International establishment of doctoral study programme
in the field of biotechnology and food sciences – MeD-BioFood

MeD-BioFood

1st International online workshop for PhD. students

BOOK OF ABSTRACTS

28th October 2021

Institute of Applied Biology
Faculty of Biotechnology and Food Sciences
Slovak University of Agriculture in Nitra

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LIST OF ABSTRACTS

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High amylose wheat flours for the development of healthy cereal based foods

Martina Angelicola, Maria Cristina Messia, Emanuele Marconi

Dipartimento Agricoltura, Ambiente e Alimenti, Universita` Degli Studi Del Molise Via F. De Sanctis Snc, 86100, Campobasso, Italy

Wheat is a versatile food ingredient to produce finished products of superior eating quality and widespread appeal. The main component of wheat grain is starch that occurs naturally packed in granules. From a biochemical point of view, starch is a glucose polymer comprising of amylose (20-30%), a linear chain, and amylopectin (80-70%), a highly branched chain.
In this study a complete characterization of an elevated amylose content soft wheat (58%) in comparison with a control wheat genotype was carried out. Several milling diagrams with both soft and hard wheat mill were assessed, and chemical and rheological characteristics were evaluated on milling products to test technological performances.
Flours obtained by conventional grinding of high amylose soft wheat has lower yield (~50%) compared to control line (65%) and higher ash content (>0.80% d.w.). Debranning of high amylose wheat at 6% increased yield and refining level of some fractions and the further application of a hard-wheat milling diagram improved milling performance (yield = 66.3%), giving a semolina-like flour that can be conveniently employed to produce semolina-based products.
During the evaluation of technological characteristics of the flours, in particular gluten index, Chopin alveograph indices and falling number, it was emerged that official methods of analysis used for conventional soft wheat might be adapted for the modified samples, because the higher content of amylose modifies water absorption and gelatinization properties of the dough. Overall, rheological features of high amylose wheat flours exhibited higher water absorption, stability, and altered starch pasting properties.
The high amylose flours presented high nutritional characteristics, in particular due to the high content of resistant starch (up to 28.9% of total starch). As a consequence, they could be used as raw materials for the production of innovative foods with high content of resistant starch which might bear nutritional and healthy claims according to EU Reg. 432/2012 and EC Reg. 1924/2006.

Keywords: Milling performance, dough rheology, high amylose flours, resistant starch.

Valorization of Ficus carica by-products: peel, seeds, no-optimal fruits and leaves

Candela Teruel Andreu

Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Ctra. Beniel, Km 3.2, 03312 Orihuela, Spain

According to the Food and Agriculture Organization (FAO) of the United Nations, the world production of fig fruit is stable. The cultivation of the fig tree is part of traditional Mediterranean agriculture. In the province of Alicante (Spain), the Colar variety is the most widely grown. Due to economic reasons, 50% of the fig production is not marketed. There is a need to reduce the fruit's environmental impacts (zero waste), and bioactive compounds from fig fruit present a high added value as functional ingredients.
The use and valorization of the waste material should be further investigated, since this could offer financial benefits to farmers and solve environmental issues by ensuring the sustainable management of these materials and, bringing benefits to consumers' health and well-being. Focusing on fig by-products (peel, seeds, no-optimal fruits and leaves), individual bioactive compounds and/or extracts can increase the functional, nutritional and techno-functional properties of food products such as additives.
It is essential to highlight that comprehensive information about the bioactive compounds of fig fruits, their derivatives/by-products (peel, leaves and oil) and fig-based products was reported by researcher of this doctoral thesis. Although fig-based products have previously been reported such as smoothies, fig powders, colorants, fermented drinks and biscuits; other products should be researched as a part of this doctoral thesis for instance fig coffee, dried figs using novel technologies and fermented milks based on fig by-products.
Therefore, the overall aim of this doctoral thesis is to develop novel products based on bioactive compounds from fig by-products. To reach the main purpose we established specific objectives: i) to know the applications of figs, leaves and their by-products in the food industry; ii) to characterize freeze-dried powder from fig peel, pulp and leaf; and iii) to optimize the formulation and processing of fermented milk based on fig.

Keywords: Fig, by-products, Colar fig, Freeze-dried, Fermented milks.

Genetic, physicochemical and functional characterisation of commercial cultivars of artichoke for its productive and commercial optimization

