



ALIMENTI E NUTRACEUTICI: SALUTE E PREVENZIONE ATTRAVERSO IL CIBO

5° Convegno a cura delle Piattaforme Tematiche di Ateneo su
"Alimenti e Nutrizione" e "Salute Umana e Animale"



Evento *on-line*
13 luglio 2021



con il patrocinio di:



con la collaborazione di:



ALIMENTI E NUTRACEUTICI: SALUTE E PREVENZIONE ATTRAVERSO IL CIBO

5° Convegno a cura delle Piattaforme Tematiche di Ateneo su "Alimenti e Nutrizione" e "Salute Umana e Animale"
evento on-line **13 luglio 2021**
<https://unicam.webex.com/meet/eventi>

9:00 Saluto della autorità ed inizio dei lavori

PLENARY LECTURE

09:15 *Food waste and by-products as valuable sources of bioactive compounds*
Lina Cossignani, Università di Perugia

Comunicazioni orali

09:45 *Vitamins in human, cow, and donkey milk: a comparison of nutritional properties.*

Silvia Vincenzetti

10:00 *Polyphenol microbial metabolites modulate proteolysis in neuronal cells reducing amyloid-beta (1-42) levels.*

Valentina Cecarini

10:15 *A new HPLC-MS/MS method for the simultaneous determination of 36 polyphenols in blueberry, strawberry and their commercial products and determination of antioxidant activity.*

Ahmed M Mustafa

10:30 *Anti-inflammatory properties of hemp extracts in human bronchial cells.*

Oliviero Marinelli

10:45 *Hemp extract safety for dermatological application.*

Cristina Aguzzi

11:00 *Organic and antibiotic-free chicken meat: a proteomic approach.*

Laura Alessandrini

11:15 Coffee break

11:30 *Benefici e uso degli Aminoacidi essenziali.*

Emanuele Giordano

11:45 *Antioxidant effects of 'Mela Rosa dei Monti Sibillini' on Drosophila melanogaster longevity.*

Daniela Beghelli

12:00 *Assessment of cholesterol-lowering bioactive compounds in Italian-standard Espresso coffee.*

Franks Kamgang Nzekoue

12:15 *Capsaicin as new adjuvant in anti-cancer immunotherapy.*

Maria Beatrice Morelli

12:30 *Gut microbiota modulation counteracts neuroinflammation and reduces the deposition of amyloid and tau in Alzheimer's disease.*

Laura Bonfili

12:45 *Managing of the daily calcium dietary intake as a tool to contribute to a healthy lifestyle.*

Giorgia Vici

13:00 *Evening Primrose Oil effects in human Pancreatic Ductal Adenocarcinoma cell lines.*

Laura Zeppa

13:15 *Dietary metabolites and CVD biomarkers: what about trimethylamine and its oxidative product?*

Laura Bordoni

13:30 Pausa pranzo

14:30 *Exploiting the nutraceutical activities of the forage plant Onobrychis viciifolia (sainfoin) for the control of intestinal parasites in sheep doing without drugs.*

Sara Marchegiani

14:45 *The effect of chickpea flour addition levels and flour structures on quality and in vitro starch digestibility of corn-rice-based gluten free pasta.*

Xinying Suo

15:00 *Ideabril packaging capability in the preservation of raw and cooked ham: a comparative study.*

Laura Acquaticci

15:15 *Can probiotics counteract the detrimental effects of sleep deprivation?*

Yadong Zheng

15:30 *Water- and fat-soluble vitamins in milk after different thermal treatments and steam injection.*

Giuseppe Santini

15:45 *Inhibitory activity of Chlorogenic Acid and Coffee Silverskin extracts against Klebsiella pneumoniae carbapenemases.*

Sonia I Ciancia

16:00 *Characterization of milk proteins based nano-carriers: interactions with polyphenolic molecules of nutraceutical interest.*

Sofia Renzi

16:15 *In vitro assessment of prebiotic effect of coffee and its by-products on human intestinal microbiota.*

Chiara Salvesi

16:30 Coffee break

16:45 *Characterization of Panax ginseng root extracts: development of a new analytical method for the quantification of ginsenosides and biological studies.*

Simone Angeloni

17:00 *Quality of wholemeal pasta made with pigmented and ancient wheats.*

Francesca Pompei

17:15 *Short and medium chain free fatty acids in wine: extraction optimization and analysis.*

Lucia Lenti

17:30 *Cannabidiol extracted from Cannabis sativa induces cell cycle arrest, mitophagy and cell differentiation in chronic myeloid leukemia cells.*

Federica Maggi

17:45 *Sea fennel (Crithmum maritimum L.): a culinary herb with nutraceutical potential. Extraction, purification and chemical characterization of polar extracts.*

Diletta Piatti

18:00 *Consumer targeted food packages to promote pulse consumption.*

Dalia Camilletti

18:15 *Strategic change of gut microbiota composition ameliorates lipid metabolism in Alzheimer's disease.*

Chunmei Gong

18:30 *Protective effects of R(+)-Thioctic Acid treatment on the heart of spontaneously hypertensive rats.*

Proshanta Roy

18:45 *Green extraction of hemp (Cannabis sativa L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds, and cannabinoids): a central composite design optimization study.*

Jacopo Torresi

19:00 Chiusura dei lavori

Verrà assegnato un 1 CFU agli studenti delle Scuole di Scienze del Farmaco e dei Prodotti della Salute, di Scienze e Tecnologie (L-27) e di Bioscienze e Medicina Veterinaria (L-13/L-2, L-13, L-32, L-38, LM-6, LM-42) - UNICAM.

Comitato organizzatore e scientifico: Caprioli Giovanni, Cerquetella Matteo, Marchegiani Andrea, Sagratini Gianni
In collaborazione con: Area Comunicazione Ufficio Stampa e Marketing di UNICAM

INDICE

Plenary lecture

Food waste and by-products as valuable sources of bioactive compounds

Lina Cossignani, Federica Ianni, Luna Pollini, Francesca Blasi p. 6

Oral Communications

Ideabril packaging capability in the preservation of raw and cooked ham: a comparative study

Laura Acquaticci, Franks Kamgang Nzekoue, Lucia Bailetti, Simone Angeloni, Sagratini Gianni, Caprioli Giovanni p. 8

Hemp extract safety for dermatological application

Cristina Aguzzi, Laura Zeppa, Oliviero Marinelli, Massimo Nabissi p. 9

Organic and antibiotic-free chicken meat: a proteomic approach

Laura Alessandroni, Massimo Ricciutelli, Manuela Cortese, Silvia Vincenzetti, Renzo Galli, Gianni Sagratini p. 10

Characterization of Panax ginseng root extracts: development of a new analytical method for the quantification of ginsenosides and biological studies

Simone Angeloni, Giovanni Caprioli, Anna Maria Eleuteri, Valentina Cecarini, Mauro Angeletti, Oliviero Marinelli, Massimo Nabissi, Laura Bordoni, Rosita Gabbianelli, Gianni Sagratini p. 12

Antioxidant effects of ‘Mela Rosa dei Monti Sibillini’ on Drosophila melanogaster longevity

Daniela Beghelli, Joice Guileine Nkuimi Wandjou, Ilenia Cirilli, Filippo Maggi, Laura Giusti, Giulio Lupidi, Giovanni Caprioli, Cristina Angeloni p. 14

Gut microbiota modulation counteracts neuroinflammation and reduces the deposition of amyloid and tau in Alzheimer’s disease

Laura Bonfili, Valentina Cecarini, Chunmei Gong, Anna Maria Eleuteri p. 16

Dietary metabolites and CVD biomarkers: what about trimethylamine and its oxidative product?

Laura Bordoni, Irene Petracci, Iwona Pelikant-Malecka, Adriana Radulska, Marco Piangerelli, Joanna J. Samulak, Lukasz Lewicki, Leszek Kalinowski, Robert A. Olek, Rosita Gabbianelli p. 18

Exploiting the nutraceutical activities of the forage plant *Onobrychis viciifolia* (sainfoin) for the control of intestinal parasites in sheep doing without drugs

Daniele Braidot, Paride D'Ottavio, Serena Gabrielli, Annette Habluetzel, Filippo Maggi, **Sara Marchegiani**, Francesco Pagliacci, Paolo Polidori, Eleonora Spinozzi, Laura Tozzi, Valentina Zender p. 20

Consumer targeted food packages to promote pulse consumption

Dalia Camilletti, Giorgia Vici, Luca Belli, Claudia Annetti, Valeria Polzonetti p. 22

Polyphenol microbial metabolites modulate proteolysis in neuronal cells reducing amyloid-beta (1-42) levels

Valentina Cecarini, Massimiliano Cuccioloni, Yadong Zheng, Laura Bonfili, Chunmei Gong, Mauro Angeletti, Pedro Mena, Daniele Del Rio, Anna Maria Eleuteri p. 24

Inhibitory activity of Chlorogenic Acid and Coffee Silverskin extracts against *Klebsiella pneumoniae* carbapenemases

Sonia I Ciancia, Dezemona Petrelli, Giovanni Caprioli, Gianni Sagratini, Luca A Vitali p. 26

Benefici e uso degli Aminoacidi essenziali

Emanuele Giordano p. 28

Strategic change of gut microbiota composition ameliorates lipid metabolism in Alzheimer's disease

Chunmei Gong, Laura Bonfili, Massimiliano Cuccioloni, Valentina Cecarini, Mauro Angeletti, Anna Maria Eleuteri p. 29

Assessment of cholesterol-lowering bioactive compounds in Italian-standard Espresso coffee

Franks Kamgang Nzekoue, Laura Alessandrini, Luciano Navarini, Giovanni Caprioli, Gianni Sagratini p. 31

Short and medium chain free fatty acids in wine: extraction optimization and analysis

Lenti Lucia, Serena Scortichini, Dennis Fiorini p. 32

Cannabidiol extracted from *Cannabis sativa* induces cell cycle arrest, mitophagy and cell differentiation in chronic myeloid leukemia cells

Federica Maggi, Maria Beatrice Morelli, Massimo Nabissi, Oliviero Marinelli, Daniele Tomassoni, Cristina Aguzzi, Laura Zeppa, Giorgio Santoni, Consuelo Amantini p. 34

Green extraction of hemp (*Cannabis sativa* L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds, and cannabinoids): a central composite design optimization study

Filippo Maggi, Marco Cespi, **Jacopo Torresi**, Riccardo Petrelli, Eugenia Mazzara, Riccardo Carletti, Dennis Fiorini, Serena Scortichini, Stefano Dall'Acqua, Stefania Sut, Giovanni Caprioli, Simone Angeloni, Ahmed Mustafa, Jure Zekič p. 36

Anti-inflammatory properties of hemp extracts in human bronchial cells

Oliviero Marinelli, Cristina Aguzzi, Laura Zeppa, Massimo Nabissi p. 38

- Capsaicin as new adjuvant in anti-cancer immunotherapy**
Maria Beatrice Morelli, Consuelo Amantini, Matteo Santoni, Oliviero Marinelli, Cristina Aguzzi, Laura Zeppa, Massimo Nabissi, Giorgio Santoni p. 40
- A new HPLC-MS/MS method for the simultaneous determination of 36 polyphenols in blueberry, strawberry and their commercial products and determination of antioxidant activity**
Ahmed M Mustafa, Simone Angeloni, Doaa Abouelenein, Laura Acquaticci, Jianbo Xiao, Gianni Sagratini, Filippo Maggi, Sauro Vittori, Giovanni Caprioli p. 42
- Sea fennel (*Crithmum maritimum* L.): a culinary herb with nutraceutical potential. Extraction, purification and chemical characterization of polar extracts**
Diletta Piatti, Simone Angeloni, Giovanni Caprioli, Filippo Maggi, Massimo Ricciutelli, Lolita Arnoldi, Gianni Sagratini p. 44
- Quality of wholemeal pasta made with pigmented and ancient wheats**
Francesca Pompei, Matteo Bonfini, Xinying Suo, Ahmed M. Mustafa, Gianni Sagratini, Elena Vittadini p. 46
- Characterization of milk proteins based nano-carriers: interactions with polyphenolic molecules of nutraceutical interest**
Sofia Renzi, Diego R Perinelli, Alessandro Alesi, Valeria Polzonetti, Paolo Polidori, Silvia Vincenzetti, Stefania Pucciarelli p. 48
- Protective effects of R (+)-Thioctic Acid treatment on the heart of spontaneously hypertensive rats**
Proshanta Roy, Seyed Khosrow Tayebati, Ilenia Martinelli, Francesco Amenta, Daniele Tomassoni p. 49
- In vitro* assessment of prebiotic effect of coffee and its by-products on human intestinal microbiota**
Chiara Salvesi, Serena Scortichini, Giovanni Caprioli, Dennis Fiorini, Stefania Silvi p. 51
- Water- and fat-soluble vitamins in milk after different thermal treatments and steam injection**
Giuseppe Santini, Valeria Polzonetti, Yulia Klimanova, Stefania Pucciarelli, Paolo Polidori, Lauro Fioretti, Silvia Vincenzetti p. 53
- The effect of chickpea flour addition levels and flour structures on quality and *in vitro* starch digestibility of corn-rice-based gluten free pasta**
Xinying Suo, Margherita Dall'Asta, Gianluca Giuberti, Michele Minucciani, Elena Vittadini p. 55
- Managing of the daily calcium dietary intake as a tool to contribute to a healthy lifestyle**
Giorgia Vici, Dalia Camilletti, Benedetta Gibertoni, Bruno Adesso, Luca Belli, Valeria Polzonetti p. 57

Vitamins in human, cow, and donkey milk: a comparison of nutritional properties

Silvia Vincenzetti, Giuseppe Santini, Yulia Klimanova, JingJing Zhang, Natalina Cammertoni, Valeria Polzonetti, Stefania Pucciarelli, Paolo Polidori p. 59

Evening Primrose Oil effects in human Pancreatic Ductal Adenocarcinoma cell lines

Laura Zeppa, Cristina Aguzzi, Oliviero Marinelli, Massimo Nabissi p. 61

Can probiotics counteract the detrimental effects of sleep deprivation?

Yadong Zheng, Laura Bonfili, Amina Aboufares El Alaoui, Michele Bellesi, Anna Maria Eleuteri p. 63

Food waste and by-products as valuable sources of bioactive compounds

Lina Cossignani, Federica Ianni, Luna Pollini, Francesca Blasi

Department of Pharmaceutical Sciences, University of Perugia, Italy

lina.cossignani@unipg.it

Food waste and by-products are produced in all the phases of food life cycle, that is during agricultural production and industrial manufacturing, but also after processing, distribution and household activities. These wastes can be an important source of bioactive compounds, such as fibers, polysaccharides, proteins, lipids, phenolic compounds, vitamins, and carotenoids. Among these, a prominent place is occupied by phenolic compounds, which are well-known for their beneficial effects on human health, e.g., in the prevention of cancer and cardiovascular diseases [1]. Nowadays, there is an increasing interest toward the development and manufacture of functional foods and nutraceuticals, which have received great attention in the food market due to the growing consumer interest for “healthy” food. For this reason, pharmaceutical and food industries are interested in obtaining natural bioactive compounds to use as drugs, functional food ingredients, and nutraceuticals. To this aim, suitable extraction methods have to be developed for the different by-products generated from agro-food industries. Traditional methods are still in use, even if they can be time, energy, and solvent consuming and sometimes degradation of thermolabile compounds can occur. Sustainable methods are continuously developed by improving and optimizing the existing processes following the principles of green chemistry [2]. In this presentation, the results of some researches aimed to exploit the nutritional/health properties of food waste are shown. Olive leaves have been studied as they represent an attractive source of bioactive compounds, in particular oleuropein and hydroxytyrosol. The phenolic compounds and the antioxidant capacity in leaves from different *Olea europaea* L. cultivars, harvested in different times of the year, were investigated and significant differences between the selected cultivars and harvesting times were observed. Afterwards *Moringa oleifera* leaves were extracted by unconventional extraction techniques and the metabolite profiling was obtained by UHPLC/QTOF-MS analysis. The results suggested that extraction method had a significant effect on yield and profile of phenolic compounds and that the untargeted metabolomics analysis is a powerful tool for characterizing the phenolic profile of plant extracts. Also *Lycium barbarum* leaves were investigated as source of phenolics, and chlorogenic acid and rutin were found as main components. Microwave-assisted infusion of *L. barbarum* leaves showed that chlorogenic acid is particularly sensitive to chemical modification during microwave heating. To date, a research on apple pomace, the major by-product obtained during apple juice processing, is underway. This product contains several important nutrients and secondary metabolites, such as carbohydrates, phenolic compounds, dietary fiber and minerals [3]. The phenolic fraction was investigated after ultrasound-assisted extraction with aqueous or hydroalcoholic solvent. The fractionation of apple pomace extract was also performed giving free and bound phenols, and hydrolyzed fractions in acid and alkaline conditions. The total extract and the different fractions have

been characterized by UHPLC/Q-TOF MS analysis and *in vitro* antioxidant activity was measured. Among the phenolic compounds identified, the most abundant were phloridzin and different glycosilated forms of quercetin. Future research will focus on the addition of apple pomace in meat and dairy products, in order to obtain fiber and phenolic enriched foods.

References

- [1] Piccolella S, Crescente G, Candela L, Pacifico S. Nutraceutical polyphenols: new analytical challenges and opportunities. *J Pharm Biomed Anal.* 2019;175,112774.
- [2] Chemat F, Abert Vian M, Ravi HK, Khadhraoui B, Hilali S, et al. Review of Alternative Solvents for Green Extraction of Food and Natural Products: Panorama, Principles, Applications and Prospects. *Molecules* 2019;24,3007.
- [3] Coman V, Teleky BE, Mitrea L, Martău GA, Szabo K, et al. Bioactive potential of fruit and vegetable wastes. *Adv. Food Nutr. Res.* 2019;91:157-225.

