PHYTOCHEMISTRY OF OLIVE-LEAF EXTRACTS AND MEDICINAL PROPERTIES

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Keywords: phenolic compounds, secoiridoids, health claims, bioactivity, extraction, food, cosmetics, pharmaceutics

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Summary

Epidemiological and *in vivo* research studies highlight the protective effect of the olive tree products against several chronic diseases and their potentially positive effect on human health. As olive oil is the most-known olive tree product and is linked with several health effects, olive leaf extracts have also been extensively studied for health-promoting properties. Olive leaves contain a complex mixture comprising of different bioactive compounds, with the presence of oleuropein, hydroxytyrosol and their derivatives being characteristic for these extracts. There is accumulated evidence that olive leaf extracts may contribute to the reduction of many chronic diseases such as cancer, mellitus diabetes, and cardiovascular diseases. In addition, antioxidant, antimicrobial and antiviral properties of olive leaf extracts are also well-documented in the literature. Hydroxytyrosol, oleuropein and their derivatives are considered as the most active ingredients of olive leaf extracts and associated with remarkable beneficial effects. Bioactive flavonoids and phenolic acids are also present and act synergistically with hydroxytyrosol and oleuropein. The bioactive composition of olive leaf extracts depends on pre-and post-harvest factors as well as the extraction methods. Conventional and modern extraction techniques have been adopted to yield olive leaf extracts that are rich in targeted bioactive compounds. The olive leaf extracts have been successfully used to produce several pharmaceutical, cosmetic and nutraceutical products for diverse purposes. Furthermore, researchers recommend the use of olive leaf extract as antimicrobial and antioxidant agents in the food industry providing novel products and applications. The objective of the present chapter is to present and a critical review the health effects of olive leaf extracts and to correlate them with specific phytochemicals. Furthermore, it summarizes the methods for preparation and potential applications of olive leaf extracts.

1. Introduction

Mediterranean diet has generally been associated with a decreased risk of developing different chronic diseases thereby promoting life expectancy. Olive fruit and its products are considered a substantial element of Mediterranean diet. The disease-preventing effects of olive products are mainly attributed to their unique fatty acid profile and the presence of some bioactive components such as tocopherols, carotenoids, triterpenic acids and polyphenols. In the EU, there is an authenticated health claim that olive oil polyphenols contribute to the protection of blood lipids from oxidative stress based on human studies showing significantly reduced levels of oxidized LDL in plasma after virgin olive oil consumption. Apart from studies focused on olive oil, other studies have considered the constituents present in olive leaf extracts, mainly because of the availability and low cost of raw material in the region of the Mediterranean basin. This natural product is advertised as an active ingredient in complementary alternative medicines and nutritional supplements in western countries. Furthermore, studies and clinical trials indicate the protective effects of olive leaf extract against chronic diseases and correlate these effects with the presence of polyphenols. This accumulated evidence leads to the production of many pharmaceutical and nutraceutical supplements and the development of innovative applications for olive leaf extracts.

The present chapter aims to present the beneficial effects of olive leaf extracts giving special attention to clinical trials. In an attempt to review critically the health-promoting effects of olive leaf extracts, first the bioactive composition is presented, followed by specific effects on human health. Important health effects such as cancer, cardiovascular disorders, mellitus diabetes, antimicrobial activity, antiviral activity and antioxidant activity are reviewed. Taking into consideration the fact that the bioactivity of olive leaf extracts is strongly correlated with their polyphenolic composition, the impact of extraction techniques and parameters is also discussed. Finally, the last section of the chapter is devoted to the possible potential applications in the pharmaceutical, cosmetic and food industries.

2. Bioactive Chemical Components in Olive Leaf Extracts

The olive leaf extracts contain significant amounts of phytochemicals, which are correlated with an array of health effects. Pre-and post-harvest factors influence the bioactive compound contents in olive leaves. More specifically, cultivar, maturity stage, geographical origin, agricultural practices, climate and tree age are significant factors for the accumulation of phytochemicals in olive leaves. The drying and extraction methods also have a significant effect on the qualitative and quantitative composition of olive leaf extracts. Polyphenols are the most well-known group of bioactive compounds in olive leaf extracts. They are a group of heterogeneous compounds that refer to naturally occurring secondary metabolites largely found in the plant kingdom. Their molecular structure is based on a linkage between hydroxyl groups (-OH) and one aromatic ring. Methoxy groups ($-OCH_3$), carboxyl groups (-COOH), and glucosides are often linked to the aromatic ring. The oleuropein and hydroxytyrosol are the most characteristic phenolic compounds in olive leaf extracts. More specifically, olive leaf

polyphenols can be categorized into four major subgroups: (i) secoiridoids, (ii) phenolic alcohols (iii) phenolic acids and (iv) flavonoids.

