin were used, namely: fishmeal (4 samples) and sea mammal meals (4 reference samples). Specifically, two fish meals were from pelagic catch (FM Latin America and FM Scandinavia), one was Mackerel meal and one Salmon meal. Sea mammal meals were obtained from Dolphin, Porpoise, Seal or Whale carcass provided by the Walloon Agricultural Research Centre - CRA-W, Belgium, European Reference Laboratory for Animal Proteins. Samples were analyzed by the microscopic method, according to Annex VI of Regulation 152/2009. Sediment fractions of each sample were observed with a compound microscope at X40. Bone fragment lacunae (n. 625) images from 8 samples (FM Latin America, FM Scandinavia, Mackerel, Salmon, Dolphin, Porpoise, Seal or Whale) were recorded and processed through an IA software. Accordingly, on each lacunae 30 geometric variables have been obtained and measured. The geometric variables have been grouped in two main families, namely: size descriptors and derived shape descriptors. Considering the size descriptors, 11 of them have shown higher mean values in pelagic and farmed fish samples (FMs, mackerel and salmon) than in sea mammal pure meals. By contrast axis minor, diameter min, radius min, size width, ferret min were lower in other fish than in sea mammals. Of note, some differences within the group of fish (FM Latin America, FM Scandinavia, mackerel and salmon) have been also observed: three size descriptors referred to lacunae area were higher in salmon lacunae than in the other fish meals. Therefore, it can be concluded that combining microscopy and image analysis can contribute in distinguishing fish material vs marine mammals. Among fish, salmon seems to be characterized by certain bone lacunae size features.

FEASIBILITY STUDY ON THE USE OF MORPHOLOGICAL ANALYSIS FOR INVESTIGATING THE NUTRITIONAL ASPECTS OF PASTA AND BREAD

A. Panato[1], F. Bortolotti[1], P. Rodegher[1], P. Ninfali[2]

[1]Department of Diagnostic and Public Health, University of Verona; [2]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: anna.panato@univr.it

The present study has been focused on implementing a procedure for the morphological analysis of pasta and bread made with cereal and legume flours. In particular, the procedure was applied to whole-wheat pasta, refined-wheat pasta, black bean-pasta, red lentil-pasta, chickpea/millet/flax-pasta, whole-wheat bread and refined-wheat bread. In order to better evaluate the nutrient content of pasta and bread, also the seeds of the different flours underwent the morphological analysis. All the materials (pasta, bread and seeds) were included in paraffin. The resulting blocks were cut by microtome (Leica RM 2255, Wetzlar, Germany) in 4 um

slices. The slides were stained by using a quadri-chromic staining (Azan Trichrome + Lugol's solution). The microscopical analysis was performed with an optical microscope (Leica DM 2500, Wetzlar, Germany) at 5X and 20X, and with a fluorescence microscope (Axioskop 2, Carl Zeiss, Oberkochen, Germany). The optical microscopy analysis and fluorescence microscopy analysis allowed for the identification of the different nutrients (starch, proteins, lipids, poliphenols) in seeds, pasta and bread. A preliminary evaluation of the morphological data led to find significant differences, in terms of nutrient content, among the different type of flours, encouraging the use of the morphological approach for studying the nutritional quality of pasta and bread.

NATURAL ANTIOXIDANTS *VS* UVB DAMAGE IN HUMAN KERATINOCYTES I*N VITRO*

S. Salucci, S. Burattini, G. Zappia, M. Battistelli

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: sara.salucci@uniurb.it

Recently, food antioxidants, have attracted much interest for their relevant applications in chronic degenerative diseases, in skin inflammation and cancer.[1] In particular, polyphenols, present in virgin olive oil, well tolerated by organism after oral administration, show a variety of pharmacological and clinical benefits such as anti-oxidant, anticancer, anti-inflammatory, and neuro-protective activities[2]. In this study, the effects of oil compounds against UV-induced apoptosis have been investigated in a human keratinocyte model *in vitro*. Hacat cells were pre-treated with antioxidants before UVB exposure and their actions have been evaluated by means of ultrastructural and functional analyses. After UVB radiation typical apoptotic features such as blebbing, chromatin condensation, micronuclei presence, absent in control condition, appear. These apoptotic patterns numerically decrease in oil polyphenol-treated samples, which show a morphology similar to those of control condition. Moreover, a high number of TUNEL positive nuclei, absent in control cells, and suggestive of DNA fragmentation presence, can be observed in UVB-treated cells. When natural antioxidants have been supplied before cell death induction, a significant reduction of TUNEL fluorescent nuclei can be detected. Finally, molecular analyses demonstrate caspase involvement in UVB-induced apoptosis and the ability of polyphenols, administrated before radiation, to down-regulate their activity. In conclusion, this study highlights the oil antioxidant ability to prevent apoptotic cell death in human keratinocytes exposed to UVB, suggesting, for these molecules, a potential role in preventing skin damage.

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WINE POLYPHENOLS COUNTERACT *IN VITRO* OXIDATIVE DAMAGE

S. Salucci, S. Burattini, S. Lucarini, G. Diamantini, E. Falcieri

Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy

E-mail: sara.salucci@uniurb.it

Antioxidant nutrients can counteract the oxidative damage and play a key role in preventing the onset and ameliorating the course of degenerative conditions which appear after excessive exposure to reactive oxygen species.[1] Wines are one of the most important sources of phenolic compounds, and a moderate wine consumption is often associated with various health advantages, such as the reduction of cardiovascular, neurodegenerative, infective, bone and metabolic disorders, as well as cancer prevention.[2] Here, the content of red and white wines, provided by an italian vinery (Marche region), has been analyzed through chemical approaches useful to extract polyphenol components and to evaluate their antioxidant properties *in vitro*. Then, wine extracts have been administrated to human myeloid cells exposed to a pro-oxidant agent to verify, by means of morpho-functional analyses, their beneficial effects in preventing oxidative damage. Cromatographic analyses evidence that red wines exhibit the major content in polyphenols, while white wines present a low content of antioxidants. Morphological observations show polyphenol ability in protecting cells from damage and death. This favorable condition can be attributed to extracts present in red wine and, surprisingly, to those of wine too. In conclusion, these data enhance the use of wine polyphenols in a variety of degenerative conditions which involve oxidative stress increase.

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