IDEABRILL PACKAGING CAPABILITY IN THE PRESERVATION OF RAW AND COOKED HAM: A COMPARATIVE STUDY



Laura Acquaticci¹, Franks Kamgang Nzekoue¹, Lucia Bailetti², Simone Angeloni¹, Gianni Sagratini¹, Caprioli Giovanni¹

¹*School of Pharmacy University of Camerino, via Sant'Agostino 1, 62032 Camerino (MC), Italy*

²*Italian Center for Sensory Analysis, Matelica (MC), Italy*

laura.acquaticci@unicam.it

Food packaging contributes to the preservation and shelf-life of food. In literature, several studies demonstrate that active packaging, enriched of bioactive compounds like the essential oil of *Rosmarinus Officinalis* [1], and modified atmosphere packaging can positively influence the preservation of food. The study of new types of packaging is continuously increasing, mostly in terms of environmental impact and food preservation. In this study two types of packaging provided by Esseoquattro company were tested in order to assess their capability in the preservation of food. *Ideabrill*® packaging, a three layers pack of polyethylene high density layer, metallic layer and cellulose with long fiber layer, combined with *Ideabrill*® sachetto salvafreschezza was compared to paper coupled with wings. The study was conducted on raw and cooked ham preserved in the packaging described above through the quantification of biogenic amines (BAs) at day 0, 3, 5 and 7. BAs can be considered markers to evaluate the freshness and the quality of food. In particular, a higher concentration of BAs is related to a higher deterioration degree of food. BAs were extracted, derivatized with dansyl chloride, purified with a SPE C-18 and then analysed with an HPLC-DAD method. This study, in combination with sensorial study, shows that *Ideabrill*® packaging combined with *Ideabrill*® sachetto salvafreschezza showed the best conservation capability for raw and cooked ham when compared to the other one. Moreover, from an eco-friendly point of view, *Ideabrill*® packaging layers can be easily separated in order to encourage recycling.

References

[1] Sirocchi V, Caprioli G, Cecchini C, Coman MM, Cresci A, Maggi F, Papa F, Ricciutelli M, Vittori S, Sagratini G. Biogenic amines as freshness index of meat wrapped in a new active packaging system formulated with essential oils of *Rosmarinus officinalis*. *International journal of food sciences and nutrition*. 2013;64(8):921-928.

Hemp extract safety for dermatological application



Cristina Aguzzi¹, Laura Zeppa¹, Oliviero Marinelli¹, Massimo Nabissi¹

¹*School of Pharmacy, University of Camerino, 62032 Camerino (MC), Italy*

cristina.aguzzi@unicam.it

The skin immune response is regulated by epidermal keratinocytes, lymphocytes and dendritic cells, by the releasing of cytokines, chemokines, growth factors and activation of specific transcription factors. An aberrant regulation of this immune response causes an inflammatory condition [1]. Many studies suggest the use of cannabinoids for the management of different inflammatory dermal conditions because the endocannabinoid system is present in the skin and has a role in maintaining its homeostasis, by regulating inflammation, immune modulation, cell proliferation and differentiation [1,2]. Cannabinoids showed anti-inflammatory and anti-pruritic activity through CB1 and CB2 receptors dependent mechanisms [1,2]. So, the endocannabinoid system is being studying to treat many inflammatory skin conditions, such as atopic dermatitis, contact dermatitis, psoriasis, acne, with pharmacological products available on the market [1,2]. We analysed the effect of CBD on human keratinocyte cell line (HaCaT) and human dermal fibroblasts (NHF A12) to assess its safety on an in vitro skin model. CBD not only showed a safety profile since did not increase the expression of the main inflammatory markers, but it reduced their basal expression. On the bases of these results, we also tested some essential oils (EOs) derived from hemp (*Cannabis sativa*) with low fraction of cannabinoids and their effect on the inflammatory markers. Results showed that also the EOs did not increase the basal expression of the inflammatory markers on the same cell lines [3]. These results suggest that the use of purified compounds as well as EOs derived from hemp would be safety for a topical use, and the next step should be the evaluation of their anti-inflammatory activity in a condition of dermal inflammation.

References

- [1] Milando R, Friedman A. Cannabinoids: Potential Role in Inflammatory and Neoplastic Skin Diseases. *Am J Clin Dermatol*. 2019;20(2):167-180. doi: 10.1007/s40257-018-0410-5. PMID: 30542832.
- [2] Sheriff T, Lin MJ, Dubin D, Khorasani H. The potential role of cannabinoids in dermatology. *J Dermatolog Treat*. 2020;31(8):839-845. doi: 10.1080/09546634.2019.1675854. Epub 2019 Oct 10. PMID: 31599175.
- [3] Rossi P, Cappelli A, Marinelli O, Valzano M, Pavoni L, Bonacucina G, Petrelli R, Pompei P, Mazzara E, Ricci I, Maggi F, Nabissi M. Mosquitocidal and Anti-Inflammatory Properties of The Essential Oils Obtained from Monoecious, Male, and Female Inflorescences of Hemp (*Cannabis sativa* L.) and Their Encapsulation in Nanoemulsions. *Molecules*. 2020;25(15):3451. doi: 10.3390/molecules25153451. PMID: 32751258; PMCID: PMC7435401.

Organic and antibiotic-free chicken meat: a proteomic approach



Laura Alessandroni¹, Massimo Ricciutelli¹, Manuela Cortese¹, Silvia Vincenzetti², Renzo Galli³,
Gianni Sagratini¹

¹*School of Pharmacy, (University of Camerino) Italy*

²*School of Bioscience and Veterinary Medicine, (University of Camerino) Italy*

³*Fileni S.p.A., Località Cerrete Collicelli 8, 62011 Cingoli, Italy*

laura.alessandroni@unicam.it

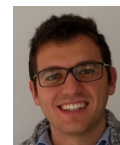
Antibiotics have been extensively used in animal husbandry, mostly as growth promoting agents or for prophylactic purposes to reduce bacterial pathogens. Their continuous use in animal production raised concern associated with emergence of resistance in meat consumers [1]. In 2018, "antibiotic-free" meat attracted the attention of more than 43% of consumers, an always growing percentage [2]. Consequently, farmers adopted several strategies such as antibiotic-free and organic methods. Antibiotic-free animals are not treated with any kind of antibiotic, while organically raised animals cannot be fed with non-natural or synthetic substances, and cannot be treated with synthetic chemotherapeutic drugs, only phytotherapy and vaccinations are allowed. A deepening research of biomarkers to differentiate antibiotic-free, organic, and conventional meat is increasingly necessary. Proteomic is the identification, quantification, and functional characterization of the proteome of a cell, tissue, or organism under a specific, defined conditions [3]. The aim of this work is to compare the proteome of organically and antibiotic-free produced poultry meat. Proteomic analysis was performed on sarcoplasmic proteins combining two-dimensional electrophoresis for separation and ultra high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-QTOF MS) and MASCOT protein database search for characterization. Two-dimensional electrophoresis (2DE) allows protein separation based on isoelectric point (pI) using an immobilized pH gradient in first dimension, and then on molecular weight using SDS-gel in the second dimension. After 2DE separation, each spot was in-gel digested with trypsin according to the protocol of Shevchenko et al. [4] and then injected in UHPLC-QTOFMS. Resulting spectra were extracted and data files were submitted to MASCOT database, to characterize protein spots. Preliminary results showed that most of identified proteins are glycolytic enzymes and qualitative analysis revealed that they were the same for both organic and antibiotic-free samples. Quantitative analysis was performed on SDS-gels using PDQuest software, it underlined statistically significant differences in expression of specific proteins, such as pyruvate kinase, β -enolase, and creatine kinase. These results were compared with several recent studies which use the same proteomic approach. They underline how over or under expressions of specific proteins affect meat quality in terms of tenderness and color [5-7]. Further studies need to be performed to confirm these preliminary results, furthermore, the next step would be the study of membrane proteins using the same proteomic method.

Acknowledgement: This research was performed at University of Camerino and supported by Fileni® industry.

References

- [1] Gilani SMH, Rashid Z, Galani S, Ilyas S, Sahar S, Zahoor-ul-Hassan et al. Growth Performance, Intestinal Histomorphology, Gut Microflora and Ghrelin Gene Expression Analysis of Broiler by Supplementing Natural Growth Promoters: A Nutrigenomics Approach. *Saudi J Biol Sci.* 2021;28(6):3438-3447.
- [2] Consumer Reports Survey Group. Natural and Antibiotic Labels Survey. *2018 Nationally Representative Survey*, 2018, <https://advocacy.consumerreports.org/research/naturalandantibioticlabelssurvey/>
- [3] Chou CJ, Affolter M, Kussmann M. A Nutrigenomics View of Protein Intake: Macronutrient, Bioactive Peptides, and Protein Turnover. In Bouchard C, and Ordovas JM. *Prog Mol Biol Transl*, Academic Press. 2012;108:51-74.
- [4] Shevchenko A, Tomas H, Havli J, Olsen JV, Mann M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat Protoc.* 2006;1(6):2856.
- [5] Malheiros JM, Braga CP, Grove RA, Ribeiro FA, Calkins CR, Adamec J, Chardulo LAL. Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Sci.* 2019;148:64-71.
- [6] Yu Q, Wu W, Tian X, Hou M, Dai R, Li X. Unraveling proteome changes of Holstein beef M. semitendinosus and its relationship to meat discoloration during post-mortem storage analyzed by label-free mass spectrometry. *J Proteom.* 2017;154:85-93.
- [7] Wu W, Yu Q, Fu Y, Tian XJ, Jia F, Li XM, Dai RT. Towards muscle-specific meat color stability of Chinese Luxi yellow cattle: A proteomic insight into post-mortem storage. *J Proteom.* 2016;147:108-118.

Characterization of *Panax ginseng* root extracts: development of a new analytical method for the quantification of ginsenosides and biological studies



Simone Angeloni^{1,2}, Giovanni Caprioli¹, Anna Maria Eleuteri³, Valentina Cecarini³, Mauro Angeletti³, Oliviero Marinelli⁴, Massimo Nabissi⁴, Laura Bordoni⁵, Rosita Gabbianelli⁵, Gianni Sagratini¹

¹*School of Pharmacy, University of Camerino, Camerino, Italy*

²*RICH – Research Innovation Coffee Hub, Belforte del Chienti, Italy*

³*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

⁴*School of Pharmacy, Experimental Medicine Section, University of Camerino, Camerino, Italy*

⁵*Unit of Molecular Biology and Nutrigenomics, School of Pharmacy, University of Camerino, Camerino, Italy*

simone.angeloni@unicam.it

The term *Panax* derived from the word “panacea” which means a cure of all disease. In fact, this medicinal plant has been used in the Traditional Chinese Medicine for the treatment of various illnesses. Recent investigations have reported that extracts prepared from the roots of ginseng possess diverse biological activities such as anticancer, antioxidant, anti-cardiovascular diseases, antidiabetic, and anti-obesity [1,2]. The aim of the present research was to develop an efficient and fast method for the characterization of ginsenosides in ginseng root extracts by using HPLC-MS/MS system with the scope to produce dried extracts rich in ginsenosides and to further evaluate them for their anti-inflammatory and anti-obesity activities. For this purpose, a new analytical method for ginsenoside quantification in ginseng roots has been developed and validated by using HPLC-MS/MS triple quadrupole. In addition, several extraction procedures such as three diverse mechanisms of extraction (liquid-solid extraction (LSE), LSE-assisted by Naviglio extractor and ultrasound-assisted extraction (UAE)) both with various solvents (ethanol, methanol, and a mixture of them with water), have been evaluated studying the extraction efficiency and recovery capacity. The most performing procedures have been applied to prepare dried extracts which have been and will be biologically investigated. The new analytical method was characterized by a good linearity ($R^2 \geq 0.9984$ for all analytes), repeatability (intraday, RSD = 2.9–6.3%; interday, RSD = 8.3–12.5%) and sensitivity (LOQ = 15 $\mu\text{g}/\text{kg}$ for all ginsenosides). The best extraction procedures considering the extraction efficiency and recovery were those prepared with ethanol:water (50:50, v/v) and methanol:water (70:30, v/v) both with SLE and UAE. All the dried extracts were constituted by high level of ginsenosides since their total content varied from 2.6 to 3.3% of dried weight extract (DWE) and the best one, in term of bioactive compound content, was that prepared with SLE with a mixture of ethanol and water. The most abundant ginsenosides in ginseng extracts were Rg1 (10.0–13.1 g/kg of DWE) and Rb1 (6.2–7.8 g/kg of DWE). The individual standards of ginsenosides did not show a strong activity against cytokines except for interferon- γ . In addition, the tested ginsenosides and the extracts similarly inhibit the activity of the NOS enzymes in TPH-1 cell line treated with LPS. The best dried extracts will be further characterized for other anti-inflammatory and anti-obesity activities in the perspective of their application in nutraceutical formulation.

References

- [1] Wee JJ, Park KM, Chung AS. Biological activities of ginseng and its application to human health. *Herbal Medicine: Biomolecular and Clinical Aspects* (2nd ed.). 2011; Chapter 8, CRC Press, Boca Raton.
- [2] Lee MR, Kim BC, Kim R, Oh HI, Kim HK, Choi KJ, Sung CK. Anti-obesity effects of black ginseng extract in high fat diet-fed mice. *J Ginseng Res.* 2013;37:308.

Antioxidant effects of ‘Mela Rosa dei Monti Sibillini’ on *Drosophila melanogaster* longevity

Daniela Beghelli¹, Joice Guileine Nkuimi Wandjou², Ilenia Cirilli², Filippo Maggi², Laura Giusti², Giulio Lupidi², Giovanni Caprioli², Cristina Angeloni²

¹*School of Bioscience and Veterinary Medicine, University of Camerino, Italy*

²*School of Pharmacy, University of Camerino, Italy*

daniela.beghelli@unicam.it

Aging could be considered as the result of a time-dependent accumulation of cell damages that inexorably brings to functional cellular impairments and increased vulnerability to chronic-degenerative diseases. The "Free Radical Theory of Aging", developed by Harman in 1956 [1], and also known as 'the oxidative damage theory of aging', has undergone several variations over the last years, including the suggestion of a central role for reactive oxygen species (ROS) produced by both environmental or endogenous factors [2]. Indeed, free radicals, by damaging biomolecules, compromise cellular homeostasis. Therefore, it is clear that natural dietary compounds with high content of antioxidants represent an extraordinary potential tool in the major healthcare challenge of delaying aging. Among fruits, great attention has been paid to apple, as it is a rich source of bioactive compounds, especially polyphenols. In the last years, an ancient variety of apple, whose cultivation had been abandoned for a long time, has been rediscovered: “la mela rosa dei Monti Sibillini” [3]. Aim of this work was to study the effect of the supplementation of two different extracts obtained from mela rosa dei Monti Sibillini on both longevity and the endogenous antioxidant defence system of *Drosophila melanogaster* (wild type, Canton strain). These fruit flies represent one of the most commonly used animal model for studying the complexity of the aging process because they not only share with humans many metabolic pathways, but also have functional homologs to nearly 65% of human disease-causing genes [4]. In this study, flies, separated by sex (n.200 for both males: M, and females: F; collected in seven tubes/gender/treatment), were lifelong supplemented (0.5% diluted in water) or not (CTR group) with two extracts: one obtained from the whole apples (A group) and the second one from their peels (B group). At two different intervals (at 15 and 45 days for F and at 15 or 30 days for M), some flies (n5/tube for three replicates), after sedation, were collected and, immediately, frozen (-80°C) till the evaluation of the expression of some genes related to antioxidant defences, such as: heme oxidase-1 (HO1); thioredoxin reductase (TRX); superoxide dismutase (SOD) and glutathione peroxidase (GpX) by RT-PCR assay. The longevity in supplemented female flies was significantly increased (by 10 and 20% in A and B groups, respectively) and also the mean half-life of CTR flies was significantly lower than the ones of A and B group. Furthermore, the B groups presented a much higher increase of half-life vs A groups (A vs B; $p < 0.0001$). In male flies, no significant effects were observed for longevity, although the average half-life of CTR group was significantly lower than the ones of A and B group. Furthermore, in males, the different extract supplementation (A vs B) did not differently modulate the half-life of fruit flies. A different trend over time of antioxidant gene expressions was observed in the two genders following the A or B supplementations. Female flies, physiologically characterized by higher antioxidant defences [5], in the early stage of supplementation showed a down regulation of all the antioxidant genes here

evaluated; whereas, at 45 days, the gene expression increased for GpX, HO1 and TxR in B group. Male flies, instead, showed a modulation of antioxidant gene expression since the early stages, where upregulation of SOD and HO1 were observed in A group at 15 days. At 30 days, the GpX and HO1 gene expressions resulted increased in B group, whereas TxR in A group. The data obtained from this study suggest that the oral supplementation of the extracts of mela rosa dei Monti Sibillini starting from birth is able to prolong the average life of both female and male flies, while an effect on maximum longevity was registered only in females. Also with regard to the modulation of the antioxidant enzymes, different results were obtained in the two genders. In particular, in females, a down regulation of the expression of SOD, GpX, HO1 and TxR genes in the early stages of life was observed, which was not found in males. During aging, however, when the endogenous antioxidant defences decrease also in females, there was an up-regulation of antioxidant genes, modulated by supplementation in both sexes, although with a higher increase in female specimens. These data suggest that the apple is a potential functional food whose chronic consumption during life might prevent the onset of those chronic-degenerative diseases in which oxidative stress plays a key role. Moreover, these results sustain the cultivation of this ancient variety of apple to promote the economic growth in this area.

References

- [1] Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1956;11: 298-300.
- [2] Pomatto LCD, Davies KJA. Adaptive homeostasis and the free radical theory of ageing. *Free Radic Biol Med.* 2018;124:420-430.
- [3] Wandjou JGN, Lancioni L, Barbalace MC, Hrelia S, Papa F, Sagratini G, Vittori S, Dall'Acqua S, Caprioli G, Beghelli D, Angeloni C, Lupidi G, Maggi F. Comprehensive characterization of phytochemicals and biological activities of the Italian ancient apple 'Mela Rosa dei Monti Sibillini'. *Food Res Intern.* 2020;109422.
- [4] Berrak U, Chen K, Bellen HJ. *Drosophila* tools and assays for the study of human diseases. *Dis Mod and Mech.* 2016;9:235-244.
- [5] Niveditha S, Deepashree S, Ramesh SR, Shivanandappa T. Sex differences in oxidative stress resistance in relation to longevity in *Drosophila melanogaster*. *J Comp Physiol B: Bioch Syst Envir Phys.* 2017;187,7,899-909.