The secoiridoids are biosynthesized with the coupling of phenylpropanoid and mevalonic acid pathways. Oleuropein is an ester of elenolic acid and hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and is responsible for the bitter taste of olive leaf and green olives. Oleuropein is the most abundant secoiridoid in olive leaf extracts as its content can reach up to 14% in weight. It is noteworthy that oleuropein is not usually found in olive oil due to its hydrolysis and hydrophilicity. However, some studies reported the oleuropein content in olive oil ranges between 0.005% and 0.12%. Olive leaf extracts are also a rich reservoir of oleuropein derivatives as oleuropein aglycone, demethyloleuropein, oleuroside and oleuropein-aglycone di-aldehyde. Ligstroside and its derivatives are also found in olive leaf extracts. As Figure 1 demonstrates a hydroxyl group in an aromatic ring is the difference between oleuropein and ligstroside. Tyrosol and hydroxytyrosol are also important components of olive leaf extracts and belong to the group of phenolic alcohols. They are produced by the hydrolysis of oleuropein. Vanillin, a phenolic aldehyde, is also present in olive leaf extracts at low concentrations.

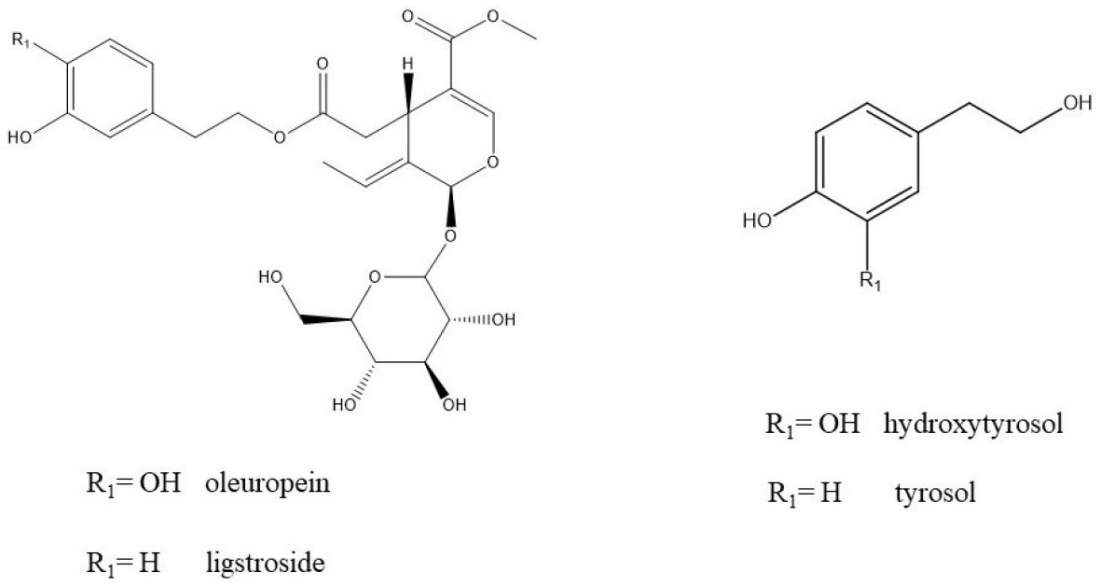

Figure 1. Structures of most common secoiridoids and phenol alcohols present in olive leaf extracts.

Phenolic acids found in olive leaf extracts are derivatives of hydroxycinnamic, and hydroxybenzoic acids (Figure 2). The most abundant phenolic acids in olive fruits are caffeic acid, 5-caffeoylquinic acid, p-coumaric acid, gallic acid, and vanillic acid. Furthermore, the presence of verbascoside, a derivative of hydroxycinnamic acid, in olive leaf extract has been reported. In addition, olive leaf extracts comprise flavonoids, which are widely distributed in nature. Olive leaf extracts mainly contain flavone glycosides; apigenin, diosmetin, kaempferol, luteolin, and quercetin are conjugated with glucose and rutinose and play a critical role in the bioactivity of olive leaf extracts. The presence of other flavonoids such as catechin and hesperidin has been reported less often.