Marina Giménez Berenguer

Miguel Hernández University of Elche, Spain

Globe artichoke [Cynara cardunculus L. var. scolymus (L.) Fiori] is an herbaceous perennial plant native from the Mediterranean region, where its production is mostly located. In the recent years, its consumption has spread all over the world, being highly appreciated for its high content of bioactive compounds. Artichoke is a rich source of antioxidant compounds and contains high amounts of total phenolic compounds, mainly hydroxycinnamics and flavonoids such as luteolin. Nevertheless, this high content of polyphenols makes artichoke a vegetable very susceptible to browning, which may difficult the use during processing and in ready-to-eat products. Quantitative and qualitative phenols profile is influenced by various factors and in previous works, significant differences have been found in the polyphenol content depending on artichoke cultivars, as well as the type of flower heads (main or guides, secondary or second and tertiary or third, according to their development order). Thus, the main objective of this research is to improve the competitiveness of the artichoke in all phases of the production chain, from seeds or plant material commercialization, passing through agricultural production and industry, to commercialization stage. To achieve that, two different articles have been published at the moment. In the first one, the main aim was to study the influence of flower head order and treatment on the individual phenolic content of globe artichoke cultivars obtained by different propagation methods. Our results report that the levels of individual phenolic compounds identified in globe artichoke cultivars are highly influenced by both, the flower head order and gibberellic acid treatment. Tertiary head orders showed the highest individual phenolic content, followed by secondary and main heads, and this effect was cultivar-dependent. In the second research, the main objective was to carry out a physical-chemical and functional characterization, studying the polyphenol content of the artichoke heads (main, secondary and tertiary) after the commercial harvest of Blanca de Tudela, Lorca, Tupac and Green Queen artichoke cultivars. This experiment was made in an experimental plot of Miguel Hernandez University (Alicante, southern Spain) under Mediterranean climate conditions and conventional agronomic practices. Results showed that tertiary heads had the highest polyphenolic content with respect to secondary and tertiary artichokes, being this difference significantly noticeable in Lorca cultivars. In conclusion, a greater knowledge in this field will allow the selection of artichoke cultivar based on their phenolic profile and know the adaptability of each one for fresh consumption and industrial processing as ready-to-eat product.

Keywords: flower head, bioactive compounds, browning, minimally processed artichoke, phenolic content.

Valorization of prickly pear [Opuntia ficus-indica (L.) Mill: Study of its phytochemical, nutraceutical and functional properties

Lucía Andreu-Coll

Grupo de Investigación en Fruticultura y Técnicas de Producción, Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Carretera de Beniel, km 15 3.2, 03312-Orihuela, Alicante, Spain

Opuntia ficus-indica (L.) Mill., usually named prickly pear or nopal cactus, is the Cactaceae plant with the greatest economic relevance in the word. This plant is mainly known by their fruits, popularly named "figs" or "tunas", but their cladodes are also consumed, mainly in Mexico, which is the country with the largest area under cultivation. In addition to their use for human consumption, the prickly pear botanical parts (fruits, cladodes, and seeds) can be used for other purposes, such as in the pharmaceutical and cosmetic industries, animal feeding, biofuel production and phytoremediation of soils, among other uses.
The objective of this Doctoral Thesis, whose development began in 2017, was to determine the phytochemical, nutraceutical and functional qualities of the different parts of Opuntia ficus-indica (L.) Mill. (fruits, cladodes and seeds) in order to evaluate their use for human diet, animal feeding or industrial use.
To reach the main purpose, four specific objectives were determined:
• Objective 1: Phytochemical, nutraceutical, and functional characterization of the fruit, cladodes, and seeds. About this objective, four publications were published, in which antioxidant activity, bioactive compounds, (poly)phenolic profile, fatty acids and nutritional composition of prickly pear were analyzed.
• Objective 2: Sensory analysis. Regarding this objective, volatile compounds were analyzed in prickly pear fruit pulp and results were published.
• Objective 3: Evaluation of the quality parameters of prickly pear fruits during their conservation under different conditions. Concerning this objective, a publication about changes in fruit quality, antioxidant activity, bioactive compounds and ethylene and respiration rate under cold and shelf life storage was published.
• Objective 4: Economic estimation of prickly pear production and its feasibility in Spain. About this objective, a publication which analyzed cost production structure, economic evaluation regarding bioactive compounds and considering environmental issues was published.

Keywords: prickly pear, antioxidant activity, nutritional composition, bioactive compounds, cost production.

Evaluation of the chicken meat quality after application of grape pomace into their diet

Matej Čech, Peter Haščík

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Products Evaluation and Processing, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

Chicken meat is of a great importance in human nutrition worldwide, as it contributes to global food security thanks to their availability. It provides an excellent source of proteins, fats, essential amino acids, minerals, vitamins, and other nutrients. Chicken meat is suitable for quick and undemanding preparation, which is popular especially nowadays, when people tend to spend less and less time preparing meals at home. The great advantage of chicken meat is also that, unlike other types of meat, there are no religious and social restrictions for its consumption, it has a short produce period and is also relatively cheap.

The wine industry generates a huge amount of waste, which consists of stems, grape pomace (GP), sewage waters and yeast sludge, which treatment or disposal can have a toxic impact on the environment. For every 6 l of wine, about 1 kg of GP is produced. Thanks to the various organic acids (tartaric, malic and citric acid), oils, alcohol, fiber, proteins, and especially various polyphenols, GP can also be used to produce value-added products; but traditionally, GP is incorporated into the soil or as feed for livestock.

The aim of our work was to apply this voluminous agricultural by-product into the complete feed mixture of broiler chickens Ross 308 as a source of many nutritive compounds in the amount of 1, 2 and 3% (red GP, variety Alibernet). After 42 days feeding period, chickens were slaughtered, and their carcasses and meat was subjected to the analyzes – selected indicators of meat performance; basic chemical composition, amino acids and fatty acids profile of breast and thigh muscle; oxidative stability, technological and organoleptic properties of chicken meat.