Gut microbiota modulation counteracts neuroinflammation and reduces the deposition of amyloid and tau in Alzheimer's disease



Laura Bonfili, Valentina Cecarini, Chunmei Gong, Anna Maria Eleuteri

School of Biosciences and Veterinary Medicine, University of Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italy

laura.bonfili@unicam.it

Alzheimer's disease (AD) is a big socioeconomic burden, since effective prevention strategies and definitive treatment are not currently available. In AD, amyloid β aggregation, tau hyperphosphorylation and neuronal death are strictly associated with neuroinflammation. Protein aggregates initiate microglial activation and stimulate the release of inflammatory mediators that determine AD severity [1]. Additionally, alterations of gut microbiota composition play a crucial role in the development of AD. In fact, intestinal bacteria can influence brain and immune system function by the production of lipopolysaccharides and amyloid. Dysbiosis and increased abundance of pro-inflammatory bacteria are frequently observed in the gut of AD patients causing both local and systemic immune-mediated inflammation, oxidative stress, and impaired intake and metabolization of nutrients [2]. Recently, probiotics have emerged as a promising and safe strategy to manipulate gut microbiota composition and increase the host health status through a multi-level mechanism that is currently under investigation [3]. In this study, a multi-strain probiotic formulation of lactic acid bacteria and bifidobacteria (SLAB51) was chronically administered to a triple-transgenic mouse model of AD, B6;129-Psen1tm1Mpm Tg (APP^{Swe}, tau^{P301L})1Lfa/J (named 3xTg-AD), in order to investigate the potential beneficial effects on AD progression through behavioural tests, biochemical and immunohistochemical analyses, metabolomic and lipidomic studies. Probiotics exerted multiple effects in AD mice by modulating gut microbiota composition. They favoured the proliferation of anti-inflammatory bacterial species and caused metabolic changes, such as the increase of anti-inflammatory short chain fatty acids (SCFAs), able to directly act in the brain, positively interfering with inflammatory cytokines and increasing the concentration of gut hormones that are extremely important in the regulation of energy homeostasis, food intake and nervous functions [4,5,6]. Cholesterol homeostasis was improved in AD mice supplemented with probiotics, involving pathways mediated by the well-recognized anti-inflammatory liver X receptors. Plasma lipidomic analyses highlighted dynamic interactions among numerous actors of a complex inflammatory response, in which polyunsaturated fatty acids can compete each other and simultaneously co-operate in the resolution of inflammation. The downstream effects of these changes were the modulation of neuronal proteolysis, the reduction of amyloid β and tau aggregates and the improvement of cognitive abilities, with consequent slowdown of AD progression. Concluding, probiotics supplementation represents a promising multi-target intervention strategy able to positively shift gut microbiota composition, ameliorating neuroinflammation and metabolic alterations, finally counteracting cognitive decline and successfully reducing AD main hallmarks.

References

- [1] Onyango IG, Jauregui GV, Čarná M, Bennett JP Jr, Stokin GB. Neuroinflammation in Alzheimer's Disease. *Biomedicines*. 2021;9(5):524.
- [2] Cerovic M, Forloni G, Balducci C. Neuroinflammation and the Gut Microbiota: Possible Alternative Therapeutic Targets to Counteract Alzheimer's Disease? *Front Aging Neurosci*. 2019;11:284.
- [3] Bonfili L, Cecarini V, Gogoi O, Gong C, Cuccioloni M, Angeletti M, Rossi G, Eleuteri AM. Microbiota modulation as preventative and therapeutic approach in Alzheimer's disease. *FEBS J*. 2021;288(9):2836-2855.
- [4] Bonfili L, Cecarini V, Berardi S, Scarpona S, Suchodolski JS, Nasuti C, Fiorini D, Boarelli MC, Rossi G, Eleuteri AM. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. *Sci Rep*. 2017;7(1):2426.
- [5] Bonfili L, Cecarini V, Cuccioloni M, Angeletti M, Berardi S, Scarpona S, Rossi G, Eleuteri AM. SLAB51 Probiotic Formulation Activates SIRT1 Pathway Promoting Antioxidant and Neuroprotective Effects in an AD Mouse Model. *Mol Neurobiol*. 2018;55(10):7987-8000.
- [6] Bonfili L, Cecarini V, Gogoi O, Berardi S, Scarpona S, Angeletti M, Rossi G, Eleuteri AM. Gut microbiota manipulation through probiotics oral administration restores glucose homeostasis in a mouse model of Alzheimer's disease. *Neurobiol Aging*. 2020;87:35-43.

Dietary metabolites and CVD biomarkers: what about trimethylamine and its oxidative product?



Laura Bordoni¹, Irene Petracci², Iwona Pelikant-Malecka^{3,4}, Adriana Radulska^{3,4}, Marco Piangerelli⁵, Joanna J. Samulak⁶, Lukasz Lewicki⁷, Leszek Kalinowski^{3,4,8}, Robert A. Olek⁹ and Rosita Gabbianelli¹

¹*Unit of Molecular Biology and Nutrigenomics, School of Pharmacy, University of Camerino, Camerino, MC, Italy*

²*School of advanced studies, University of Camerino, Camerino, MC, Italy*

³*Department of Medical Laboratory Diagnostics, Medical University of Gdansk, 80-211 Gdansk, Poland*

⁴*Biobanking and Biomolecular Resources Research Infrastructure Poland (BBMRI.PL), 80-211 Gdansk, Poland*

⁵*Computer Science Division and Mathematics Division, School of Science and Technology, University of Camerino, 62032 Camerino, Italy*

⁶*Doctoral School, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland*

⁷*University Center for Cardiology, 80-211 Gdansk, Poland*

⁸*Department of Mechanics of Materials and Structures, Gdansk University of Technology, 80-223 Gdansk, Poland*

⁹*Poznan University of Physical Education, Krolowej Jadwigi 27/39, 61-871 Poznan, Poland*

laura.bordoni@unicam.it

Recent studies indicate a potential role of trimethylamine (TMA) and its oxidative form, trimethylamine N-oxide (TMAO), in the development of cardiovascular diseases (CVD). Since TMA is produced by the gut microbiota from dietary precursors (e.g., carnitine and choline), this association has a direct impact on dietary advices aimed at preventing CVD. TMAO has been proposed as a promising biomarker of cardiovascular health, despite a clear mechanistic explanation of this association is still missing. Together with classical CVD predictors, another novel molecular biomarker for CVD is the measurement of circulating mitochondrial DNA copy number (mtDNA_{cn}). Since mtDNA_{cn} changes have been associated with both intrinsic and extrinsic factors, it has been proposed as a potential biomarker for complex multifactorial diseases, as it is an early indicator of damage and mitochondrial dysfunction. In a population of 389 coronary artery disease (CAD) patients and 151 healthy controls, we investigated TMA, TMAO and mtDNA_{cn}, in association with established risk factors for CVD (gender, age, hypertension, smoking, diabetes, glomerular filtration rate (GFR)), with the aim to identify biomarkers predictive of CVD and clarify the impact of TMA and TMAO on health. Results showed that neither TMA nor TMAO were able to predict CAD in our population. On the contrary, a biomarker including mtDNA_{cn}, gender, and hypertension emerged as a good predictor of CAD. MtDNA_{cn} was significantly lower in CAD patients and in hypertensive subjects; it correlates with GFR and TMA, but not with TMAO. Our findings do not support the usage of TMA and TMAO as CAD biomarkers, and suggest that dietary restrictions or supplementations aimed at reducing the concentration of these metabolites in the circulation should be considered with

caution. On the other hand, mtDNAcn might be a new interesting plastic biomarker suitable to monitor the exposure to risk factors and the efficacy of preventive interventions for a personalized CAD risk reduction.

Exploiting the nutraceutical activities of the forage plant *Onobrychis viciifolia* (sainfoin) for the control of intestinal parasites in sheep doing without drugs



Daniele Braidot¹, Paride D'Ottavio², Serena Gabrielli³, Annette Habluetzel¹, Filippo Maggi¹, **Sara Marchegiani**¹, Francesco Pagliacci⁴, Paolo Polidori¹, Eleonora Spinozzi¹, Laura Trozzo², Valentina Zender¹

¹*School of Pharmacy, University of Camerino, Italy*

²*Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Italy*

³*School of Science and Technology, University of Camerino, Italy*

⁴*Department of Land, Environment, Agriculture and Forestry, University of Padova, Italy*

sara.marchegiani@unicam.it

Sainfoin (*Onobrychis viciifolia*) is traditionally used in the mountainous areas of the Marche and Umbria Regions as a forage for sheep. A large bulk of literature demonstrates the plant's various nutraceutical properties in small ruminants. These are related to the presence of secondary metabolites and specifically to condensed tannins. Beneficial responses of animals fed on sainfoin hay or green forage include improved growth, milk and wool production, fertility, and reduced methane emissions and ammonia volatilization from dung or urine. Most important is the plant's capacity to reduce gastro-intestinal nematodes of small ruminants which is related to condensed tannins, namely to prodelphinidins and procyanidins and their degree of polymerisation [1]. This study exploits the available knowledge on the plant's anthelmintic effects to develop sainfoin based alimentary schemes for local sheep farms, allowing breeders to control intestinal parasites without the employment of anthelmintic drugs. In addition, the known nutraceutical effects of sainfoin on various animal husbandry parameters may lead to an increase in profitability of the family-based farm holdings. Specifically the project aims to i) assess the cultivation practices and the use of sainfoin by sheep breeders in the 'Alto Maceratese' area; ii) provide a comprehensive chemical characterization of tannins and other secondary metabolites of local sainfoin samples (ecotype 'lupinella nostrale' vs. ecotype 'lupinella quarantina'); iii) measure *in vitro* the anthelmintic activity of sainfoin extracts against the sheep parasite *Strongyloides papillosus*; iv) elaborate with sheep breeders nutraceutical sainfoin feeding protocols and evaluate the impact on intestinal parasite densities and animal husbandry parameters such as lamb growth and milk quality. From the first interviews (n=7) held with local farmers, emerges that breeders recognize the nutritional benefits of sainfoin stating that sheep 'like it very much', 'produce more milk' and 'the milk has a pleasant smell'. None of the farmers interviewed up to now has heard about effects on sheep parasites. Farmers retain the ecotype 'lupinella nostrale' very adapted for cultivation on 'poor soils' in the mountainous area of the 'Alto Maceratese' due to the plant's capacity to resist to adverse climatic conditions such as droughts. The ecotype 'lupinella quarantina' on the other hand, is more adapted to hilly areas as it requires more fertile soils similar to 'erba medica' (lucerne: *Medicago sativa*). Usually, farmers employ various forages according to the soil characteristics and altitude of their cultivatable land. Thus, during winter sheep are kept on hay from sainfoin and other forage plant species and during summer flocks graze

on fields after hay harvest (sainfoin or other) and on permanent semi-natural pastures. Therefore, the quantity of sainfoin and condensed tannins assumed by sheep flocks varies considerably from one farm to another and according to the season. Infection with gastro-intestinal nematodes is a common problem in small ruminants, impacting negatively on animal health and production parameters, if left uncontrolled. Usually, anthelmintic drugs are administered once a year (Nov / Dic) to the entire flocks. Data collected on 6 farms in 2018 revealed a mean number of 304 eggs / g of fecal material (EPG) ranging from 76 to 885 EPG. An egg density of 300 to 400 EPG is likely to have an impact on production parameters. Periodic monitoring effected in one farm at ‘Sant’Ilario’, on which sheep are held traditionally on sainfoin hay and sainfoin pasture to about 50 % of the total feed, revealed very low egg counts of 17, 6 and 68 EPG in spring 2020, autumn 2020 and spring 2021 respectively, values that convinced the breeder not to give any drug treatments since spring 2020. Whether these low parasite densities can be ascribed to the sainfoin rich diet of the flock will tell us the quantitative analysis of condensed tannins present in the sainfoin samples from that farm (analysis in progress). Work planned for the next months will focus on the chemical characterisation of sainfoin samples taken from various sites of the area and on the assessment of their anthelmintic activity *in vitro* against free-living stages (larvae and adults) of the sheep parasite *Strongyloides papillosus*. With these data at hand, antiparasitic sainfoin feeding schemes will be elaborated with interested breeders and the outcome in terms of parasite control and production parameters monitored.

References

- [1] Mueller-Harvey I, et al. Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. *Crop Science*. 2019;59;1-25.

Consumer targeted food packages to promote pulse consumption



Dalia Camilletti¹, Giorgia Vici¹, Luca Belli¹, Claudia Annetti², Valeria Polzonetti¹

¹*Scuola di Bioscienze e Medicina Veterinaria – Università di Camerino, Italia*

²*Fertitecnica Colfiorito Srl - Colfiorito (Pg), Italia*

dalia.camilletti@unicam.it

Pulse consumption is today a critical aspect in the diet, despite the known health benefits derived from their intake [1,2]. Consumers do not choose pulses in their diet for several reasons, such as long cooking time, flatulence connected to their intake and the shortage of innovative products [3,4]. Our studies are in line with literature data. One of our on-line surveys conducted on 789 subjects shows that according to the 62.3% of the participants pulses are not consumed for the long preparation and cooking time they require and according to the 45.9% for their unpleasant taste. Considering the health benefits due to pulse intake in the diet, it is fundamental to promote their consumption and in particular to conceive non-conventional strategies to try satisfying consumer needs and requests on pulses [5]. In this context, in collaboration with Fertitecnica Colfiorito s.r.l. company it was developed a project aimed to promote a healthy lifestyle through pulse consumption among consumers. For the project 5 specific targets of consumers (sportsmen, families, fast cooking consumers, traditional foods consumers and organic foods consumers) were identified and 5 specific product packages were designed starting from the evaluation of company products. Company food products were selected according to their nutritional characteristics (i.e. carbohydrates, proteins and fibre content) in accordance to target consumers. For example, in sportsmen the attention was focused on nutrient timing, while for families on both adults and children nutritional needs. In each package both traditional pulses and innovative food formulations were proposed (i.e. dry beans and novel pulses-based products). Products were picked out also taking into account meal occasions and time for meal preparation and consumption, in order to guarantee healthy food choices even when time for cooking is not available. In addition, general dietary suggestions were provided to promote pulses use and to guide consumers in understanding how frequently they should be consumed and how to combine them to get a balanced plate. Furthermore, the project wished to support informed food choices as it is worth knowing that healthy dietary behaviours allow to contribute to a more sustainable future.

References

- [1] Ferreira H, Vasconcelos M, Gil AM, Pinto E. Benefits of pulse consumption on metabolism and health: A systematic review of randomized controlled trials. *Crit Rev Food Sci Nutr.* 2021;61.1:85-96.
- [2] Becerra-Tomás N, Papandreou C, Salas-Salvadó J. Legume consumption and cardiometabolic health. *Adv Nutr.* 2019;10(Suppl_4): S437-S450.
- [3] Schneider AVC. Overview of the market and consumption of pulses in Europe. *Br J Nutr.* 2002;88.S3: 243-250.

- [4] Thirunathan P, Manickavasagan A. Processing methods for reducing alpha-galactosides in pulses. *Crit Revs Food Sci.* 2019;59.20:3334-3348.
- [5] Curran J, McLachlan M, Black R, Widders I, Manary M. Collaboration among sectors to increase pulse consumption. *Ann NY Acad Sci.* 2017;1392: 3-5.

Polyphenol microbial metabolites modulate proteolysis in neuronal cells reducing amyloid-beta (1-42) levels



Valentina Cecarini¹, Massimiliano Cuccioloni¹, Yadong Zheng¹, Laura Bonfili¹, Chunmei Gong¹, Mauro Angeletti¹, Pedro Mena^{2,3}, Daniele Del Rio^{2,3}, Anna Maria Eleuteri¹

¹*School of Biosciences and Veterinary Medicine, University of Camerino, Italy*

²*Human Nutrition Unit, Department of Food and Drugs, University of Parma, Italy*

³*Microbiome Research Hub, University of Parma, Parma, Italy*

valentina.cecchini@unicam.it

Alzheimer's disease (AD) is a progressive neurodegeneration characterized by extensive protein aggregation and deposition in the brain, associated with defective proteasomal and autophagic-lysosomal proteolytic pathways [1]. Since current drugs can only reduce specific symptoms, the identification of novel treatments is a major concern in AD research. Among natural compounds, (poly)phenols and their derivatives/metabolites are emerging as candidates in AD prevention due to their multiple beneficial effects [2-4]. Interestingly, phenyl- γ -valerolactones (PVLs), products of intestinal microbial metabolism of flavan-3-ols, constitute a rich portion of phenolic metabolites in the circulation of subjects exposed to the widely spread dietary sources of this subclass of compounds [5]. Here, we investigated the ability of a selection of PVLs to modulate the functionality of cellular proteolytic pathways and to counteract the production and release of the toxic A β (1-42) peptide, at levels readily achievable in body fluids upon moderate consumption of flavonoid-rich foods or beverages. Neuronal SH-SY5Y cells transfected with either the wild-type or the 717 valine-to-glycine amyloid precursor protein mutated gene were used as an AD model and treated with 5-(4'-hydroxyphenyl)- γ -valerolactone, 5-(3',4'-dihydroxyphenyl)- γ -valerolactone and 5-(3'-hydroxyphenyl)- γ -valerolactone-4'-sulfate. Combining *in vitro* and *in silico* studies, we observed that the phenyl- γ -valerolactones of interest modulated cellular proteolysis via proteasome inhibition, through the action of their lactone group, and consequent autophagy upregulation as a compensatory protective mechanism. Furthermore, the metabolites inhibited cathepsin B activity, a lysosomal protease associated with the amyloidogenic processing of the amyloid precursor protein and the release of amyloid peptides [6], eventually reducing the amount of intra- and extracellular A β (1-42) peptides. Our findings establish, for the first time, that these metabolites exert a neuroprotective activity by regulating intracellular proteolysis and confirm the role of autophagy and cathepsin B as possible targets of AD therapeutic strategies.