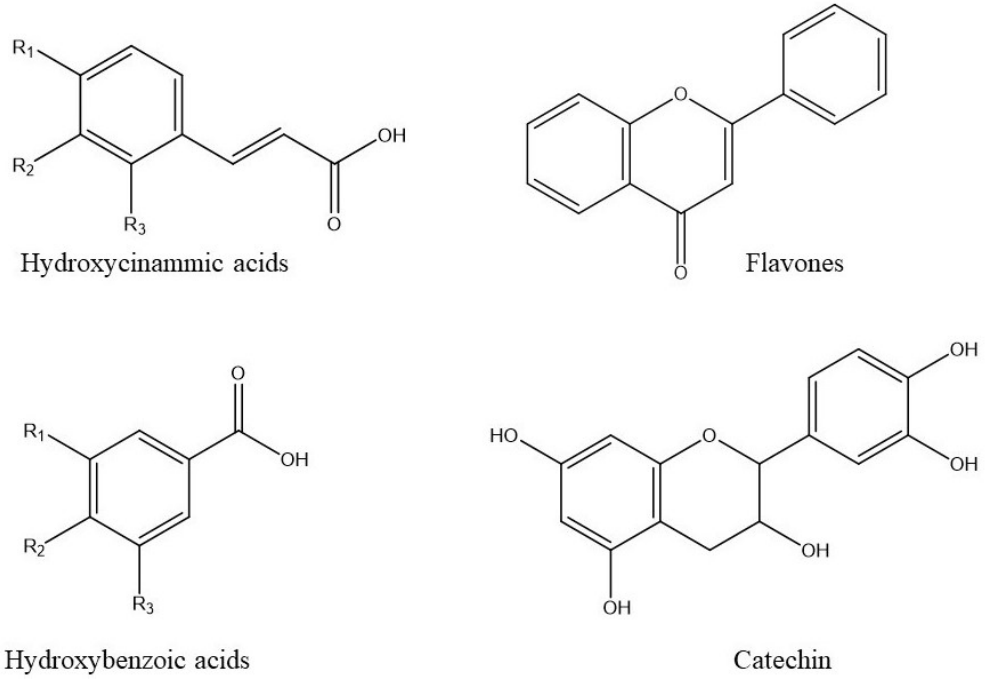

Figure 2. Typical structure of phenolic acids and flavonoids

Triterpenic acids are another group of phytochemicals in olive leaf extracts. From a chemical view, they are pentacyclic terpene acids present diverse biological activities (Figure 3). The most abundant triterpenic acid is oleanolic acid, which represents up to 79–89%, followed by maslinic acid with 14–20% of triterpenic acid content. Ursolic acid, uvaol and erythrodiol have also been identified in olive leaf extracts.