From the results it seems that GP positively affected ($P \leq 0.05$) live body weight, proportion of valuable meat parts, but negatively affected the carcass yield (also confirmed by other authors) and increased the weight of internal fats. Red GP did not have a significant effect on the chemical composition of the breast muscle, but slightly improved the protein content and reduced the water content in the thigh muscle ($P \leq 0.05$). The selected dietary supplement generally increased ($P > 0.05$) the fat content in the experimental groups but without a significant increase in cholesterol content and lipid oxidation. Mentioned results were recorded namely after 3% supplementation. Other results are yet to be processed.

To conclude, any nutritional supplement should not have a negative impact on the final quality of the chicken, but also on the health of the consumer. From this point of view, red grape pomace as a voluminous agricultural by-product appears to be a suitable candidate for broiler chicken nutrition.

Key words: broiler chickens, meat performance, meat quality, grape pomace.

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Effects of deficit irrigation in olive oil quality and composition

José Miguel García-Garví

Miguel Hernández University, Higher Polytechnic School of Orihuela. Agrifood and Agroenviromental Research and Innovation Centre (CIAGRO-UMH), Spain

The expansion of super high-intensive cultivation of olive (Olea europaea) groves requires irrigation techniques compatible with the increasing scarcity of water due to climate change and olive oil production. For this, the effect of two regulated deficit irrigation treatments (RDI) and a sustained deficit irrigation (SDI) treatment in an Arbequina olive variety orchard located in Carmona (Seville, Spain) were applied. Control treatment supplied 100% of the water lost by evapotranspiration, the Optimal RDI treatment with a 54% reduction, the Confederation RDI treatment and the Confederation SDI treatment, both with a 72 % reduction in water consumption. To determinate the effects of these irrigation treatments, official quality parameters stablished by International Olive Council (IOC) (free acidity and peroxide indexes, K values and descriptive sensory analysis), fatty acids and volatile compounds, total antioxidant activity and total phenolic content were analyzed. In this sense, the irrigation treatments did not affect at official quality parameters, but some many changes can be observed in the other parameters analyzed. In sensory analysis, deficit irrigation treatments generate green and fruity olive oils than full irrigated treatment. Respect on volatile profile, reducing water intake made changes in mayor volatile compound (trans-2-hexenal), related with green flavors. Fatty acid profile showed changes respect control, increasing oleic acid and total content of monounsaturated fatty acids and total phenolic content increased when hydric stress was applied. Antioxidant activity, measured by ABTS+• and DPPH•, did not show changes between treatments and control. On the other hand, irrigation deficit treatments applied in this study did not generate changes at olive oil yield.

Keywords: Olea europaea, GC-MS, high-intensive olive orchard, olive oil quality, hydric stress.

Physico-chemical, nutritional and aromatic characterization and establishment of the descriptive and affective sensory profiles of *Fondillón*

Hanán Issa-Issa[1], David López-Lluch[2], Ángel A. Carbonell-Barrachina[1]

[1]*Research Group "Food Quality and Safety", Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Carretera de Beniel, km 3.2, 03312 Orihuela, Spain*
[2]*Departamento de Economía Agroambiental, Ingeniería Cartográfica y Expresión Gráfica en la Ingeniería, Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Carretera de Beniel, km 3.2, 03312 Orihuela, Spain*

Fondillón is a unique type of Alicante wine, and its protected under the Alicante Designation of Origin. It is the first wine to have its own name and is recognized by the European Union in its E-bacchus database. Its main characteristics are: (i) It is made with grapes of the Monastrell variety which are overripe on the vine; (ii) Its minimum alcohol content is 16 percent; and, (iii) Its minimum aging time is 10 years. The overall aim of this research was to carry out a physico-chemical and sensory characterization of *Fondillón* from different wineries and soleras/vintages, to evaluate its acceptance by national consumers and to determine the main buying and satisfaction drivers. The main results obtained showed that 25 phenolic compounds were identified and quantified, being the protocatechuic and gallic acids the predominant ones. In addition, 56 volatile compounds were identified, with esters and alcohols predominating (e.g., ethyl lactate and isoamyl alcohol). Of these 56 compounds, 22 were identified as aromatic active compounds, e.g., phenylethyl alcohol (floral) and diethyl succinate (fruity). Finally, it was observed that this wine was highly appreciated by Spanish consumers when having intense fruity, alcoholic, bitter and balsamic notes.

Keywords: Alicante wine, active aromatic compounds, Monastrell, phenolic compounds, descriptive sensory analysis.

Formulation of gelled emulsions with healthy oils and pseudocereal flours for fat replacement in meat products

Carmen María Botella-Martínez.