References

- [1] Cecarini V, Bonfili L, Cuccioloni M, Mozzicafreddo M, et al. The fine-tuning of proteolytic pathways in Alzheimer's disease. *Cell Mol Life Sci*. 2016;73:3433-3451.
- [2] Bensalem J, Dudonne S, Gaudout D, Servant L, et al. Polyphenol-rich extract from grape and blueberry attenuates cognitive decline and improves neuronal function in aged mice. *J Nutr Sci*. 2018; 7:e19.

- [3] Bensalem J, Servant L, Alfos S, Gaudout D, et al. Dietary Polyphenol Supplementation Prevents Alterations of Spatial Navigation in Middle-Aged Mice. *Front Behav Neurosci.* 2016;10:9.
- [4] Philip P, Sagaspe P, Taillard J, Mandon C, et al. Acute Intake of a Grape and Blueberry Polyphenol-Rich Extract Ameliorates Cognitive Performance in Healthy Young Adults During a Sustained Cognitive Effort. *Antioxidants (Basel).* 2019;8:650.
- [5] Mena P, Bresciani L, Brindani N, Ludwig IA, et al. Phenyl-gamma-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity. *Nat Prod Rep.* 2019;36:714-752.
- [6] Hook V, Toneff T, Bogyo M, Greenbaum D, et al. Inhibition of cathepsin B reduces beta-amyloid production in regulated secretory vesicles of neuronal chromaffin cells: evidence for cathepsin B as a candidate beta-secretase of Alzheimer's disease. *Biol Chem.* 2005;386:931-940.

Inhibitory activity of Chlorogenic Acid and Coffee Silverskin extracts against *Klebsiella pneumoniae* carbapenemases



Sonia I Ciancia¹, Dezemona Petrelli², Giovanni Caprioli¹, Gianni Sagratini¹, Luca A Vitali¹

¹*School of Pharmacy, University of Camerino, Italy*

²*School of Biosciences and Veterinary Medicine, University of Camerino, Italy*

soniailaria.ciancia@studenti.unicam.it

Coffee processing produces numerous waste by-products (around two billion tonnes), including silverskin (SS). A possible reuse of these by-products is desirable, as they are not only an environmental but also an economic burden. Based on the observation that chlorogenic acid has anti-Extended-Spectrum- β -Lactamase activity [1] and on the presence of this compound in SS [2], the aim of this work was to evaluate the inhibitory activity of chlorogenic acid and SS extracts against *Klebsiella pneumoniae* type-two carbapenemase (KPC2). Using four extraction solvents, H₂O, MeOH, MeOH:H₂O (50:50) and EtOH:H₂O (70:30), four SS extracts were obtained. The inhibition activity of the carbapenemase-dependent resistance mechanism was measured against *Klebsiella pneumoniae* clinical strains KP-187 (KPC2-positive), KP-5 (KPC2-positive) and KP-ATCC13823 (carbapenem-susceptible control strain). *Escherichia coli* ATCC25922 was used as tester strain. In all experiments, imipenem was the indicator carbapenem antibiotic. To detect anti-carbapenemase activity, an adaptation of the Carbapenem Inactivation Method (CIM) [3] was developed and referred to as reverse Carbapenem Inactivation Method (rCIM). The protocol requires that produced carbapenemases are released from the cells after lysis, which was achieved by suspending 10 μ l of bacterial culture in 200 μ l of a Lysis Buffer. A disc preloaded with imipenem with or without putative inhibitors (i.e. chlorogenic acid or SS extracts) was added to the cell lysate. The highest concentrations of chlorogenic acid and extracts were 25 μ g/ml and 500 μ g/ml, respectively. After incubation at 37°C for 20 minutes, the disc was placed on an agar plate previously seeded with a 3 McFarland units suspension of the carbapenem-susceptible *E. coli* tester strain. Incubation at 37°C followed and results were recorded at two endpoints: 3 hours and 24 hours. The presence of a halo around the disc was an indication that the carbapenemase was not present or inhibited. To exclude that lack of carbapenemase activity could depend on inefficient bacterial cell lysis, experiments were carried out using a modified Lysis Buffer, wherein the concentration of the components with lytic activity was doubled. The SS extracts and chlorogenic acid showed no carbapenemase inhibitory activity irrespective to the Lysis conditions applied. Contrary to the observation against extended-spectrum beta lactamases (ESBL), pure chlorogenic acid did not show activity against KPC2 under the experimental conditions used. Also SS extracts did not inhibit the enzyme.

References

[1] Sahare P, et al. Molecular and Computational Analysis of β -Lactamases: Comprehensive Approach Towards Combating Drug Resistance by Chlorogenic Acid. *Int J Pharm Sci Rev Res.* 2016;37:104-109.

[2] Nzekoue FK, Angeloni S, Navarini L, Angeloni C, Freschi M, Hrelia S, Vitali LA, Sagratini G, Vittori S, Caprioli G. Coffee silverskin extracts: Quantification of 30 bioactive compounds by a new HPLC-MS/MS method and evaluation of their antioxidant and antibacterial activities. *Food Research International*. 2020;133:109128.

[3] van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The Carbapenem Inactivation Method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. *Plos ONE*. 2015;10:e0123690.

Benefici e uso degli Aminoacidi essenziali

Emanuele Giordano

emanuelegiordano057@gmail.com

Gli aminoacidi essenziali sono molecole totipotenti [1] che possono essere usati per scopo clinici [2], nello sport e attività fisica [3], e per aumentare il benessere e la salute. Gli aminoacidi essenziali sono molecole che stimolano al massimo i processi anabolici, contribuendo al massimo effetto di sintesi proteica, rivestendo un particolare ruolo positivo negli anziani sani [4]. Il muscolo scheletrico umano è un serbatoio di aminoacidi per i tessuti e organi vitali in assenza di assorbimento di aminoacidi dall'intestino e fornisce precursori gluconeogenici epatici. Inoltre, l'alterato metabolismo muscolare gioca un ruolo chiave nella genesi, e quindi nella prevenzione, di molte comuni condizioni patologiche e malattie croniche [5]. Evidenze recenti su modelli animali sembrano indicare un aumento della longevità e salute a seguito di particolari rapporti tra aminoacidi essenziali e non essenziali [6]. Miscele particolari di aminoacidi essenziali sono in grado di aumentare la biogenesi mitocondriale, ridurre i marker dell'inflammatione, avere effetti benefici sul peso corporeo e grasso corporeo, ridurre l'incidenza delle infezioni, migliorare la funzione cognitiva [7].

References

- [1] Pasini E, Corsetti G, et al. Protein-Amino Acid Metabolism Disarrangements: The Hidden Enemy of Chronic Age-Related Conditions, *Nutrients*. 2018;10(4):391.
- [2] Markofski MM, et al. Effect of Aerobic Exercise Training and Essential Amino Acid Supplementation for 24 Weeks on Physical Function, Body Composition, and Muscle Metabolism in Healthy, Independent Older Adults: A Randomized Clinical Trial, *J Gerontol A Biol Sci Med Sci*. 2019;74(10):1598-1604.
- [3] Tipton KD, Wolfe RR. Exercise, protein metabolism, and muscle growth, *Int J Sport Nutr Exerc Metab*. 2001;11(1):109-132.
- [4] Wolfe RR, Volpi E. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults, *Am J Clin Nutr*. 2003 Aug;78(2):250-258.
- [5] Wolfe RR. The underappreciated role of muscle in health and disease. *Am J Clin Nutr*. 2006;84(3):475-482.
- [6] Dioguardi FS, Corsetti G. Influence of Diets with Varying Essential/Nonessential Amino Acid Ratios on Mouse Lifespan, *Nutrients*. 2019;11(6):1367.
- [7] Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: an evolutionary perspective, *Aging (Albany NY)*. 2011;3(5):464-478.

Strategic change of gut microbiota composition ameliorates lipid metabolism in Alzheimer's disease



Chunmei Gong, Laura Bonfili, Massimiliano Cuccioloni, Valentina Cecarini, Mauro Angeletti, Anna Maria Eleuteri

School of Biosciences and Veterinary Medicine, University of Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italy

chunmei.gong@unicam.it

Alzheimer's disease (AD) and age-related dementias represent a major global health challenge. Unhealthy diet and lifestyle can unbalance the intestinal microbiota composition and, consequently, energy metabolism, contributing to AD pathogenesis. Impairment of cerebral cholesterol metabolism occurs in both aging and AD, and lipid-lowering agents have been associated to a lower risk of neurodegenerative diseases [1], but the link between blood lipid profile and AD is a matter of debate [2]. Previous studies demonstrated that oral supplementation with a multi-strain probiotic formulation (SLAB51) reduced amyloid beta aggregates and brain damages in a triple transgenic mouse model of AD (3xTg-AD) [3]. Treated mice showed improved cognitive functions in response to an enrichment of gut anti-inflammatory metabolites, increased plasma concentrations of neuroprotective gut hormones, and ameliorated glucose uptake and metabolism [3,4]. Considering the contradictory data on the role of deregulated lipid homeostasis in AD and in light of the promising effects of probiotics on energy metabolism, 3xTg-AD mice and their wild type counterpart were chronically treated with SLAB51 with the aim of deeply dissecting the role of microbiota modulation in ameliorating AD pathology by affecting lipid homeostasis. On this purpose, 8 week-old 3xTg-AD mice were orally administered with SLAB51 for 4 and 12 months to analyze the plasma lipid profile (using lipidomic analyses and enzymatic colorimetric assays), along with the cerebral and hepatic expression levels of key regulators of cholesterol metabolism (through Western blotting and ELISA). As expected, dyslipidemia was observed in older 3xTg-AD mice but not in wild type animals. Interestingly, upon probiotics administration, cholesterol biosynthesis was inhibited in AD mice with a process involving sterol regulatory element binding protein 1c and liver X receptors mediated pathways. Decreased plasma and brain concentration of 27-hydroxycholesterol and increased brain expression of cholesterol 24S-hydroxylase indicated that alternative pathways of bile acid synthesis are influenced. These data, together with the hypocholesterolemic effects and the ameliorated fatty acids profile successfully demonstrated that microbiota modulation through probiotics can positively change lipid composition in AD mice, with arachidonic acid representing one important hub metabolite in the interactions among probiotic-induced lipid profile changes, insulin sensitivity, and inflammation. Concluding, our data definitely demonstrate that gut microbiota modulation with probiotics successfully counteract AD progression influencing multiple pathways including lipid metabolism. The effective prevention of AD through the fine regulation of the associated risk factors will remain a constant goal of research and present findings should be inevitably considered in future preventative and therapeutic AD protocols.

References

- [1] McGuinness B, Passmore P: Can statins prevent or help treat Alzheimer's disease? *J Alzheimer's Dis* 2010;20:925-933.
- [2] Liu Y, Zhong X, Shen J, Jiao L, Tong J, Zhao W, Du K, Gong S, Liu M, Wei M. Elevated serum TC and LDL-C levels in Alzheimer's disease and mild cognitive impairment: A meta-analysis study. *Brain research*. 2020;1727:146554.
- [3] Bonfili L, Cecarini V, Berardi S, Scarpona S, Suchodolski JS, Nasuti C, Fiorini D, Boarelli MC, Rossi G, Eleuteri AM. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. *Sci Rep*. 2017;7(1):2426.
- [4] Bonfili L, Cecarini V, Gogoi O, Berardi S, Scarpona S, Angeletti M, Rossi G, Eleuteri AM. Gut microbiota manipulation through probiotics oral administration restores glucose homeostasis in a mouse model of Alzheimer's disease. *Neurobiol Aging*. 2020;87:35-43.

Assessment of cholesterol-lowering bioactive compounds in Italian-standard Espresso coffee



Franks Kamgang Nzekoue¹, Laura Alessandrone¹, Luciano Navarini², Giovanni Caprioli¹, Gianni Sagratini¹

¹*School of Pharmacy, University of Camerino, via Sant' Agostino 1, 62032 Camerino, Italy*

²*illycaffè S.p.A., Via Flavia 110, 34147 Trieste, Italy*

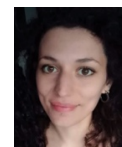
astride.kamgang@unicam.it

Espresso Coffee (EC) is one of the most popular coffee brews in the world, obtained from the percolation of hot water under pressure through compacted cakes of roasted ground coffee [1]. To our knowledge, among all the bioactive compounds investigated in coffee-based products, phytosterol (PS) content has never been assessed in coffee brews such as EC. This class of bioactive compounds is known for their LDL cholesterol-lowering properties, which contrast with the blood cholesterol increment effect of diterpenes, another class of phytochemicals present in coffee [2,3]. This study aims thus, to assess for the first time the content of PS in EC to improve the knowledge on the phytochemicals and health potentials of coffee brews. 14 EC samples produced with coffee cultivated in 13 different countries were studied. PS were extracted by hot saponification followed by derivatization for HPLC-DAD analysis. β -sitosterol (48.1-178 mg L⁻¹) was the most abundant followed by stigmasterol (11.4-46.3 mg L⁻¹) and campesterol (9.9-48.8 mg L⁻¹). Total PS fraction ranged from 69.8 mg L⁻¹ to 275.0 mg L⁻¹ with an average level of 145.4 ± 52.7 mg L⁻¹. Therefore, a standard cup of EC (25 mL) could provide 3.7 ± 1.4 mg of PS. This quantity is not enough for blood-cholesterol reduction but is a quantity that could inhibit the potential unhealthy physiological effects of diterpenes in coffee brews.

References

- [1] Illy A, Viani R, Suggi Liverani F. Espresso coffee: the science of quality, 2nd ed.; Elsevier: Amsterdam, Netherlands 2005.
- [2] Nzekoue FK, Alesi A, Vittori S, Sagratini G, Capriol, G. Development of a functional whey cheese (ricotta) enriched in phytosterols: Evaluation of the suitability of whey cheese matrix and processing for phytosterols supplementation. LWT, 2021;139,110479.
- [3] Cornelis MC, E-Sohemy A. Coffee, caffeine, and coronary heart disease. Curr Opin Lipidol 2007;18(1):13-19.

Short and medium chain free fatty acids in wine: extraction optimization and analysis



Lenti Lucia, Serena Scortichini, Dennis Fiorini

School of Science and Technology, Chemistry Division, University of Camerino, Via S. Agostino 1, Camerino, Italy

lucia.lenti@unicam.it

The composition of free fatty acids (FFAs) in food matrices is an important parameter in the definition of quality. Depending on the matrix under consideration, their presence and amount may give positive but also negative contributions. In cheese, for example, they are known to afford the characteristic aroma, but they are able to produce an undesired defect of cheese-like off-flavor in other products such as milk or cream [1,2]. When considering wine, acetic acid (AA) plays a very important role in this context. It is the main constituent of wine volatile acidity, and when present above certain levels it is associated with an uncontrolled proliferation of acetic acid bacteria. This results in the typical vinegar defect in the aroma and the consequent wine spoilage [3]. Short and medium chain free fatty acids (SCFFAs and MCFFAs) different from AA are also found in wine, being by-products of yeasts fermentation. Their amount is important since they can significantly impact the sensory quality when present above their olfactory thresholds [4]. Starting from these premises, the analysis and quantification of SCFFAs and MCFFAs in wine samples has a key importance in the definition of wine quality. The aim of the present study was to optimize and validate an analytical method to quantify FFAs in wines after their liquid-liquid extraction (LLE) by diethyl ether and a subsequent analysis by gas chromatography coupled with flame ionization detection. The extraction optimization has been performed by considering different conditions: starting from the acidification of wine samples with aqueous sulphuric acid solution, to the use of different salts and salts mixtures. Promising preliminary results have been obtained using the mixture $\text{NaH}_2\text{PO}_4/(\text{NH}_4)_2\text{SO}_4$, a salt combination resulted to improve the extraction of SCFFAs and MCFFAs also by headspace solid phase microextraction as reported in a previous study [5].

References

- [1] Pinho O, Ferreira I M P L V O, Ferreira M A. Solid-phase microextraction in combination with GC/MS for quantification of the major volatile free fatty acids in ewe cheese. *Anal Chem.* 2002;74:5199-5204.
- [2] Gonzales-Cordova A F, Vallejo-Cordova B. Quantitative determination of short-chain free fatty acids in milk using solid-phase microextraction and gas chromatography. *J Agric Food Chem.* 2001;49:4603-4608.
- [3] Bartowsky E J, Henschke P A. Acetic acid bacteria spoilage of bottled red wine- A review. *Int J. Food Microbiol.* 2008;125:60-70.
- [4] Olivero S J P, Tryjillo J P P. A new method for the determination of short chain fatty acids from the aliphatic series in wine by headspace solid phase microextraction-gas chromatography-ion trap mass spectrometry. *Anal Chim Acta.* 2011;696:59-66.

[5] Fiorini D, Pacetti D, Gabbianelli R, Gabrielli S, Ballini R. A salting out system for improving the efficiency of the headspace solid-phase microextraction of short and medium chain free fatty acids. *J Chromatogr A*. 2015;1409:282-287.

Cannabidiol extracted from *Cannabis sativa* induces cell cycle arrest, mitophagy and cell differentiation in chronic myeloid leukemia cells

Federica Maggi^{1,2}, Maria Beatrice Morelli², Massimo Nabissi², Oliviero Marinelli², Daniele Tomassoni³, Cristina Aguzzi², Laura Zeppa², Giorgio Santoni² and Consuelo Amantini³

¹*Department of Molecular Medicine, Sapienza University, 00185 Rome, Italy*

²*School of Pharmacy, University of Camerino, 62032 Camerino, Italy*

³*School of Biosciences and Veterinary Medicine, University of Camerino, 62032 Camerino, Italy*

federica.maggi31@gmail.com

Cannabidiol (CBD) is one of the phytochemicals extracted from *Cannabis sativa* lacking of psychoactive effects whose use as herbal medicine dates back to 500 BC. It displays several effects such as anti-inflammatory, antioxidant, analgesic, anxiolytic, anticonvulsive and neuroprotective [1]. Recently, different companies started to make products, containing hemp-derived CBD, for body care purposes, as oil for salad dressings, or to be taken directly as a dietary supplement [2]. In addition, it is now well accepted that CBD can be also considered a promising new anti-cancer strategy, alone or in combinations with conventional chemotherapies. In fact, several findings demonstrated that CBD is able to induce cell death, inhibit cell migration in vitro, decrease tumor size and vascularization in glioma models [1,3,4]. Moreover, similar interesting results have been obtained in breast [5], lung [6], colon rectal [7] and prostate [8] cancers. CBD acts as an agonist for the Transient Receptor Potential Vanilloid (TRPV) channels 1 and 2 while it has no affinity for the endocannabinoid CB1 and 2 receptors [9]. Although, successful results were obtained in Chronic Myeloid Leukemia (CML) with TKI inhibitors, several patients show TKI resistance. Thus, this study investigated the effects induced by CBD treatment via activating TRPV2 in CML cell lines. The effects of CBD treatment, via TRPV2, were assessed by cell viability, BrdU proliferation and cell cycle assays. The stimulation of mitophagy and the expression of differentiation markers was evaluated by western blot, FACS analysis and confocal microscopy. The involvement of TRPV2 was studied by gene silencing. CBD treatment inhibited cell proliferation and cell cycle in CML cells. It also promoted mitochondria dysfunction and mitophagy via TRPV2 activation. These effects are associated with changes in OCT-4 and PU.1 differentiation markers. Finally, a synergistic effect by combining CBD with Imatinib was found. CBD, by promoting inhibition of cell proliferation, mitophagy and the reduction in stemness, could be a new strategy to enhance conventional therapy and improve the prognosis of CML patients developing TKIs resistance. Overall, these results supported previous findings about the idea that hemp-derived products could be used as supplements able to ameliorate the healthy status.