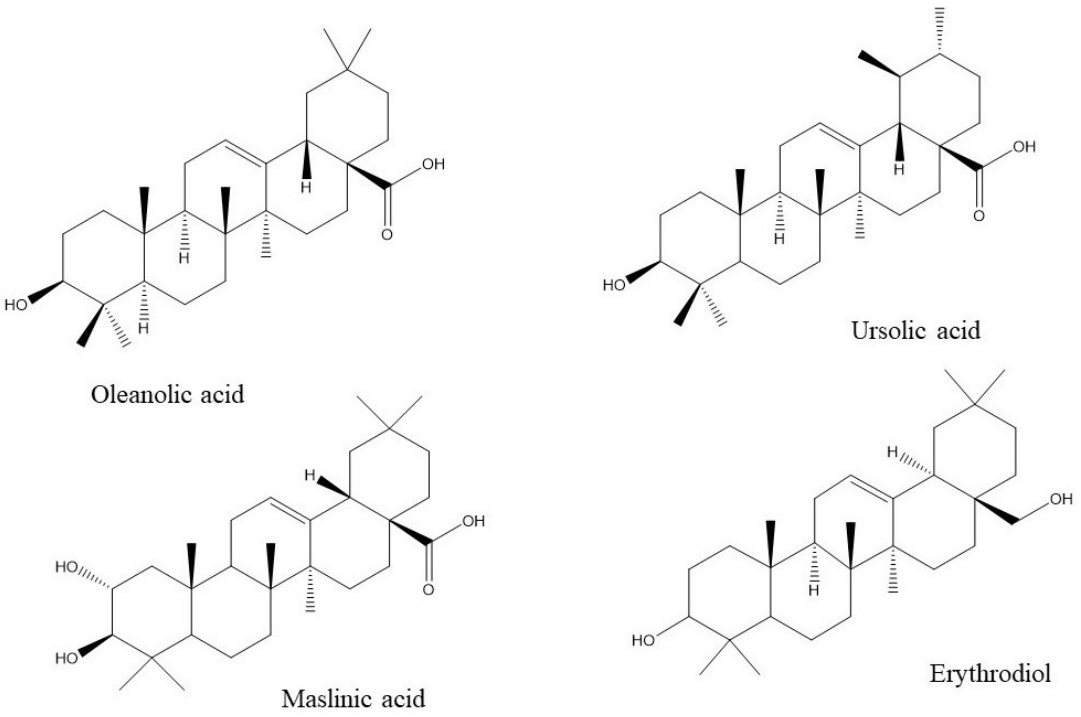

Figure 3. Structures of most abundant triterpenic acids present in olive leaf extracts

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Bibliography

Aouidi, F., Okba, A., Hamdi, M. (2017). Valorization of functional properties of extract and powder of olive leaves in raw and cooked minced beef meat. *Journal of the Science of Food and Agriculture*, 97(10), 3195–3203. [This article presents the potential of olive leaf extract as inhibitor of lipid oxidation in meat products]

Bao, J., Zhang, D. W., Zhang, J. Z. H., Huang, P. L., Huang, P. L., Lee-Huang, S. (2007). Computational study of bindings of olive leaf extract (OLE) to HIV-1 fusion protein gp41. *FEBS Letters*, 581(14), 2737–2742. [This work discusses the antiviral potency of olive leaf extracts]

Barbouti, A., Kanavaros, P., Kitsoulis, P., Goulas, V., Galaris, D. (2020). Olive oil–contained phenolic compounds protect cells against H_2O_2-induced damage and modulate redox signaling by chelating intracellular labile iron. In *Olives and Olive Oil in Health and Disease Prevention*. Eds. Preedy, V., Watson, R. Academic Press. [This book chapter explains the cellular antioxidant activity of olive polyphenols]

Borjan, D., Leitgeb, M., Knez, Ž., Hrnčič, M. K. (2020). Microbiological and antioxidant activity of phenolic compounds in olive leaf extract. *Molecules*, 25(24). [Microbiological and antioxidant activities of olive leaf polyphenols are demonstrated]

Boss, A., Bishop, K. S., Marlow, G., Barnett, M. P. G., and Ferguson, L. R. (2016). Evidence to support the anti-cancer effect of olive leaf extract and future directions. *Nutrients*, 8(8). [This review summarizes the anticancer activity of olive leaf extracts]

Celano, M., Maggisano, V., Lepore, S. M., Russo, D., Bulotta, S. (2019). Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: A critical analysis of experimental studies. *Pharmacological Research*, 142, 77–86. [The anticancer activity of secoiridoids is demonstrated]

da Rosa, G. S., Vanga, S. K., Gariepy, Y., Raghavan, V. (2020). Development of Biodegradable Films with Improved Antioxidant Properties Based on the Addition of Carrageenan Containing Olive Leaf Extract for Food Packaging Applications. *Journal of Polymers and the Environment*, 28(1), 123–130. [This research article indicates the utilization of olive leaf extracts to develop food packaging materials]

de Bock, M., Derraik, J. G. B., Brennan, C. M., Biggs, J. B., Morgan, P. E., Hodgkinson, S. C., Hofman, P. L., Cutfield, W. S. (2013). Olive (*Olea europaea* L.) Leaf Polyphenols Improve Insulin Sensitivity in Middle-Aged Overweight Men: A Randomized, Placebo-Controlled, Crossover Trial. *PLoS ONE*, 8(3). [This study presents a clinical trial for antidiabetic potency of olive leaf extracts]