Miguel Hernández University, Higher Polytechnic School of Orihuela. Agrifood and Agro-environmental Research and Innovation Center (CIAGRO-UMH), Spain

There is a direct relationship between the consumption of saturated and trans fats, with an increased risk of suffering from various cardiovascular diseases, which is the leading cause of death worldwide. Pork back fat is the most usual fat source (solid) in meat products for both technological and sensorial properties but with an unhealthy lipid profile (high content of saturated fatty acids). Achieving its replacement in an adequate way, mimicking the properties of solid fat in the formulation of meat products, is the priority objective of many investigations. One of the recent and successful strategies for this replacement (partial or total) is the use of structured vegetable oils (with a healthy lipid profile) as gelled emulsions.

For the preparation of these gelled emulsions, pseudo-cereal flours such as amaranth, buckwheat, white quinoa and teff were used. On the other hand, the use of vegetable oils with a healthier profile of fatty acids was proposed, such as those obtained from hemp, chia, flax seeds and sesame. These emulsions will be used as substitutes for fat at different concentrations in the preparation of different fresh meat products such as hamburgers and cooked ones such as frankfurters.

Therefore, the main objectives of this research project are: (i) to develop gelled emulsions, by combining vegetable oils and pseudo-cereal flours; (ii) to optimize the technological process for preparing fresh meat products (hamburger), adapting it to the fat replacement for gelled emulsions, and characterizing the new meat product; (iii) to optimize the technological process for preparing cooked meat products (frankfurters), adapting it to the substitution of fat for gelled emulsions and characterizing the products made with the new formulations; and (iv) to determine the sensory stability and food safety of new meat products during their preservation.

There was great variability in the behaviour of the properties of the gelled emulsions produced and in the properties of the products to which they have been added. In general, fat replacement improved lipid profile with reduction of saturated fatty acids and increasing polyunsaturated fatty acids, depending on the percentage of substitution achieved. The substituted meat products have not showed great changes in texture, lipid oxidation and acceptability respect to traditional meat products. In some cases, 100% substitution level was successfully achieved.

Keywords: fat replacement, gelled emulsion, healthier lipid profile, meat products.

Potential effects of quercetin on human ovarian granulosa and cancer cells *in vitro*

Michal Mihaľ[1], Simona Baldovská[2], Adriana Kolesárová[1]

[1]*Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*
[2]*AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Nitra, Slovakia*

Quercetin, a major representative of the flavonol subclass, has received considerable attention. It is present in a wide variety of fruits and vegetables for example onions, shallots, grapes, tea and sea buckthorn. It is well known that the quercetin stimulated cell proliferation and increased total antioxidant capacity of the cells and also can decreased cell survival and viability cancer cells. The aim of this study was to determine the effect of quercetin at concentrations 5; 10; 20; 50; 100 µmol/mL (24 h) on the viability of human ovarian carcinoma cell line (OVCAR-3) and human ovarian granulosa cell line (HGL5), as well as secretion of steroid hormones (17ß-estradiol and progesterone). Cell viability was evaluated by *AlamarBlue*^{TM} cell viability assay, and the release of steroid hormones was assayed by ELISA methods. In our research, we did not observe any changes in viability of ovarian cells HGL5. On the other hand, we did observe significant decrease ($P \leq 0.001$) in viability of ovarian carcinoma cells OVCAR-3 at 100 µmol/mL quercetin concentration. Progesterone release by HGL5 cells was not significantly affected. However, a significant ($P \leq 0.05$) increase of the 17ß-estradiol secretion by quercetin at concentration 50 µmol/mL by HGL5 cells was observed. According to our research, quercetin did decrease cell viability of cancer ovarian cells but did not affect healthy cells. We can assume, quercetin may play a role in protective and pro-apoptotic effect and also steroidogenesis, however further study is needed to safely determine its bioactivity.

Key words: quercetin, ovarian, HGL5, OVCAR-3

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Brewers' spent grain as a functional food ingredient: the trend and the challenges

Joncer Naibaho

Department of Functional Food Products Development, Faculty of Biotechnology and Food Sciences, Wroclaw University of Environmental and Life Sciences, Poland

The potential of brewers' spent grain (BSG) as a functional food ingredient has been studied extensively due to the presence of certain nutritional value. Several compounds such as phenolic compounds, protein and the isolate, dietary fiber, fatty acids and other minor compounds are responsible for remarkable biological activities including antioxidant, DNA protection, antimicrobial and prebiotic potential. The addition of BSG as a food ingredient improved the nutritional value while it tended to intensify the textural properties due to the high amount of dietary fiber. This phenomenon led to 2 research possibilities: the potential of BSG in regulating the flow behaviour of semi-solid food (yogurt) and modification of the dietary fiber composition of BSG.

BSG and its protein extracts (prepared with three different enzymes treatment) were applied in yogurt and plant-based yogurt alternatives production. The study revealed that BSG and its derivatives modified the textural formation of yogurt during the fermentation process, modified the microstructural properties and preserved lactic acid production, the growth of lactic acid bacteria, syneresis and rheological behaviour during the refrigerated storage. The same phenomenon was also observed in plant-based yogurt alternatives (soybean- and coconut based).