References

- [1] Seltzer ES, Watters AK, MacKenzie D, Granat LM, Zhang D. Cannabidiol (CBD) as a Promising Anti-Cancer Drug. *Cancers (Basel)*. 2020;12:3203, doi:10.3390/cancers12113203.
- [2] Rupasinghe HPV, Davis A, Kumar SK, Murray B, Zheljzakov VD. Industrial Hemp (*Cannabis sativa* subsp. *sativa*) as an Emerging Source for Value-Added Functional Food Ingredients and Nutraceuticals. *Molecules* 2020;25.doi:10.3390/molecules25184078.

- [3] Solinas M, Massi P, Cinquina V, Valenti M, Bolognini D, Gariboldi M, Monti E, Rubino T, Parolaro D. Cannabidiol, a Non-Psychoactive Cannabinoid Compound, Inhibits Proliferation and Invasion in U87-MG and T98G Glioma Cells through a Multitarget Effect. *PLoS One*. 2013;8:e76918, doi:10.1371/journal.pone.0076918.
- [4] Deng L, Ng L, Ozawa T, Stella N. Quantitative Analyses of Synergistic Responses between Cannabidiol and DNA-Damaging Agents on the Proliferation and Viability of Glioblastoma and Neural Progenitor Cells in Culture. *J Pharmacol Exp Ther*. 2017;360:215-224, doi:10.1124/jpet.116.236968.
- [5] Sultan AS, Marie MA, Sheweita SA. Novel mechanism of cannabidiol-induced apoptosis in breast cancer cell lines. *The Breast*. 2018;41:34-41, doi:10.1016/j.breast.2018.06.009.
- [6] Ramer R, Bublitz K, Freimuth N, Merkord J, Rohde H, Hausteim M, Borchert P, Schmuhl E, Linnebacher M, Hinz B. Cannabidiol inhibits lung cancer cell invasion and metastasis via intercellular adhesion molecule-1. *FASEB J*. 2012;26:1535-1548, doi:10.1096/fj.11-198184.
- [7] Jeong S, Kim BG, Kim DY, Kim BR, Kim JL, Park SH, Na YJ, Jo MJ, Yun HK, Jeong YA, et al. Cannabidiol Overcomes Oxaliplatin Resistance by Enhancing NOS3- and SOD2-Induced Autophagy in Human Colorectal Cancer Cells. *Cancers (Basel)*. 2019;11:781. doi:10.3390/cancers11060781.
- [8] De Petrocellis L, Ligresti A, Schiano Moriello A, Iappelli M, Verde R, Stott CG, Cristino L, Orlando P, Di Marzo V. Non-THC cannabinoids inhibit prostate carcinoma growth in vitro and in vivo: pro-apoptotic effects and underlying mechanisms. *Br J Pharmacol* 2013;168:79-102. doi:10.1111/j.1476-5381.2012.02027.x.
- [9] Stasiłowicz A, Tomala A, Podolak I, Cielecka-Piontek J. Cannabis sativa L. as a Natural Drug Meeting the Criteria of a Multitarget Approach to Treatment. *Int J Mol Sci*. 2021;22:778. doi:10.3390/ijms22020778.

Green extraction of hemp (*Cannabis sativa* L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds, and cannabinoids): a central composite design optimization study



Filippo Maggi¹, Marco Cespi¹, **Jacopo Torresi¹**, Riccardo Petrelli¹, Eugenia Mazzara¹, Riccardo Carletti¹, Dennis Fiorini², Serena Scortichini², Stefano Dall'Acqua³, Stefania Sut³, Giovanni Caprioli¹, Simone Angeloni¹, Ahmed Mustafa¹, Jure Zekič⁴

¹*School of Pharmacy, University of Camerino, 62032 Camerino, Italy*

²*School of Science and Technology, University of Camerino, 62032 Camerino, Italy*

³*Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 Padova, Italy*

⁴*Laboratory of Food Chemistry, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia*

jacopo.torresi@unicam.it

Industrial hemp (*Cannabis sativa* L.) is an herbaceous plant with a characteristic scent that has been used for over 5000 years for its various properties. In particular, hemp essential oil can be used in pharmaceutical, cosmetic, and nutraceutical fields. In this work, hemp Futura 75 was processed with an innovative and green extraction method, namely Microwave-Assisted Extraction (MAE) [1], using a Design of Experiment (DoE) approach [2]. After the essential oil distillation, two valuable by-products, namely the residual biomass and water enriched in hydrophilic compounds, were recovered and analyzed. The design variables analyzed were the microwave irradiation power (W/g), the extraction time (min), and the amount of water added to hemp samples (%). This research aimed to evaluate how these experimental parameters can affect the EO yield and composition together with the yield and antioxidant properties of the aqueous residue. Total phenolic and flavonoid content (TPC, TFC) and antioxidant activity (DPPH) were calculated through spectrophotometric UV-Vis experiments [3-4]; whereas phenolics and cannabinoids in the residual water and biomass, respectively, were determined by HPLC-MS analysis. GC-MS analysis was performed to study the chemical composition of essential oils obtained by MAE. At the same time, GC-FID was used to quantify the essential oil constituents and the CBD content in the residual biomass. Regarding the essential oil analysis, the responses yield and content of some compounds were well described by the mathematical models applied. In addition, in the case of the aqueous residues, the yield, polyphenols, and flavonoids content and antioxidant activity, as the four responses analyzed, suitably fit the models. On the other hand, there were no relationships between the CBD content in the residual biomass and the MAE experimental conditions, probably due to the samples' intrinsic variability. A multiple optimizing procedures based on desirability approach was employed to identify the most suitable experimental parameters able to maximize all the satisfactory responses simultaneously. In particular, high microwave power and long extraction times are needed to enhance the yield and chemical profile of essential oil, along with a medium-high amount of water, required to improve the aqueous extract yield and antioxidant capacity. HPLC-MS analysis put in evidence the abundance of flavone glycosides and CBD in the residual water and biomass, respectively. Our work showed that

hemp biomass is a multipurpose matter able to provide different fractions employable in several fields such as nutraceuticals, pharmaceuticals, cosmetics, and pest science.

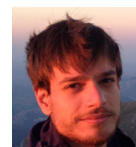
Acknowledgment

This research was supported by the University of Camerino as part of the project BIOCANAPA. Cofounded by PSR Marche 2014-2020, Misura 16.1, Azione 2. *C. sativa* var Futura 75 samples were kindly provided by La Biologica – Società Cooperativa Agricola.

References

- [1] Lucchesi ME, Chemat F, et Al.. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *J Chromatogr A*. 2004;1043:323-327.
- [2] Fiorini D, Scortichini S, et Al. Cannabidiol-enriched hemp essential oil obtained by an optimized microwave-assisted extraction using a central composite design. *Ind Crops Prod*. 2020;154:112688.
- [3] Mustafa AM, Maggi F, et Al. Chemical and biological analysis of the by-product obtained by processing *Gentiana lutea* L. and other herbs during production of bitter liqueurs. *Ind Crops Prod*. 2016;80:131-140.
- [4] Chen GL, Chen SG, et Al. Antioxidant capacities and total phenolic contents of 30 flowerers. *Ind Crops Prod*. 2018;111:430-445.

Anti-inflammatory properties of hemp extracts in human bronchial cells



Oliviero Marinelli¹, Cristina Aguzzi¹, Laura Zeppa¹, Massimo Nabissi¹

¹*School of pharmacy, University of Camerino, Italy*

oliviero.marinelli@unicam.it

Inflammatory lung diseases represent one of the most widespread affections associated with morbidity, mortality and health care expenditure in Europe [1]. Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disorder associated to a chronic inflammatory response. Main features are a progressive and irreversible airflow limitation, consequence of inflammatory cells infiltration and alteration in cilia structure and functionality [2-3]. The prescribed therapy consists of inhalation of bronchodilators and glucocorticoids [4], but it is historically adopted the use of traditional herbal remedies for treatment of respiratory diseases [5]. Previously, we evaluated thyme extract, obtained from *Thymus vulgaris* L., widely used as a spice, as potential remedy for airways affections. Briefly, we studied the effects of thyme extract in an *in vitro* model of lung inflammation induced by lipopolysaccharide (LPS) in primary human airway (bronchial/tracheal) epithelial cell lines (HBEpC/HTEpC) [6]. Indeed, exposure to LPS increases several pro-inflammatory mediators expression, such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF- α), involved in pathogenesis of COPD [7], and mucus production in airway epithelial cells. Results showed that thyme extract was non-cytotoxic for HBEpC/HTEpC cells and was effective in reducing inflammatory markers as NF-kB, IL-1 β and IL-8 expression in LPS-treated cells [6]. Subsequently, to further characterize the beneficial use of thyme extract in COPD treatment, it was evaluated its effects in regulating Cilia beating frequency (CBF), that is dramatically impaired in COPD, using a 3D *in vitro* model of Human Airway Epithelia, using three different bronchial primary cells derived from COPD single donors. Results show that thyme extract is involved in stimulating CBF, through an increase of $[Ca^{2+}]_i$ in a TRPV4 channel-dependent manner [8]. Hemp (*Cannabis sativa* L.) represents a sustainable crop multipurpose with a low environmental impact which can be useful in the agricultural field, phytoremediation and for nutrition, cosmetic, building, and pharmaceutical application. In this regard, the hemp essential oil (EO), which is a mixture of volatile compounds with a lower concentration of the cannabinoid fraction, is gaining interest by pharma companies, for its potential anti-inflammatory properties [9-10]. Despite plant EOs have shown several biological properties, it has been scarcely investigated. On this basis, the aim of this study was to investigate if the EOs obtained from inflorescences of hemp may be useful for airways affection management. For the purpose, we selected two different hemp varieties, namely, USO31 and Carmagnola CS and human normal bronchial cells as model of airway inflammation *in vitro*. Preliminary results obtained in human bronchial cells show that hemp essential oils displayed a moderate cytotoxic effect in a dose-dependent manner, with an IC_{50} of $34.2 \pm 1.1 \mu\text{g mL}^{-1}$ for Carmagnola CS essential oil and $40.62 \pm 1.8 \mu\text{g mL}^{-1}$ for USO31 essential oil. Then, to evaluate the anti-inflammatory potential of these EOs, we treated bronchial cells with Etoposide that several studies have indicated to trigger cytokines production in human cell lines *in vitro* [11]. Hemp EOs, used at non-cytotoxic doses, did

not cause an inflammatory state but, additionally, were able to revert an inflammatory condition, reducing the release of the cytokines induced by Etoposide. In conclusion, these preliminary results evidenced a potential use of EOs for bronchial inflammatory diseases.

References

- [1] Weigand LA, Udem BJ. Allergen-induced neuromodulation in the respiratory tract. *Chem Immunol Allergy*. 2012;98:142-162.
- [2] Cukic V, Lovre V, Dragisic D, Ustamujic A. Asthma and Chronic Obstructive Pulmonary Disease (COPD) - Differences and Similarities. *Mater Sociomed*. 2012;24(2):100-5.
- [3] Yaghi A, Dolovich MB. Airway Epithelial Cell Cilia and Obstructive Lung Disease. *Cells*. 2016;5(4):40.
- [4] Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *N Engl J Med*. 2005;353(16):1711-1723.
- [5] WHO Regional Office for the Western Pacific. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine. 1993. Manila, Republic of the Philippines.
- [6] Oliviero M, Romilde I, Beatrice MM, Matteo V, Giovanna N, Consuelo A, Claudio C, Giorgio S, Filippo M, Massimo N. Evaluations of thyme extract effects in human normal bronchial and tracheal epithelial cell lines and in human lung cancer cell line. *Chem Biol Interact*. 2016;256:125-133.
- [7] Cheng DS, Han W, Chen SM, Sherrill TP, Chont M, Park GY, Sheller JR, Polosukhin VV, Christman JW, Yull FE, Blackwell TS. Airway epithelium controls lung inflammation and injury through the NF-kappa B pathway. *J Immunol*. 2007;178(10):6504-6513.
- [8] Nabissi M, Marinelli O, Morelli MB, Nicotra G, Iannarelli R, Amantini C, Santoni G, Maggi F. Thyme extract increases mucociliary-beating frequency in primary cell lines from chronic obstructive pulmonary disease patients. *Biomed Pharmacother*. 2018;105:1248-1253.
- [9] Farinon B, Molinari R, Costantini L, Merendino N. The seed of industrial hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. *Nutrients*. 2020;12(7):1935.
- [10] Fiorini D, Molle, A, Nabissi M, Santini G, Benelli G, Maggi F. Valorizing industrial hemp (*Cannabis sativa*L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation. *Ind. Crops Prod*. 2019;128:581–589.
- [11] Kawagishi C, Kurosaka K, Watanabe N, Kobayashi Y. Cytokine production by macrophages in association with phagocytosis of etoposide-treated P388 cells in vitro and in vivo. *Biochim Biophys Acta*. 2001;1541(3):221-230.

Capsaicin as new adjuvant in anti-cancer immunotherapy



Maria Beatrice Morelli¹, Consuelo Amantini², Matteo Santoni³, Oliviero Marinelli¹, Cristina Aguzzi¹, Laura Zeppa¹, Massimo Nabissi¹, Giorgio Santoni¹

¹*School of pharmacy, University of Camerino, Italy*

²*School of biosciences and biotechnologies, University of Camerino, Italy*

³*Medical Oncology Unit, Hospital of Macerata, Italy*

mariabeatrice.morelli@unicam.it

Red chili (*Capsicum frutescens*) is widely used as a spice for flavoring foods worldwide. Accumulating evidence has shown multiple pharmacological effects of Capsicum on a variety of physiological systems [1,2]. Pungent capsaicinoids (capsaicin, dihydrocapsaicin), antioxidant vitamins (ascorbic acid, vitamin E), carotenoids (β -carotene, β -cryptoxanthine) and several organic acids and minerals are the major active chemical substance found in *Capsicum frutescens* [2]. Capsaicin (CPS) (8-methyl-N-vanillyl-6-nonenamide) is an irritant for mammals, including humans. The burning and painful sensations associated with CPS result from its chemical interaction with sensory neurons. CPS, is a derivative of vanillyl amide (8-methyl-N-vanillyl-6-nonenamide). It binds to a receptor called the vanilloid receptor subtype 1 (VR1 or TRPV1) belonging to the Transient Receptor Potential Vanilloid subfamily [3]. CPS has been used medicinally for centuries because it can reduce cholesterol, blood lipid content, blood sugar content and it also has properties of anti-oxidative, anti-inflammatory, anti-obesity and analgesic [4]. At present, it has been demonstrated that CPS exerts anti-cancer activity and immunomodulatory functions [5]. Indeed, the engagement of TRPV1 on dendritic cells by CPS modulates their function by upregulating antigen-presenting and costimulatory molecules, in addition to initiating their migration to draining lymph nodes [6]. Moreover, CPS induces damage-associated molecular patterns of immunogenic cell death in human bladder cancer cells [7]. Bladder cancer has been characterized as a tumor group in which the immunological response is relatively well preserved [8]. A number of immune checkpoint inhibitors have been approved as first-line therapy in case of cisplatin-ineligible patients or as second-line therapy for patients with metastatic urothelial carcinoma of the bladder. However, only 30% of patients with metastatic urothelial carcinoma will respond to this type of immunotherapy [9]. Among the immune checkpoints, programmed death-ligand 1 (PD-L1) seems to predict response to immune checkpoint inhibitors in such patients. PD-L1 is the transmembrane protein ligand of PD-1, expressed on the cell membrane in T and B lymphocytes, antigen presenting cells and can be induced in tumor cells within the tumor microenvironment. The PD-1/PD-L1 pathway negatively regulates T cell activation, thus it plays an important role in controlling anti-tumor immunity response [10]. Recently, several clinical trials targeting PD-1/PD-L1 pathways using anti-PD-1 or anti PD-L1 antibodies demonstrated the clinical benefit for the patients with bladder cancer and one of these antibodies, atezolizumab, was approved by Food and Drug Administration in USA [11]. Therefore, compounds able to affect the expression of PD-L1 deserve further study. Herein, we evaluated the ability of CPS, at not cytotoxic dose, to influence PD-L1 expression, both at mRNA and protein levels, in 5637 and

T24 bladder cancer cell lines. We found that the exposure of both cell lines to CPS at 50 μ M for 12 h and 24 h can increase the expression of PD-L1 evaluated by RT-PCR, western blot and immunohistochemistry analyses. Preliminary results showed that the mechanism involved in CPS-mediated upregulation of PD-L1 expression is ROS-independent and TRPV1-mediated. Moreover, the involvement of interferon γ 1/ γ 1, through the activation of the NME/NM23 nucleoside diphosphate kinase 4 (NME4)/STATs signaling pathway has been evaluated. Further studies *in vivo* and *in vitro* are definitely required to completely address the CPS immunomodulatory capability in bladder cancer and to elucidate the related anti-tumor effects.