Difonzo, G., Pasqualone, A., Silletti, R., Cosmai, L., Summo, C., Paradiso, V. M., Caponio, F. (2018). Use of olive leaf extract to reduce lipid oxidation of baked snacks. *Food Research International*, 108, 48–56. [Uses of olive leaf extract in food industry]

El, S.N., Karakaya, S. (2009). Olive tree (*Olea europaea*) leaves: Potential beneficial effects on human health. *Nutrition Reviews,* 67, 632-638. [This review summarizes the oleuropein content in olive leaf extracts]

Flamminii, F., Di Mattia, C. D., Nardella, M., Chiarini, M., Valbonetti, L., Neri, L., Difonzo, G., Pittia, P. (2020). Structuring alginate beads with different biopolymers for the development of functional

ingredients loaded with olive leaves phenolic extract. *Food Hydrocolloids*, 108, 105849. [Novel food packaging materials are demonstrated]

González-Hedström, D., García-Villalón, Á. L., Amor, S., de la Fuente-Fernández, M., Almodóvar, P., Prodanov, M., Priego, T., Martín, A. I., Inarejos-García, A. M., Granado, M. (2021). Olive leaf extract supplementation improves the vascular and metabolic alterations associated with aging in Wistar rats. *Scientific Reports*, 11(1), 1–16. [Protective effects of olive leaf extracts against chronic diseases are reported]

Goulas, V., Exarchou, V., Troganis, A. N., Psomiadou, E., Fotsis, T., Briasoulis, E., Gerothanassis, I. P. (2009). Phytochemicals in olive-leaf extracts and their antiproliferative activity against cancer and endothelial cells. *Molecular Nutrition and Food Research*, 53(5). [This work demonstrates the bioactive composition and anticancer activity of olive leaf extracts]

Hermans, M. P., Lempereur, P., Salembier, J. P., Maes, N., Albert, A., Jansen, O., and Pincemail, J. (2020). Supplementation effect of a combination of olive (*Olea europea* L.) leaf and fruit extracts in the clinical management of hypertension and metabolic syndrome. *Antioxidants*, 9(9), 1–15. [Protective effects of olive leaf extracts against chronic diseases are reported]

Jolayemi, O. S., Stranges, N., Flamminii, F., Casiraghi, E., Alamprese, C. (2021). Influence of free and encapsulated olive leaf phenolic extract on the storage stability of single and double emulsion salad dressings. *Food and Bioprocess Technology*, 14(1), 93–105. [Potential use of olive leaf extracts in food emulsions]

Khemakhem, I., Fuentes, A., Lerma-García, M. J., Ayadi, M. A., Bouaziz, M., Barat, J. M. (2019). Olive leaf extracts for shelf life extension of salmon burgers. *Food Science and Technology International*, 25(2), 91–100. [Olive leaf extracts as food additive]

Liu, Y., McKeever, L. C., Malik, N. S. A. (2017). Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. *Frontiers in Microbiology*, 8, 1–8. [The article presents the antimicrobial activity of olive leaf extract]

Lockyer, S., Corona, G., Yaqoob, P., Spencer, J. P. E., Rowland, I. (2015). Secoiridoids delivered as olive leaf extract induce acute improvements in human vascular function and reduction of an inflammatory cytokine: A randomised, double-blind, placebo-controlled, cross-over trial. *British Journal of Nutrition*, 114(1), 75–83. [A clinical trial for protective effect of olive leaf extract against cardiovascular disorders]

Ma, S. C., He, Z. D., Deng, X. L., But, P. P. H., Ooi, V. E. C., Xu, H. X., Lee, S. H. S., Lee, S. F. (2001). *In vitro* evaluation of secoiridoid glucosides from the fruits of *Ligustrum lucidum* as antiviral agents. *Chemical and Pharmaceutical Bulletin*, 49(11), 1471–1473. [The antiviral activity of secoiridoids is discussed]

Markhali, F. S., Teixeira, J. A., Rocha, C. M. R. (2020). Olive tree leaves-A source of valuable active compounds. *Processes*, 8(9). [This review demonstrates the polyphenolic composition and extraction methods]