Autoclave treatment (AT) was conducted in order to evaluate the dietary fiber modification on BSG and its impact on physical and biological properties. The results showed that AT degraded the insoluble dietary fiber into soluble dietary fiber. Therefore, a modification of water holding capacity and oil holding capacity was identified. Furthermore, AT also changed the phenolic composition of BSG and its antioxidant activity. Higher temperatures induced the formation of benzoic acid and (+)-catechin.

Quality management in agro-food product development and technology: Relevant synthesis with analytical-qualitative case studies

Charles Odilichukwu R. Okpala

Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences 25 Norwida St. 50-375 Wrocław, Poland

Quality operates by a pathway of people>process>product within the agro-food technological system. Despite the massive information/literature on the quality of agro-food products, there appears to be much less emphasis about their quality management. Specifically, the compulsory quality management aspects of small-scale agro-food production processes particularly in developing countries remains scanty. To supplement existing information, the aim of this work is to qualify and quantify the impact of compulsory quality management aspects specific to small-scale agro-food production processes, using case studies, particularly in developing countries, prior to product development. Thus far, areas already covered include a) The concept, content, the context of quality that leads consumers to purchase a given food product; b) Thorough synthesis of the knowledge surrounding compulsory quality management aspects of the agro-food product industry; and c) Case studies investigating the compulsory quality management aspects, involving people, and processes within the agro-food product development industry, and to help actualize cleaner (food) production. In near future, we hope to formulate a food product, conduct detailed experimental analysis, and determine its pre-and post-quality evaluation.

Keywords: agro-food technological system; compulsory quality management aspects; small-scale agro-food production processes; product development.

Elicitation Strategies in Preharvest to increase Quality of Table Grape and Pomegranate Fruit at Harvest and during Storage

María Emma García Pastor

Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Alicante, Spain

Pomegranate and table grape are fruit with high acceptation by consumers due to their organoleptic quality and their content in both nutritive and bioactive compounds related to human health. However, the table grape cultivars, 'Crimson' and 'Magenta', and the 'Mollar de Elche' pomegranate show an important lack of red intense colour at harvest and some others alterations that determine their quality losses during postharvest storage. Fungal decay in table grape and in the case of pomegranate its sensitivity to develop chilling injury (CI) when they are stored under cold conditions are key factors which compromise fruit storage, transport and marketing.

In recent years, research has been performed aimed to find preharvest treatments with natural compounds to increase fruit quality at harvest and to maintain it during storage, due to consumers' concerns and legal restrictions regarding the use of postharvest chemical treatments. In this sense, the application of naturally occurring plant compounds as preharvest treatments to delay ripening and senescence, preserving fruit and vegetable quality, has received considerable attention.

Therefore, the main aim of this PhD Thesis is to provide solutions to the pomegranate and table grape quality problems through preharvest treatments with methyl jasmonate (MeJa), salicylic acid (SA) and its derivatives; acetyl salicylic acid (ASA) and methyl salicylate (MeSa), and oxalic acid (OA).

The results of this PhD Thesis have shown that 0.1 mM MeJa, 0.1 mM MeSa and 5 mM OA in table grape, as well as 5 mM MeJa, 10 mM SA and 10 mM OA in pomegranate fruit improve fruit quality attributes, mainly colour, at harvest and during storage of table grape and pomegranate fruit. These results would help to the horticultural companies since they would provide fruits with higher quality standards at harvest and after their postharvest handling, storage and marketing.

Keywords: anthocyanins, bioactive compounds, colour, elicitors, postharvest.

Genomics and Machine Learning for Biodiversity Studies

Giovanna Salvatore[1], Mariasilvia D'Andrea[1]

[1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, via De Sanctis snc, 86100 Campobasso, Italy*