References

- [1] Srinivasan K. Biological activities of red pepper (*Capsicum annuum*) and its pungent principle capsaicin: a review. *Crit Rev Food Sci Nutr*. 2016;56:1488-1500.
- [2] Antonious GF. Capsaicinoids and Vitamins in Hot Pepper and Their Role in Disease Therapy. In book: *Capsaicin and its Human Therapeutic Development*. 2018.
- [3] Yang F, Zheng J. Understand spiciness: mechanism of TRPV1 channel activation by capsaicin. *Protein Cell*. 2017;8:169-177.
- [4] Chapa-Oliver AM, Mejia-Teniente L. Capsaicin: from plants to a cancer-suppressing agent. *Molecules*. 2016;21(8):931.
- [5] Zhang S, Wang D, Huang J, Hu Y, Xu Y. Application of capsaicin as a potential new therapeutic drug in human cancers. *J Clin Pharm Ther*. 2020;45:16-28.
- [6] Basu, S, Srivastava, P. Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. *Proc Natl Acad Sci. USA*. 2005;102:5120-5125.
- [7] D'Eliseo D, Manzi L, Velotti F. Capsaicin as an inducer of damage-associated molecular patterns (DAMPs) of immunogenic cell death (ICD) in human bladder cancer cells. *Cell Stress Chaperones*. 2013;18:801-808.
- [8] Joseph M, Enting D. Immune Responses in Bladder Cancer-Role of Immune Cell Populations, Prognostic Factors and Therapeutic Implications. *Front Oncol*. 2019;9:1270.
- [9] Lopez-Beltran A, Cimadamore A, Blanca A, Massari F, Vau N, Scarpelli M, Cheng L, Montironi R. Immune Checkpoint Inhibitors for the Treatment of Bladder Cancer. *Cancers*. 2021;13:131.
- [10] Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol Immunother*. 2007;56:1173-1182.
- [11] Black PC, Tangen C, Singh P, McConkey DJ, Lucia S, Lowrance WT, Koshkin VS, Stratton KL, Bivalacqua T, Sharon E, Kassouf W, Porten SP, Bangs RC, Plets M, Lerner SP, Thompson IM. Phase II trial of atezolizumab in BCG-unresponsive non-muscle invasive bladder cancer: SWOG S1605 (NCT #02844816). *J Clin Oncol* 2021 - Abstract

A new HPLC-MS/MS method for the simultaneous determination of 36 polyphenols in blueberry, strawberry and their commercial products and determination of antioxidant activity



Ahmed M Mustafa^{1,2}, Simone Angeloni^{1,3}, Doaa Abouelenein^{1,2}, Laura Acquaticci¹, Jianbo Xiao⁴, Gianni Sagratini¹, Filippo Maggi¹, Sauro Vittori¹, Giovanni Caprioli¹

¹*School of Pharmacy, University of Camerino, via Sant' Agostino 1, 62032 Camerino, Italy*

²*Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt*

³*RICH – Research and Innovation Coffee Hub, Via E. Betti 1, 62020 Belforte del Chienti, (MC), Italy*

⁴*Faculty of Food Science and Technology, University of Vigo, Spain*

ahmed.mustafa@unicam.it

Berry fruits consumption has increased in recent years because they are rich sources of polyphenols with reported health benefits [1,2]. Only few reports described the quantitative determination of few phenolic compounds by HPLC-MS/MS, and they only examined anthocyanins or phenolic acids [3,4]. The aim of the present work was to develop a new comprehensive and fast HPLC-MS/MS method for simultaneous determination of 36 phenolic compounds (7 anthocyanins, 9 flavonols, 4 flavan-3-ols, 2 dihydrochalcones, 2 flavanones and 12 phenolic acids) present in blueberry, strawberry, and their commercial products; and to evaluate their antioxidant activities. To our knowledge, this is the first time that HPLC-MS/MS was used for simultaneous quantification of all these compounds of interest taken together in strawberry and blueberry fruits. Blueberry fruits showed higher contents of anthocyanins, flavonols and phenolic acids, while strawberry fruits exhibited higher contents of flavan-3-ols, dihydrochalcones and flavanones. Anthocyanins were the main phenolic constituents in both berries. Furthermore, the higher total phenolic content in the blueberry fruit and jam justified their greater antioxidant capacity measured by DPPH free radical assay [5], compared to strawberry. In conclusion, this new HPLC-MS/MS method is useful and reliable for quality control and authentication analyses of blueberry and strawberry fruits and their commercial food products, such as jams.

References

- [1] Mikulic-Petkovsek M, Slatnar A, Stampar F, Veberic, R. HPLC–MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species. *Food Chem.* 2012;135(4):2138-2146.
- [2] Miller K, Feucht W, Schmid M. Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. *Nutrients.* 2019;11(7):1510.
- [3] Kang J, Thakali KM, Jensen GS, Wu X. Phenolic acids of the two major blueberry species in the US Market and their antioxidant and anti-inflammatory activities. *Plant foods hum nutr.* 2015;70(1):56-62.

- [4] Stein-Chisholm RE, Beaulieu JC, Grimm CC, Lloyd SW. LC–MS/MS and UPLC–UV evaluation of anthocyanins and anthocyanidins during rabbiteye blueberry juice processing. *Beverages*. 2017;3(4):56.
- [5] Mustafa AM, Maggi F, Ozturk N, Ozturk Y, Sagratini G, Torregiani E, Vittori S, Caprioli G. Chemical and biological analysis of the by-product obtained by processing *Gentiana lutea* L. and other herbs during production of bitter liqueurs. *Ind Crops Prod*. 2016;80:131-140.

Sea fennel (*Crithmum maritimum* L.): a culinary herb with nutraceutical potential. Extraction, purification and chemical characterization of polar extracts



Diletta Piatti¹, Simone Angeloni¹, Giovanni Caprioli¹, Filippo Maggi¹, Massimo Ricciutelli¹, Lolita Arnoldi², Gianni Sagratini¹

¹School of Pharmacy, University of Camerino, Via S. Agostino 1, 62032 Camerino, Italy;

²Research and Development Department, Indena SpA, viale Ortles 12, 20139 Milan, Italy

diletta.piatti@unicam.it

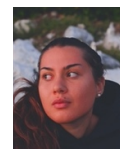
Crithmum maritimum L., also known as sea fennel, rock samphire and locally as ‘paccasassi’, is a perennial halophyte, thrives on maritime rocks and sometimes in sandy beaches along the Mediterranean, Pacific and Atlantic coasts. Its potential for commercial cultivation has not yet been fully exploited though it is considered a promising biosaline crop [1]. Sea fennel has been used since ancient times in folk medicine as antiscorbutic, tonic, carminative, diuretic, depurative and vermifuge. Today it is believed as an emerging edible vegetable crop, famous for its culinary and not culinary properties [2]. *C. maritimum* has lots of food uses due to its aromatic traits, for instance to make pickles and to flavour and season soups, sauces and salads. The plant is reported in the *Belfrit* list, including plants that could be used for food supplements due to sufficient scientific evidence. The use of the plant in the diet is also confirmed by its presence in the “List of Traditional Agri-Food Product” by the Italian Ministry of Agriculture as a traditional agri-food product of Puglia [3]. According to the medicinal use, sea fennel is also considered a source of human health compounds, namely fatty acids such as linoleic (C18:2 ω6) and linolenic (C18:3 ω3) acids [4], and phenolics compounds with well documented antioxidant effects [5]. Due to the growing interest in this vegetable as a source of nutraceutical compounds [6], different studies have been performed to understand the total chemical profile of the vegetable. This work aims to provide a complete quantitative and qualitatively overview of the extract obtained from the aerial parts of *C. maritimum*. For this purpose: aerial parts were dried, ground and extracted by percolation with ethanol 70% at 60°C. The purification was done using an Amberlite® XAD7HP sorbent resin. For the quantification, HPLC-DAD-MS/MS system, equipped with electrospray ionization (ESI) and a Zorbax ODS (250 x 4.6 mm, 5 μm) column, was used. The extract was mainly composed of phenolic compounds, among which hydroxycinnamic acids and flavonoids were the two main chemical classes. Among the former, chlorogenic acids, including 5-O-caffeoylquinic acid (32.04-166.21 mg g⁻¹), 3,5-di-O-caffeoylquinic acid (8.44-38.86 mg g⁻¹) and 4,5-di-O-caffeoylquinic acid (5.70-25.85 mg g⁻¹), were the most abundant ones. Among flavonoids, rutin (1.60-4.33 mg g⁻¹) and kaempferol-3-O-rhamnoside (0.07-0.33 mg g⁻¹) were the main constituents. These results support the use of this plant as a functional food or in nutraceuticals due to the abundance of antioxidant compounds.

References

[1] Zenobi S, Fiorentini M, Zitti S, Aquilanti L, Foligni R, Mannozi C, Mozzon M, Orsini R. *Crithmum maritimum* L.: First Results on Phenological Development and Biomass Production in Mediterranean Areas. *Agronomy*. 2021.

- [2] Renna M. Reviewing the Prospects of Sea Fennel (*Crithmum maritimum* L.) as Emerging Vegetable Crop. *Plants* (Basel). 2018;7(4):92.
- [3] Renna M, Gonnella M, Caretto S. et al. Sea fennel (*Crithmum maritimum* L.): from underutilized crop to new dried product for food use. *Genet. Resour. Crop Evol.* 2017;64:205-216.
- [4] Gnocchi D, Del Coco L, Girelli C.R. et al. ¹H-NMR metabolomics reveals a multitarget action of *Crithmum maritimum* ethyl acetate extract in inhibiting hepatocellular carcinoma cell growth. *Sci Rep.* 2021;11:1259.
- [5] Meot-Duros L, Magné C. Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiol Biochem.* 2009;47(1):37-41.
- [6] Pereira AG, Fraga-Corral M, García-Oliveira P, Jimenez-Lopez C, Lourenço-Lopes C, Carpena M, Otero P, Gullón P, Prieto MA, Simal-Gandara J. Culinary and nutritional value of edible wild plants from the northern Spain rich in phenolic compounds with potential health benefits. *Food & Function.* 2020;11(10):8493-8515.

Quality of wholemeal pasta made with pigmented and ancient wheats



Francesca Pompei¹, Matteo Bonfini², Xinying Suo¹, Ahmed M. Mustafa^{3,4}, Gianni Sagratini³, Elena Vittadini¹

¹*School Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy*

²*CIAS Innovation, Matelica (MC), Italy*

³*School of Pharmacy, University of Camerino, Camerino (MC), Italy*

⁴*Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt*

francesca.pompei@studenti.unicam.it

Pasta, one of the most popular products in the world, has been subjected in recent years to an important revitalization of its ingredients to respond to the market request for “new”, healthier and more sustainable products. Ancient wheat varieties have played an important role in this innovation as they are expected to have higher nutritional quality, to increase biodiversity, and to be more sustainable than a traditional wheat variety [1]. Pigmented wheats have also gained attention due to their antioxidant properties [2]. The objective of this study was to investigate consumer’s acceptability and the chemical and physical quality of wholemeal pasta made with two different wheat varieties: an ancient wheat, called Senatore Cappelli (SC), and a pigmented wheat, named Granomischio (GM). SC and GM wholemeal pastas (“paccheri” shaped) were acquired by the same producer (Pastificio Marella Srl, Gioia del Colle, BA) to ensure uniformity of production and were characterized for consumers’ acceptability with a 60 consumers’ panel. The panelists tested the products in a domestic setting (home use test) and responded to an on-line questionnaire with the aim to evaluate their perception of pasta attributes (colour, flavour, taste, texture and overall acceptability) and their products’ preference. Pastas were also characterized for cooking quality at optimal cooking time (solid loss, water uptake, dimensional changes, hardness, adhesiveness [3]), as well as for antioxidant activity (AOA) and total polyphenolic content (TPC) [4]. SC and GM paccheri were very well received by consumers who judged the products very positively with an average of 6.7 (SC) and 6,9 (GM) points on a 9 scale (8-9: 53%SC and 60%GM), and willingness to buy the product in the future of 65% (SC) and 61% (GM). Colour was one the major drivers of liking for both types of pasta (90% liking in GM and 92% in SC). GM drivers of satisfaction were appearance (95%), texture (85%), with an overall satisfaction of 75% of the total number of consumers. SC was very well perceived for appearance, taste and smell, while texture was less liked (55% found product too hard), but this did not hinder overall product appreciation (70%). Finally, comparing the two samples more than the 63 % of the tested population GM over SC. During cooking SC was found to lose less solids ($5.0\pm 0,3\%$ vs $5.9\pm 0,1\%$ g solids/100g water) in the cooking water as compared to GM. Cooked SC pasta was found to have higher moisture content ($48.9\pm 0.4\%$ vs $51.8\pm 0.47\%$ g water /100 g product), to be slightly harder ($35.3 \pm 2.5N$ vs $31.4\pm 2.2N$) and more adhesive ($2.3\pm 1.0 \cdot 10^{-3}$ J vs $2.7 \pm 1.4 \cdot 10^{-3}$ J) than GM pasta. Cooked SC had also lower TPC (28.6 ± 1.3 vs 59.3 ± 1.0 mg gallic acid equivalents/100 g of dry weight) and AOA (10.6 ± 8.1 vs 79.7 ± 1.0 mg trolox equivalent/100 g of dry weight) than GM. This work showed that wholemeal pasta produced with ancient and pigmented

wheat varieties was very well perceived by consumers and had a good cooking quality. Pigmented wheat conferred to product interesting levels of polyphenols and antioxidant activity higher than the unpigmented variety, that may have also an impact on consumers wellbeing.

References

- [1] Boukid F, Folloni S, Sforza S, Vittadini E, Prandi B. Current trends in ancient grains-based foodstuffs: Insights into nutritional aspects and technological applications. *Comp Rev Food Sci Food Saf.* 2018;17(1):123-136.
- [2] Zanoletti M, Abbasi Parizad P, Lavelli V, Cecchini C, Menesatti P, Marti A, Pagani MA. Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production, *LWT - Food Sci Tech.* 2017;75:663-669.
- [3] Morreale F, Boukid F, Carini E, Federici E, Vittadini E, Pellegrini N. An overview of the Italian market for 2015: cooking quality and nutritional value of gluten-free pasta. *Int J Food Sci Tech.* 2018;54(3):780-787.
- [4] Mustafa AM, Maggi F, Ozturk N, Ozturk Y, Sagratini G, Torregiani E, Vittori S, Caprioli G. Chemical and biological analysis of the by-product obtained by processing *Gentiana lutea* L. and other herbs during production of bitter liqueurs. *Industrial Crops and Products.* 2016;80,131:140.

Characterization of milk proteins based nano-carriers: interactions with polyphenolic molecules of nutraceutical interest



Sofia Renzi¹, Diego R Perinelli², Alessandro Alesi³, Valeria Polzonetti¹, Paolo Polidori², Silvia Vincenzetti¹, Stefania Pucciarelli¹

¹*School of Biosciences and Veterinary Medicine, University of Camerino, Italy*

²*School of Pharmacy, University of Camerino, Italy*

³*Sabelli S.p.A., Zona Ind.le Basso Marino, Ascoli Piceno, Italy*

sofia.renzi@unicam.it

Milk proteins are able to bind or encapsulate hydrophobic and amphipathic substances behaving as nano-carrier systems for bioactive substances with nutraceutical properties. Among the milk proteins, β -lactoglobulin (β -LG) is an excellent candidate for nanoparticle formation, acting as transport vehicle for bioactive compounds such as vitamins, antioxidants, fatty acids, which when bound or encapsulated increase their stability and bioavailability [1]. Resveratrol, as well as many plant derived polyphenolic compounds, although poorly soluble in water, is transported by human serum albumin to the tissues, where it can exert its bioactivity (antioxidant, anti-inflammatory, and anticancer activity). Designing safe and cheap systems to encapsulate resveratrol in a functional food can be a suitable strategy to increase its bioavailability. In the 3D structure of β -lactoglobulin it is possible to identify the main site for specific hydrophobic ligand binding in the β -barrel delimited internal cavity, where, buried within the calyx, the Trp19 is positioned [2]. Exploiting Trp intrinsic fluorescence we have monitored the ability of cow's milk β -lactoglobulin to form complexes with resveratrol and other polyphenolic compounds, in order to elucidate the binding and self-assembly properties of this whey protein as a carrier system of bioactive molecules. At pH 6.4 the binding affinity for the formation of the complex between β -LG and resveratrol has been determined ($K_d = 4.52 \pm 1.16 \mu M$) and resulted to be higher with respect to other tested bioactive molecules like trigonellin (no measurable binding), caffeine ($K_d = 43.78 \pm 3.99 \mu M$), and chlorogenic acid ($K_d = 10.41 \pm 3.8 \mu M$). Binding of resveratrol by β -LG resulted to be protective with respect to *trans-cis* isomerization process to which resveratrol is subjected (mainly under UV-Vis light), which can have a detrimental impact on its biological activity [3]. Nano-carriers based on milk proteins can represent suitable and safe systems providing food with micronutrients of great potential on health.

References

- [1] Shafaei Z, Ghalandari B, Vaseghi A, Divsalar A, Haertlé T, Saboury AA, Sawyer L. β -Lactoglobulin: An efficient nanocarrier for advanced delivery systems. *Nanomedicine: NBM* 2017;13:1685-1692.
- [2] Liang L, Subirade M. Study of the acid and thermal stability of β -lactoglobulin–ligand complexes using fluorescence quenching. *Food Chemistry* 2012;132:2023-2029.
- [3] Pantusa M, Bartucci R, Rizzuti B. Stability of trans-Resveratrol Associated with Transport Proteins *J Agric Food Chem.* 2014;62:4384-4391.