Mohammadi, A., Jafari, S. M., Esfanjani, A. F., Akhavan, S. (2016). Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil. *Food Chemistry*, 190, 513–519. [The ability of olive leaf extract to improve the oxidative stability]

Sánchez-Gutiérrez, M., Bascón-Villegas, I., Rodríguez, A., Pérez-Rodríguez, F., Fernández-Prior, Á., Rosal, A., Carrasco, E. (2021). Valorisation of *Olea europaea* L. Olive Leaves through the Evaluation of Their Extracts: Antioxidant and Antimicrobial Activity. *Foods*, 10(5), 966. [This work describes the bioactivity of olive leaf extracts]

Shalaby, A. R., Anwar, M. M., Sallam, E. M. (2018). Improving quality and shelf-life of minced beef using irradiated olive leaf extract. *Journal of Food Processing and Preservation*, 42(11), 1–10. [The use of olive leaf extracts as antioxidant additive in foods]

Sudjana, A. N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T. V., Hammer, K. A. (2009). Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. *International Journal of Antimicrobial Agents*, 33(5), 461–463. [This study explores the antimicrobial properties of olive leaf extracts]

Testa, B., Lombardi, S. J., Macciola, E., Succi, M., Tremonte, P., Iorizzo, M. (2019). Efficacy of olive leaf extract (*Olea europaea* L. cv Gentile di Larino) in marinated anchovies (*Engraulis encrasicolus*, L.) process. *Heliyon*, 5(5), e01727. [Antioxidant and antimicrobial potency of olive leaf extracts in food science]

Toulabi, T., Delfan, B., Rashidipour, M., Yarahmadi, S., Ravanshad, F., Javanbakht, A., Almasian, M. (2021). The efficacy of olive leaf extract on healing herpes simplex virus labialis: A randomized double-blind study. *Explore*, 000. [The clinical trial highlights the antiviral activity of olive leaf extract]

Wani, T.A, Masoodi, F.A., Gani, A., Baba, W.N., Rahmanian, N., Akhter, R., Wani, I.A., Ahmad, M. (2018). Olive oil and its principal bioactive compound: Hydroxytyrosol–A review of the recent literature. *Trends in Food Science and Technology,* 77*,* 77-90. [This review summarizes the oleuropein content in olive oils]

Zemheri-Navruz, F., Acar, Ü., and Yılmaz, S. (2020). Dietary supplementation of olive leaf extract enhances growth performance, digestive enzyme activity and growth-related genes expression in common carp *Cyprinus carpio*. *General and Comparative Endocrinology*, 296. [The study describes the benefits of the use of olive leaf extracts as feed for fishes]

Zemheri-Navruz, F., Acar, Ü., Yılmaz, S. (2019). Dietary supplementation of olive leaf extract increases haematological, serum biochemical parameters and immune related genes expression level in common carp (Cyprinus carpio) juveniles. *Fish and Shellfish Immunology*, 89, 672–676. [The impact of olive leaf extract on fish nutrition is discussed]

Zribi, A., Gargouri, B., Jabeur, H., Rebaï, A., Abdelhedi, R., Bouaziz, M. (2013). Enrichment of pan-frying refined oils with olive leaf phenolic-rich extract to extend the usage life. *European Journal of Lipid Science and Technology*, 115(12). [This study reports the valorization of olive leaf extracts to prevent lipid oxidation in vegetable oils]

Biographical Sketch

Vlasios Goulas received his bachelor degree from the University of Ioannina, Department of Chemistry, majoring in the field of 'Food Chemistry'. He continued his post-graduate studies in the same University. He was awarded his PhD degree in 2009 after completing his thesis entitled 'Development of hyphenated LC-NMR techniques for the exploitation of bioactive components in nature products'. Subsequently, he worked for two years as post-doc research fellow in Cyprus University of Technology/ Department of Agricultural Sciences, Biotechnology and Food Science. and currently holds an academic position at the same Institution. Dr Goulas performs research with state-of-the-art equipment in the field of natural products analysis and processing. Particular attention is given in three areas of specific expertise: (i) elucidation of the composition of natural products and plant origin foods (ii) discovering new bioactive compounds and/or extracts for industry, and (iii) the impact of processing on stability of bioactive compounds. To date, he has been the author of 44 research papers and 7 review papers (h-index =19) in peer-reviewed journals and three book chapters.