Thanks to the Next Generation Sequencing, nowadays massive datasets have been produced and this poses difficult computational challenges. In this context statistical and bioinformatic tools have to be more and more responsive to analyze these, so called, big data. The analysis of Single Nucleotide Polymorphism, possible thanks to the sequencing, changed drastically the research approach for association and biodiversity studies. In this context, the aim of this research is to study and characterize the trout that inhabits two main rivers of Molise region. Mediterranean trout (*Salmo cettii syn. Salmo macrostigma*) is a freshwater fish of particular interest with economic significance, and it is seriously threatened by the introduction of commercial hatchery strains for recreation activities, which hybridizing with the native one, has compromised the genetic integrity of this latter. The selection of hybrids based only on morphomeristic characteristics is insufficient to provide a clear taxonomic information, thus genetic methods are traditionally adopted. Recently a couple of high-density SNP chips have been developed for commercially important salmonid species, such as rainbow trout and Atlantic salmon; however, they have not yet been tested on Mediterranean trout. 14 interesting sites were identified for each basin and 288 individuals were enrolled in the study. A portion of adipose fin tissue was collected from live animals and individual genomic DNA was isolated. Genotyping was performed by the 57K rainbow trout Axiom SNP array (Affymetrix). All 288 samples were also genotyped for 16S rDNA, and LDH-C1* genes as a further control analysis for 100% non-native trout identification. Pairwise weighted F_{ST} and overall F-statistics were estimated in Arlequin v.3.5.2.2. Genetic distance was investigated by Principal Coordinated Analysis (PCoA) with GenAlex v.6.503. Genetic ancestry was estimated using ADMIXTURE v.1.3.0. Clear genetic differentiation between Biferno and Volturno samples was detected. Overlapping region was identified, representing Atlantic specimens detected in both rivers (as confirmed by PCR-RFLP and ADMIXTURE). Allochthonous genome is widely spreading into the wild of both rivers but a different pattern of introgression seemed to be recognizable within the two basins. Furthermore, in this context and with a slightly different population we decided to perform other analysis to obtain a reduced panel of markers with the most informative SNPs, useful to differentiate Atlantic (ATL) and Mediterranean (MED) trout populations. Among the others the use of Machine Learning (ML) is recently gaining attention in many fields for several pilot application. Random Forest (RF) especially is a supervised algorithm that builds a complex forest of decisional trees that allows to classify a sample. A Random Forest algorithm was fitted for marker selection, along with other statistics such as F_{ST}, Delta and Principal Component Analysis. Additionally, a new Random Forest Classifier was fitted for the validation step and, in detail, we passed to the algorithm each of the panels of 96 SNPs obtained by the selection methods and we recorded the Out Of Bag error. Furthermore, we used the SNPs, resulted in common to all methods, to classify our population and we highlighted that up to 50% of introgression rate we could classify with a relatively low OOB error, 4.96%. Our study demonstrated that rainbow trout SNP array can be successfully used for Mediterranean trout genotyping. In particular, our findings allowed us to select candidate sites for adult collection, needed for the production of genetically pure juvenile trout, and sites to carry out the eradication of alien trout and successive re-introduction of native trout. Furthermore, SNP-array technology and ML approach were combined for the first time to select most informative

markers for ATL and MED trout identification. The obtained reduced panel of SNP may represent an interesting and cost-effective tool for monitoring the level of introgression between native and alien trout population for conservation purpose.

Keywords: Mediterranean trout; genetic diversity; SNP; introgressive hybridization; biodiversity preservation; machine learning; reduced panel of SNP

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Pre- and postharvest treatments to increase quality of the whole and minimally processed green pepper fruit

Alicia Dobón Suárez

Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Alicante, Spain

Pepper fruit (Capsicum annum L.) is a highly demanded crop and has a great importance worldwide production. The increase of this crop is mainly due to its high content of vitamin C, carotenoids, phenolic compounds and capsaicins. The consumption of these bioactive compounds provides human beneficial effects to consumers, related to their antioxidant properties. Pepper fruits are harvest and consumed at different phenological and ripening stages, which determine their phytonutrients and antioxidants content. In addition, pepper fruit is a very perishable vegetable which needs a proper handling to maintain the postharvest fruit quality. The main problems that affect its shelf-life during transport, storage and marketing are mainly the fungal decay incidence, weight losses, softening and chilling injury (CI) symptoms when they are stored at temperatures lower than 7 ^{o}C.

Currently, due to the economic and nutritional importance of pepper fruit, it is intended to investigate and develop new alternatives that, applied in pre- or postharvest, provide a solution to problems triggered during postharvest storage, which reduce the quality of the whole green pepper fruit and minimally processed.

Therefore, the main aim of this PhD Thesis is to elucidate the influence of the phenological stage and harvest date throughout the crop cycle of green pepper fruit, 'Lamullo' type, on the bioactive compounds content, as well as provide solutions to green pepper fruit quality problems through pre- and postharvest treatments with salicylic acid (SA), methyl salicylate (MeSa), methyl jasmonate (MeJa) and oxalic acid (OA).

The results of this PhD Thesis that have been obtained until now have shown that the phenological stages and harvest dates are two key factors that significantly influence the bioactive compounds content and the antioxidant activity of this type of green pepper fruit. Thus, it is advisable to harvest the green pepper fruit at the most advanced phenological stage (S12) and on the latest harvest date (in April) studied under this PhD Thesis. Moreover, the preharvest application of SA at 0.5 mM by foliar spray to green pepper plants has a significant effect on improving crop yield, fruit quality parameters and bioactive compounds content at harvest and after 21 days of storage at 7 ^{o}C. Other treatments and results are still being studied and analysed throughout this PhD Thesis.

Keywords: antioxidants, chilling injury, elicitors, phenological stage, storage.