PROTECTIVE EFFECTS OF R (+)-THIOCTIC ACID TREATMENT ON THE HEART OF SPONTANEOUSLY HYPERTENSIVE RATS



Proshanta Roy¹, Seyed Khosrow Tayebati², Ilenia Martinelli², Francesco Amenta², Daniele Tomassoni¹

¹*School of Biosciences and Veterinary Medicine, University of Camerino, 62032 Camerino, Italy*

²*School of Pharmacy; University of Camerino, 62032 Camerino, Italy*

proshanta.roy@unicam.it

Hypertension represents a multifactorial disease characterized by elevated blood pressure. It is a risk factor for cardiovascular diseases [1,2]. Increasing evidence attributed the main role of oxidative stress for what concerns hypertension and cardiovascular damage mechanisms because it promotes endothelial dysfunction, vascular remodeling, and inflammation, leading to vascular damage [3]. Excess bioavailability of reactive oxygen species (ROS) often accompanied hypertension-induced structural abnormalities on mitochondria of the cardiomyocyte [4]. Even if the data of clinical trials are discordant, for the management of hypertension, the supplementation of natural antioxidants could also be a promising therapeutic tool with the administration of antihypertensive drugs [5]. Thiocctic acid (TIO) is an antioxidant existing in nature and expressed in two optical isomers. R(+)-TIO is the naturally occurring eutomer, whereas the most used formulation of the compound in clinical practice is the mixture of (+) and (-)-enantiomers. Previously, we demonstrated that the TIO treatment reduced oxidative stress and prevented adhesion molecule expression in the cardiac vascular endothelium of spontaneously hypertensive rats (SHR) [6]. The present study was designed to investigate the effect of treatment with R (+)-TIO on the heart of SHR, using immunochemical and immunohistochemical techniques. R (+)-TIO (125 μ mol/Kg/day) was administered intraperitoneally for 30 days in 24-weeks-old SHR. Hypertensive rats were compared to age-matched normotensive Wistar Kyoto (WKY) rats. After 4 weeks of treatment with R (+)-TIO, blood pressure values were significantly decreased in SHR. This is possibly related to the effects at the levels of the endothelial vessels that determine vasodilation. The results confirmed that the oxidative mechanisms of organ alterations due to hypertension were characterized by left ventricular cardiomyocytes hypertrophy, fibrosis, proteins oxidation accompanied by inflammation with an increased expression of IL-1 beta, IL-6, and tumor necrosis factor-alpha. R (+)-TIO reduced the development of oxidative stress, cardiomyocyte hypertrophy, and inflammatory processes. Taken all together, R (+)-TIO may be considered as one of the antioxidant candidate molecules to prevent cardiac alterations associated with hypertension. The effects observed after treatment with R (+)-TIO could open new perspectives for countering heart injury, which represents a common feature in hypertensive end-organs damage.

References

- [1] Sliwa K, Stewart S, Gersh BJ. Hypertension: a global perspective. *Circulation*. 2011;123:2892-2896.
- [2] Messerli FH, Rimoldi SF, Bangalore S. The Transition From Hypertension to Heart Failure: Contemporary Update. *JACC Heart Fail*. 2017;5:543-551.

- [3] Eirin A, Lerman A, Lerman LO. Mitochondrial Injury and Dysfunction in Hypertension-Induced Cardiac Damage. *Eur Heart J*. 2014;35:3258-3266.
- [4] Rubattu S, Pagliaro B, Pierelli G, Santolamazza C, Di Castro S, Mennuni S, Volpe M. Pathogenesis of Target Organ Damage in Hypertension: Role of Mitochondrial Oxidative Stress. *Int J Mol Sci*. 2014;16:823-839.
- [5] Baradaran A, Nasri H, Rafieian-Kopaei, M. Oxidative Stress and Hypertension: Possibility of Hypertension Therapy with Antioxidants. *J Res Med Sci*. 2014;19:358-367.
- [6] Tayebati SK, Tomassoni D, Di Cesare Mannelli L, Amenta, F. Effect of Treatment with the Antioxidant Alpha-Lipoic (thioctic) Acid on Heart and Kidney Microvasculature in Spontaneously Hypertensive Rats. *Clin Exp Hypertens*. 2016;38:30-38.

***In vitro* assessment of prebiotic effect of coffee and its by-products on human intestinal microbiota**



Chiara Salvesi^{1,2}, Serena Scortichini^{1,3}, Giovanni Caprioli⁴, Dennis Fiorini³, Stefania Silvi²

¹*School of Advanced Studies, University of Camerino, Camerino, Italy*

²*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

³*School of Science and Technology, University of Camerino, Camerino, Italy*

⁴*School of Pharmacy, University of Camerino, Camerino, Italy*

chiara.salvesi@unicam.it

The human intestinal microbiota has long been known to influence human health and disease, but recently, its improvement by modulation of gut microbiota is an upcoming evolving strategy [1]. The microbiota present in the human body provides numerous benefits, including enhancement of overall well being and also protection against pathogenic microorganisms. Gut microbiota exerts its protective role through the production of different signaling molecules which can modify the functionality of other organs. When the gut bacterial composition is altered (dysbiosis), there is a strong association with the pathogenesis of many inflammatory diseases and infections. Prebiotics supplementation could be a good strategy to improve intestinal microbiota of the target host, conferring health benefits. Currently, other substances beside carbohydrates, i.e. polyphenols, might fit the updated definition of prebiotics presenting evidence of beneficial effects in the host [2]. Coffee and its by-products are very interesting substrates in this sense, because they contain plenty of polyphenols and other essential compounds like melanoidins and chlorogenic acid, which make them potential prebiotic food components. The aim of the present study was to characterize and assess the potential prebiotic properties of seven coffee by-products, studying *in vitro* the modifications on human gut microbiota and its metabolic products. The coffee by-products, all from *Coffea arabica*, were selected and studied to evaluate compounds and bioactive substances, responsible for their prebiotic activities on the intestinal microbiota. The selection of coffee by-products was made of: Green Coffee Bean (coarsely ground), Silver Skin (generated from the roasting process of the bean), Spent Coffee Ground (generated from the coffee preparation), two extracts from Silver Skin and from Spent Coffee, finally the Roasted Coffee and an Espresso freshly made. A fermentation system (batch culture) in anaerobic conditions was used to mimic the human intestine. During all fermentation cycles, the biodiversity of gut microbiota was analysed and selected bacterial groups were enumerated using Real-Time PCR analysis. At the same time points the detection and quantification of short chain fatty acids (SCFA) was also performed. Inulin was chosen as well-defined prebiotic substrate, to compare the results obtained from all the considered coffee substrates. The general trend is an increase in the bacterial counts of beneficial bacterial groups (*Lactobacillus* spp. and *Bifidobacterium* spp.), and a decrease for *Clostridium coccooides-Eubacterium rectale* group. These data are supported also by the SCFA that resulted to accumulate during fermentation especially acetic and propionic acids. The valorisation as prebiotics of these by-products highlights that their recovery from the coffee industry is of great importance, not only because of their significant properties, but also because it could exploit a part of industry wastes.

References

- [1] Bibbò S, Ianiro G, Giorgio V, Scaldaferrì F, Massucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. *European Review for Medical and Pharmacological Sciences*. 2016;20:4742-4749.
- [2] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews, Gastroenterology & Hepatology*. 2017;14:491-502.

Water- and fat-soluble vitamins in milk after different thermal treatments and steam injection



Giuseppe Santini, Valeria Polzonetti¹, Yulia Klimanova¹, Stefania Pucciarelli¹, Paolo Polidori², Lauro Fioretti³, Silvia Vincenzetti¹

¹*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy*

²*School of Pharmacy, University of Camerino, Camerino (MC), Italy*

³*Simonelli Group, Belforte del Chienti (MC), Italy*

giuseppe.santini@unicam.it

To prepare a good cappuccino, it is preferable to use fresh whole milk as its high-fat content contributes to the flavour and its protein content is useful for a good whipping of the milk contributing to its creaminess. The foam is produced by the nozzle of the coffee machine, which is necessary to obtain a compact cream, but it is also important that the temperature of the milk must not exceed 65-70°C, to avoid an unpleasant aroma and the lack of foam formation. Furthermore, the nutritional quality of the milk should remain unchanged after the steam injection treatment. In the present work, we focused the attention on the water- and fat-soluble vitamins content in pasteurized bovine milk samples (HQ-milk) treated at different temperatures (40-80°C) and steam injected pressure using a nozzle of Simonelli Group coffee machine. The treatment time was 20, 30, 40, 50, and 60 seconds. Water- and fat-soluble vitamins content on the treated HQ-milk has been determined by RP-HPLC using a C18 column (HiQSil C18 HS, 5 µm, 4.6 mm i.d, 250 mm, Kya Tech Corporation), connected to an HPLC Agilent 1260 Infinity LC System (Agilent Technologies), according to two different protocols from Albalá-Hurtado and co-workers [1, 2]. Quantification was achieved by a calibration curve obtained relating the concentrations (mM) of each vitamin standard loaded in the column to the peak area corresponding to each concentration. The results showed that if the only temperature is applied, vitamin B₂, nicotinic acid, and nicotinamide were not affected by the increasing temperature because the exposure time from 20 to 60 seconds is too short to cause a decrement in the vitamins content. However, when the HQ-milk is treated with increasing temperature together with steam injection, an interesting result regarding the nicotinic acid concentration was obtained. In fact, the concentration of nicotinic acid increases by about 6.5 folds starting from 7.5 mM at 25°C to reach a mean value of 45 mM at a temperature range of 50-70°C. It is also interesting to note that the increase of nicotinic acid concentration is time-dependent: if the HQ-milk is treated for 20 seconds with the steam injection (time normally used to heat the milk during the preparation of the cappuccino), nicotinic acid reaches its highest concentration (54 mM) at 70°C, but if the milk is treated for 60 seconds, the highest concentration (43 mM) is reached at 50°C. Nicotinic acid (Niacin) is used to prevent and treat niacin deficiency that may result from alcohol abuse, malabsorption syndrome, Hartnup disease, poor diet. Furthermore, nicotinic acid is used to treat dyslipidemias since reduces triglycerides and LDL cholesterol and raises HDL cholesterol levels [3]. In our experiments, we observed that the increase in nicotinic acid dependent on the steam injected and the treatment time is accompanied by a simultaneous decrease in quinolinic acid. The latter is generated within the kynurenine pathway resulting from L-tryptophan catabolism and may be present in the milk since it

is related to the composition of the cows' diet [4]. Quinolinic acid may be converted to nicotinic acid by a decarboxylation reaction induced by the temperature and the steam injected pressure [5]. However, this hypothesis needs to be further confirmed. On the contrary, nicotinamide and B₆ decrease when temperature and steam injection increased, whereas vitamin B₂ resulted not affected by the treatment. Among fat-soluble vitamins, the results showed that vitamin E and vitamin D₃ did not undergo variations after temperature and steam injection treatment while vitamin A was slightly affected. In conclusion, these results indicate that the nutritional quality of HQ-milk, from the point of view of the concentration of vitamins, seems not influenced by the thermal treatment and by the injected steam. It was observed, after this treatment, an increase in milk nicotinic acid content, very interesting considering the beneficial effects on human health exerted by this vitamin.

References

- [1] Albalá-Hurtado S, Veciana-Nogués M.T, Izquierdo-Pulido M, Mariné-Font A. Determination of water-soluble vitamins in infant milk by high performance liquid chromatography. *J Chromatogr A*. 1997;778:247-253.
- [2] Albalá-Hurtado S, Rodriguez SN, Veciana-Nogués T, Mariné-Font A. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography. *J Chromatogr A*. 1997;778:243-246.
- [3] Bogan KL, Brenner C. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD⁺ precursor vitamins in human nutrition. *Annu Rev Nutr*. 2008;28:115-130.
- [4] Rouge P, Cornu A, Biesse-Martin AS, Lyan B, Rochut N, Graulet B. Identification of quinoline, carboline and glycinamide compounds in cow milk using HRMS and NMR. *Food Chem*. 2013;141:1888-1894.
- [5] Fu J, Savage PE, LuInd X. Hydrothermal Decarboxylation of Pentafluorobenzoic Acid and Quinolinic Acid. *Eng Chem Res*. 2009;48:10467-10471.

The effect of chickpea flour addition levels and flour structures on quality and in vitro starch digestibility of corn-rice-based gluten free pasta



Xinying Suo^{1,2}, Margherita Dall'Asta³, Gianluca Giuberti³, Michele Minucciani⁴, Elena Vittadini^{1*}

¹*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy*

²*School of Food and Bioengineering, Collaborative Innovation Centre of Food Production and Safety, Zhengzhou University of Light Industry, Zhengzhou, China*

³*Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy*

⁴*Massimo Zero srl, Merano (BZ), Italy*

xinying.suo@unicam.it

Gluten-free (GF) pasta in the Italian market is produced using corn and rice starch as main ingredients [1], resulting in a product rich in available carbohydrates with high postprandial glycemic response [4], low in protein and fiber, and with poor cooking quality [2-3]. Inclusion of chickpea flour into GF pasta formulation may positively affect product's nutritional profile by increasing protein and fiber, and reducing the glycemic impact while increasing environmental sustainability [5-10]. Chickpea flour pre-treatment (e.g. pre-gelatinization, milling degree) may affect both cell integrity and degree of starch gelatinization that may have a profound impact of both product's quality and nutrients accessibility [11-14]. The present work aims to produce corn-rice-chickpea GF pasta to investigate the effect of different chickpea addition levels and chickpea flour structural attributes on product's cooking quality and in vitro starch digestibility. Pasta was produced with heat-treated white corn and rice flours at a mass ratio of 3:2 in all formulations, while conventional chickpea flour was included at level of 0%, 25%, 50%, 75% and 100% to investigate the effect of chickpea addition levels, and the effect of chickpea flours structural attributes (pre-gelatinization, milling degree [fine and coarse]) was tested at 50% inclusion. Nutritional facts, cooking quality, texture quality, and starch digestibility of products were investigated. GF pasta produced with increasing chickpea flour additions showed a high increase in protein and fiber content, a shorter optimal cooking time and reduced solid loss during cooking as compared to the control corn-rice. Color of chickpea pasta shifted towards brown/dark, while texture was found to be softer and less adhesive. More importantly, chickpea addition significantly reduced the content of total starch, available starch, rapidly and slowly digestible starch but increased content of resistant starch which suggests a reduced glycemic load and glycemic index in vivo. However, no significant differences were found in respect to chickpea flour structural attributes. Current results demonstrate that chickpea flour is able to impart corn-rice-based GF pasta with desired quality and attenuated starch digestibility. Flour structural attributes had no significant influence on GF pasta quality and in vitro starch digestibility.

References

[1] Morreale F, Boukid F, Carini E, Federici E, Vittadini E, Pellegrini N. An overview of the Italian market for 2015: cooking quality and nutritional value of gluten-free pasta. *International Journal of Food Science and Technology*. 2018;54(3):780-786.

- [2] Calvo-Lerma J, Crespo-Escobar P, Martínez-Barona S, Fornés-Ferrer V, Donat E, Ribes-Koninckx C. Differences in the macronutrient and dietary fibre profile of gluten-free products as compared to their gluten-containing counterparts. *European Journal of Clinical Nutrition*. 2019;73(6):930-936.
- [3] Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values. *American Journal of Clinical Nutrition*. 2002;76:5-56.
- [4] Trevisan S, Pasini G, Simonato B. An overview of expected glycaemic response of one ingredient commercial gluten free pasta. *Lwt*. 2019;109:13-16.
- [5] Gan Y, Liang C, Hamel C, Cutforth H, Wang H. Strategies for reducing the carbon footprint of field crops for semiarid areas. A review. *Agronomy for Sustainable Development*. 2011;31(4): 643-656.
- [6] Güngörmüşler M, Başınhan İ, Üçtuğ FG. Optimum formulation determination and carbon footprint analysis of a novel gluten-free pasta recipe using buckwheat, teff, and chickpea flours. *J Food Process Preserv*. 2020;44:e14701.
- [7] Kaur R, Prasad K. Technological, processing and nutritional aspects of chickpea (*Cicer arietinum*) - A review. *Trends in Food Science and Technology*. 2021;109:448-463.
- [8] Khrisanapant P, Kebede B, Leong SY, Oey I. A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. *Foods*. 2019;8(12):6-9.
- [9] Attia RS, Aman ME, El-Tabey Shehata AM, Hamza MA. Effect of ripening stage and technological treatments on the lipid composition, lipase and lipoxygenase activities of chickpea (*Cicer arietinum* L.). *Food Chemistry*. 1996;56(2):123-129.
- [10] Saget S, Costa M, Barilli E, Wilton de Vasconcelos M, Santos CS, Styles D, Williams M. Substituting wheat with chickpea flour in pasta production delivers more nutrition at a lower environmental cost. *Sustainable Production and Consumption*. 2020;24:26-38.
- [11] Lai HM. Effects of hydrothermal treatment on the physicochemical properties of pregelatinized rice flour. *Food Chemistry*. 2001;72(4):455-463.
- [12] BeMiller JN, Huber KC. Physical Modification of Food Starch Functionalities. *Annual Review of Food Science and Technology*. 2015;6(1):19-69.
- [13] Marti A, Seetharaman K, Pagani MA. Rice-based pasta: A comparison between conventional pasta-making and extrusion-cooking. *Journal of Cereal Science*. 2010;52(3):404-409.
- [14] Boukid F, Vittadini E, Lusuardi F, Tommaso G, Carini C, Morreale F, Pellegrini N. Does cell wall integrity in legumes flours modulate physiochemical quality and in vitro starch hydrolysis of gluten-free bread? *Journal of Functional Foods*. 2019;59:110-118.
- [15] Padalino L, Mastromatteo M, Lecce L, Spinelli S, Conte A, Alessandro Del Nobile M. Optimization and characterization of gluten-free spaghetti enriched with chickpea flour. *International Journal of Food Sciences and Nutrition*. 2014;66(2):148-158.

Managing of the daily calcium dietary intake as a tool to contribute to a healthy lifestyle



Giorgia Vici, Dalia Camilletti, Benedetta Gibertoni, Bruno Adesso, Luca Belli, Valeria Polzonetti

School of Biosciences and Veterinary Medicine – University of Camerino, Italy

giorgia.vici@unicam.it

Calcium is an essential element for human health for growth, maintenance and repair of bone tissue, muscle contraction, nerve conduction and blood clotting [1,2]. The Reference Levels of Nutrients and Energy Intake for the Italian Population defines the recommended daily requirements depending on specific needs related to factors as age, gender, pregnancy and menopause [3]. In athletes, calcium intake is also important to decrease the risk of low bone-mineral density and stress fractures [2]. However, studies conducted on the Italian population reported intake ranges often lower than those recommended [4,5]. Also in athletes, inadequate intakes have been reported both in male and female [6,7]. In this regard a questionnaire was proposed to 503 subjects, aged 12-85, to evaluate their calcium intake. It was observed that 81% of female and 74% of male do not reach the recommended values. The highest percentages were obtained in female aged 15-17 (89%) and in male and female older than 60 years (83%). In physically active subjects (63% of the studied population), the 76% shows inadequate calcium intakes. This percentage results close to the one of sedentary that do not reach recommended values (80%). Calcium intake deficiency is common in all age stages both in sedentary and in physically active people. In this context, right foods choice represents a fundamental aspect. Milk and dairy products are the most relevant source of dietary calcium in terms of both quantity and bioavailability [8]. By evaluating daily calcium intake in a group of non-elite athletes using 500mL of skimmed milk as post-exercise recovery drink, it was possible to observe that calcium intakes were in line with recommended values. Moreover, an improvement in body composition and, in particular, on hydration status was highlighted. This underlined milk's role as recovery drink but also as a coadjutant to improve calcium intake. Thus, these findings support the necessity of design specific dietary interventions that should start at early ages and continue through adulthood. Strategies should be developed to sensitize on calcium importance and to improve its daily intake. Right food choices should be considered taking into account naturally calcium-rich foods and novel calcium-enriched products.

References

- [1] Muscariello R, Rendina D, Giannettino R, Ippolito S, Romano O, Coretti, De Vita S, Martino M, Sepe C, Nuzzo V, et al. Calcium daily intake and the efficacy of a training intervention on optimizing calcium supplementation therapy: A clinical audit. *Nutr Metab Cardiovasc Dis.* 2021;31:354-360.
- [2] Thomas DT, Erdman KA, Burke LM. American college of sports medicine joint position statement. Nutrition and athletic performance. *Med Sci Sports Exerc.* 2016;48:543-568.
- [3] Società Italiana di Nutrizione Umana (SINU). LARN—Livelli di Assunzione di Riferimento di Nutrienti ed Energia Per la Popolazione Italiana; IV Revisione; SICS: Milano, Italy, 2014.

- [4] Vannucci L, Masi L, Gronchi G, Fossi C, Carossino AM, Brandi ML. Calcium intake, bone mineral density, and fragility fractures: evidence from an Italian outpatient population. *Arch Osteoporos*. 2017;12:40.
- [5] Sette S, Le Donne C, Piccinelli R, Arcella D, Turrini A, Leclerc C. The third Italian national food consumption survey, INRAN-SCAI 2005–06–part 1: nutrient intakes in Italy. *Nutr Metab Cardiovasc Dis*. 2011;21:922-932.
- [6] Matt SA, Barrack MT, Gray VB, Cotter JA, Van Loan MD, Rauh MJ, McGowan R, Nichols JF. Adolescent Endurance Runners Exhibit Suboptimal Energy Availability and Intakes of Key Nutrients. *J Am Coll Nutr*. 2021;25:1-8.
- [7] Gogojewicz A, Śliwicka E, Durkalec-Michalski K. Assessment of Dietary Intake and Nutritional Status in CrossFit-Trained Individuals: A Descriptive Study. *Int J Environ Res Public Health*. 2020;17(13):4772
- [8] Angelino D, Rosi A, Vici G, Dello Russo M, Pellegrini N, Martini D, and SINU Young Working Group. Nutritional quality of plant-based drinks sold in Italy: the Food Labelling of Italian Products (FLIP) study. *Foods*. 2020;9(5):682.

Vitamins in human, cow, and donkey milk: a comparison of nutritional properties



Silvia Vincenzetti¹, Giuseppe Santini¹, Yulia Klimanova¹, JingJing Zhang¹, Natalina Cammertoni¹, Valeria Polzonetti¹, Stefania Pucciarelli¹, Paolo Polidori²

¹*School of Biosciences and Veterinary Medicine, University of Camerino, Italy*

²*School of Pharmacy, University of Camerino, Italy*

silvia.vincenzetti@unicam.it

Donkey milk (DM) has been used since ancient times in the feeding of infants who, for various reasons, could not be breastfed. Furthermore, the health benefits of DM have been tested in several clinical trials for the treatment of debilitated and convalescent subjects [1]. Today we know that DM is a valuable source of nutraceutical substances beneficial to human health, including bioactive peptides, essential fatty acids, and vitamins [2]. The present work focuses precisely on this last aspect, the identification and determination of the water- and fat-soluble vitamins in DM and their comparison with the vitamins content in human milk and cow's milk. Donkey and cow's milk water-soluble vitamins were determined by a chromatographic method [3], using an HPLC Agilent 1260 Infinity LC System (Agilent Technologies), connected to a C18 HiQSil HS column (5 μm , 4.6 mm i.d, 250 mm, Kya Tech Corporation, Tokyo, Japan). The obtained results in cows' and donkey milk were compared to the vitamins content of human milk taken from the published literature. DM is a good source of nicotinic acid ($18.75 \pm 1.92 \mu\text{M}$), this value is higher compared to human milk ($4.64 \mu\text{M}$, [4]) but lower than the one found in cow's milk in the present work (27.52 ± 4.91). Nicotinic acid (niacin) is a member of the B3 vitamin group that reduces the release of fatty acid from the adipocytes, thus showing a lipid-lowering effect, this molecule is used to treat the progression of atherosclerotic cardiovascular disease. Folic acid (vitamin B9) is present in DM with a concentration ($0.83 \pm 0.08 \mu\text{M}$) higher than human milk ($0.365 \mu\text{M}$) and about half of that found in cow's milk in our determinations ($1.93 \pm 0.53 \mu\text{M}$). Milk represents an excellent source of folic acid that is involved in nucleic acid synthesis, accelerates cell division, and for this reason, very important for children's growth. Pyridoxine (vitamin B6) content is $5.38 \pm 3.35 \mu\text{M}$, this value is very similar to that found in bovine milk both from our determinations ($2.5 \pm 0.61 \mu\text{M}$) and in the literature ($1.0-11.23 \mu\text{M}$; [5]). Vitamin B6 is involved in several metabolic processes such as lipid and amino acid metabolism, gluconeogenesis, the regulation of the immune system, and the development of the nervous system. Donkey milk also contains thiamine ($0.66 \pm 0.26 \mu\text{M}$), riboflavin ($0.168 \pm 0.04 \mu\text{M}$), with levels very close to those found in the literature for bovine milk [5] but higher compared to human milk [4]. Very interesting is the content of vitamin C, a vitamin with well-known antioxidant properties which in donkey milk has a value of 0.32 mM , similar to the value found in human milk (0.28 mM) but much higher than the quantity found in cow's milk ($28.4 - 170.3 \mu\text{M}$). Among fat-soluble vitamins, it is known by the literature that the vitamin D2 and D3 content in donkey milk ($0.042 \mu\text{M}$ and $0.016 \mu\text{M}$, respectively) is higher than the values found for this vitamin in human and bovine milk ($0.008 \mu\text{M}$) [6]. Furthermore, it has been shown that vitamin D levels in donkey milk are related to seasonality since they show the highest value in summer due to the greater sun exposure of the animals [6].

Donkey milk vitamin E amount is about 3.4 μM [6], higher than the value found in the literature [5] in bovine milk (1.4 μM , respectively), but lower concerning the amount found in the human milk (8.0 ± 3.8 μM). Vitamin E is a potent antioxidant that neutralizes free radicals, protecting tissues and organs from oxidative damage. These results show that DM, besides the presence of the well-known nutraceutical compounds, is also a valuable source of vitamins, indicating that this milk can represent a functional food for infants but also for elderly consumers.

References

- [1] Tafaro A, Magrone T, Jirillo F, Martemucci G, D'Alessandro A, Amati L. Immunological Properties of Donkeys Milk: Its Potential Use in the Prevention of Atherosclerosis. *Curr. Pharm. Des.* 2007;13:3711-3717.
- [2] Vincenzetti S, Pucciarelli S, Polzonetti V, Polidori P. Role of Proteins and of Some Bioactive Peptides on the Nutritional Quality of Donkey Milk and Their Impact on Human Health. *Beverages.* 2017;3:34.
- [3] Albalá-Hurtado S, Veciana-Nogués T, Izquierdo-Pulido M, Mariné-Font A. Determination of water-soluble vitamins in infant milk by high performance liquid chromatography. *J Chromatogr A.* 1997;778:247-253.
- [4] Ren X, Yang Z, Shao B, Yin S-A, Yang X B. Vitamin Levels in Human Milk among Different Lactation Stages and Areas in China. *PLoS ONE.* 2015;10:e0133285.
- [5] Haug A, Høstmark AT, Harstad OM. Bovine milk in human nutrition-A review. *Lipids Health Dis.* 2007;6:25.
- [6] Martini M, Altomonte I, Licitra R, Salari F. Short communication: Technological and seasonal variations of vitamin D and other nutritional components in donkey milk. *J Dairy Sci.* 2018;101:8721-8725.

Evening Primrose Oil effects in human Pancreatic Ductal Adenocarcinoma cell lines



Laura Zeppa¹, Cristina Aguzzi¹, Oliviero Marinelli¹, Massimo Nabissi¹

¹*School of Pharmacy, University of Camerino, 62032 Camerino (MC), Italy*

laura.zeppa@unicam.it

Evening Primrose (*Oenothera* L.) is a medicinal plant and the most numerous species in the *Oenothera* L. family is the *Oenothera biennis*. Evening Primrose Oil (EPO), obtained from its seeds, is rich in triacylglycerols and the main ones are linoleic acid (LA) (70–74%) and γ -linolenic acid (GLA) (8–10%), polyunsaturated fatty acids (PUFAs), belonging to omega-6 acids group [1]. The presence of GLA makes EPO a valid dietary supplement to reduce triacylglycerols and low-density lipoprotein (LDL) in plasma and an interesting compound for the treatment of rheumatic and arthritic conditions and for atopic dermatitis [1,2]. LA and GLA are precursors of dihomo-linolenic acid (DGLA), that is metabolized in series 1 prostaglandins and is oxidized in 15-hydroxyeicosatrienoic acid (15-HETrE) with anti-inflammatory and anti-proliferative effect, but at the same time, they can be also converted in arachidonic acid (AA), inducing pro-inflammatory series 2 prostaglandins and series 4 leukotrienes [1]. EPO is also composed by unsaponifiable compounds and among these, the sterols have important role in the reduction of pro-inflammatory mediators [2]. Moreover, several studies demonstrated GLA antitumoral activities in different cancer cell types and its ability to enhance chemotherapeutic drugs activity [3]. Pancreatic Ductal Adenocarcinoma (PDAC) is an infiltrating neoplasm with glandular differentiation derived from the pancreatic ductal tree. Among the solid cancers, it is considered one of the most aggressive, with five years survival of 5-7% [4-6]. It was demonstrated a family genetic predisposition, but also some precursor lesions within pancreatic tissue and somatic mutations of *KRAS* oncogene and *CDKN2A*, *TP53* and *SMAD4* suppressor genes are implicated in PDAC pathogenesis [4,7,8]. In the last years scientific interest in using molecules of natural origins as adjuvant in cancer therapy is grown [9]. Several phytochemicals demonstrated anticancer properties, thanks to their effects in suppressing cancer cell survival and proliferation, in inducing cancer cell death and for their synergistic/adjuvant interactions with different molecular pathways targets of chemotherapeutical drugs. [10]. Particularly, a study evidenced a reduction of pancreatic cancer cells growth, migration and invasion by treatment with DGLA [11]. So, since LA and GLA are the most important triacylglycerols present in EPO, we decided to analyze the effects of EPO in two human pancreatic ductal adenocarcinoma cell lines (PANC-1 and MIAPaCa-2). Data showed a reduction of cancer cell vitality after administration of EPO and an induction of cancer cell death as confirmed by Western Blot analysis. Then, the effects of EPO were also evaluated on its ability to modulate the main pathways involved in PDAC aggressiveness. In conclusion, these preliminary data suggest a potential role of EPO in PDAC aggressiveness.

References

[1] Timoszuk M, Bielawska K, Skrzydlewska E. Evening Primrose (*Oenothera biennis*) Biological Activity Dependent on Chemical Composition. *Antioxidants* (Basel). 2018;7(8):108.

- [2] Montserrat-de la Paz S, Fernández-Arche A, Angel-Martín M, García-Giménez MD. The sterols isolated from Evening Primrose oil modulate the release of proinflammatory mediators. *Phytomedicine*. 2012;19(12):1072-6.
- [3] Kong X, Ge H, Chen L, Liu Z, Yin Z, Li P, Li M. Gamma-linolenic acid modulates the response of multidrug-resistant K562 leukemic cells to anticancer drugs. *Toxicol In Vitro*. 2009;23(4):634-9.
- [4] Luongo M, Marinelli O, Zeppa L, Aguzzi C, Morelli MB, Amantini C, Frassinetti A, di Costanzo M, Fanelli A, Santoni G, Nabissi M. Cannabidiol and Oxygen-Ozone Combination Induce Cytotoxicity in Human Pancreatic Ductal Adenocarcinoma Cell Lines. *Cancers (Basel)*. 2020;12(10):2774.
- [5] Luchini C, Capelli P, Scarpa A. Pancreatic Ductal Adenocarcinoma and Its Variants. *Surg Pathol Clin*. 2016;9(4):547-560.
- [6] Adamska A, Domenichini A, Falasca M. Pancreatic Ductal Adenocarcinoma: Current and Evolving Therapies. *Int J Mol Sci*. 2017;18(7):1338.
- [7] Ansari D, Tingstedt B, Andersson B, Holmquist F, Stureson C, Williamsson C, Sasor A, Borg D, Bauden M, Andersson R. Pancreatic cancer: yesterday, today and tomorrow. *Future Oncol*. 2016;12(16):1929-46.
- [8] McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol*. 2018;24(43):4846-4861.
- [9] Brunetti A, Marinelli O, Morelli MB, Iannarelli R, Amantini C, Russotti D, Santoni G, Maggi F, Nabissi M. Isofuranodiene synergizes with temozolomide in inducing glioma cells death. *Phytomedicine*. 2019;52:51-59.
- [10] Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. *Front Pharmacol*. 2020;10:1614.
- [11] Yang X, Xu Y, Gao D, Yang L, Qian SY. Dihomo- γ -linolenic acid inhibits growth of xenograft tumors in mice bearing human pancreatic cancer cells (BxPC-3) transfected with delta-5-desaturase shRNA. *Redox Biol*. 2019;20:236-246.

Can probiotics counteract the detrimental effects of sleep deprivation?



Yadong Zheng, Laura Bonfili, Amina Aboufaires El Alaoui, Michele Bellesi, Anna Maria Eleuteri

School of Biosciences and Veterinary Medicine, University of Camerino, Italy

yadong.zheng@unicam.it

Sleep is a key physiological process in maintaining health and wellbeing and it is considered an important modulator of the immune response. Although sleep exact function is not fully elucidated, an increasing number of studies has focused on the evaluation of the detrimental effects of sleep deprivation. This condition leads to a range of brain deficits, including impaired memory and concentration, and increased risk for neuropsychiatric disorders. The brain responds to sleep loss with rapid and extensive changes at molecular, biochemical, electrophysiological, and structural levels. Research in humans and rodents has repeatedly described low-grade inflammation in association with sleep loss, as reflected by increased inflammatory cytokines and by the presence of other markers of cerebral inflammation and oxidation [1-3]. Moreover, the impact of sleep deprivation on gut microbiota composition and the role of gut dysbiosis in triggering both peripheral and central inflammatory processes have recently emerged [4]. Considering that probiotics have well documented anti-inflammatory and antioxidant properties [5,6] and that supplementation with *Lactobacilli* improved sleep quality [7], the aim of the present study is to evaluate the ability of chronic probiotic treatment to counteract the deleterious effects of insufficient sleep by reducing neuroinflammation and oxidative stress in an animal model of sleep deprivation. In detail, 8-week-old B6129SF2 mice were chronically administered with SLAB51 multi-strain formulation or water. Upon treatment, the animals were sleep deprived for one week and sacrificed. Plasma and brain inflammatory and oxidative markers were measured through immunometric assays. Interestingly, a decreased expression of ionized calcium-binding adaptor molecule 1 (Iba1) and a reduction of pro-inflammatory cytokines, together with decrease of dityrosine and nityrotyrosine residues in the brain of sleep deprived mice treated with probiotics were detected, demonstrating an amelioration of both inflammatory and oxidative status in treated animals. These data strengthen the link between gut-microbiota and sleep quality and support the use of probiotics as a potential aid against the negative consequences of insufficient sleep.

References

- [1] Robert P. Smith, Cole Easson, Sarah M. Lyle. Gut microbiome diversity is associated with sleep physiology in humans. PLOS ONE. 2019;14(10).
- [2] Alkadhi K, Zagaar M, Alhaider I, Salim S, Aleisa A. Neurobiological consequences of sleep deprivation. Curr Neuropharmacol. 2013;11(3):231-49.
- [3] Fatin Atrooz and Samina Salim. Sleep deprivation, oxidative stress and inflammation. Advances in Protein Chemistry and Structural Biology. Volume 119 ISSN 1876-1623.
- [4] Yuanyuan Li, Yanli Hao, Fang Fan, Bin Zhang. The Role of Microbiome in Insomnia, Circadian Disturbance and Depression. Front Psychiatry. 2018;9:669.

- [5] Bonfili L, Cecarini V, Gogoi O, Gong C, Cuccioloni M, Angeletti M, Rossi G, Eleuteri AM. Microbiota modulation as preventative and therapeutic approach in Alzheimer's disease. *FEBS J.* 2021;288(9):2836-2855.
- [6] Bonfili L, Cecarini V, Berardi S, Scarpona S, Suchodolski JS, Nasuti C, Fiorini D, Boarelli MC, Rossi G, Eleuteri AM. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. *Sci Rep.* 2017;7(1):2426.
- [7] Dos Reis Lucena L, Terra Loyola V, Leopoldino de Bortolli C, Levy Andersen M, Tufik S, Hachul H. Effects of Supplementation with *Lactobacillus* Probiotics on Insomnia Treatment. *Altern Ther Health Med.* 2021:AT6040. Epub ahead of print. PMID: 33609341.

ISBN: 978-88-6768-049-8