Effect of the bee bread on liver enzymes of male Japanese quails (*Coturnix japonica*)

Nikoleta Šimonová, Anna Kalafová, Rudolf Dupák, Monika Schneidgenová, Emília Hanusová, Cyril Hrnčár, Juraj Čuboň, Peter Haščík, Marcela Capcarová

Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

Bee bread is characterized by its antibacterial, hepatoprotective, and antitumor properties, but has not been well studied yet. Since legislation does not allow the addition of growth promoters to animal feed, bee bread as natural product might have positive effect on stimulating growth. Nutrients from bee bread are well absorbed while digestion due to the process of fermentation. The aim of this study was to evaluate effect on liver enzymes by adding bee bread into feed of Japanese quails in different concentrations, which allows a better understanding of it´s possible effects. A total of 40 male Japanese quails (*Coturnix japonica*) were randomly divided into four groups according to administered bee bread into feed mixture HYD 11, which was given *ad libitum*, as follows: P1 (n = 10) 2 $g.kg^{-1}$ bee bread, P2 (n = 10) 4 $g.kg^{-1}$, P3 (n = 10) 6 $g.kg^{-1}$, and the control without additives (K) for 8 weeks. The groups were kept under the same conditions and parameters were analysed using an automatic clinical device Biolis 24i Premium. We noticed slight increase in alanine aminotransferase (ALT) in P2 (0.06 ± 0.043 µkat/l) compared with P3 (0.03 ± 0.015 µkat/l), and in enzyme aspartate aminotransferase (AST) between P3 (4,64 ± 1,06 $\mu kat/l^{-1}$) compared to P2 (7,85 ± 1,64 $\mu kat/l^{-1}$), although not statistically significant ($P > 0.05$). We can conclude that bee bread did not have a significant ($P > 0.05$) effect on the liver enzymes by addition into feed, nevertheless, the results can be used for further examination. All the selected liver enzymes were within the normal range. We can conclude that bee bread did not have a significant ($P > 0.05$) effect on liver enzymes by addition into feed, nevertheless, the results can be used for further examination. Despite the inconclusive results, bee bread might have positive effect on overall health by adding into the feed.

Keywords: bee bread, beehive products, Japanese quails, liver enzymes.

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Effect of the Equilibration time on post-thaw quality of manually freezed ram sperm

Jakub Vozaf[1], Alexander Makarevich[2], Andrej Baláži[2], Jaromír Vašíček[1,2], Andrea Svoradová[2,3], Linda Dujíčková[2], Lucia Olexíková[2], Peter Chrenek[1,2]

[1] *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Science, Institute of Biotechnology, Tr. A. Hlinku 2, 94901, Slovakia*
[2] *NPPC, Research Institute for Animal Production Nitra, Hlohovecka 2, 95141 Lužianky-near-Nitra, Slovakia*
[3] *Department of Animal Morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic*

The aim of our research was to improve ram sperm post-thaw quality through optimization of cryopreservation regimen. We tested different lengths of equilibration (EL; 0, 2, 4, 6 and 8 hours) of ram sperm cryopreserved using manual slow freezing technique in the liquid nitrogen (LN2) vapours. The ejaculates were collected from clinically healthy and sexually mature rams (n = 12) of Wallachian sheep breed (2.5-5 years) twice a week by an electro-ejaculation within the autumn season. Sperm doses were extended in a Triladyl® diluent containing glycerol, egg yolk and antibiotics. Following thawing, the sperm samples were analysed for the motility (CASA), viability (SYBR-14/PI) and *in vitro* penetration/fertilization (P/F) assays. It was found that the EL of 6 hours (EL-6) ensured higher post-thaw sperm total (76.6±2.3%) and progressive (65.0±4.18%) motility compared to other lengths tested. Using a SYBR-14/PI assay, the proportion of viable versus dead spermatozoa (47.67% vs. 31.11%, resp.) in the cryopreserved group significantly differed from those in the control group (79.86% and 14.07%, resp.). Fertilizing ability of cryopreserved sperm (EL-6), examined by *in vitro* heterologous P/F test on bovine mature oocytes (62.00%), was significantly lower compared to the control group (72.82%). However, in the percentage of fertilized zygotes the EL-6 group (54.67%) did not differ from the control (52.26%). These results provide evidence that extension of equilibration time up to 6 h can improve post-thaw quality of ram sperm.

Keywords: sheep, sperm, manual freezing, equilibration, motility, viability.

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Effect of genotype and diet on carcass and meat quality traits of broiler chickens

Mengjun Wu, Siria Tavaniello, Sanije Zejnelhoxha, Antonella Fatica, Marisa Palazzo, Elisabetta Salimei, Giuseppe Maiorano

Dipartimento Agricoltura, Ambiente e Alimenti, University of Molise, 86100 Campobasso, Italy

Soybean is the main source of plant-derived protein in the diet of poultry and other farm animals Its rising price every year has led to increased production costs and reduced sector's profitability. One source of protein that can replace soybean (*Glycine max* L) to some extent in the poultry diet is pea (*Pisum sativum* L.). A study with 120 male broilers (60 Kabir Rosso Plus and 60 New Red) was conducted in a family farm to evaluate the effects of diet: soybean (S) or pea seeds (P). Diets were isoenergetic and isonitrogenous. Birds were housed in 20 floor pens, 6 birds per pen with 5 replications per each of four groups. At 83 days of age, all birds were weighed and 40 of them, randomly chosen (2 for each replication), were slaughtered. At slaughter, weights of the carcass, pectoral muscle (PM), thighs and wings were recorded, and the respective yields calculated. On the right PM, pH, color and water holding capacity (WHC) were measured at 24 h post mortem. The left PM was vacuum packaged and stored frozen (–20°C) until chemical analysis. Data were analyzed by two-way ANOVA, including diet and genotype with interactions. Neither live weight at slaughter nor slaughter performance (carcass and commercial cuts as weights and yields) as well as pH, color, WHC and total lipid and cholesterol content of PM were affected by diet. Taking into account the fatty acid profile of PM, P diet increased total content of saturated fatty acids (SFA), monounsaturated fatty acids ($P<0.05$), and n3/n6 ratio ($P<0.01$); while, S diet increased polyunsaturated fatty acids (PUFA), PUFA/SFA ratio and n6/n3 ratio ($P<0.01$), while reduced atherogenic and thrombogenic indexes ($P<0.05$). Regarding genotype, New Red showed higher ($P<0.05$) values of final live weight and carcass traits than Kabir. A significant interaction ($P<0.05$) was found between factors for PM weight and yield: Kabir showed greater values when fed soybean; New Red higher values when fed pea seeds. Meat from New Red had a higher ($P<0.05$) pH value compared to Kabir. Genotype did not affect ($P>0.05$) color, total lipid, cholesterol content and fatty acid profile of PM. In conclusion, replacing soybean with pea seeds in the chicken diet could represent an interesting farm management strategy, especially in those agronomic realities where it is produced. As expected, chicken genotype affected slaughter traits: New Red chickens showed greater productive capacity.

Novel carrot-based food products with desired health benefits

Emel H. Yusuf, Paulina Nowicka, Aneta Wojdyło

Department of Fruit, Vegetable and Nutraceutical Plant Technology, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wroclaw, Poland

Carrot is one of the most consumed vegetables all around the world. The first cultivated carrots were purple and yellow coloured, then followed by white and orange carrots. In the present study, twelve carrot varieties with different sizes and colours were analysed besides juices, smoothies and carrot snacks are going to be prepared and chemical analyses be made as well. Normal-sized carrots were between 20-45 mm and 50-150 g, mini-sized carrots were 10-20 mm and 8-50 g, finally, micro-sized carrots were smaller than10 mm and lighter than 8 g; all three sizes of carrots were purple, orange, yellow and white coloured. Moreover, the study aims that to investigate physicochemical characteristics, bioactive compounds and in-vitro biological activities for all materials. According to raw carrot materials, purple carrot samples in all sizes were the richest for the total polyphenols; normal yellow and mini orange carrots exhibited the highest contents of carotenoids. The highest α-glucosidase and cholinesterase inhibition activities were observed in mini purple and micro purple carrots respectively. Thus, analyses on carrot juices, smoothies and snacks are pursued to suggest more carrot-based food products to the food industry and consumers to boost healthy body functions.

Keywords: colourful carrots, carrot juices, carrot-based smoothies, carrot snacks.

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Effect of carcass weight on fatty acid composition and nutritional indices in meat of Nero d'Aspromonte pigs

Sanije Zejnelhoxha[1,2], Mengjun WU[1], Siria Tavaniello[1], Marisa Palazzo[1], Giuseppe Maiorano[1]

[1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy*
[2]*Food Research Center, Faculty of Biotechnology and Food, Agricultural University of Tirana, Tirana, Albania*

In light of the increased concern of consumers towards the impact of meat consumption on human health, this study aimed to evaluate the effect of carcass weight on fatty acid profile of meat from Nero d'Aspromonte pigs, a native genotype reared in Calabria region, Italy. At slaughter, twenty *longissimus thoracis (LT)* muscles were collected from ten low weight (LW) (106.5 ± 4.8 kg) and ten high weight (HW) (144.8 ± 4.2 kg) carcasses. Total lipid and fatty acid composition were assessed through Folch method. Data were analysed by one-way ANOVA. Intramuscular fat and saturated fatty acid (SFA) contents were similar among groups ($P>0.05$). Meat from HW carcasses showed a higher ($P<0.01$) content of monounsaturated fatty acids (+3.2%), mainly in the form of oleic acid (C18:1n9; $P<0.01$) and palmitoleic acid (C16:1n7; $P<0.05$); while, levels of polyunsaturated fatty acids (PUFA) and n-6 PUFA were higher ($P<0.01$) in LW group (+3.1% and +3.0%, respectively). In particular, meat from LW group showed a higher content of linoleic acid (C18:2n6, $P<0.01$), dihomo-γ-linolenic (C20:3n6, $P<0.05$), as well as of eicosapentaenoic (C20:5n3, $P<0.05$) and docosapentaenoic (C22:5n3, $P<0.01$) acids which were present in very small amount (less than 0.2%). As for nutritional indices, the n6/n3 and PUFA/SFA ratios were higher ($P<0.05$) in LW group, while atherogenic and thrombogenic indices were similar ($P > 0.05$) among groups. In conclusion, meat from heavy carcasses results more suitable for technological transformation (lower PUFA, more susceptible to oxidative process) and with a better n6/n3 ratio from the nutritional point of view.

Keywords: autochthonous pig, meat quality, fatty acids, nutritional indices.

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